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Abstracts of the 3rd International & 15th Iranian Genetics Congress

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The Scientific Responsibilities of Abstracts and Author’s Details Rest Upon Authors and The 3rd International and 15th National Genetics Congress Committees.
I am so proud to serve once again as the scientific secretary in 3rd international and 15th Iranian Genetics congress. Rapid advances in the field of Genetics and related disciplines of sciences have been amazing and unimaginable in recent years which is known as post-genomics era. Decoding the human genome is by sure the most important scientific achievement of human being. However, this accomplishment did not stop there, but proceeded towards development of technologies used in next generation sequencing (NGS). NGS not only opened the way for decoding the genomes of other creatures, but also made it possible to decode the genomes of extinct species, including our close relatives (Neanderthals and Denisovans). Identification of various disease-causing genes, developing medicinal biotechnology, expanding bioinformatics applications, production of transgenic plants and animals, personalized medicine, and genome editing by means of CRISPR-Cas are the most important cutting-edge researches nowadays. In this context, it is necessary to work harder and to draw a new road map for Genetics progress in our country, coordinated by the rapid progress of Genetics worldwide. In order to achieve this goal, we are following two major strategies in recent congress. 1- To create a network of genetic researchers and students nation-wide to coordinate the education and research activities in different universities and research institutions. Creating the provincial cores of Genetics Society of Iran was the first step to achieve this aim. 2- Expanding international academic cooperation. Holding up the current meeting as a joint program with German Society of Genetics is a novel feature of the current congress. On the sidelines of this cooperation, we hope to expand and develop scientific collaborations with the European Union. Finally, I hope that the 3rd international and 15th national congress of Iranian genetics provides a dynamic and productive environment to exchange a variety of opinions, and experiences among students and researchers.

With best regards,
Seyed Javad Mowla
Vice-President and Co-Scientific Secretary of the 3rd International and 15th Iranian Genetics Congress
Animal Genetics

O-1: Investigation of molecular evolution and epidemiological links related to circulating Avian Avulavirus 1 isolates between 1995 and 2016 in Iran

Mayahi V, Esmaelizad M

Central Laboratory Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Alborz, IRAN.
vafamayahi@yahoo.com

Evolution of Avian Avulavirus 1 isolates and emergence of genetic variants are related to different agents i.e., host adaptation, immune response evasion and selective pressures. Hence, investigation of epidemiological links between circulating viruses in different regions and their evolutionary relatedness are of the major issues for biosecurity strategies, yet many bottlenecks remain unsolved. In this study, we have conducted molecular investigation of circulating Avian Avulavirus 1 isolates in Iran (1995-2016) based on virus classification methods. On the basis of evolutionary divergences, sub-genotype Vl9, Vlj, VIIj, VIIId, XIIa and XIIIid isolates have been circulated in the Iran during 21-year period. Data analysis revealed that Vl9 isolates shared highest similarity to Vlj sub-genotype isolates (i.e., Russian and Polish viruses). The lowest difference of Vlj sub-genotype isolates (2012) was from a virus isolated in 2015 in Iran. Furthermore, evolutionary divergences indicated that Chinese and Ukrainian viruses may have played crucial role in emergence of VIIj isolates. Our study also showed that XIIa isolates circulating in Iran may have resulted in the emergence of adapted variants known as XIIIid sub-genotype isolates. Here, we suggest the evolutionary and epidemiological study of virulent Avian Avulavirus 1 isolates could help providing accurate molecular data in the region, and result in designing more efficient recombinant vaccines.

Keywords: Avian Avulavirus 1, Epidemiology, Newcastle disease virus, Fusion protein, Classification, Evolution

O-2: A Novel miRNA Located in the GATA4 Gene, Targets the Cardiac Master Regulator MESP1

Medlej A†, Mohammad Soltani B‡, Mowla SJ†, Baharvand H‡

1. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
abdullahmedlej@gmail.com

GATA4 is an important member of the GATA family of zinc-finger transcription factors. It plays various roles in cardiac specification, differentiation and morphogenesis through binding to cardiac super-enhancers and promoting cardiac gene expression. MicroRNAs are single-stranded, small (20-24 nt) non-coding RNAs that regulate the expression of most protein-coding genes post-transcriptionally. MiRNAs control important cellular processes such as proliferation, differentiation, apoptosis, and tumorigenesis. In the current study, we scanned the GATA4 gene using various bioinformatic tools to search for novel miRNAs. From the distinct stem-loop structures that we obtained, we selected the one that has the best scores as an important miRNA candidate. We detected the endogenous expression of the mature candidate miRNA in cardiac samples and confirmed our results by sequencing. Additionally, overexpression of the stem-loop sequence resulted in upregulation of the mature miRNA form named GATA4-miRNA1. The expression pattern of GATA4-miRNA1 showed a minimal expression at the beginning and after the sixth day of in vitro cardiomyocyte differentiation, while it exhibits a maximal expression from day two to four. Using bioinformatic tools, we predicted MESP1, a master regulator of cardiomyogenesis, as a potential target of GATA4-miRNA1. The overexpression of GATA4-miRNA1 in SW480 cells resulted in downregulation of MESP1 at the transcript level. The discovery of GATA4-miRNA1 introduces a novel miRNA that has important regulatory effects during cardiomyogenesis.

Human Genetics and Medicine

O-3: Genotype-phenotype correlations among Iranian patients with cystic fibrosis: Clinical manifestations of CFTR mutations.


1. Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
2. Department of Genetics and Biotechnology, School of Biological Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
3. Department of Pediatrics, Shahid Beheshti University of Medical Sciences, Tehran, Iran
E-mail: Sama.abbasib66@yahoo.com

Cystic fibrosis (CF) is a highly lethal genetic disorder characterized by clinical symptoms such as pulmonary complications, pancreatic deficiency, and abnormal electrolytes in sweat. The type of mutations varies between different ethnicities and geographic regions. The aimed of this study is to clarify the correlations between the type of gene mutations (genotype) and the manifestation of clinical symptoms (phenotype) in Iranian CF patients. Methods: Our study included 21 CF patients and their parents referred to the Noor Human Genetics Center. All the participants had the clinical symptoms and positive sweat test (>60mmol/l); however, six cases had borderline sweat test. Therefore, the mutations described were identified by performing direct sequencing analysis of the complete coding regions and flanking intronic sequences of the CFTR gene. Results: The samples belonged to 21 unrelated families (13 male and 8 female). Noticeably, 42.85% of families had consanguine marriages. Fifteen
CFTR mutations were recognized from different Iranian races in 21 patients, including: c.2051_2052delAAinsG, c.2988+1G>A, c.1000C>T, c.3484C>T, c.1521_1523delCTT, c.744-9_744-6 del GATT, c.4389G>A, c.254G>A, c.50delIT, c.358G>A, c.2856G>C, c.1647T>G, c.2657+5G>A, c.1542_1543delAT, c.3909C>G, c.1397C>G, and c.1210-12_1210-11insGT [5T, TG12]. Depending on the mutation type, the clinical manifestations varied from a very mild phenotype (only pancreatic deficiency) to severe forms such as acute pulmonary disorders and Meconium ileus. Conclusions: Based on the literature review, it was confirmed that genotype-phenotype correlations are particularly exist for pancreatic deficiency of the Iranian patients with cystic fibrosis. Taken together, our findings demonstrated a various and complicated association between the CFTR genotype and the clinical symptoms.

Keywords: Cystic Fibrosis- CFTR- IRAN

O-4: Investigation on the mir-133, mir-199 expressions and potential target genes expression of autophagy pathway genes such as FIP200, ATG13 in breast cancer patients

Ahmadi S, safari M, Teimori H.

1. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Rahmatiyeh, Shahrekord, Iran
2. PhD Student of Medical Genetics, Department of Medical Genetics, Faculty of medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Rahmatiyeh, Shahrekord, Iran

E-mail: saman.ahmadi_89@yahoo.com

Autophagy, a highly conserved self-digestion process, is one of the mechanisms that may play a dual role in the development or inhibition of cancer. Different signaling pathways have been involved in the up regulation or down regulation of autophagy. MicroRNAs are small noncoding RNAs that have important roles in regulation of gene expression. This study evaluated the expression levels of mir-133 and mir-199 and potential target genes expression of autophagy pathway such as FIP200 and ATG13 in breast cancer patients. Methods: this case-control study was performed in 47 cancerous tissue samples and paired matched adjacent non-cancerous tissues were obtained from breast cancer patients. The samples were collected with ethical principles of Imam Khomeini hospital cancer institute using American Joint Committee on Cancer (AJCC) guideline. After RNA extraction, cDNA synthesis and qRT-PCR, the expression levels of FIP200, ATG13 and mir-133, mir-199 was measured and results were confirmed by western blot. Result: our results have shown that the expression levels of FIP and ATG13 decreased in tumor samples versus non-tumor samples. Also, irrelevance of mir-133, mir-199 expression have been shown by qRT-PCR. Conclusion: We suggested that mir-133, mir-199 play pivotal roles in regulation of FIP200 and ATG13 that involve in autophagy process. Accordingly, it can be said that mir-133, mir-199 expression levels can be used as novel biomarkers for progression of breast cancer.

Keywords: Breast cancer, Autophagy, mir-133, mir-199, FIP200, ATG13

O-5: Evaluation of MBP, TCF4, EGR1 genes expression and methylation in patients with schizophrenia and its psychopathology, intelligence and cognitive impairments

Alizadeh F1, Shahsavand Ananloo E2, Bozorgmehr A3, Tavakkoly-Bazzaz J1
1. Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran
2. Department of Genomic Psychiatry and Behavioral Genomics (DGPG), Roozbeh Hospital, School of Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran
3. Department of Neuroscience, Faculty of Advanced technologies in Medicine, Iran University of Medical Sciences (IUMS), Tehran, Iran

f35552a@gmail.com

Schizophrenia (SCZ), with a prevalence of 0.5 to 1 percent among the population, is considered as one of the most serious and debilitating mental disorders. The diagnosis of SCZ is essentially based on phenotypic examinations and trying to find the most reliable biomarkers is still going on. In this study, we evaluated the association of MBP, TCF4 and EGR1 genes mRNA level in peripheral blood with psychopathology, cognitive and intellectual impairments of Iranian SCZ patients. 70 unmedicated schizophrenia patients were selected as the case group, alongside with 72 healthy subjects as the control group. Using real-time PCR, MBP, TCF4 and EGR1 mRNA levels were compared between the case and the control groups. Additionally, all subjects were assessed in terms of psychopathology cognitive and intelligence abilities, using PANSS, WMS, Stroop and WCST measurements.

Our results revealed that the MBP mRNA level is significantly higher and TCF4 mRNA level is significantly lower in the case group. Moreover, it was found that patients have a weaker function in all psychopathology, cognitive and intellectual assessments. Further analysis showed that MBP mRNA level is negatively correlated with scores of WAIS and WMS, and is positively correlated with Stroop and WCST errors and PANSS score.

In conclusion, the mRNA level of MBP and TCF4 seems to be associated with SCZ, its psychopathology and intellectual and cognitive impairments in Iranian patients. A correlation between reduction in TCF4 expression and DNA hyper methylation of the TCF4 promoter in SCZ patients suggests that an epigenetically defined hypo-activity of TCF4 may be linked to SCZ pathogenesis. Furthermore, this epigenetic mark in DNA extracted blood can be considered as one of the key determinants in a panel of diagnostic and/or therapeutic biomarkers for SCZ.

Keywords: Schizophrenia, TCF4, MBP, PANSS, psychopathology, Methylation

O-6: MicroRNA_143 inhibits cell migration and invasion of lung cancer cell line
Asghari azar V1,2, Baradaran B1, Sakhinia E1

1. Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
2. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
E-mail: vahid.asghariasar@yahoo.com

Background: MicroRNAs (miRNAs) are an extensive family of small (~24 nucleotide), endogenous, single-stranded non-coding RNAs which target the 3’-untranslated region (3’-UTR) of specific mRNAs to promote their degradation or repression of translation. miRNAs regulate important cellular processes such as proliferation, apoptosis, mobility, cell cycle progression, and differentiation, and their altered expression is associated with various cancers. Among those miRNAs, miR-143 shows tumor-suppressive activity in some human cancers. Lung cancer is the leading cause of cancer deaths worldwide and metastasis is the major cause of death in lung cancer patients.

Methods: Human lung cancer cells (A549) were cultured at 37 °C in 5% CO2 with RPMI 1640 media supplemented with 10% fetal bovine serum (FBS). Transient transfection of miRNA precursors or inhibitors was carried out using Lipofectamine 2000 according to the manufacturer’s protocol. Migration of human lung cancer cells in culture was determined by the “scratch” assay. For this, cells were seeded into a six well tissue culture dish. Cells in monolayers were scratched in a single straight line using a pipette tip. The migratory distance was measured under a microscope equipped with a camera.

Results: Intrinsic miR-143 expression was significantly decreased in lung cancer cells compared to non-cancerous epithelial cells. Restoration of miR-143 led to inhibit migration and invasion of human lung cancer cells. These data suggest that miR-143 suppress pathways relevant to tumorigenicity and cancer progression.

Conclusion: The existing experimental evidence suggests that it is worth testing MiRna-143 as a cancer therapeutic agent since the results of this study demonstrate that MiRna-143 has the ability to inhibit migration and invasion of human lung cancer cells.

Keywords: Lung cancer, MicroRNAs, MiRna-143, Scratch assay

O-7: Frequency of polymorphisms of CYP2C9 and VKORG1 genes in warfarin user

Bagheri Moghadam M, Soveizi M, Amiri F, Maleki M, Mahdieh N

Genetic Reaserch laboratory, Rajaie Cardiovascular Medical and Reaserch Center, Iran University of Medical Sciences, Tehran, Iran
E-mail: mahi.bagheri@gmail.com

Warfarin is usually prescribed for preventing thrombosis especially in patients with cardiovascular disease. Different drug-responses are observed among patients. Some genetic variations have been described to be involved responses of individuals to drug. In this study, the prevalence of common variants of CYP2C9 and VKORG1 genes is investigated. Materials and Methods: A total of 58 people with cardiovascular disease who treated by warfarin were recruited in this study. Clinical features and the history of drug usage were documented. DNA was extracted from peripheral blood samples. PCR-RFLP was performed for detecting common variants. Results: The mean age of patients was 41 years, ranging from 3 years to 80 years. The CYP2C9 variant gene was observed in 27 individuals. The heterozygous variant had a high frequency. The VKORG1 variant gene was observed in 22 individuals. It was found homozygously in 12 patients. Discussion and Conclusion: Environmental and genetic factors may have a significant effect on treatment; therefore, the study of genetic variations could be helpful to determine the dose of warfarin. Keywords: Warfarin-CYP2C9-VKORG1-Genotype-PCR-RFLP

O-8: Long noncoding RNA, ANCR, is upregulated in esophageal squamous cell carcinoma


1. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Cardiogenetic research lab, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
3. Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran
4. Tehran University of Medical Sciences, Tehran, Iran
E-mail: b119.balalei@yahoo.com

Long noncoding RNAs (lncRNAs) are a major class of RNA molecules with emerging roles in stem cell pluripotency, cellular reprogramming, cellular transformation, and tumorigenesis. Anti-differentiation non-coding RNA, ANCR, is suppressed upon terminal differentiation of multiple cell types. The broad expression pattern of ANCR, in combination with its reduced expression levels in a number of terminally differentiated cell types raised the possibility of a potential functional role for ANCR in the tumorigenesis. At the present study, To identify a potential expression alteration of ANCR during tumorigenesis, we initially have examined the expression pattern of lncRNA-ANCR in esophageal squamous cell carcinoma (ESCC). The quantitative real-time RT-PCR results revealed a significant upregulation of ANCR (P value = 0.0161) in tumor samples of ESCC and in high-grade tumor samples, in comparison to the low-grade ones (P value = 0.0371). Moreover, the presence of amplified fragment of ANCR was detected in serum samples and we found a significant up-regulation of ANCR in serum samples with ESCC compared to normal samples (P value = 0.0392). Furthermore, based on the results of sequencing, We found two variants of ANCR in ESCC samples and KYSE-30 cells. In conclusion, our data suggest a possible role of ANCR in tumorigenesis of esophageal tissues and demonstrate the expression different variants of ANCR in ESCC samples.

Keywords: esophageal squamous cell carcinoma, non-coding RNA, lncRNA-ANCIR, variants
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Bidram M, Asghari SM

Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran
E-mail: S.maryam33@yahoo.com

Angiogenesis is the formation of new blood vessels from the pre-existing ones, involved in malignancy, tumor growth, invasion, and metastasis. This process is mainly regulated by increased activity of angiogenic factors such as vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs) on the surface of endothelial cells. VEGFs, including VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placenta growth factor (PIGF), are selective cytokines, acting exclusively on vascular endothelial cells through VEGF receptors, including VEGFR-1, VEGFR-2, and VEGFR-3. VEGFR1 and VEGFR2 mediated signaling pathways regulate activation PAK (p21-activated kinase) function indirectly, followed by deposition of angiogenic growth factors. PAKs are serine/threonine-specific intracellular protein kinases that are located at the intersection of several signaling pathways that are required for oncogenesis. Overexpression or mutational activation of PAK isoforms frequently occurs in various human tumors. The aim of this study is investigating of PAK expression on the breast cancer bearing model mice in the two groups, PBS and anti-angiogenic peptide-treated mice. The expression level of PAK gene was determined by Real-time PCR using PAK specific primers, and was evaluated the quantitative expression of PAK against GAPDH as the reference. To this end, extraction of total RNA was performed by Trizol reagent (Invitrogen), and subsequently cDNA was synthesized. We found that reduced expression of PAK by blockage of VEGFR was mediated by antagonistic peptide. These findings suggest that PAK expression may be a useful strategy to address the downregulation of angiogenic signals.

Keywords: anti-angiogenic therapy, antagonistic peptide, PAK, Real-time PCR

O-10: Investigating the role of the LncRNA (NR-024058) in colorectal cancer

Bjeije H, Soltani B, Behmanesh M, Nouri Nir B

Dr. Bahram Soltani- Associate professor at Tarbiat Modares University
Dr. Mehrdad Behmanesh- Professor at Tarbiat Modares University
Dr. Babak Nouri Nir-Medical Doctor at Mehrad Hospital
bjeije_hassan@hotmail.com

The multifunctional cytokine transforming growth factor beta (TGFβ) plays a dual contrasting behavior in colorectal cancer. In non-cancerous and premalignant colorectal cells, it exerts tumor-suppressive role by inducing cell-cycle arrest and apoptosis. However, in advanced colorectal cancer cells it promotes tumorigenesis and progression. The molecular mechanism mediated by TGFβ signaling pathway is largely clear, however, the involvement of lncRNAs in TGFβ signaling pathway is still unknown. LncRNAs can act as miRNA sponges causing miRNA inactivation that will indirectly modulate the expression pattern of miRNA target genes. Therefore, lncRNAs orchestrate a complex intracellular signaling network and the identification of their molecular functions has a critical importance. RNA seq data shows that NR-024058 has distinct variations in colorectal cancer samples. Our bioinformatic studies showed that this lncRNA has the ability to sponge three miRNAs (miR-532-5p, miR-323a-3p and miR-105-5p) which are probably interfering with TGF-β signaling. In the current study, we found that NR-024058 overexpression in HCT116 cell line promotes K-Ras and Smad6 upregulation which have been shown to inhibit TGF-β signaling pathway through modulating the activity of smad3/4 complex. Furthermore, NR-024058 overexpression induces upregulation of c-myc and downregulation of p21 genes which are TGF-β downstream target genes. Finally, we concluded that NR-024058 is a potential oncogenic factor that inhibits TGF-β tumor suppressor activity through a crosstalk between TGF-β and MAP Kinase signaling pathways.

Keywords: Colorectal cancer, TGF-Beta, NR-024058, miRNAs

O-11: Investigation of Leukocyte Telomere Length (LTL) in a cohort of Iranian subjects


1. Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
2. General Surgery Department, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran
3. Cardiology Department, Imam Khomeini Complex Hospital, Tehran University of Medical Sciences, Tehran, Iran
4. Cellular and Molecular Research Center, Sabzevar University of Medical Sciences, Sabzevar, Iran
5. Department of Biochemistry, Genetic and Nutrition, Faculty of Medicine, Alborz University of Medical Sciences, Karaj, Iran
E-mail: shima.dehdas@gmail.com

We investigated relative LTL in a cohort of 334 subjects (17-85 years, 69% women) from different ethnicities in Iran. The cohort included 127 unrelated nonsmoker healthy subjects (73% female, 78% Persian) that were clinically examined to have no sign of chronic infections, cancers, or cardiovascular diseases, and lived at least 10 years in their current city. We also studied 53 morbidly obese subjects, and 154 samples with unknown health condition from Genetics Research Center DNA bank. All samples quantified against a 5 point standard curve using a mixture of 5 genomic DNA with different chronological ages as reference DNA. Quantitation was done based on relative standard curve using standards to determine the relative quantity of telomere for each sample. The ratio of telomere to single copy gene was calculated and normal- ized by this ratio for K562 genomic DNA from the same plate. Primary analysis of 127 healthy subjects showed that telomere length decreased as age increased, women had longer telomeres than men (p-value = 0.033), and individuals from Persian ethnicity had longer telomeres than other ethnicities.
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(O-12): A Novel Mutation in the OFD1 Gene Causes Oral-Facial-Digital Syndrome Type 1 in an Iranian Family

Dehghan Tezerjani M, Maroofian R, Vahidi Mehrjardi M.Y., Dehghani M.R

Medical Genetics Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
E-mail: mmvahidi@yahoo.com

Oral-facial-digital syndrome as heterogeneous developmental conditions is characterized by abnormalities in the oral cavity, facial features and digits. Furthermore, central nervous system (CNS) abnormalities can also be part of this developmental disorder. At least 13 forms of OFDS based on their pattern of signs and symptoms have been identified so far. Type 1 which is now considered to be a ciliopathy accounts for the majority of cases. It is transmitted in an X-linked dominant pattern and caused by mutations in OFD1 gene which can result in embryonic male lethality Case Report: Patient 1 (III-VII) The first patient was a 9-year-old girl with a birth weight of 3200 g who was born to a family with non-consanguineous parents at 37 wk gestation, because of the fourth pregnancy. Facial abnormalities that could be seen in the patient were dolichocephaly, macrocephaly (54.3 cm- 88 percentile), saddle nose deformity, low set ears, downsllant palpebral fissures, and thin hair and eyebrows. Patient 2 (III-III) She had the following abnormalities: dolichocephaly, macrocephaly (51 cm- 66 percentile), multiple and malformed dentition, cleft lip and palate, asymmetric, bifid and lobulated tongue, macroglossia, multiple hyperplastic frenulum, ankyloglossia, low set ears, downsllant palpebral fissures, and thin hair and eyebrows. Mother of the patients (II-IV) The mother of patients 1 and 2 was 29 yr old. She had ah history of five abortions of malformed male fetuses (III-I, III-II, III-IV, III-V and III-VI). The abortions of all male fetuses happened during their third month of pregnancy. She was born with bifid tongue that was surgically repaired. In addition, her dry and thin hair is remarkable Method: We extracted genomic DNAs from the peripheral blood samples using the ReliaPrep® kit (Blood gDNA Miniprep System, Promega). The mutational hotspot within 8 exons of OFD1 (including exons 2, 3, 7, 8, 9, 12, 13 and 16) were amplified based on standard protocols. Primer sequences are available upon request. Then, the study employed 3730 DNA Analyser and BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) for sequencing of the PCR products in both directions.

Results: Our genetic studies identified a novel 2-base pair deletion (c.1964-1965delGA) in exon 16 of OFD1 leading to a frame shift (p.Arg654X) in two patients and their mother. The mutation has not been previously reported, nor is present in The NHLBI Exome Sequencing Project Exome Variant Server (September 2013), Complete Genomics (February 2012), dbSNP (134&“137), 1000 Genomes (May 2012) and Exome Aggregation Consortium (ExAC), Cambridge, MA and as it was expected it was absent in the father and the brother of mother. Conclusions: We identified a novel truncating mutation in OFD1 in three female members of a family displaying variable symptoms and severity of clinical manifestation of OFDS type 1. As observed in previous cases with OFD1, phenotypic variability even within a family is possibly a rule rather than the exception. Hence, this report emphasizes importance as well as the challenges of genetic counseling for OFD1 patients and their relatives. In cases of OFDS, thorough physical examination, collecting the family history and genetic screening of the affected individuals and their female relatives, along with monitoring of renal function are mandatory.

Keywords: OFD1, Oral-facial-digital syndrome, X-linked dominant, Miscarriage

(O-13): Whole exome sequencing identified a novel ALDH5A1 variant associated with SSADH Deficiency in an Iranian family with autism

Fattahi M, Alavi A, Ghasemi Firouzabadi S, Nozari A, Farajzadeh Vaillou S, Karimian J, Crosby A, Behjati F.

1. Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
2. Department of Molecular Genetics, RILD Institute, University of Exeter, Royal Devon and Exeter NHS Hospital, Wonford, Exeter, UK
E-mail: mahshid.fattahi@gmail.com

Next Generation Sequencing with the application of Whole Exome Sequencing (WES) has fundamentally revised the concept of disease etiology and classification, and promisingly proposes novel therapeutic interventions. In Iran, this approach seems to be much more promising, due to the different ethnic background and probably to the involvement of novel genes. Material and methods: We performed WES in 2 sibship with Autism Spectrum Disorder whose parents were first cousins. The main clinical features included prominent expressive language deficit, infantile onset hypotonia, hyporeflexia and severe ataxia. Electrodiagnostic studies were suggestive of flaccid CP, and biochemical studies showed absence of metabolic acidosis with increased glycine concentration in plasma. No specific Metabolic disorder was identified. Results: Having filtered the WES data against genes associated with IEM, a homozygous novel variant was identified in ALDH5A1 which was a GABA Metabolic and Autism associated gene. Co-segregation analysis, furthermore, validated the variant as the causative mutation. Clinical reassessment supported the diagnosis of SSADH deficiency. Conclusion: This study is intended to discuss the impact of molecular genetics on precise diagnosis. In addition, WES is the most favorable and preferred method once approaching single gene disorders in Iranian families because of the distinctness of our genetic background in comparison to...
western countries, as well as the low yield of genetic testing of known pathogenic variants.

Keywords: Whole exome sequencing, Autism spectrum disorder, ALDH5A1 novel mutation, SSADH deficiency

O-14: The first investigation of leukocyte telomere length in Iranian subjects with morbid obesity

Ghorashi T, Safafi S, Larti F, Najmabadi H, Larti F

1. Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
2. General Surgery Department, Firoozgar Hospital, Iran University of Medical Sciences, Tehran, Iran
3. Cardiology Department, Imam Khomeini Complex Hospital, Tehran University of Medical Sciences, Tehran, Iran
E-mail: tghorashi71@gmail.com

Morbid obesity can accelerate normal aging. Leukocyte Telomere Length (LTL) is a biomarker of aging which shows high variability in different ethnicities and has an association with body mass index. In this matched-pairs study, we examined LTL of 53 morbidly obese subjects (18?65 years old, 85% women). Individuals with BMI>40 kg/m2, who were candidates for bariatric surgery, and had a sibling from the same sex with age difference about ±5 years, were selected before surgery. By selecting siblings as controls, most important factors affecting LTL (age, genetic background, ethnicity, and sex) were highly adjusted for cases and controls. All siblings (17?61 years old) were nonsmokers and clinically examined to have no sign of chronic infections, cancers, or cardiovascular diseases. LTL was measured by qPCR, based on relative standard curve method, using a mixture of 5 genomic DNA with different chronological ages as reference DNA, and K562 as quality control. Ratio of telomere to single copy gene (albumin) quantity was calculated and normalized by K562 for each sample. Data analysis showed that LTL was negatively correlated with age in both cases (r=-0.163) and controls (r=-0.157). We compared LTL between 53 cases and 53 completely matched non-obese siblings by paired-samples T-test. Results showed that obese subjects have significantly shorter LTL than non-obese individuals (P value=0.002). According to well matching criteria between obese and non-obese subjects, these results strongly suggest that high body mass index can cause shorter telomeres, and increase the incidence of age related disorders.

Keywords: Leukocyte Telomere Length, Morbid obesity, BMI, qPCR

O-15: Investigation of mutation sites on the type I neurofibromatosis gene that caused the mild, moderate, and severe phenotypes in three non-relative families with NF1

Hajibabaei P, Bazyar R, Ghasemi H, Dastneshan Sh, Behpour S, Vatanmakian N, Tavallaei M, Ghada Sh

1. Department of Genetics and Biotechnology, School of Biological Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
2. Human Genetics Research Center, Baqiyatalah University of Medical Sciences, Tehran, Iran

Medical Sciences, Tehran, Iran.
3. Department of Hematology, Faculty of Allied Medicine, Tehran University of Medical Sciences, Tehran, Iran
E-mail: payamhajibabaei@gmail.com

Neurofibromatosis type 1 is the most common autosomal dominant genetic disorders which is caused by loss-of-function mutations of NF1 gene (OMIM #162200). The most common manifestations of this disorder are cutaneous and subcutaneous neurofibromas, cafe-a-lait and freckling. In the case of severe mutations, bone abnormalities and learning disability are also observed. The aim of the study was to determine the sites and types of mutations associated with neurofibromatosis type 1 in the NF1 gene in three non-relative families with NF1 patients who referred to Noor Human Genetics Research Center (NGRC). Methods: Peripheral blood samples were taken from affected individuals of three families and an informed consent was obtained in all patients. In this research, we used the polymerase chain reaction (PCR) and DNA sequencing survey to characterize the NF1 gene mutations in affected individuals. Results: By direct sequencing revealed a novel heterozygote frame shift mutation (c.1458-1459 del AA) in exon 13 of NF1 gene in affected members of first family with mild phenotype. In second family, we also found a heterozygote frame shift mutation c.1541_1542 del AG in exon 14, which caused severe form of NF1 disorder. In last family, the missense single base substitution in the exon 37 (C.52354G>A, plys1745lys) in one NF1 allele was identified. The mentioned mutation caused a moderate form of NF1 disorder in affected members of third family. Conclusions: This study shows mutation phenotypes that offer a valuable tool for clinicians and diagnostic medical genetics labs to determine disease risks and modify screening programs.

Keywords: NF1, Neurofibromatosis type 1, Genetics, neurofibroma, Iran

O-16: Study of the P53 gene polymorphisms of gastric cancer patients from northern and north-western parts of Iran

Heidary sheikh M, Barzegar A, Zahri S.

1. Department of Biology, Faculty of Sciences, University of Mohaghegh Ardabili, Ardabil, Iran
2. Department of Basic Sciences, Agricultural Sciences and Natural Resources University of Sari, Iran
E-mail: mohamadkazem_hs@yahoo.com

P53 is a tumor suppressor gene that acts as a molecular or cellular protector. Previous reports have revealed P53 gene mutation rates of 0% to 70% in gastric cancer patients of different regions and populations. Gastric cancer is the second most common malignancies worldwide. Hotspot regions of P53 gene are located on exons 5-8, in which more than 90% of point mutations occur in this region. In this study, the single nucleotide polymorphisms of exons 5-8 of P53 gene were investigated in patients with gastric cancer in northern and north western parts of Iran. The endoscopic biopsy specimens were dissected from 30 gastric cancer patients from Aras Gastroenterology clinic at Ardabil’s Imam-Khomeini hospital as well
O-17: Prevalence of common mutations of HFE gene in patients with beta thalassemia in HAMEDAN

Jalilian M, Azizi Jalilian F, Mahdieh N

1. Genetic Research Laboratory, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.
2. Department of Microbiology, Faculty of Medicine, Hamedan University of Medical Sciences, Hamedan, Iran.
E-mail: masoomejalilian@yahoo.com

Hemochromatosis is an autosomal recessive disorder of iron metabolism. The HFE gene in this disorder has been identified on chromosome 6 (6p21.3). HFE gene mutations are the main cause of Hemochromatosis. Thalassemia patients are at risk of hemochromatosis because of blood transfusions. In this study, two common mutations of the HFE gene is presented among thalassemia patients. Material and Methods: In this study, 41 patients with β-thalassemia in Hamadan province were studied. DNA was extracted from blood samples. PCR-RFLP method was used to determine the common variants of HFE gene. Result: p.H63D polymorphism was detected in heterozygously in 8 patients and polymorphism p.C282Y was not seen in the studied patients. Discussion and Conclusion: According to studies, it is likely that in Iran, H63D mutation is common, so their frequency in the healthy population of Iran should also be studied.

Keywords: Beta Thalassemia, Hemochromatosis, HFE, H63D, C282Y.

O-18: Evaluation of common mutations in K-RAS gene in suspected gastric cancer biopsy samples

Jamshid Abbadi Sh, Zolfaghar M, Javid M, Rafiee S, Saber S, Ebrahim A

1. Department of Molecular and Cellular Biology, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran -Iran (IAUPS ).
2. Department of Genetics, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran -Iran (IAUPS).
3. Department of Genetics, University campus2 university of guilan, Rasht, Iran.
4. Yas Human Medical Genetics Laboratory, Tehran, Iran.
E-mail: shahinjamshidabadi@yahoo.com

Gastric cancer is the second most common type of cancers. There are some evidences that show the correlation of genetic changes with the risk of gastric cancer. The mutation of K-ras genes in the lumbar, lung and colon cancer is common. K-RAS gene belongs to the ras family and is in charge of the production of the protein which plays a crucial role in human tissue signaling such as reproduction, differentiation and aging. The purpose of this study was to analyze the pathological data as well as K-ras gene mutation in patients susceptible to gastric cancer through Real Time-PCR and sanger sequencing.

Methods: The samples were collected from 100 patients susceptible to gastric cancer as target group. DNA was extracted using VIOGENE kit. Real-Time PCR was done using Roche Light Cycler 96 and REAL QUALITY K-RAS MuST RI-11-30 kit. The finding mutations by Real Time PCR confirmed by Sanger Sequencing. Results: The mutation was reported on the K-ras gene in 8 biopsy cases, which 5 were at the D12G region and 2 at the V12G region and 1 at the D13G region. Conclusion: The results of the study showed mutations in the K-ras gene in suspected cases of gastric cancer.

Keywords: Gastric cancer, K-RAS, Real-Time PCR, Sanger Sequencing

O-19: Identification of novel genes in ten patients with neuromuscular disease who have no mutations in known genes by reanalyzing data of whole exome sequencing

Javadi Golrodbari F, Alavi A, Kazemi Nasab S, Kahrizi K, Najmabadi H
E-mail: sara.shvh@yahoo.com

Inherited neuromuscular diseases (NMDs) are a heterogeneous group of genetic disorders which affect neurons, nerves, muscles, and/or neuromuscular junctions and present clinical and genetics heterogeneity. Regarding to the numbers and size of NMD-causative genes, molecular diagnosis by direct sequencing is time consuming and expensive. This leads to diagnostic delays. Nowadays, the application of whole exome sequencing (WES) has made significant progress into solving this problem. Here, we used WES for disease-causing gene identification in ten NMD-families with autosomal recessive mode of inheritance and no mutations in known genes. DNAs of the probands were extracted, enriched and sequenced on the Illumina HiSeq2000 platform. Sequence alignment and variant callings were performed. SNP variations with a reported MAF (<1%) in public databases were filtered. Then variants with a distance of +/-2 bp from protein-coding genes and synonymous variants were removed. Subsequently, only variations that were present in the homozygous or compound heterozygous states, and that segregated with disease status, were further considered as candidate causes of disease. Finally, the remaining variants were evaluated by bioinformatics tools. Data analysis led to identification of candidate causal variants for six out of 10 patients (60%). Homozygous mutations in a known gene (CLTCL1)
and six novel causative genes ASPH, KCNE4, BCORL1, MTMR8, SLC12A3, and EXO1 were detected. Variations in BCORL1, MTMR8 genes were detected in one proband and both presented appropriate criteria as disease-causing genes. The present study demonstrated the power of WES for diagnosis and identification of novel causative genes in this group of diseases.

Keywords: Neuromuscular diseases (NMDs), Whole Exome Sequencing (WES), Molecular diagnosis

O-20: Synthesis, characterization and anticancer effect of Gemini-curcumin nanoformulation on human Breast cancer cell lines

Karimpour M1, Hosseinpour Feizi M1, Babaei E1, Mahdavi M1, Najafi F2

1. Department of Biology, School of Natural Science, University of Tabriz, Tabriz, Iran
2. Department of Resin and Additives, Institute for Color Science and Technology, Tehran, Iran
Babaei2539@gmail.com

Introduction: Curcumin is the polyphenolic constituent of turmeric which has been recognized as an effective anticancer agent due to modulation of multiple intracellular signaling pathways. However, the poor bioavailability of curcumin triggers the efforts for improving the cellular stability of curcumin. To overcome this drawback, we used Gemini Surfactant biodegradable nanocarrier and studied its anticancer effect on breast cancer cells.

Materials and Methods: Gemini-curcumin polymersomes (Gs-Cu) were synthesized through nanoprecipitation method and their physicochemical properties were determined using DLS and SEM techniques. Subsequently, the anticancer effect of Gs-Cu nanoparticles was examined on three different breast cancer cell lines including MCF-7, SkBr-3 and MDA-MB-231 through uptake kinetics, MTT, Hoechst staining assays as well as Cell cycle distribution and effector caspases activity analysis. Furthermore, qRT-PCR was performed to study the expression of p16INK4a, p14ARF, Bax and Bcl-2 genes involved in cell cycle and apoptosis.

Results: Regarding to the enhanced cellular uptake of sphere shaped Gs-Cu nanoparticles, our data showed that apoptosis is significantly induced in treated cells. Also, qRT-PCR analysis showed that Gs-Cu could effectively up-regulate the expression of p16INK4a, p14ARF and Bax, while significantly decrease the Bcl-2 expression in these breast cancer cells.

Conclusion: The current data demonstrate that Gs-Cu might exert its antitumor effects through apoptosis induction and cell cycle arrest. Taken together, our results demonstrate that the novel formulation of curcumin has the potential to be considered as anticancer agent. However, extensive studies are needed to investigate the therapeutic characteristic of Gs-Cu that some of them are under work in our lab.

Keywords: Gemini-curcumin, Breast cancer, p16INK4a, p14ARF, Bax and Bcl-2

O-21: Association and in silico studies of ENPP1 gene variants with type 2 diabetes mellitus in a Northern Iranian population

Keshavarz P, Sharafshah A

Cellular and Molecular Research Center, School of Medicine (Department of Genetics), Guilan University of Medical Science, Guilan, Iran
parvan1372@yahoo.com

Introduction: In the current study, a sample population of Northern Iranians was selected to investigate the association of K121Q, rs1799774, rs7754561, and rs997509 ENPP1 gene variants and their haplotypes with T2DM.

Methods: Genomic DNAs of 978 samples were extracted by Salting Out standard technique-and then genotyped by the TaqMan assay.

Results: The results show significant differences between study groups for K121Q (P=0.0004) under a Dominant and rs7754561 (P=0.002) under a co-dominant hereditary model. Based on allele frequency, there was a significant difference between two study groups at K121Q and rs7754561 variants (P=0.010 andP=0.01, respectively). There was no evidence for an association between ENPP1 haplotypes and overall risk of T2DM. Genotype-phenotype sub-analyses showed no significant relationship of four studied polymorphisms with age, gender, FBS, and systolic and diastolic blood pressures. Homology modeling and molecular docking of ENPP1 in K173 and Q173 models with ATP, AMP, and 2’3’-cGAMP as ligands revealed that all ligands had a more binding affinity to Lys173 protein model, and 2’3’-cGAMP had a higher affinity to both ENPP1 protein models compared to ATP and AMP.

Conclusions: These findings suggest that ENPP1 gene variants may have a potential impact on the occurrence of T2DM in Northern Iranians.

Keywords: Polymorphism, ENPP1, type 2 diabetes mellitus, homology modeling, docking

O-22: Dysregulation of miR-9 and miR-143 expression in urine specimens of sulfur mustard exposed patients

Khafaei M, bahmani H, amini A, Tavallai M

Human Genetic Research Center, Baqiyatallah Medical Sciences University, Tehran, Iran
Mn.khafaei@yahoo.com

Sulfur mustard (SM) or mustard gas is a chemical alkylating agent that causes blisters in the skin (blister gas), burns the eyes and causes lung injury. Some major cellular pathways are involved in the damage caused by mustard gas such as NF-κb signaling, TGF-β signaling, WNT pathway, inflammation, DNA repair and apoptosis. MicroRNAs are non-coding small RNAs (19â€“25 nucleotides) that are involved in the regulation of gene expression and are found in two forms, extracellular and intracellular. Changes in the levels of extracellular microRNAs are directly associated with many diseases, it is thus common to study the level of extracellular microRNAs as a biomarker to determine the pathophysiologic status. In this
O-23: Study of hypericin effects on mRNA expression pattern in breast cancer cells

Khaki Sanati Z, V.Shariati J, Tajadod G, Javidi MA

1. Department of cell and Molecular Biology, College of Biosciences, Islamic Azad University North Tehran Branch, Tehran, Iran.
2. Genome Center, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.
3. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

E-mail: khaki_sanati@yahoo.com

Breast cancer is one of the most common mortal cancers among the women in the world and is also a heterogeneous and complex disease. In the recent years, herbal medicine has played an important role in treating breast cancer cells. The purpose of this study is to investigate the cytotoxic and apoptotic effects of Hypericin, which is obtained from the plant Hypericum Perforatum, on the expression and regulation level of mRNA genes on MCF-7 cell line. The MCF-7 cell line was cultured and incubated with various concentrations of hypericin for 24 and 48 h. MTT assay was performed to determine cytotoxicity activity and the viability of cells was measured by ELISA Reader in 570 nm. Moreover, apoptosis was confirmed by Annexin V/Propidium Iodide Assay. Afterwards, we used TRIZOL reagent to isolate total RNA and the isolated mRNA was sent for RNA Sequencing (Next Generation Sequencing). Differential gene expression analysis revealed the role of key oncogenes and induced apoptotic genes, pathway enrichment analysis uncovered significant alteration in signaling pathways.

Keywords: breast cancer, mRNA, RNA Sequencing, hypericin, MCF-7

O-24: Identification of causative genes for the neurodegenerative diseases ALS, BVVL, Fazio Londe, CMT2 and HMSN-P

Khani M1, Elahi E-1, Nafissi Sh2, Alavi A4, Shamshiri H3, Taheri H1, Tolou Ghani M6, Moazzeni H6

1. School of Biology, College of Science, University of Tehran, Tehran, Iran.
2. Department of Biotechnology, College of Science, University of Tehran, Tehran, Iran.
3. Department of Neurology, Tehran University of Medical Sciences, Tehran, Iran.
4. Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran.
5. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.
6. Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran.

E-mail: marzieh.khany@gmail.com

Introduction: Neurodegenerative diseases are debilitating and often incurable conditions. Amyotrophic lateral sclerosis (ALS), is the most prevalent neuromuscular disorder and BVVL, Fazio Londe, Charcot-Marie-Tooth Type 2 (CMT2) and HMSN-P are four disorders whose symptoms are related to and sometimes overlap with ALS. Although causative genes for all the disorders are known, mutations in these do not account for diseases in most affected individuals. Our goal was to identify causative genes for these related neurodegenerative diseases in Iranian patients.

Materials & Methods: The most common causative genes were screened for mutations in the probands of 125 families. Subsequently, whole-genome exome sequencing was performed for 30 members of 13 families without mutations in said genes. Novel candidate causative genes were identified and segregation analysis, and screenings of ethnically matched control individuals were performed.

Results: Twenty probands had mutations in known causative genes. Exome sequencing identified four novel genes in ALS, BVVL and Fazio Londe families. Also, we found SPG11 mutations in two juvenile ALS families.

All mutations segregated with diseases status and mutations in the novel genes were not observed in 1000 control individuals. Genetic analysis is ongoing in some of the families without mutations in known genes.

Conclusion: Four candidate new genes for neurodegenerative diseases were identified, three of which have roles in apoptosis. Identification of these will enhance our understanding the etiology of the diseases. Our findings substantiates the contribution of SPG11 to ARJALS and emphasizes potential commonalities among the etiologies of the five neurodegenerative disorders.

Keywords: Amyotrophic Lateral Sclerosis ;BVVL ;Fazio Londe ;HMSN-P ;Charcot-Marie-Tooth Type ;2 Exome sequencing

O-25: Report of two Marfan patients in Iranian population: application of next generation sequencing

Masoumi T, Soveiz M, Shakerian F, Maleki M, Mahdieh N

Genetic Reasearch laboratory ,Rajae Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran

E-mail: tannazmasoumi1369@gmail.com

Marfan syndrome is a rare genetic disorder with an autosomal dominant pattern of inheritance. The disease is caused by the mutation of the FBN1 gene. In this study, two Iranian patients with Marfan syndrome are reported due to mutations in the gene. Materials and methods: Clinical evaluations and cardiac
examinations were performed. Genetic testing was carried out. NGS gene panel of Marfan syndrome and Marfan-like syndromes were done for the patients. In silico analyses were performed for pathogenicity prediction of the variant using available software tools such as MutationTaster, Provean, SIFT and polyphen-2. Results: clinical evaluations were consistent with Marfan syndrome. Two pathogenic variants were found in our patients including c.18897C>T and c.7783G>A. In silico analyses predicted that these mutations are probably pathogenic. Discussion: NGS gene panel tests could be helpful to find the causal variants in Marfan-like syndromes. FBN1 mutations may be common among our population.

**Keywords**: Marfan syndrome, NGS, FBN1, Bioinformatics

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**O-26: Investigation of molecular evolution and epidemiological links related to circulating Avian Avulavirus 1 isolates between 1995 and 2016 in Iran**

**Mayahi V, Esmaelizad M**

Central Laboratory Department, Razi Vaccine and Serum Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Alborz, IRAN.

vafamayahi@yahoo.com

Evolution of Avian Avulavirus 1 isolates and emergence of genetic variants are related to different agents i.e., host adaptation, immune response evasion and selective pressures. Hence, investigation of epidemiological links between circulating viruses in different regions and their evolutionary relatedness are of the major issues for biosecurity strategies, yet many bottlenecks remain unsolved. In this study, we have conducted molecular investigation of circulating Avian Avulavirus 1 isolates in Iran (1995-2016) based on virus classification methods. On the basis of evolutionary divergences, sub-genotype VIg, VIj, VIIj, VIIId, XIIa and XIIId isolates have been circulated in the Iran during 21-year period. Data analysis revealed that VIg isolates shared highest similarity to VIg sub-genotype isolates (i.e., Russian and Polish viruses). While, the lowest difference of VIj sub-genotype isolates (2012) was from a virus isolated in 2015 in India. Furthermore, evolutionary divergences indicated that Chinese and Ukrainian viruses may have played crucial role in emergence of VIIj isolates. Our study also showed that XIIa isolates circulating in Iran may have resulted in the emergence of adapted variants known as XIIId sub-genotype isolates. Here, we suggest the evolutionary and epidemiological study of virulent Avian Avulavirus 1 isolates could help providing accurate molecular data in the region, and result in designing more efficient recombinant vaccines.

**Keywords**: Avian Avulavirus 1, Epidemiology, Newcastle disease virus, Fusion protein, Classification, Evolution

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**O-27: A Novel miRNA Located in the GATA4 Gene, Targets the Cardiac Master Regulator MESP1**

**Medlej A\(^1\), Mohammad Soltani B\(^2\), Mowla SJ\(^1\), Baharvand H\(^2\)**

1. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

GATA4 is an important member of the GATA family of zinc-finger transcription factors. It plays various roles in cardiac specification, differentiation and morphogenesis through binding to cardiac super-enhancers and promoting cardiac gene expression. MicroRNAs are single-stranded, small (20-24 nt) non-coding RNAs that regulate the expression of most protein-coding genes post-transcriptionally. MiRNAs control important cellular processes such as proliferation, differentiation, apoptosis, and tumorigenesis. In the current study, we scanned the GATA4 gene using various bioinformatic tools to search for novel miRNAs. From the distinct stem-loop structures that we obtained, we selected the one that has the best scores as an important miRNA candidate. We detected the endogenous expression of the mature candidate miRNA in cardiac samples and confirmed our results by sequencing. Additionally, overexpression of the stem-loop sequence resulted in upregulation of the mature miRNA form named GATA4-miRNA1. The expression pattern of GATA4-miRNA1 showed a minimal expression at the beginning and after the sixth day of in vitro cardiomyocyte differentiation, while it exhibits a maximal expression from day two to four. Using bioinformatic tools, we predicted MESP1, a master regulator of cardiomyogenesis, as a potential target of GATA4-miRNA1. The overexpression of GATA4-miRNA1 in SW480 cells resulted in downregulation of MESP1 at the transcript level. The discovery of GATA4-miRNA1 introduces a novel miRNA that has important regulatory effects during cardiomyogenesis.

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**O-28: Genes implicated in intellectual disability associated with mitochondrial dysfunction**

**Mehvari S, Najmabadi H, Kahrizi K.**

Department of Biology, Faculty of Basic Science, University of Mazandaran, Babolsar, Iran

E-mail: sepideh.mehvari@yahoo.com

Mitochondrial dysfunction correlates strongly with neurodevelopmental disorders, in which the underlying cause can arise from insults to either mitochondrial DNA (mtDNA) or non-coding mitochondrial genes. The spectrum of phenotype consequences vary between single-organ or multisystem manifestations, with the most effect on those tissues with high-energy demand. The next generation sequencing (NGS) era has yielded a significant number of genes associated with mitochondrial diseases, estimated to be more than 250 hitherto. Through the research into the elucidation of genetic causes underlying hereditary intellectual disability (ID) in two separate cohorts of 136 and 404 families “largely with two or more affected individuals” we identified potentially disease-causing variants in 78 families (73 genes) and 219 families (184 genes) respectively. Of these, a combined total of approximately 20 genes have been identified to harbor a likely causal variant in regard to mitochondrial dysfunction, among which there are
a number of recently reported ID candidate genes, including HEMK1, PGAM5, SLC25A23, and TMEM135. Moreover, for two of the known genes, namely L2HGHD and SURF1, allelic mutations have been noticed in more than one single family. In sum, by the use of NGS in 540 predominantly Iranian families, potentially ID-causative variants in 257 genes â€“ 297 families â€“ have been detected in our study, from which ~20 are mitochondrial-related genes giving rise to ID in almost 22 families that indicate a proportion of 7.7%. These findings highlight the important contribution of mitochondrial derangements towards cognitive impairment.

**Keywords:** intellectual disability, mitochondrial dysfunction, next generation sequencing

### Relationship between the expression level of miR-135a and the HOXA10 gene in eutopic and ectopic endometrium

**Mirabutalebi SH, Karami N, Montazeri F, Kalantar S M**

Genetics Department, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

E-mail: hr_mirabutalebi@yahoo.com

The study of microRNA expression changes, can effective in diagnosing and treating the disease. miR-135a is one of the most important micro-ribonucleic acids involved in endometriosis, which is associated with endometriosis. Among the genes that become the target of the miR-135a and are subjected to changes in the endometrium of patients with endometriosis is the HOXA10 gene. The HOXA10 gene is expressed in the endometrium in respond to steroid hormones. Objective: The aim of this study was to evaluating the expression of miR-135a and its relationship with the level of HOXA10 gene expression in both endometrial ectopic and eutopic tissues in patients with endometriosis. Materials and Methods: In this study, both case-eutopic and case-ectopic tissue samples were obtained from 17 women with endometriosis and the eutopic tissue was also sampled from 17 non-infected women as the control group. The genes expression of miR-135a and HOXA10 were investigated using q-RT PCR. Results: The significant decrease in the expression of HOXA10 gene detected in case-ectopic during the luteal phase compared to the control samples, while in the case-ectopic, the expression of this gene increased. Also, the expression miR-135a in the luteal phase of patient samples compared to controls showed a remarkable increase in the case-ectopic as well as significant decrease in the case-ectopic. Conclusion: Considering the inverse relations between the over-expression of miR-135a and the reduction of HOXA10, it concluded that miR-135a can be applied as an endometrial diagnostic and therapeutic biomarker

**Keywords:** Endometriosis, Gene expression, Micro-ribonucleic acid, HOXA10, miR-135a

### O-29: The long non-coding RNA, PSORS1C3, is regulated by glucocorticoids

**Mirzadeh Azad F1, Malakootian M2, Mowla SJ1**

1. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran

E-mail: ft.mirzadeh@yahoo.com

The long non-coding RNA (lncRNA) PSORS1C3 is located in HLA-C locus, and overlapped with POU5F1 (OCT4) gene. Former studies showed that PSORS1C3 had strong linkage to psoriasis, an immune mediated disease of skin. However, the molecular mechanism of action of PSORS1C3 remained unclear. In our latest work we showed that PSORS1C3 was vigorously spliced to generate 24 novel spliced variants (Malakootian et al., 2017). Herein, we used bioinformatics tools to predict PSORS1c3 promoter and its binding transcription factors. Our promoter-reporter assays showed that PSORS1C3 expression was affected by glucocorticoids treatment. We also identified that PSORS1C3 entangled gene, OCT4, was affected by dexamethasone treatment without having a glucocorticoid responsive element (GRE) in its promoter. This observation might be due to a possible regulatory effect of PSORS1C3 on OCT4 transcription. However, to confirm the regulatory effect of PSORS1C3 on OCT4 further investigation would be needed

**Keywords:** PSORS1C3, lncRNA, glucocorticoids

### O-30: Attenuation of inflammation genes expression by Juglans regia extract in an Osteoarthritis-like invitro model.

**Mohamadi**

Biology teacher at education & training ministry, Kermanshah, Iran.

E-mail: eqbal.mohamadi@gmail.com

Todays, the use of herbal medicine in common disease control and treatment, such as inflammatory diseases; is increasing. The present study aimed to assess anti-inflammatory effects of Juglans regia extract in an osteoarthritis-like model on synoviocytes cells cultured from Holstein cows. the extract cytotoxicity was determined as 1mg/dL by MTT, LDH and SDH assay. Then LC50 was determined as 1µg/ml. The pro-inflammatory cytokines TNF-α, IL-18 and IL-1 genes expression treated with NSAIDs, Steroids, and the extract was assessed in groups as followings; Cell, Cell+LPS, Cell+LPS+NSAIDs, Cell+LPS+Steroids and ell+LPS+Juglans regia extract. Then using real-time PCR technique, analyzed with Pfaff, F static and ANOVA methods and contrasting with REST 2009 software. The results showed down-regulating expression of the inflammatory genes (p<.05), due to Juglans regia extract and anti-inflammatory effects of the extraction. In the present study, it proved that effects of Persian walnut extract on synoviocytes decreased the expression of genes involved in inflammation 42.37%\(\pm\)47.53%, and 42.81% respectively for TNF-α, IL-1, and IL-18; contrasting to control group. So Persian walnut ingredients can be used as an alternative treatment and it can be offered as well as an effective recommended.

**Keywords:** anti-inflammatory effects, Juglans regia extract, cytokines, synoviocytes, gene expression, qPCR.
O-31: MicroRNA-34a inhibit breast cancer proliferation and migration

MohammadiA, mansoori B, Baradaran B

1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

E-mail: ali_mohammadi6868@yahoo.com

miR-34a has been reported in several cancers, the cellular and molecular mechanism of miR-34a in breast cancer remains unclear. A recent study suggested that miR-34a could be one of the key regulators of breast cancer. The aim of this study is to investigate the role of miR-34a in breast cancer and its ehanisms. Methods: The miR-34a mimic mature sequence was transfected into MCF-7 cells. To evaluate the effect of miR-34a on the breast cancer cell proliferation and migration, MTT and scratch wound healing assay was performed, respectively. Then, the expression level of miR-34a and the inhibitory effect of miR34a on caspase3 expression were evaluated by the qRT-PCR. Results: The induction of miR-34a in MCF-7 cells was confirmed by GFP channel imaging system and the 10 fold increasing in miR-34a expression in stable cells. MTT and wound healing assays showed that miR-34a have anti-proliferation and cell migration in stable miR-34a breast cancer cell line compared to control group. The result of qRT-PCR on caspase3 expression showed miR-34a could decrease that expression level of miR-34a in stable miR-34a cells compared to control cells. Conclusions: As the results displayed miRNA-34a could regulate the breast cancer proliferation and migration by targeting the caspase3 mRNA. Also the result suggested that miR-34a can be a therapeutic molecule in target therapy of breast cancer.

Keywords: miR-34a, Breast cancer, Proliferation, Migration, Caspase3


Mosanan farsi Sh, Abdollahi L, Dehghani fard, Kalantar E

Department of Genetics, Faculty of Science University of Maragheh, Maragheh, Iran

E-mail: Shamimfa2@gmail.com

Factor V Leiden) FVL (G169A, factor II) prothrombin (G2010, and methylenetetrahydrofolate reductase) MTHFR (C677T and A1298C mutations are three most common inherited disorders of blood clotting. FVL is the most common genetic cause of venous thrombosis, and is mutation of coagulation. MTHFR mutation is a risk factor for vascular disease. And elevation of FII mutation increases venous thrombosis risk. In this study, we aimed to investigate these mutations as risk factors in coronary artery disease) CAD. (For this purpose, genomic DNA was extracted from peripheral blood sample and PCR-RFLP was performed for genotyping each mutation. The genotypes were determined in 182 patients and 280 normal controls.

FVL, MTHFR, and FII heterozygous and homozygous mutations in the control group were 30.7%, 2.3% for C677T, 35.5%, for A1298C, and 0% respectively. Frequency of FVL mutation in patients with coronary artery disease (15.6%) was higher than that of the control group. The FII mutation is associated with an increase in cardiovascular and coronary artery disease. The frequency of this mutation in patients was 6.4% MTHFR mutation rate was 52.6% for C677T and 62% for A1298C in patients group. Accordingly, increased frequency of mutations in patients demonstrates a meaningful association between occurrence of these mutations and coronary artery disease. These results are considered to be statistically significant and meaningful.

Keywords: risk factor, polymorphism, genetics, thrombophilia

O-33: The association of Endothelial Nitric Oxide Synthase (eNOS) Gene polymorphism with Risk of Male Infertility in Mazandaran province.

Mousavi-Nasab F s, Hosseinzadeh-Colagar A.

1. Department of Molecular and Cell Biology, Faculty of Basic Sciences, Islamic Azad University-Tonekabon Branch
2. Department of Molecular and Cell Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar

E-mail: Faeze.mousavi2015@gmail.com

Recent studies have demonstrated that making the sorts of oxygen reactive, such as nitric oxide can cause oxidative lipid damage, protein damage and damage to the DNA of cells. Sperm DNA damage result to the reduction in the mobility of sperm, damage of Acrosome membrane lead to inability of sperm to fertilize the Oocyte. Increasing expression of endothelial Nitric Oxide Synthase (eNOS) gene, is involved in various diseases such as cardiovascular and infertility diseases. The aim of this study was to assess the association between eNOS gene single nucleotide polymorphism (SNP) (rs2070744) with risk of male infertility and the quality of sperm parameters in a population of Mazandaran, Iran. Material and methods: In this case-control study, 100 infertile men were enrolled as patients group. Control groups consisted of 100 fertile men. eNOS genotype (CC, TT and TT) was determined using Polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Results: Analyzing the results demonstrated that the frequency of Heterozygous sick, was more compared to group control but this difference was not significant (P > 0.05). Data also demonstrated that the T allele in the sick group was seen less compared to the T allele that was seen among the control group but this difference was not significant among any levels and groups (P > 0.05). Conclusion: The findings of this study suggested that rs2070744 SNP couldn’t be applied as an appropriate genetic risk factor for risk of male infertility. However more comprehensive studies in different populations are required to confirm our data.

Keywords: Male, Infertility, eNOS, Polymorphism, PCR-RFLP

O-34: Detection and Functional Analysis of Calumenin-Re-
Abstracts of the 3rd International & 15th Iranian Genetics Congress

3rd O-35: Antagonistic peptide of VEGF-A and VEGF-B attenuated angiogenesis through blockade of endothelial cell migration: investigation of FAK gene expressions in 4T1 mammary carcinoma bearing Balb/c mice

Nazarian A, Asghari S.M

Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran
E-mail: nazarian.atefeh@gmail.com

CREC is a family of calcium-binding proteins, with multiple EF-hand motifs, that are widely distributed in different cellular compartments including; nucleus, cytosol, and endoplasmic network, etc. One of the member of this protein family is Calumenin (CALU), for which alteration in expression pattern is reported in cancer and a number of other diseases. However, the underlying signaling pathways for its regulation has not been elucidated yet. Considering the key role of miRNAs in cancer progression, we aimed to detect the miRNAs that are associated with the CALU regulation. With this aim, the miRBase and miRmap algorithms were used to identify the CALU related miRNAs, which led to detection of Mir30 family as the most relevant microRNAs which are related to calumenin with high scores. Taking a quantitative RT-PCR approach, as an in vitro invasive cancer model, the levels of identified miRNAs were evaluated in a colorectal cancer (CRC) SW480 cell line. Taking advantage of the miRanda database in the subsequent step, the most upregulated and downregulated signaling pathways, correlated with CALU, were designed. The obtained data will be applicable in identification of the signaling intermediates that have crucial roles in cancer development, towards adopting suitable therapeutic strategies.

Keywords: Calumenin, Colorectal cancer, microRNA

3rd O-36: Evaluation of the ErbB3 Gene Expression and its Li-gand, NRG1 in Patients with Major Depression

PARKASH R1, MALEKZADEH K2, SHARIFI M1, FALLAH S4

1. Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Yazd, Iran
2. Molecular Medicine Research Center@OEHormozgan Health Institute@OEHormozgan University of Medical Sciences@OEHormozgan University of Medical Sciences
3. Department of Psychiatry, Ebne-Sina Psychiatric Hospital, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
4. Fertility and Infertility Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
parkash.biology@gmail.com

Introduction: Major depressive disorder (MDD), also known simply as depression, is a mental disorder characterized can negatively affect a person’s personal, work, and general health. The disease is the fourth major cause of the burden of diseases in the world, which alone accounts for the largest share of non-lethal diseases. Between 2.6~7% of adults with major depression die by suicide. Several genetic linkage analysis and genome wide association studies tried to identify susceptible genes relevant to MDD, to find reliable biomarkers. ErbB3 is a tyrosine kinase receptor, implicated in myelinization processes, controlling the growth and development of Schwann cells that wrap around nerve axons to provide electrical insulation. Also it binds members of the neuregulin family, such as neuregulin-1, and plays an important role in promoting oligodendroglia differentiation. NRG1 has role in neurodevelopment, glutamate, and other neurotransmitter receptor expression regulation.

Aims & Objectives: Evaluation the impact of ErbB3 and NRG1 genes in MDD patients.

Methods: The present study was approved by the Ethics and Human Rights Committee of Hormozgan University of Medical Sciences (HUMS). 60 persons (20 severe, 20 moderate and 20 age-sex matched healthy controls, which are all confirmed by Bek Test) were collected from Shahid Mohammadi Hospital, Bandar Abbas. Briefly, 1ml blood was collected before and after standard and conventional treatment. The mRNA and protein expression in blood was measured by Real-Time PCR and ELISA methods, respectively.

Results: The NRG1 mRNA and protein level was significantly decreased for 90% in both severe and moderate depressed pa-
tients as comparison with controls. The expression of ErbB3 was significantly increased 2.5 fold in moderate and 3.3 fold in severe patient. (p-values shows in graphs). Increasing the expression level of ErbB3 and decreasing in level of NRG1 showed a convergent with severity of depression. The level of NRG1 was significantly increase even to 130% in moderate depressed people and 50% in severe group after conventional treatments. Conventional treatment could decrease ErbB3 level to normal range.

Conclusions: The obtained data confirmed pathologic role of these studied genes in depression and can be considered as a pre-diagnostic (even before symptom emerging), as well as prognostic biomarker to evaluate treatment efficiency. These genes particularly ErbB3 as receptor can be considered as therapeutic target for drug designing.

Keywords: ErbB3-NRG1-Moderate Depression-Severe Depression

O-37: Transcriptome-wide analysis of lncRNAs in hypericin treated breast cancer cells

Pourvali B, V.Shariati J, Tajadod G, Javidi M.A

1. Department of cell and Molecular Biology, College of Biosciences, Islamic Azad University North Tehran Branch, Tehran, Iran.  
2. Genome Center, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran  
3. Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (IAUPS)

E-mail: pourvali_behnaz@yahoo.com

lncRNAs or non-coding RNAs are one of the most important sequences in the genome and it is added to their importance everyday. Long non-coding RNAs or lncRNAs (over 200 bp) have a role in central cellular processes vital for survival, including transcriptional regulation, post-translational processes, and their potential effects on tumor suppressor pathways. However, the effects of these sequences on the conversion of natural tissue to cancer have not been determined yet. Breast cancer is one of the most common malignant cancers among women with high mortality rates and affects a large number of women each year. Hypericin, which is extracted from Hypericum perforatum, has apoptotic or cytotoxic effects on tumor cell lines and prevents angiogenesis resulting from the tumor. Therefore, we study the effects of hypericin apoptosis and toxicity on lncRNAs on MCF-7 cell line, which is a human breast cell line. MCF-7 cells were cultured in DMEM medium with specific conditions and incubated with different concentrations of hypericin for 24 to 48 hours. We used the MTT kit to obtain appropriate concentration of cancer cells, and cell viability was measured by ELISA reader at 570 nm. Subsequently, we examined the apoptosis induction in these cells by testing the Annexin V / Propidium Iodide cells. Total RNA purification with TRIzol was performed. We extracted the total RNA for RNA Sequencing. Using RNA-depletion approach and the data was analyzed for lncRNAs expression by multiple softwares and paths, resulting in identification of several novel lncRNAs.

Keywords: lncRNA, breast cancer, MCF-7, RNA Sequencing.

O-38: Departement of Immunology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.

Rezapour-Firouzi S1,2,3, Shahabi Sh1, Mohammadzadeh A1, Mehranfar S1,2,3, Tehrani AA1, kheradmand F3, Mazloomi E1, Mohammadian M1, Sadeghzadeh M0

1. Department of Immunology and Genetics, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran.  
2. Cellular and Molecular Research Center, Urmia University of Medical Sciences, Urmia, Iran.  
3. Faculty of Nutrition, University of Medical Sciences at Tabriz, Iran, Iran.

4. Department of Pathobiology, Faculty of Veterinary Medicine, Urmia University, Urmia, Iran.  
5. Department of Biochemistry, School of Medicine, Urmia University of Medical Science, Urmia, Iran.  
6. Department of Physiology, Faculty of Medicine, Urmia University of Medical Science, Urmia, Iran.

S.firooz@gmail.com

Background: Experimental autoimmune encephalomyelitis (EAE) is a murine model that the most commonly used experimental model for the human multiple sclerosis (MS). Because of limited efficacy and adverse side effects of the current treatments, identifying novel therapeutic agents is important. We investigated whether hemp seed/ evening primrose oils (EPO/HSO) in comparison with rapamycin (RAPA) plays a role in MS treatment.

Methods and materials: Chronic-EAE was induced by myelin basic protein (MOG) in C57/BL6 mice that were assigned to three groups (6/group). To evaluate the therapeutic effects of EPO/HSO in comparison with RAPA, Group A: mice were co-administered with EPO/HSO + RAPA; group B: mice were injected with RAPA; C group: mice were fed with EPO/HSO. Results were compared to two control groups. Mice were euthanized on day 28 of post-immunization. The weight, clinical score and histological findings were evaluated. The immunological factors (genes expression of mTORC1, mTORC2, IFN- ?, and IL-10 of spleen cells) were assessed.

Results: EPO/HSO was able to attenuate the severity of EAE and delay the disease progression. Treatment with only EPO/HSO significantly inhibited the genes expression of mTORC1 and IFN-? and promoted the genes expression mTORC2 and IL-10.

Conclusion: EPO / HSO treatment improved and reduced various parameters of EAE severity in the mice, including clinical score, immunological, and histological findings. These results suggest that The EPO/HSO is likely participating in demyelination in the spinal cord the MS development, and that it could serve as an effective therapeutic target for the treatment of MS.

Keywords: Spleen cells, Oenothera biennis L, Cannabis sativa L, PUFA, Adjuvant

O-39: Identification of mitochondrial tRNA Ile, Gln & Met genes mutations in Iranian patients with idiopathic recur-
O-39: A novel mutation in the BRCA1 gene in an Iranian family with hereditary breast cancer

Sadat fatemi K, Amin M, sabeghi S, abiri M , Zeinali S

1. Dr. Zeinali’s Medical Genetics Laboratory, Kawsar Human Genetics Research Center, Tehran, Iran
2. Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
3. Department of Medical Genetics, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
E-mail: kiyana_ac@yahoo.com

Breast cancer (B.C) is the most common female malignancy and is the major cause of death in middle-aged women. Therefore, early detection can play an important role in disease prevention. About 5 to 10% of the cases are due to an inherited mutation in two major genes, BRCA1 and BRCA2 which transmit as an autosomal dominant form. Genetic testing enables us identifying patients at increased risk of developing B.C. The aim of the study was to identify the causative mutation of early B.C in a family with 9 affected members. Methods: Linkage analysis was performed with the help of STR markers linked the BRCA1 and BRCA2 genes to indirectly track the mutation. Then the candidate gene was subsequently sequenced to find the mutation. Results Linkage analysis showed that BRCA1 gene is segregating with the disease. Sequencing results showed a novel heterozygote (c.3607 C>T, P.R1203 X) variant in BRCA1 gene. The variant was heterozygote in all affected members and was not present in healthy members of the family. Conclusions: The newly identified variant caused a truncated protein which is not active and cause disease. Genetic testing is useful for the preventive interventions for families with high risk of the disease. Identification of these novel mutations helps in developing a mutation to program for early breast cancer screening. Early-onset B.C (less than 45 years) and a limited family history are sufficient to justify mutation screening with a detection rate of over 25% in this group.

Keywords: breast cancer, BRCA1 gene, sequencing, Iran

O-40: A novel mutation in the BRCA1 gene in an Iranian family with idiopathic recurrent miscarriage

Sadat fatemi K, Amini M, sabeghi S, abiri M , Zeinali S

1. Department of Biology, Faculty of Science, Yazd University, Yazd, Iran
2. Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
E-mail: sabet6494@gmail.com

Repeated pregnancy loss (RPL) is defined as three or more consecutive pregnancy failures and is estimated to affect ~1% of couples trying to conceive. The cause of RM remains unknown in approximately 50% of cases although much work has been done in the past to identify the underlying mechanisms. Mitochondrial transfer RNAs (tRNA) genes are essential components of protein biosynthesis. These genes are hotspots for mutations. These mutations are associated with a wide spectrum of human diseases. Many genetic factors are known in assessment RPL. The aim of this study was identification of mitochondrial tRNA Isoleucine, Glutamin and Methionine gene mutation in Iranian patients with idiopathic recurrent miscarriage (RM). The nucleotide variations of tRNA Isoleucine, Glutamin and Methionine were investigated in 80 women with idiopathic repeated pregnancy loss. The related mitochondrial area was amplified using a polymerase chain reaction (PCR). The PCR products were demonstrated by 2% agarose gel electrophoresis, all PCR products were run on SSCP gels and samples showed a considerable shifts due to a probable mutation were sent for DNA sequencing. Nucleotide variations were analyzed in different bioinformatics websites and softwares. The sequence analysis revealed 2 novel mutations in tRNA Ile in 5 cases; 4263A>T in 1 case and 4265delA in 4 cases. These variations may increase the risk of RM in carrier women. Further studies would, however, be required to confirm the phenotypic effect of these variations.

Keywords: Repeated pregnancy loss (RPL), Mitochondrial genomes (mtDNA), Mitochondrial transfer RNAs (tRNA), Isoleucine,Glutamin and Methionine tRNA, Mutation,PCR-SSCP
Keywords: microRNAs, breast cancer, hypericin, miRNA Sequencing, MCF-7

O-42: Isolation and Characterization of Cell Free DNA from CRC patients in Plasma
Shahabi E*, Krachian MA2, Vaziri HR1

1. Department of Biology, University of Guilan, Rasht, Iran
2. University of mashhad, Faculty of Medicine, Mashhad, Iran

ehse.shahabi1991@gmail.com

Since patients with mutations in the KRAS gene do not respond to Anti-EGFR antibody therapies, the presence or absence of mutations in this gene for rapid detection those who are prone to colorectal cancer (CRC) and the choice of a suitable therapeutic approach are inevitably. In recent years, the attention of researchers has been drawn to the use of cell free DNA (cfDNA) as a non-invasive biomarker. Studies have shown that genetic and epigenetic changes in cfDNA are a reflection of the genetic and epigenetic properties of tumor cell DNA. The purpose of this study was to detect mutations in codons 12 and 13 of KRAS gene in cfDNA and DNA of the tumor tissue, and then to assess the degree of coordination between the mutations detected in plasma and tissue. This study included 10 subjects with colorectal cancer and 5 healthy controls. DNA was extracted from the tissue and plasma. The mutation tracking was done by HRM and Allele Specific qPCR techniques. Finally, sequencing was performed by Sanger method to ensure the accuracy of the results. The results showed mutations in 8 plasma samples, which was identical with the mutations detected in the tissue. The highest percentage of mutations included the nucleotide change of c.35G> A (G12D). The results indicate that the gene mutations in the primary and metastatic tumors can also be detected in the plasma cfDNA. Although more studies in larger populations are needed to confirm these findings.

Keywords: cell free DNA, CRC, KRAS mutations, Cancer biomarker

O-43: Lessons Learnt from Genetic Studies of Autosomal Recessive Non-Syndromic Hearing Loss: Implications for Molecular Diagnostics in Iran

Tabatabaiefar MA1, Hashemzadeh Chaleshtori M2

1. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan Iran
2. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran

mamintab@yahoo.co.uk

Introduction: Hearing loss (HL) is the most frequent sensory birth defect in human. Autosomal recessive non-syndromic HL (ARNSHL) is the most common type of hereditary HL. Iran, with a high rate of consanguinity has a much higher rate of ARNSHL than expected. Its extreme genetic heterogeneity, with over 70 known loci is still a big challenge for molecular diagnostics Methods: over 250 Iranian multiplex families from several provinces which were negative for GJB2, as the most common cause of HL, were recruited. They were screened for up to 15 DFNB loci using genetic linkage analysis. In a subset of the remaining families, next-generation sequencing (NGS) and genome-wide homozygosity mapping were performed, Necessary steps such as Sanger sequencing and co-segregation studies were followed: Results: GJB2 is the first gene to be investigated in ARNSHL (16% in average) followed by mutations in SLC26A4 (DFNB4) (8-10%). Although a variety of other loci are involved in the rest of cases, 6-7 of them are among the ones that are worth being screened including DFNB2 (MYO7A), DFNB3 (MYO15A), DFNB21 (TECTA), DFNB7/11(TMC1), DFNB9 (OTOF) and DFNB24 (RDX). NGS is very promising in clarification of the etiology and pathogenic variants in several rare genes as well as novel genes have been identified. To minimize costs, it would be logical to define a panel of genes for Iran. Conclusion: Advancement in technologies combined with population-specific profiles of genes could set the stage for genetic diagnostics of HL.

Keywords: molecular diagnosis, deafness, genetic linkage analysis, Next-generation sequencing, Iran

O-44: Exome sequencing identified pathogenic variations in genetic forms of skeletal disorders

Zeighami J1*, Zamani M1,2, Seifi T1,2, Mazaheri N1,2, Sedighzadeh S1,2, Negahdari S1, Shariati Gh1, *Sedaghat A1,4, Saberi A1,2, Hamid M1,3, Galehdari H1

1. Narges Genetics Diagnostic Laboratory, Ahvaz, Iran.
2. Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
3. Department of Genetics, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran.
4. Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran.
5. Departments of Biotechnology, Institute Pasteur, Tehran, Iran. zeitghami@yahoo.com

INTRODUCTION: Genetic disorders that related to the skeletal system are contributed to the disturbances in the complex processes of skeletal development, growth and homeostasis. The symptoms and etiologies of genetic forms of skeletal disorders are very heterogeneous, so it complicates the differential diagnosis of such diseases. Nowadays, whole-exome sequencing (WES) provides the ability of rapid and cost-effective molecular diagnosis of inherited disease.

METHODS: 17 cases represented the hallmark symptoms of skeletal disorder referred to Narges Genetics Diagnostic Laboratory from 2014 to 2018. The history and physical condition of the patients considered and they subjected to the whole-exome sequencing. The obtained genetic profile analyzed by bioinformatics tools. The candidate variants determined and then their validation and allele segregation confirmed by Sanger sequencing.

RESULT: 4 cases diagnosed as Osteogenesis imperfecta FKBP10 COL1A1 and COL1A2 were the causative genes in 3 cases 1 case remained unknown. In 2 cases
the CHST3 gene revealed as mutated gene that is responsible for Spondyloepiphyseal dysplasia with congenital joint dislocations syndrome. Mutations in NEK1 gene cause Short-rib thoracic dysplasia 6 with or without polydactyly/syndrome that found in 2 patients. The other genes including BHLHA9, CY-P27B1, PCNT, GPX4, and PYCR1 were responsible respectively for Camptosynpolydactyly, complex/Syndactyly, meso-axialsynostotic with phalangeal reduction, Vitamin D-dependent rickets, type I, Microcephalohypoplastic syndromal dwarfism, Spondylometaphyseal dysplasia, Sedaghatian type and Cutsi laxa, autosomal recessive, type IIB found among patients. The genetic cause of 4 cases remained unknown.

CONCLUSION: WES can be used to confirm the diagnosis of the genetic disorders. In complicated conditions which there are overlapping presentations while other molecular testing is time and cost consuming, WES is the most helpful strategy.

Keywords: exome sequencing, skeletal disorders

O-45: Topoisomerase II inhibitor, epirubicin, down-regulates HMGB1 and hTERT expression and leads MDA-MB231 cells to HMGB1 release

Zia AA, Rabbani-chadeegani A, Sargolzaei J

Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran
alizia91@yahoo.com

The high mobility group box 1] IHMGB1 (is an abundant and ubiquitous chromatin associated protein in mammals, acting as a DNA chaperone in transcription, recombination, and repair. HMGB1 dissociates from chromosomes during mitosis and take part in telomere maintenance and modulates telomerase activity which can be detected in the vast majority of cells that require an increased ability to replicate, such as cancer cells. Epirubicin (EPI) is a potent inhibitor of topoisomerase II, the mode of action is stabilization of cleavable complexes between Topo II and DNA. In this study MDA-MB231 breast cancer cells were exposed to EPI for 24 h, the HMGB1 protein was extracted from drug treated and the controls, run on SDS-PAGE, and then detected by western blot. The expression of HMGB1 and hTERT mRNA were examined by real-time RT-PCR. The results showed that intracellular HMGB1 protein and its mRNA expression level were decreased in concentration dependent manner. Upon addition of EPI, hTERT mRNA level remained unchanged) p (0.05<up to 20 Åg/ml of EPI, whereas at higher concentrations the hTERT expression was significantly decreased (p<0.001). The results also revealed that HMGB1 appear in the media of late apoptotic and necrotic MDA-MB 231 cells. From the result it is concluded that, EPI, down-regulates the mRNA expression of HMGB1 which is correlated with suppression of hTERT mRNA level demonstrating that in the presence of EPI, HMGB1 can modulate hTERT expression and induces apoptosis/necrosis cell death in breast cancer cells.

Keywords: HMGB1, Epirubicin, Topoisomerase, Telomerase, Apoptosis

O-46: The pathway of auxin biosynthesis in halotolerant plant growth-promoting rhizobacterium Pseudomonas sp. J8

Ahmadabadi S1, Saghafl K2, Shariati JV3, Hosseini-Mazinani M1

1. Department of plant biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
2. Imam Khomeini International University, Qazvin, Iran
safs.2018@gmail.com

Diverse bacterial species possess the ability to produce the auxin phytohormone indole-3-acetic acid (IAA). Different biosynthesis pathways have been identified and redundancy for IAA biosynthesis is widespread among plant-associated bacteria. Interactions between IAA-producing bacteria and plants lead to diverse outcomes on the plant side, varying from pathogenesis to phyto-stimulation. This property is best documented for bacteria that interact with plants because bacterial auxin can cause interference with the many plant developmental processes regulated by auxin. Auxin biosynthesis in bacteria can occur via multiple pathways as has been observed in plants. In this research, Pseudomonas sp. J8 was isolated from olive rhizosphere in Zanjan province. Whole genome of this strain was sequenced and the genes known to be involved in auxin biosynthesis pathways were studied. Two major IAA biosynthetic pathways were found in this strain. Indole-3-acetic acid is biosynthesized from tryptophan (Trp) via two proposed routes according to their key intermediates, namely acetamide (IAM) and tryptamine (TAM). Based on molecular and bioinformatics studies and evolutionarily conserved core mechanisms, it is thought that every two pathways, IAM and TAM, are active for auxin biosynthesis in this bacteria.

Keywords: Auxin, auxin biosynthesis, IAA, plant growth-promoting bacteria, phytohormone

O-47: Epitope mapping, codon optimization, cloning and expression of Phlebotomus papatasi SP15 salivary protein in Lactococcus lactis

Davarpanaha E, Seyed N, Rafati S, Safaralizadeh S, Taheri T

Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
Department of Immunotherapy and Leishmania Vaccine Research, Pasteur Institute of Iran, Tehran, Iran.
elahedavarpanah@yahoo.com

Introduction: Leishmaniasis is one of the neglected diseases in the world that is transmitted through the bite of female sand fly of Phlebotomus genus. No treatment or vaccine has been introduced until now. Here, we used Lactococcus lactis as a nonpathogenic expression system to present one of the most important Phlebotomus papatasi salivary proteins, SP15 pro-
tein, to BALB/c immune system.

**Methods:** SP15 epitope prediction was accomplished with different online software (SYFPEITHI, RANKPEP, NetMHC, IEDB, EpiJen and NetCTL) to clarify existing immunogenic T-cell epitopes. For prediction of any exposed domain of SP15 to immune system, second and third structures predicted with RaptorX property and Swiss-model, respectively. Whole SP15-EGFP and EGFP genes were codon optimized, synthesized to express in L. lactis. Both genes were cloned at downstream of PrtP signal peptide sequence in pNZ8121 vector to be presented on bacterial cell-wall. The expressed protein was confirmed by western blotting using anti-GFP antibody, fluorescence microscopy and flow-cytometry.

**Results:** Some of online software with different algorithms confirmed the presence of immunogenic epitopes that are able to trigger the immune responses through HLA-I (IMHECAKK-V, AIQYEYDKTI, YQYYGFAM, SLKADIRKI) and HLA-II (IMHECAKKV, FVAMDNNIA, YYGFVAMDN, IRTFSNVLI). Secondary and tertiary structure of SP15 has shown that none of the epitopes are cryptic in SP15 protein. SP15 expression in L. lactis was monitored and confirmed through different tools.

**Discussion:** SP15 has a high score immunogenic epitope and can consider as an epitope base-or whole antigen vaccines candidate. In addition, SP15 expressing L. lactis is developed to be used in vaccination experiments.

**Keywords:** Leishmaniasis, SP15, Phlebotomus papatasii, Lactococcus lactis, pNZ8121, HLA I/II

**O-48:** Analysis of transcriptome profiling of responsive somatic cells to UV-B irradiation in the colonial green alga *Volvox carteri*

Ekhtari S, Razeghi J, Hasanpur K, Kianianmomeni A, Movafeghi A

Department of Plant Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran

Department of Animal Science, Faculty of Agriculture, University of Tabriz, Tabriz, Iran

Department of Cellular and Developmental Biology of Plants, Faculty of Natural Sciences, University of Bielefeld, Bielefeld, Germany

Ekhtari.soulmaz@yahoo.com

Light is one of the most important environmental signal that affects the physiology and action of Volvox carteri. However, there are limited reports about the effects of UV-B irradiation on genome-wide transcriptional regulation in this multicellular green alga. *V. carteri* has only 2 cell types includes mortal, motile somatic cells and immortal, immotile reproductive cells. In addition, it is a suitable organism for transcriptomics studies since its compact genome with a relatively small number of genes. Here, using RNA-seq data, we surveyed the overall transcriptional responses of two cell-types, somatic cells and reproductive cells, to continuous UV-B irradiation. Gene expression analysis was carried out in order to elucidate the effect of UV-B radiation on whole transcriptional modification of physiological mechanisms. The results showed that, as compared to control group, there were no differentially expressed genes in reproductive cells under treatment. However, treating the somatic cells with UV-B radiation resulted in at least 126 differential genes as compared to untreated control group. Our results showed that there is light-specific transcriptional regulation in this organism. So that, different pathways (i.e., metabolic process, cellular process, response to stimulus, localization, cellular component organization or biogenesis, biological regulation, regulation of biological process) were co-regulated by UV-B irradiation via the transcriptional regulation of genes encoding key enzymes in these pathways.

**Keywords:** Volvox carteri, UV-B radiation, RNA-seq, Transcriptomics, Gene regulatory

**O-49:** Mating-type loci can be used for identification of strains from the mycophenolic acid producer *Penicillium brevicompactum.*

Mahmoudjanlou Y, Dahlmann Tim A., Ulrich KÄ¼ck.

Ruhr-University Bochum, Lehrstuhl für Allgemeine und Molekulare Botanik, University street 150, Building ND7, 44801 Bochum, Germany

E-mail: yasaman.mahmoudjanlou@rub.de

In heterothallic ascomycetes, mating and sexual propagation are controlled by two non-allelic idiomorphs, designated as mating-type loci (MAT1-1 or MAT1-2). They carry genes encoding transcription factors, which have either an alpha- or a high mobility group- DNA-binding domain. After cloning of MAT loci by using PCR primers for conserved sequences flanking the MAT loci, we discovered the genomic organization of the MAT1-2-1 and MAT1-1-1 open reading frames from at least 20 strains of filamentous fungus *Penicillium brevicompactum.* This fungus is one of the substantial fungi in the pharmaceutical industry used for the large-scale production of the immunosuppressant mycophenolic acid (MPA). The open reading frames were verified by cDNA cloning and sequencing. Comparing MAT amino acid sequences with those from other Penicillium species revealed a high homology in the DNA binding domains. However, other regions of the proteins were less similar. Beside 2 molecular markers, Internal transcribed spacer (ITS), ?-tubulin, MAT loci were also used for taxonomic characterization of 37 wild type strains provided from different culture collections. Remarkably, from 36 strains, previously described as *P. brevicompactum* 16 were identified as another related species. Further, we identified an almost equal number of MAT 1-1 and MAT1-2 strains, suggesting that sexual reproduction occurs between *P. brevicompactum* strains in nature. Our data suggests that MAT loci can be used as a novel molecular marker to identify strains from *P. brevicompactum* and point to the potential of this gene for the taxonomic identification of other Penicillium species.

**Keywords:** Mating-type Loci

**O-50:** Expression of recombinant Human Serum Albumin (rHSA) in E. coli strain BL21 (DE3)

Rezaiy P¹, Vaziri H¹, Mousavi SH²

Abstracts of the 3rd International & 15th Iranian Genetics Congress
1. Biology department, University of Guilan, Guilan, Rasht, Iran
2. Molecular bank, Iranian Biological Resource Center, ACECR, Karaj, Iran
Poria.rezaiy@gmail.com

Human serum albumin (HSA) is a multifunctional protein exclusively synthesized by human liver hepatocytes and continuously secreted into the circulation. Escherichia coli, were considered to be one of the efficient platforms for rHSA production due to well-established molecular tools, high growth rate and cultivation capacity. In the present study the coding sequence of HSA gene was codon optimized by Optimizer server and then synthesis and cloned in PET-21 expression vector a by foreign company. The synthesis gene was sequenced to check the T7 promoter and HSA sequences accuracy. This recombinant vector was transformed in E.coli (BL21) and subsequently IPTG was used for induction of recombinant serum albumin protein (rHSA) in E.coli and total protein was assayed by SDS-PAG. The SDS-PAG band for rHSA was not seen even after changing in temperature and time for induction. To understand and resolved this problem the coding sequence of HSA was removed from initial recombinant vector by NdeI & NotI restriction enzymes and subsequently were cloned in 3 bacterial expression vectors (PET-28a, PET-21a and pARA-28a). The strong SDS-PAG band was seen for E.coli strains that harbor recombinant pET-28a and pARA-28a with molecular weight almost equal to 66KD and we didn’t see this result for recombinant PET-21a. The recombinant HSA was purified by Ni-NTA recombinant protein purified kit from Qiagen co. and then evaluated by SDS-PAG. After purification one protein band was seen near to 66KD protein weight marker. Most likely this SDS-PAG band belong to our recombinant HAS.

Keywords: Human Serum Albumin, Recombinant Protein, E.coli

O-51: Production of Gamma-Aminobutyric Acid(GABA) by Bifidobacterium animalis-lactis

Taheri SS1, Shafiee M2,3, Khosravi A4,5, Saghaeian M6

1. Islamic Azad University of Damghan, Damghan, Iran
2. Department of Medical Genetics, Golestan University of Medical Sciences, Gorgan, Iran
3. Department of Molecular Medicine, Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran
4. Stem Cell Research Center, Golestan University of Medical Sciences, Gorgan, Iran
5. Biochemistry and Metabolic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran
mohshaf@gmail.com

Background: Gamma-Amino butyric acid (GABA) is an inhibitory neurotransmitter produced by irreversible decarboxylation of the glutamate by the glutamic acid decarboxylase (GAD) enzyme. It has been shown that GABA can be useful in colorectal cancer prevention. Here, we aimed to construct a recombinant Bifidobacterium animalis-lactis (BB-12) to produce GABA as a potential probiotic supplement.

Materials and methods: The glutamate decarboxylase gene was synthesized and propagated in the pET-32a (+) expression vector. The plasmid was then purified and sub-cloned to BB-12 by electroporation. The E.coli was also transformed with recombinant plasmid by cold CaCl2 method as positive control. The recombinant colonies were confirmed by Colony-PCR and finally the culture medium was used for final determination of the GABA by HPLC.

Results: Our findings showed that electroporation delivery of the recombinant GAD-pET-32a (+) to the BB-12 can result to GABA production in the culture medium after 72h of incubation equal to 19.774 ppm.

Conclusion: Colonizing of GAD gene was performed for the first time in this bacterium. The recombinant BB-12 encoding GAD can produce GABA which maybe useful as a genetically modified probiotic bacteria for local delivery of GABA in gut microbiome. However, further studies are needed.

Keywords: Gamma-Amino butyric acid (GABA), Glutamate decarboxylase, Bifidobacterium animalis , Lactis, Probiotic

O-52: Implication of the adaptive laboratory evolution method to improve recombinant Pichia pastoris productivity

Tavassoli Z, Arjmand S, Ranaie Siyadat S O

Protein Research Center, Shahid Beheshti University, G.C, Tehran, Iran
z.tavassoli1991@gmail.com

INTRODUCTION: Adaptive laboratory evolution (ALE) is a common methods in biological studies for improving growth and production of different cells in a long term duration and under the specified conditions.

OBJECTIVE: The aim of present study was to investigate the adaptive laboratory evolution of Pichia Pastoris yeast cells, cultured in medium containing 1% methanol, in order to enhance the expression of recombinant human vascular endothelial growth factor (VEGF) protein.

MATERIALS AND METHODS: The X33 strain of yeast Pichia pastoris containing human VEGF gene under the control of alcohol oxidase 1 (AOX1) promoter, was used. The generation time of yeast cells in a complex (YPM) and defined (FM22) media, and with methanol as the carbon source, was calculated. Four repeat for each medium were cultured for 490 and 230 generations in complex and defined media, containing 1% methanol, respectively. The efficiency of final cells for production of recombinant VEGF was measured using ELISA.

RESULTS: The generation time for Pichia pastoris cells in YPM and FM22 was 3.4 and 7.3 h/generation respectively. In compared with the prototype, the obtained clones after 490 generations in YPM showed no significant differences in growth and VEGF production. While, the production of recombinant VEGF increased 1.3 fold in one of the clones grown in the FM22 medium.

Keywords: Adaptive laboratory evolution, Pichia pastoris, VEGF, generation time
Genetic Resources

O- 53: Venom gland Transcriptomic analysis of Iranian yellow scorpion á¢€œOdontobuthus doriaeľ revealed some new findings by medical purposes

Naderi Soorki M1, Galehdari H1, Baradaran M2, Jalali A3

1. Department of Genetics, School of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Pharmacology and Toxicology, School of Pharmacy, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.
3. Department of Pharmacology and Toxicology, School of Pharmacy and Toxicology Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran.

Introduction: Scorpion venom contains mixture of biologic molecules including selective toxins with medical capability. Odonthubuthus doriae (O.doriae) belonged to Buthidae family of scorpions and gained more interest among Iranian dangerous scorpion since 2005. The envenomation of this scorpion causes usually neurological signs because of existence of toxins affecting on ion channels.

Material & Methods: Total RNA was isolated from yellow Iranian scorpion glands. A cDNA library was achieved by synthesize and insertion of dscDNA into special vectors and subsequent transformation to chemical competent E. coli as host. Library was screened by culturing of the liquid library on LB-agar plates. Analysis of positive clones was performed by plasmid extraction and sequencing of inserts. Finally, sequences have been analyzed and characterized by bioinformatics software each.

Results: Analysis showed that toxins (42% of ESTs) had more venom transcripts than other venom components (antimicrobial peptide (10%), cell proteins (11%) and venom peptide (13%)) that may have capacity for medical used. Two EST didn't have any similarity by known scorpion peptides and may be new.

Conclusion: For the first time; we report a comprehensive study of an Iranian scorpion with interesting and novel findings and characterized a new putative sodium channel modifier and a new iron transporter in scorpions by some bioinformatics software, and then predicted their structures and functions.

Keywords: Transcriptome analysis; cDNA library; Venom gland; Iranian scorpion; Odonthobuthus doriae

New Technologies and Technological Advances in Genetics

O- 54: CRISPR/Cas9 targeting of SNHG15 as an oncogenic lncRNA inhibits cell proliferation capacity in colorectal cancer

Saeinasab M, Matin M, Gonzalez J, Bahrami A, Mowla SJ, Huarte M

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
3. Department of Gene Therapy and Regulation of Gene Expression, Center for Applied Medical Research, University of Navarra, Pamplona 31008, Spain
4. Institute of Health Research of Navarra (IdiSNA), Pamplona, Spain
5. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

maryam_naderi_soorki@yahoo.com

Recently, thousands of long noncoding RNAs (lncRNAs) have been found to be aberrantly expressed in various types of cancers. Since colorectal cancer (CRC) is the third most common human malignancy worldwide, we investigated lncRNAs misregulated in this type of cancer, and identified SNHG15 as a potentially oncogenic lncRNA. By analyzing expression data from CRC patients available on TCGA, we observed that SNHG15 upregulation is an early event in colorectal cancer promotion and its expression is maintained at high levels until later stages. As SNHG15 sequence has two E-box binding motifs for MYC, we analyzed colorectal adenocarcinoma RNA-seq data from TCGA and revealed SNHG15 is upregulated in the samples with high levels of MYC expression. After silencing MYC in a CRC cell line, the level of SNHG15 was decreased significantly. So SNHG15 transcription could be regulated by MYC. Using CRISPR-Cas9 system, we deleted the region containing exons 3 to 5 from SNHG15 and several clones were obtained with decreased expression of SNHG15. Results showed that these clones have low proliferation and colony formation capacity. However, we did not observe any significant changes in cell cycle and percentage of apoptotic cells. To investigate the effects of SNHG15 in vivo, we injected clones to immunodeficient mice and found that the tumors formed by knock-out cells were obviously smaller and lighter than wild type cells. In summary, these results describe for the first time an important role of SNHG15 in promoting colon cancer and suggest a novel prognostic marker and target for RNA-based therapies.

Keywords: Long non-coding RNA, Colorectal Cancer, SNHG15, CRISPR-Cas9 system

O-55: Targeted next-generation sequencing revealed novel variants in Iranian families with hereditary loss of hearing

Yari A1,2, Saleh-Gohari N1,2, Babasalari M1

1. Department of Medical Genetics, Kerman University of Medical Sciences, Kerman, Iran
2. Saleh Gohari Medical Genetic Laboratory, Samen Alhojaj Charity Center, Kerman, Iran
3. Student Research committee, Kerman University of Medical Sciences, Kerman, Iran

salehgohari@yahoo.co.uk

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ing is the most common sensory defect. This disorder is genetically heterogeneous. In recent years, efforts have been made to identify genetic variants associated with this disorder. Now, with the advent of next-generation sequencing (NGS) technology, provides a quick and low-cost approach to the genetic diagnosis of hearing impairments. Here, we use NGS to examine all of 127 known deaf-related genes in 12 Iranian families.

Methods: In this study, we examined 12 Iranian families with hereditary loss of hearing. Mutation screening was performed in 127 known deaf-related genes using NGS. The identified areas were also examined by direct sequencing. Finally, the identified variants were analyzed by SIFT and PolyPhen-2 to predict the effect of variants on protein function.

Results: By Using this approach, we were able to identify the causative gene variants in 7 of 12 families. The pathogenic role of these variants has already been reported. In addition, 7 novel variants were specifically identified in 6 deaf-related genes. These gene variants were included ADGRV1-c.12786C>G, GIPC3-c.265-266insAG, USH1C-c.1659T>A, LOXHD1-c.6463C>T, OTOF-Ex2-Ex47Dup, ALMS1-c.8665_8669delCAAAG and ALMS1-c.9457A>T. Direct sequencing co-confirmed the presence of these novel variants in patients. Analysis of these variants showed that GIPC3-c.265-266insAG and ALMS1-c.8665_8669delCAAAG are likely pathogenic.

Conclusions: In this study, we successfully used NGS technology to screen mutations in the deaf-related genes. Based on our findings, 7 novel variants were found in the deaf-related genes for the first time in Iranian population. These rare hereditary variants should be considered in genetic diagnosis and counseling.

Keywords: Hearing loss, Deafness, Novel variants, deaf-related genes, Next generation sequencing (NGS)

Stem Cell

O-56: Curcumin promote osteogenic differentiation of human bone marrow

Ghorbaninejad M¹, Hosseini S, Baghaban Eslaminejad M R, Shahhoseini M²

1. Department of Molecular Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and culture, ACECR, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran

mahsaa.land@gmail.com

Mesenchymal Stem Cells (MSCs) are considered as therapeutic target for cell-mediated regenerative medicine to treat human metabolic bone diseases. Numerous efforts have been made to promote efficient differentiation of MSCs into osteoblast lineage. Accordingly, epigenetic signatures emerge as a key conductor of cell differentiation. Enhancer of Zeste Homolog 2 (EZH2), a histone methyltransferase appeared to suppress osteogenesis. Curcumin is an osteoinductive natural polyphenol compound supposedly modulates epigenetic mechanisms. The current study aims to address the role of EZH2 epigenetic factor in osteogenic activity of human bone marrow-derived MSCs (hMScs) after treatment with curcumin. We isolated MSCs from aspirated bone marrow and characterized for differentiation to mesodermal lineages and cell surface markers. The optimum concentration of curcumin was achieved by MTT assay. The effects of curcumin on cellular behavior of viability and osteogenic differentiation were evaluated at different time points under in vitro condition. Moreover, the expression level of EZH2 was assessed using quantitative real-time polymerase chain reaction (qRT-PCR) after 14 and 21 days. MTT results showed that curcumin at concentrations of 10 and 15 M had no cytotoxic effect and the cells were survived up to 70%. Quantitative-PCR results demonstrated that curcumin significantly enhanced the expression level of osteogenic markers including Runx2, osterix, collagen type I, osteopontin and osteocalcin at day 21. Interestingly, we observed that expression level of EZH2 gene down-regulated in the presence of curcumin compared to control group during osteogenesis. It is proposed that curcumin acts as an epigenetic switch to regulate osteoblast differentiation via decreasing histone methyltransferase EZH2 activity.

Keywords: Curcumin, Epigenetic, EZH2, Mesenchymal Stem Cell, Osteogenesis

O-57: Differential Expression of Long Non-Coding RNA SOX2OT in Gastric Adenocarcinoma.

Khalili M¹.², Farhangiyan P¹.², Jahandoust S¹, Mowlaj SA²

1. Department of Medical Genetics and Molecular Medicine, Zanjan University of Medical Sciences, Zanjan, Iran
2. Student Research Center, Zanjan University of Medical Sciences, Zanjan, Iran
3. Cancer Gene Therapy Research Center (CGRC), Zanjan University of Medical Sciences, Zanjan, Iran
4. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

mirakhali@yahoo.com

Gastric cancer (GC) is the third leading cause of cancer-related death in the world. Dysfunction of long noncoding RNAs (lncRNAs) in GC biogenesis are approved by increasing evidence. SOX2 overlapping transcript (SOX2OT) lncRNA, which harbors SOX2 transcription factor, is aberrantly expressed in different cancers. Materials and Methods: In this study, the expression of SOX2OT was evaluated in 33 matched pair tumor and non-tumor gastric samples and AGS and MKN45 gastric and NTERA2 embryonic carcinoma cell lines by real time PCR.

Results: Our finding revealed a significant decrease in the expression of SOX2OT in gastric tumor samples compared to their matched non-tumor samples (P<0.05). Also SOX2OT showed a lower expression in high grade compared to low grade of gastric malignancy. Furthermore, SOX2OT expres-
sion showed higher expression in NT2 compared to AGS and MKN45 cell lines. Conclusion: Simultaneous expression of SOX2 and SOX2OT was reported in some cancers. Regarding to the decreased expression of SOX2OT in the present study in concurrent with downregulation of SOX2 in our previous study, it seems that SOX2OT plays a tumor suppressor role in GC.

*Keywords:* lncRNA, SOX2OT, gastric cancer, SOX2

**O-58: Circulating Endometriosis Related microRNA Relation with Metabolic Alteration**

Majidi Zolbin M

1. Anatomy Department, Tehran University of Medical Sciences
2. Department of Obstetrics, Gynecology & Reproductive Sciences, Yale School of Medicine

masoumeh.majidizolbin@gmail.com

Endometriosis is clinically described as the growth of endometrial glands and stroma outside the uterus and inside the peritoneum cavity. This disease often leads to dysmenorrhea, dyspareunia, episodic abdominal pain, and bowel symptoms and sometimes it linked with symptoms unconnected to the reproductive tract, for instance, women with endometriosis have been shown frequently to have subordinate body mass index (BMI) than those without the disease. Although there is not enough information regarding the pathophysiology of this disease may be dysregulation of expressed genes can be the principal reason for these clinical symptoms. Regulations of expressed genes are related to circulating microRNA and are known to be differentially expressed in the sera of women with endometriosis compared to the healthy group. Here we sought to determine whether endometriosis-related differential miRNA expression could induce changes to normal adipocyte metabolic gene expression in women with endometriosis as well as stem cell content of fat tissue in mice model of endometriosis. These findings may explain the clinically-observed low body mass index of patients with endometriosis and contribute to our understanding of endometriosis as a complex and systemic disorder.

*Keywords*: endometriosis, BMI, microRNA, fat, stem cell

**O-59: Induction of Sox17-expressing Endoderm Cells Generated from Wharton’s jelly Mesenchymal Stem Cells Using Small Molecules on 3-Dimensional Nanofiber Scaffolds**

Shaffaf T¹, Kazemi Nezhad SR¹, Hoveizi E²

1. Department of Genetics, Faculty of Science, Shahid Chamran University, Ahvaz, Iran
2. Department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz, Iran

Tinashaffaf@yahoo.com

Introduction: Development and subsequent differentiation of definitive endoderm (DE) (form many of the major organs including the liver, pancreas, lungs and intestines, Mesenchymal stem cells) MSCs (are multipotent stromal cells with the capacity to self-renewal and differentiate into various lineages. In the present study, we aim to evaluate the proliferation and differentiation of WJ-MSCs to DE cells using electrospun nanofiber scaffold and small molecules.

**Materials and methods:** Poly lactic acid/(WAX) PLA/WAX electrospun nanofiber scaffold was utilized for 3 D culture. CHIR99021 and ITS were used to increase the rate of the differentiation: The expression of Sox17, FoxA2, Sox1, Sox7, and brachyury were investigated in both 2 D and 3 D treated and control samples using qRT-PCR. The differentiation of WJ-MSCs to DE was also evaluated at the protein level using Immunocytochemistry for SOX17 and FOXA2 proteins.

**Results:** The results showed that WJ-MSCs could prosperously differentiate into DE on PLA/WAX. Immunocytochemistry and qRT-PCR results revealed that key endoderm transcription factors SOX17 and FOXA2 were expressed in significantly higher levels in 3 D cultures in comparison to 2 D cultures and control samples. In addition, these data support that the small molecule efficiently drives the differentiation of WJ-MSCs towards an endodermal phenotype.

**Conclusion:** In conclusion, our research confirmed the positive effect of 3 D cultures on endoderm commitment of WJ-MSCs. The result of this study may have impacts on the therapy of diabetes patients and hepatic disorders by cell replacement therapy in future.

*Keywords*: definitive endoderm, differentiation, nanofibrous scaffold, small molecules, WJ-MSCs

**Ethics, Forensic Genetics and Human Identification**

**O-60: Application of mtDNA in identification of human bone remnants**

Bahmani H', Kiani E, Miri A, Habibi S, Mohamadi A, Khafaei M, Amini A, Tavallaee M

Human Genetics Research Center Baqiyatallah University of Medical Sciences, Tehran, Iran

Behroz_bahmani@yahoo.com

The most common methods used to Identification today are the use of STRs, but the use of these methods is not always possible. In some cases, the samples have been degraded so much that the use of genomic DNA is not possible to identify, or in cases where access to the ancestors is only possible. In cases where there is only access to mother tent, in which case it is possible to use mtDNA as an endorsement test. Given the specific features of mtDNA, such as greater stability than damage and higher copying rates than nDNAs, it can be used as a suitable model for paleontology, identification and Identifying offenders through genetically used. In 1991, in Russia, studies on mtDNA resulted in several bone bodies discovered in a mass grave for the identification of members of the Romanov family Tsar Nicholas II, and clarified the relevance of family members. In this study, DNA was first extraction from the bone and blood samples. Then, by sequencing of the hyper variable regions HV I and HV II in the Dloop region of the mitochon-
drial genome, and software analyzes, the relationship between the old bones of an anonymous body through genetic linkage of the mother’s native relative to the mother, sister and brother, who had previously been identified in other ways. It was observed that both regions are identical in all SNPs in the bone sample, mother, sister and brother.

**KEYWORDS:** mtDNA, Identification, PCR, Sequencing, Bone

**O-61:** Genetic variability of the SNP for ID 49-plex marker in north and south west Iranian populations


1. Human Genetic Research Center, Baqiyatallah University of Medical Science, Tehran, Iran
2. Department of Genetics & Biotechnology, School of Biological Science, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
3. Associated professor, Human Genetic Research Center, Baqiyatallah University of Medical Science, Tehran, Iran
4. Assistant professor, Human Genetic Research Center, Baqiyatallah University of Medical Science, Tehran, Iran

Email: rezabazayar1001@gmail.com

Single nucleotide polymorphisms (SNPs) are being increasingly used by forensic laboratories. In Iranian population, few studies have been reported with aim of allelic frequencies investigation and determination of genetic variation based on SNPs. The goal of this study is to evaluate population genetic diversity by genotyping of 49 autosomal SNPs based on SNPforID in the north (Gilakis) and southwest (Arabs) population, using the SNaPshot assay. Methods: In the current research, we analyzed 196 unrelated Iranian samples volunteer from the north and southwest regions of Iran for genotyping of 49 SNPs based on SNPforID by using SNaPShot method on the 3110xl ABI Genetic Analyzer were performed. Statistical analysis calculated using the Arlequin 3.5, GeneAlex 6.5 and GeneMapper 5.0 software. Results: The mean heterozygosities were calculated 0.407 for Gilakis and 0.44 for Arabs, also the minimum heterozygosity’s was 0.010 for rs2056277 and 0.83 for rs354439 in Gilakis and Arabs, respectively. Minor allele frequency in both Gilakis and Arabs was rs205627. There was the significant deviation in allelic frequencies from Hardy-Weinberg equilibrium for all the studied SNPs except rs727811 in Gilakis and rs251934 in Arabs. The calculated FST values presented among Gilakis and Arabs with three Iranian ethnic groups including Kurd, Persian and Lurs were not significantly different. Conclusion: Evaluation of the 49 autosomal SNPs simultaneously enables to discriminate between ethnic groups within the population. The results presented Gilakis and Arabs ethnicity genetically are similar relatively, may cause of immigration or close origin. The SnapShot method has appropriate sensitivity and specificity in order to simultaneously SNPs genotyping.

**Keywords:** Single nucleotide polymorphism(SNP), SNPforID, SnapShot, Iran

**O-62:** STR Markers and Challenges of Interpretation in the Genetic Identification

Miri A*, Habibi Azarian S, Bahmani H, Kiani E, Tavallaei M

Human Genetics Research Center, Baqiyatallah University of medical sciences, Tehran, Iran
alimiri1391@gmail.com

Short tandem repeat (STR) markers have been used by the forensic genetics community since the mid-1990s to produce DNA profiles to answer questions in criminal investigations and relationship testing. We will examine the STR markers commonly used by the forensic genetics community along with configurations of commercial STR kits that impact interpretation in Genetic Identification. Many applications of forensic genetics deal with degraded DNA in small quantities. This issue happens especially in the extraction and analysis of DNA from skeletal samples such as bones or teeth. In this paper we focused on specific challenges and solutions to genetic data analysis of old, degraded bone samples. Kinship analysis such as Disaster Victim Identification and Familial Searching involve very large numbers of comparisons. We will discuss the particularities such as the possible search strategies, diagnosis of mutation, ways to handle partial or mixed profiles affected by allelic dropout, and drop-in.

**Keywords:** Short tandem repeat (STR), Genetic Identification, Kinship analysis

**O-63:** Genetic Analysis and Genealogy of Ancient Bone Samples

Mohammadi A1, Zargari P2, Ramezani M3, Ahmadi K, Tavallai M

NOOR Genetic center
sepehre3293@gmail.com

Ancient DNA analysis can inspire both public and scientific community. Knowing about ancient human genome and comparing with modern human being genome can give us new perspective about evolution and migration of human during the history. Ancient DNA (aDNA) is DNA isolated from ancient specimens. It can be also loosely described as any DNA recovered from biological samples that have not been preserved specifically for later DNA analyses. Examples include the analysis of DNA recovered from archaeological and historical skeletal material, mummified tissues, archival collections of non-frozen medical specimens, preserved plant remains, ice and permafrost cores, Holocene plankton in marine and lake sediments, and so on. Due to the considerable anthropological, archaeological, and public interest directed toward human remains, they have received considerable attention from the DNA community.

Ancient DNA analysis can inspire both public and scientific community. Knowing about ancient human genome and comparing with modern human being genome can give us new perspective about evolution and migration of human during the history. Ancient DNA (aDNA) is DNA isolated from ancient specimens. It can be also loosely described as any DNA recovered from biological samples that have not been preserved specifically for later DNA analyses. Examples include the analysis of DNA recovered from archaeological and historical skeletal material, mummified tissues, archival collections of non-frozen medical specimens, preserved plant remains, ice and permafrost cores, Holocene plankton in marine and lake sediments, and so on. Due to the considerable anthropological, archaeological, and public interest directed toward human remains, they have received considerable attention from the DNA community.

In the very beginning of 1980, a group of Chinese researches proved that DNA is preserved in the tissues of ancient bodies. At 1984 researchers succeeded in the extraction DNA from quagga, an extinct member of the horse family, and in 1985 they could extract DNA from mammoth remains. At 1984 paabo demonstrated that DNA was present in a mummified in-
fantom from an Egyptian dynasty. Genetic genealogy is the use of DNA testing in combination with traditional genealogical methods to infer relationships between individuals and find ancestors. Genetic genealogy involves the use of genealogical DNA testing to determine the level and type of the genetic relationship between individuals. In this method we using of DNA markers such as autosomal SNPs, Y SNPs and mtDNA SNPs. By analyzing the sequence of mtDNA and chromosome Y, we can identify the path of human migration throughout history and the common ancestor of humans.

**Keywords:** Ancient DNA, Genealogy, DNA testing, DNA markers, mtDNA, SNPs

**O-64: The Successful DNA Extraction from Skeletal Remains for Genetic Identification**

Tabkhi R, Habibi S, chavoshi S, tavallaie M.

human Genetic Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

s.habibi28@yahoo.com

DNA identification using human remains is often necessary in cases of unidentified persons or in special cases that go through decades from unknown skeleton, in these cases, DNA extraction is performed to determine the identity of people from hard tissues such as bone and tooth, if the tooth and bone have long been in the environment and under different conditions, the work becomes much harder and reduce the possibility of extracting the DNA to be used in the next steps. This work has been accomplished after hands-on experiments and studies conducted over several years, both in manual and automated methods with very satisfactory results.

To use bone requires preparation steps before extraction. In the manual method, a bone fragment that has a higher density and is healthier than other parts are first cut. The removal of the contamination by physical and chemical methods is carried out and then the crushing and powdering is done, then demineralization is performed and finally the DNA extraction is performed in three steps of digestion, salting out and passing through the silica column using the QIAamp® DNA Mini Kit Blood with some changes in user manual.

Based on the results obtained from accurate DNA quantification using the Quantifiler Trio Kit and the successful amplification of the AmpFLSTR “Identifier” and AmpFLSTR “Minifiler” kits, the extraction method used was very efficient and effective and is now used in the identification of missing martyrs and other missing persons.

**Keywords:** DNA extraction, identification, skeletal remnants
Animal Genetics

P-1: Gene expression of TRAF3 in jejunum of broiler chickens under experimental induction of necrotic enteritis

Ahmadian M¹, Sekhavati MH², Kermanshahi H¹, Javadamaneh A¹ Razmyar J²

1. Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
monire.ahmadian2020@gmail.com

Necrotic enteritis (NE) is an important intestinal infectious disease caused by clostridium perfringens in commercial poultry flocks. Enteric disease is a major concern in poultry industry because it increases mortality and decreases products. Recent studies of NE have focused on finding different ways to control the disease and on understanding its pathogenesis. Various factors favor the development of experimental NE, including use of coccidial vaccines, combined with netB-positive C. perfringens administration and nutritional factors such as high percentage of NSP. In the present study, 80 male broiler chickens (Ross 308) randomly assigned to a completely randomized design including 2 treatments of 4 replicates and 10 chickens in each replication. Treatments were 1) a corn-soybean meal based diet (Ctrl), and 2) Ctrl contained 250 g wheat/kg diet and simultaneously challenged with coccidiosis vaccine and Clostridium perfringens. Ioculation of Eimeria and clostridia were applied on day 19. The jejunal samples were used to determine the expression of immune-related genes in subjected chickens. Relative expression of TRAF3 gene was evaluated and GAPDH and ACTB were used as reference genes to normalize expression data. Results showed that the combination of challenging factors (i.e. high wheat inclusion plus coccidiosis vaccine plus C. perfringens) increased (P < 0.05) TRAF3 gene expression significantly.

Keywords: Necrotic enteritis, TRAF3, gene expression, broiler chickens

P-2: Analysis of some candidate genes in association with behavior in Canine

Fallahi M, Masoudi A, Vaez Torshizi R

Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
mohammad.fallahi@modares.ac.ir

The potential trainability of dog is affected by learning and memory genes. Although some researches have been done on exploring the genes affecting learning and memory in animals, but these kinds of genes have not yet been identified in canine. The results of our previous study showed that three genes BDNF, CCK and TAC1 gene could be a strong candidate associate with learning and memory in dogs. Therefore, in this research we tried to sequence the structure of these genes in Caine. To achieve this, the behavioral characteristics of a population of 80 Belgian Malinois were evaluated using questionnaire that was based on the Canine Behavioral Assessment and Research Questionnaire (C-BARQ). Then, total DNA of the samples was isolated using salting out method from blood tissue. The target regions of these genes were amplified by Polymerase Chain Reaction (PCR) using the suitable primers. In this study, 8 single nucleotide polymorphisms (SNPs) were identified in the candidate genes. Further investigation revealed that the -161 G >T mutation located in the canine TAC1 putative promoter region and affected transcription factor binding site. A statistical analysis revealed that the GG genotype at the -161G >T produced a significantly greater trainability level than that of the TT genotype (P < 0.001). Furthermore, the A (CGATAGGA) haplotype combination was significantly associated with canine trainability behavior (P < 0.001). But the results did not identify any polymorphism for the BDNF and CCK genes that can be attributed to the conservation of these genes in Canine.

Keywords: Dog, Learning and Memory, Genetics, behavior

P-3: Application of SSCP-PCR technique on immune genes of bovine

Firouzamandi M¹, Eshghi D¹, Shahbazi R², Tolouei M², Asadpour R²

1. Department of Pathobiology, Faculty of Veterinary Medicine, 5166, University of Tabriz, 616471, Tabriz, Iran.
2. Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Iran.
m.firouzamandi@tabrizu.ac.ir

Background: Bovine lymphocyte antigen (BoLA) is an important component of the immune system. BoLA genes are organized into I, II, and III classes. At present, only one class I locus (BoLA-A) is internationally accepted on the basis of serologica l testing. DRB3 is one of the class II genes which cattle express per haplotype. Objective: our objective was characterization of BoLA-DRB3 and BoLA-A in bovine. Method: In current study, blood sampling was carried out from Holstein cattle (n=50 susceptible and n=50 resistant to mastitis) and a random sampling was conducted from Iranian native cattle (Sarabi; n=50). Amplification of exon 2 of BoLA9DRB3 and BoLA-A genes by specific primers was used for SSCP technique. Results: SSCP technique able to recognize many genotypes patterns which were associated with resistance or susceptible cattle to mastitis. Also, SSCP results was showed 17 genotypes pattern which were associated with resistance or susceptible cattle to mastitis. Conclusion: Thus, our finding indicated that Sarabi breed comprises completely different allelic in DRB3.2 region in compared to Holstein breed. Moreover, results of this study revealed that exon 2 of BoLA-A is monomorphic in both studied breed. This is the first study on characterization of exon 2 of BoLA-A gene using SSCP technique in bovine.

Keywords: BoLA-DRB3, BoLA-A, PCR-SSCP technique, Mastitis Disease

P-4: Effect of training population size and marker density
on genomic prediction accuracy of BaysC, BayesR and BayesL methods

Foroutanifar S

College of agriculture and natural resources, Razi University, Kermanshah, Iran
foroutanifar@gmail.com

The aim of this research was to study effects of training population size and marker density on accuracy of genomic breeding value estimated by BaysC, BayesR and BayesL methods. For creating historical generation, a population with an effective population size (Ne) of 100 (50 males and 50 females) and two traits with the heritability of 0.6 and 0.1 was simulated using stochastic model for 50 generations. In generation 51, the number of individuals increased to either 1000 or 500 with both genotype and phenotype records for both traits (Reference population). By randomly mating of individual in previous generation, validation population was generated. The simulated genome size for each animal was 10 Morgan that was equally divided between 10 chromosomes. In order to evaluate different marker density, 100 or 1000 SNP markers were evenly located on each chromosome. A total of 50 QTL loci were randomly distributed over the genome for each trait. Marker effects in reference population were estimated using BayesC, BayesR or BayesL methods. Accuracy of genomic breeding values of different methods were compared in validation population. The results showed that for all methods accuracy of breeding values increased as heritability, map density and number of individuals in reference population increased. When the number of reference population was 500, there was no differences between accuracy of different methods. Whereas, The BayesC method had higher accuracy relative to other methods, when the number of individual in reference population was increased to 1000.

Keywords: BayesC, BayesR, BayesL, Genomic selection.

P-5: Experimental verification of a predicted novel miRNA located in SPTBN4

Hosseini F, Mohammad Soltani B, Hosseinkhani S, Baharvand H
1. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.
2. Department of Biochemistry, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
3. Department of Stem Cells, Royan Institute, Tehran, Iran
fahime hosseini@gmail.com

Spectrin, beta, non-erythrocytic 4, also known as SPTBN4, is an actin crosslinking and molecular scaffold protein that links the cell membrane to the actin cytoskeleton, and functions in a number of cell process: cell cycle, cell adhesion, cell spreading, DNA repair and intracellular traffic. MicroRNAs are many small non-coding RNAs approximately 18-24nt in length that act as master regulators in many pathways and process in a cell. SPTBN4 gene was searched for miRNA like structures prediction with some softwares such as SSC profiler, MiPred, mireVal, CIDmiRNA and etc. In this study, one conserved stem-loop structures was selected and over-expressed in a human cell line to survey production of putative mature miRNA of its precursor. The result of our study confirmed the production of a novel miRNA in this gene. The novel miRNA is the first miRNA identified in SPTBN4 gene.

Keywords: miRNAs, SPTBN4, Software

P-6: Evaluation of the effect of autophagy induction and inhibition on Street rabies virus titer in NMRI murine model by real-time PCR

Hosseini Heydarabadi SF, Sheikholeslami F, Salahshouriifar I
1. Department of Genetics, Faculty of Basic science, Science and Research branch, Islamic Azad University, Tehran, Iran.
2. Rabies laboratory, Virology Department, Pasteur Institute of Iran
fatemeh hs70@yahoo.com

Background and Aim: Rabies is the most common zoonotic disease in the country. Autophagy is a vital process that maintains homeostasis by removing the harmful components of cytoplasm and can be used as an inherent defense mechanism against viruses. Increasing knowledge about the interaction between rabies virus and autophagy pathway genes and effect of host cell autophagy on rabies virus replication may lead to new antiviral treatments.

Materials and Methods: Six groups of NMRI mice were treated by 3-methyl adenine, rapamycin, sterile phosphate buffer, street rabies virus, rapamycin plus rabies virus and 3-methyl adenine plus rabies virus. Drugs and virus were injected intra¬cranially. Street rabies virus titer and Map1lc3 gene expression were detected by Real-time PCR and cell death in apoptosis was measured by TUNEL Assay and LC3B protein level was measured by Immunohistochemistry.

Results: Map1lc3 gene expression in viral specimens increased over all hours compared with normal specimens. The level of LC3B proteins were also increased during street rabies virus infection, but the number of apoptotic cells did not increase. Doses of rapamycin and trimethyl adenine drugs used in this study did not have any effect on the rabies virus titer

Conclusions: The street rabies virus induces autophagy in brain of infected mice, which could be result of the innate immune system of the cells inhibition of virus activity but autophagy induction and inhibition did not have any effect on Street rabies virus titer in brain of infected mice. The street rabies virus has no apoptotic effect

Keywords: street rabies virus, Autophagy, Map1lc3 gene, LC3B, Immunohistochemistry, Real- time PCR

P-7: Application Of STR In Genetic

Jabbari S, Mashayekhi MR, Hasanpour A
1. Department of Genetic, Tabriz Branch, Islamic Azad University, Tabriz, Iran
2. Department of Genetic, Tabriz Branch, Islamic Azad University, Tabriz, Iran

Abstracts of the 3rd International & 15th Iranian Genetics Congress
3. Department of Clinical Sciences, Tabriz Branch, Islamic Azad University, Tabriz, Iran
sarvin.shayan@yahoo.com

Introduction: The horse’s influence on human history and civilization make it one of the most important domestic animals. Among molecular markers, microsatellites are suitable for biodiversity evaluation owing to their codominant inheritance, high heterozygosity and distribution across the genome, ease and reliability of scoring. In the current work genetic diversity of the Arabian horse breed was studied using 50 individuals. Among molecular markers, microsatellites are suitable for biodiversity evaluation owing to their codominant inheritance, high heterozygosity and distribution across the genome, ease and reliability of scoring. In the current work genetic diversity of the Arabian horse breed was studied using 50 individual horses.

Material and methods: Sampling from Arabian horse was done and their DNAs extracted. Extracted DNAs were run in an agarose gel and concentration and quality of DNAs was measured by Nano-drop. We used four microsatellite markers include ASB17, LEX3, HMS1, and CA425. These loci were amplified by multiplex PCR with fluorescent dye-labeled primers. PCR products were separated and analyzed with capillary electrophoresis and the outputs were analyzed using Genmapper software.

Results and discussion: The results showed at ASB17 locus 6 alleles, observed heterozygosity in ASB17 locus was 0/62 however the expected heterozygosity was 0/715. At LEX3 locus 9 allele was seen. At this locus observed heterozygosity was 0/26 and expected heterozygosity was 0/812. At HMS1 locus 6 allele was seen also the allele, Observed and expected heterozygosity at this locus were 0/52 and 0/669. At CA425 locus 5 alleles was seen Observed and expected heterozygosity was calculated 0/52 and 0/546.

Conclusion: Results of this study showed high frequency genetic diversity in Arabian population in compare with the other horse breeds.

Keywords: Arabian horse, Microsatellite, Genetic diversity, Multiplex PCR

P-8: Aquatic transgenic technology in aquaculture

Jamshidi Sh.

Agricultural Biotechnology Research Institute of Iran, Gilan Branch, Agricultural Research, Education and Extension Organization (AREEIO), Rasht, Iran.
jamshidi99@yahoo.com

Aquatic transgenic organism is an organism with a foreign gene or non-coding deoxyribonucleic acid (DNA) fragment is artificially introduced and stably integrated in their genomes. Since the first report in 1985, a wide range of transgenic fish and marine bivalve mollusks and crustaceans have been produced by microinjecting or electroporating homologous or heterologous transgenes into newly fertilized or unfertilized eggs and sperm. For producing transgenic organism many factors has been included. The first and the most important factor is aquatic species for gene transfer. In species selecting for gene transfer the target of research and species preservation with a high safety conditions and facilities should be concerned. The second factor is construct of target gene should be programmed for make it and need for these studies. For example gene construct in gene transfer method should have open reading frame of gene target. Also gene construct should have regulatory factors for regulating stable or transient gene expression or affect growth and development in organism. In the third step, recombinant construct should be transfer into sperm or embryo for stable gene expression. In the end stage, individuals received target gene should be traced. In addition, application of transgenic technology for producing transgenic fish and other aquatic organisms with beneficial traits such as somatic body growth, resistance to diseases and producing useful product for human, domesticated animal, poultry and aquatic organisms have been increased by biotechnology.

Keywords: Aquatic transgenic organisms, gene transfer, construct, transgenic fish, biotechnology.

P-9: Study the protective effect of curcumin-linoleic acid on anxiety and NT4 gene expression changes in male rats stricken with Multiple Sclerosis


Department of Animal Biology, Faculty of Natural sciences, University of Tabriz, Tabriz, Iran.
Maryam_kh7425@yahoo.com

Introduction: Anxiety is perhaps the most taxing effect of living with MS. Neurotrophins can have a potential role in the disease pathology. The purpose of this study is to assess the antioxidant effects of curcumin-linoleic acid on anxiety and NT4 gene expression alteration in brains of MS rats.

Material and Methods: 35 adult male rats were randomly divided into five groups including: control, sham, lesion or MS group MS+curcumin-linoleic acid (5 and 10 microgram/rat). One week after MS induction treated groups were micro-injected by mentioned dose of curcumin-linoleic acid for 5 consecutive days. Elevated Plus Maze was used for studying the anxiety related behavior. Gene expression was measured by RT-PCR with the use of GapDH as a house keeping gene.

Results: The results showed that the level of open arm time (OAT) and open arm entries (OAE) significantly decreased in multiple sclerosis group in comparison to control or sham groups respectively (p<0.05) and (p<0.01). Treatment of MS group with curcumin-linoleic acid (5 and 10 microgram/rat) significantly increased the level of open arm time (OAT) and open arm entries (OAE) in comparison to MS group (p<0.001). Furthermore, the level of NT4 gene expression significantly decreased in multiple sclerosis group in comparison with control and sham groups (P<0.05).

Discussion and Conclusion: Treatment of MS group with curcumin-linoleic acid in both doses has anxiolytic effect. Curcumin-linoleic acid reduced the level of anxiety in MS group. The alteration levels of NT4 gene expression has a role in pathology of MS disease.

Keywords: Multiple Sclerosis, Anxiety, NT4 gene expression, curcumin-linoleic acid, Rat
Water and River buffalo (Bubalus bubalis) are important domestic animals distributed in the tropical and subtropical regions. Recent studies have reported that DGAT1, GH, GHR, PRL, CSN and PRLR genes localized near to quantitative trait loci (QTL) are associated with milk traits (fat and protein) in dairy cattle and buffalo. DGAT1 in buffaloes is approximately 8.3 kb long, contains 17 exons, and is located on the 14 chromosome. Two single-nucleotide polymorphisms (SNPs) in the 8 exon of DGAT1 that cause the substitution of lysine with alanine (K232A) were considered to significantly affect variation in milk fat content (1). CSN3 polymorphism has been investigated during the last decade using nucleotide sequence analysis. Two nucleotide variants at codons 135 Thr (ACC)/Ile (ATC) and 136 Thr (ACC/ACT) (silent mutation), have been reported in Italian Bulgarian and water buffalo genomic library (1). Growth hormone (GH) is an anabolic hormone synthesized and secreted by the somatotroph cells, and plays an important role in postnatal tissue growth, lactation, reproduction, and also, protein, lipid and carbohydrate metabolism in buffalo (2). Prolactin (PRL) is one of the most versatile hormones of the pituitary gland in terms of biological activities. The main functions of PRL are regulation of production, promotion of lactation in mammals, synthesis of milk (lactogenesis) and maintenance of milk secretion (galactopoiesis) (2). The aim of the present study was to molecular bioinformatics analysis of Candidate genes and milk production in the Buffalo (Bubalus bubalis) whit Focus on the DGAT1, CSN, GH, GHR, PRL and PRLR genes. The nucleotide sequence of DGAT1 (AY999090), GH (AJ011533), GHR (AY940159), PRL (EF027441), PRLR (EF054878) and CSN (GCF_000471725.1) was obtained from Gen NCBI. The DNASTAR (DNASTAR Inc., Madison, WI, USA), Genomics (CLC Genomics, version 4) soft wares were used for finding cis-regulatory elements in genomic sequences, CpG islands and ORFs regions. As well as, in order to find a relationship between PIN and sequences I only used sequences, CpG islands and ORFs regions. As well as, in order to find a relationship between PIN and sequences I only used sequences, CpG islands and ORFs regions. The results indicated that there is several possible CpG islands (CGIs) in the guanine- and cytosine rich regions for epigenetic and genome studies. Additionally, the sequences of DGAT1, GH, GHR, PRL, CSN and PRLR genes showed 13, 6, 3, 7, 4 and 9 motifs at several regions. The Results herein suggest that more studies are needed to better understand of effects of candidate genes on milk product in buffalo. Keywords: Bubalus bubalis, Candidate gene, Bioinformatics.

P-11: Study of PRNP gene polymorphisms in Lori-bakhtiar sheep and it’s relation with Scrapie disease

Moradi Shahrbabak H, Samiei A, Mehrabani Yegane H, Sadeghi M, Emamjome S V
Tehran University, Iran
samiee.animalscience@gmail.com

Scrapie disease is the result of a mutation in the PRNP gene (Perion protein). Despite of some polymorphisms, only 3 codons are involved in sensitivity to Scrapie, which includes codons 136, 154, and 171. For this experiment, 53 blood samples were collected from Lori-Bakhtiar sheep and specific primers were used to amplify the gene. The PCR-RFLP method was used to detect polymorphism in these 3 positions. Only genotype AA was detected for position 136 and only RR genotype was found for position 154. There for all of animals were monomorph in the two loci. But the results show that animals in position 3 of the codon 171 were polymorph and genotypes of R, Q and H were observed. As a result, genotypes of animals for the three loci were ARQ / ARQ, ARQ / ARR, ARQ / ARH, ARR / ARH and ARH / ARH with the frequency of 0.358 (19 sheep), 0.301 (16 sheep), 0/169 (9 sheep), 0/056 (3 sheep) and 0.113 (6 sheep), respectively.allelic frequency of Q, R, and H were 0.594, 0.179, and 0.226, respectively. According to studies, Sheep with ARR / ARR genotype are very resistant and ARR / ARQ, ARR / ARH and ARR / AHQ genotypes are resistant and AHQ / ARQ, ARQ / ARQ, ARQ / AHQ, ARQ / ARH genotypes donâ€™t have a significant difference for sensitivity or resistant to the disease and ARR / VRQ and ARQ / VRQ / ARQ genotypes was sensitive and VRQ / VRQ, ARQ / VRQ, VRQ / ARQ, AHQ / VRQ and VRQ / VRQ genotypes are highly sensitive to scrapie. The most of animal in Lori-Bakhtiar were ARQ / ARQ genotypes and classified as sensitive to scrapie. We should use of the gene in selection program Due to importance of the gene about scrapie disease. Keywords: polymorphism, Lori-bakhtiar, Scrapie gene

P-12: Study of PRNP gene polymorphisms in Lori-bakhtiar sheep and it’s relation with Scrapie disease

Moradi Shahrbabak H, Samiei A, Mehrabani Yegane H, Sadeghi M, Emamjome S V
Tehran University, Iran
samiee.animalscience@gmail.com

Scrapie disease is the result of a mutation in the PRNP gene
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MAX) algorithm with the recently developed Caprine 53K study (GWAS) using an efficient mixed model analysis (EMMAX) algorithm with the recently developed Caprine 53K SNP bead-chip. An adaptive permutation testing was utilized to determine the significant SNPs as they couldn’t pass the FDR and Bonferroni cut-off thresholds. Potential candidate genes were explored using the ARS1 genome assembly on the NCBI Genome Data Viewer website. We identified genes such as LRRN3, LEO1 and SGC3 with biologically relevant roles in cell growth and muscular differentiation which could be used in breeding strategies pending further molecular validation.

Keywords: PCA, Markhoz, Goat, GWAS

P-14: Detecting Differential Usage of Exons from RNA-Seq Data of the heart and lung in sheep tissues

Nikokalam Azim F, Masuodi A, Ehsani A, Shariati P

Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
fa.nikokalam13@gmail.com

Different usage of exons of the genes in animal tissues is one of the most important genetic issues which affect a various expression of genes in organs. In the present study, we used RNA-Seq data of the heart and lung tissues of sheep to explore the differentially expressed exons between the tissue and individuals. To achieve this, three replicates of the RNA-seq data including SRR5190383, SRR5190386 and SRR5190389 of heart and SRR5190382, SRR5190385 and 5190388 of the lung were aligned usage software star and The remainder of the analysis is done in R usage introduced to DEXSeq, package. The results showed that when heart tissue was studied in different individuals, the difference in gene expression was about 25 genes, while gene difference in lungs between the individuals reached zero and actually other was no difference between gene expression in the lung of the subjects could not be seen. Next study expresses that, the difference of gene expression between the lungs and heart tissues was about 1013 genes. Finally, in conclusion, the difference between gene expression among the individuals is less than a difference between tissues in the samples.

Keywords: polymorphism, Lori-bakhtiari, Scrapie gene

P-15: The polymorphism effect of leptin gene on milk production traits in Holstein cattle

Rahmati H¹, Hosseinpour Mashhadi M¹*, Elahi Torshizi M¹

Department of Animal Science, Mashhad Branch, Islamic Azad University, Mashhad, Iran
amirkhan.2015@gmail.com

The aim of this study was to investigate the polymorphism of leptin gene and its effects on milk production traits in Holstein cattle.

In order to investigate the relationship between the polymorphism of leptin gene and some milk production traits in Holstein cows, blood samples were collected from 100 cows from a dairy farm in Khorsan Razavi province. GeNet Bio was used to extract the DNA from the commercial kit. In order to amplify a fragment of 94 pairs of the gene, the PfU PCR PreMix

Markhoz goats are one of the worthy multi-purpose breeds with the main habitat in the west of Iran, Kurdistan. They are very well-known for producing a luster fiber called “Mohair”, but meat and milk production have the second and third degree of importance by providing the main likelihood for the native residents. The latter makes the study of some growth and body size traits from the genetic aspects vital. In this study, we applied principal component analysis (PCA) for the first time to the genetic data of the Markhoz goat. The latter makes the study of some growth and body size traits from the genetic aspects vital. In this study, we applied principal component analysis (PCA) for the first time to the genetic data of the Markhoz goat.

1. Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.
2. Department of Animal Science, Cornell University, Ithaca, NY, USA
3. Department of Animal Science, Faculty of Agriculture Engineering, University of Kurdistan, Sanandaj, Iran.

anahit_nazari@ut.ac.ir

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Markhoz goats are one of the worthy multi-purpose breeds with the main habitat in the west of Iran, Kurdistan. They are very well-known for producing a luster fiber called “Mohair”, but meat and milk production have the second and third degree of importance by providing the main likelihood for the native residents. The latter makes the study of some growth and body size traits from the genetic aspects vital. In this study, we applied principal component analysis (PCA) for the first time to the genetic data of the Markhoz goat. The latter makes the study of some growth and body size traits from the genetic aspects vital. In this study, we applied principal component analysis (PCA) for the first time to the genetic data of the Markhoz goat.
(AccuPower-South Korea) was used to amplify the gene. Polymerase chain reaction (PCR) was performed for amplification of 94 pairs of games using a pair of proprietary initiators. The fragment was digested by PCR-RFLP by KPN21 restriction enzyme. Statistical data was analyzed using POPGENE software version 1.32. The genotype of each animal was determined by using RFLP method and 3% agarose gel and ethidium bromide staining. For digestion of PCR products, 1 unit KPN21 enzyme was used at 37 °C for 6 hours. The frequency of T and C alleles were 0.53 and 0.47, respectively, and the TT and CT and CC genotypes were 0.397, 0.266 and 0.337, respectively. Chi square test showed that the studied population was not in Hardy Weinberg equilibrium (p <0.05). The relationship between the polymorphisms and traits was evaluated by SAS software. The Duncan test was used to compare the average of milk production traits. The polymorphism of the leptin gene was not significantly correlated with the traits of the milk yield, fat content, and milk protein content and percentage (P> 0.05). There was a significant relationship between the leptin polymorphism and the level of S.C.C (P <0.05) the highest mean of somatic cell count was related to TT genotype. This suggests the possible role of the leptin gene in the regulation of immune responses and the occurrence of mastitis.

**Keywords:** Polymorphism, Leptin Gene, Holstein Cow, PCR-RFLP

**P-15: The Effect of Fetal Bovine Serum on Differentiation of Ovine Myogenic Satellite Cells**

Rashidian Z¹, Javadmanesh A², Dehghani H²

1. Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine and Embryonic and Stem Cell Biotechnology and Regenerative Medicine Research Group, The Research Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

javadmanesh@um.ac.ir

Satellite cells are a population of adult muscle stem cells that play a key role in mediating muscle regeneration. The established muscle-derived satellite cells model can be used to study the genes associated with muscle development, and as seed cells for animal biotechnology related studies. Sheep satellite cells have a greater similarity to human satellite cells with regard to metabolism, life span, proliferation and differentiation, than satellite cells of the rat and mouse; from this feature, can be used as an animal model for the treatment of human diseases. These cells are precursors of myoblast cells. The purpose of this study was to determine the effect of the Fetal Bovine Serum (FBS) content on the process of satellite cells growth rate and differentiation. The satellite cells were isolated from Semimembranosus and Semitendinosus Muscle tissues of 50 to 60-day-old Kurdi sheep fetuses. After Enzymatic digestion, a combination of satellite and non-myogenic cells was cultured on the flask. Flasks were replaced after 3 hours to isolate non-myogenic cells, such as fibroblasts. After 6 days, the cells differentiated. Then the cell growth were evaluated by counting for 8 continuous days in media containing 0, 5 and 10% of FBS. The analysis of cell growth showed that the differentiation of satellite cells was significantly higher in enriched medium with 10% FBS (P<0.05).

**Keywords:** Satellite cells, Sheep, Fetal bovine serum, Differentiation

P-16: Transcriptomics pathway analysis with cellular signaling pathways as a key tool to improve marbling in beef cattle

Roudbari Z¹,², Coort S L², Evelo C T³,¹

1. Department. Animal Science, University of Jiroft, Jiroft, Iran
2. Department. Bioinformatics-BiGCaT, Maastricht University, Maastricht, The Netherlands
3. Maastricht Centre for Systems Biology (MaCSBio), Maastricht University, The Netherlands

rodbari.zahra@gmail.com

Background: Red meat is as an important dietary source that provides part of the nutritional requirements such as proteins, minerals, B-complex vitamins and essential fatty acids. Intramuscular fat is located throughout skeletal muscles. It is responsible for the marbling seen in certain cuts of beef. Marbling is a trait of major economic relevance that positively influences sensory quality aspects, including flavor, juiciness and tenderness of meat.

**Objectives:** the objective of this study was to identify cellular signaling pathways regulating muscle marbling in beef cattle using microarray gene expression data.

**Methods:** publicly available preprocessed transcriptomics data (E-GEOD-46411) from a study by Sadkowski et al was used. They measured gene expression in skeletal muscle of well-marbled beef and lean-marbled beef using Agilent microarrays. Pathway analysis was performed with the pathway visualization and analysis tool, PathVisio.

**Results:** The regulation of marbling is possibly the result of interaction of signaling pathways in muscle, fat and intramuscular connective tissue, identifying these processes with pathway analysis can help to decipher the key marbling processes. Pathway analysis revealed 17 pathways that showed differences in expression (z-score > 1.96) between well-marbled and lean marbled beef. MAPK (WP998) and P38 MAPK (WP1037) signaling, two pathways well known two affect lipid metabolism, were enriched in the well marbling breed. In addition, the signaling pathways â€œhypertrophy modelâ€-, â€œmicroRNAs in cardio myocyte hypertrophyâ€- and â€œphysiological and pathological hypertrophy of the heartâ€- that play a role in tissue development were affected. Interestingly, the analyses also demonstrated that pathways related to immune response (IL signaling, TCR signaling and Toll-like receptor signaling pathways) and insulin signaling, mitochondrial gene expression and Vitamin D metabolism were enriched and might act together with the pathways related to lipid metabolism.

**Conclusion:** The present study shows that regulatory pathways of marbling in Bos taurus muscle are regulated not only for pathways related to lipid metabolism and muscle development but also for pathways involved in energy metabolism, protein synthesis and immune response.
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Keywords: Animal breeding, Marbling, Meat quality, Transcriptomics analysis

P-17: Recent and past effective population size in different horse breeds.

Sadeghi R, Moradi-Shahrbabak M, Miraei Ashtiani SR, Antczak DF
1. Department of Animal Science, University of Tehran, 4111 Karaj, Iran.
2. Baker Institute for Animal Health, College of Veterinary Medicine, Cornell University, Ithaca, New York 14853 USA.
E-mail: rsadeghi2@ut.ac.ir

Estimates of effective population size (Ne) are important in species conservation biology and in animal husbandry. This study evaluated the effective population size (Ne) from genome-wide linkage disequilibrium in three different horse breeds: Thoroughbred (n=17), Turkmen (n=11) and Persian Arabian (n=71). We used a recently developed method that estimates Ne from genome-wide linkage disequilibrium (LD) [SNP software, Barbato et al., 2015]. The samples were collected from USA (Thoroughbred horses) and Iran (Turkmen and Persian Arabians) and genotyped using the Equine 670k Affymetrix SNP chip. The relationship between LD and Ne was estimated using the Haldane recombination rate modifier under the assumption of a genome-wide linear relationship such that 1 cM ~1 Mb. Genome-wide average LD (r2) decreased with increasing genomic distance for all breeds. Persian Arabian had the lowest level of LD. The LD decay of Turkmen was lower than that of the Persian Arabian and Thoroughbred. We observed a pattern of decreasing Ne with estimated values of 54, 62 and 245 at one generation ago for Thoroughbred, Turkmen and Persian Arabian, respectively. This pattern is consistent with artificial selection by breeders with increasing usage of some specific bloodlines in more recent generations. This result can be used in analyzing population structure and designing horse breeding strategies.

Keywords: Effective population size, linkage disequilibrium, SNP, horse.

P-18: Analysis of inbreeding in the closed population of Iranian Karakul sheep

Sadeghi S1, Sheikhlou M2, Safari R2, Bahri Binabaj F3
1. Ahar Faculty of agriculture and natural resources, University of Tabriz, Iran
2. Department of Animal Science, Ahar Faculty of Agriculture and Natural Resources, University of Tabriz, Iran
3. Department of Animal Science, Faculty of Agricultural Science, University of Gonbad Kavoos, Gonbad Kavoos, Iran
pkaraji@yahoo.com

The objectives of this study were to estimate the inbreeding and parameters derived from inbreeding such as Ballou and Kalinowski ancestral inbreeding and partial inbreeding coefficients using the pedigree information collected from 1991 to 2016 in the breeding flock of Iranian Karakul sheep. Animals born between 2012 and 2016 with a pedigree completeness index of at least 0.6 were selected as a reference population to reflect the status of inbreeding in last generation. Average inbreeding in all animals and in the reference population were 0.85 and 1.36 percent, respectively. The rate of inbreeding was 0.05% per year and 0.16% per generation. Inbreeding of the animals in the reference population decomposed to the partial inbreeding from 280 founders. Only 108 founder have positive contribution to the inbreeding of the reference population. The 10 and 25 founders contributing the most to inbreeding explained 42 and 66% of the inbreeding of the reference population, respectively. Mean Ballou ancestral inbreeding in all animals and in the reference population were 1.17 and 2.12, respectively. The amount of Kalinowski ancestral inbreeding was low in this population, as mean Kalinowski ancestral inbreeding in all animals and in the reference population were 0.07 and 0.04, respectively. The estimated inbreeding and its trend were in acceptable level. The estimated partial inbreeding coefficients can be used in mating decisions to avoid inbreeding arising from founders with the greater contribution to inbreeding depression. Considering the estimated Ballous ancestral inbreeding, testing the incidence of purging of the deleterious alleles in this population are suggested.

Keywords: Inbreeding, Partial Inbreeding, Ancestral Inbreeding, Karakul sheep

P-19: Characterisation of mutations in Gonadotropin releasing hormone receptor gene (GnRHR) in Markhoz goats

Shokrollahi B1, Sedighi Z2, Saadati N2
1. Department of animal science, agriculture faculty, Sanandaj branch, Islaic Azad University, Kurdistan, Iran
2. Department of Biology, Basic sciences faculty, University of Kurdistan, Kurdistan, Iran
Borhansh@yahoo.com

Markhoz goats are raised in Kurdistan regions of Iran, Iraq and Turkey and are a high-quality mohair producing animals. The population of this breed has been decreased from 22,000 heads in 1996 to less than 1,400 heads in 2017. The variation in the kidding size of Markhoz goats makes them an interesting genetic material to study the underlying genetic mechanism of prolificacy. Reproductive traits have a high economic value in goat breeding, but the heritability of such traits is low; therefore, selective breeding as a traditional method will be a slow process for improvement of the reproductive performance. Alternatively, exploring and discovering of prolificacy genes in goat breeds could effectively increase their reproductive performances through marker assisted selection (MAS) that can propose a clarification for improvement in traits. The hypothalamic gonadotropin-releasing hormone receptor (GnRHR) is a key controller of the reproductive system, which stimulates the synthesis and release of LH and FSH in the pituitary gland. Because of the main role of GnRHR in regulating gonadotropin synthesis and release, the GnRHR gene revealed to be a good candidate for mutations associated with reproductive performance. The present study was aimed to identify mononucleo-
tide mutations in Gonadotropin-releasing hormone (GnRH) receptor gene in markhoz goat breed. Blood samples were taken from forty goats from Markhoz breed. Genomic DNA was extracted from the blood samples. A part of Gonadotropin-releasing hormone receptor gene was amplified using PCR; and sequences of PCR products were determined. Data were analyzed using NCBI_BLAST software package. The obtained sequences were aligned with GnRHR sequence (with access ID NM-001285612) using Clustalw software package. Various types of mutation, inserts and deletions were identified in different haplotypes; and results of sequencing analysis showed that 10 haplotypes, 16 mutations, 3 attachments and 4 omission cases exist in investigated sequence. This research appeared the genetic variations in GnRHR gene in Markhoz goats, these variations should be confirmed and investigated for their possible association with reproductive traits in Markhoz breed.

Keywords: GnRHR, Mutation, Reproduction, Markhoz Goat

P-20: Evaluation of the genetic diversity of Iranian maral deer (Cervus elaphus maral) based on mitochondrial and microsatellite markers

Farahvash T, Vaez Torshizi R, Masoudi AA, Rezaei HR, Tavallaei M

1. Department of Animal Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.
2. Department of Environmental Science, Faculty of Fisheries and Environmental Science, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran.
3. Human Genetic Research Center, Baqiatallah Medical University, Tehran, Iran.

Maral deer is the biggest native deer of Iran that in the past was existed in the Caspian area. During the recent decades most of the deer populations have been exposed to the size reduction due to several reasons such as disturbance of the forests, distribution of the cities and the illegal hunting. These factors have been fragmented maral populations into few small naturally reserved and one wild populations over years. The smaller are the populations the less adaptation and merit would be expected in the wild populations. This is why there should be conservation management for these populations. In order to have a successful management, there must be sufficient information of the population and genetic structure from the species. In order to evaluate the genetic structure of the Iranian maral deer populations, samples were collected from Gorgan, Gilan, Mazandaran, Semnan, Qazvin and East Azerbaijan and the genetic distance was higher than 90 percent similarity between maral populations. The D-Loop and cyt b sequences had the highest diversity. The genetic distance was higher between East-Azerbaijan and Mazandaran and between Semnan and Qazvin populations. The D-Loop and cyt b sequences had 5 and 4 haplotypes, respectively with 95 percent haplotype diversity. Tajima’s D showed that maral populations were suffered from tense natural selection at the late glacial and had founder effect. The phylogenetic analysis of maral and other red deer species showed that maral were in the same cluster with west European red deer and had low similarity with the median Asia and African-north American red deer. In order to estimate the genetic diversity of maral deer, 6 microsatellite loci were amplified. The mean observed polymorphism was 76 percent. The F was negative for the all loci indicated that there was genetic diversity within maral populations based on the microsatellite information. The AMOVA showed that 10 percent of the diversity was from between and 90 percent was from within populations. The genetic similarity matrix showed higher than 90 percent similarity between maral populations. According to the genetic distance and Fst from mitochondrial and microsatellite markers, it is recommended to transfer male deer from Gorgan and Mazandaran populations to the other reserved populations to improve the genetic diversity by gene fellow and avoiding further reduction in genetic diversity.

Keywords: Genetic diversity, Maral, Deer, Iran

P-21: Applied bioinformatics to assay genetic networks of resistance to heat stress in native chicken

Tohidi R¹, Nasiri M²

1. Dept. of Agriculture, University of Torbat-e Jam
2. Dept. of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad

tohidi75@gmail.com

Heat stress strongly affects poultry production. Selection of chickens resistant to heat stress can be a suitable strategy to reduce the negative effects of global temperature increase on chicken breeding. In this study, a gene network based on the data from previous studies about the effect of thermal stress on the expression of genes in the chickens was created. Five genetic groups including 109 genes were identified in this network. A gene network analysis showed that genes of heat-shock proteins (HSPs), cochaperones, immune genes and cell structural genes exhibited strong relationships. Then comparing the expression of Iranian native chicken genes and Ross breed showed that the three genetic families in native chickens of Iran were more strongly expressed than Ross commercial breed. Therefore, there is a potential of genetic resistance to heat stress in chicken, which needs to be investigated in an experimental study.

Keywords: Genetic network, heat stress, bioinformatics, DA-
P-22: Transcriptomic profiling to identify biological processes involved in mastitis in dairy cattle

Torabi A1, roudbari Z2, Seyedabadi H3

1. Department of Agriculture, Payam Noor University (PNU), P.O.BOX, 19395-3697 Tehran, Iran.
2. Department of Animal Science, University of Jioft, Jiroft, Iran
3. Department of Animal Biotechnology, Animal Science Research Institute of IRAN (ASRI), Agricultural Research Education and Extension Organization (AREEO), Karaj, Iran
azadehtorabi@gmail.com

Background: Mastitis is one of the health disorders with large effect on dairy farms and animal welfare it is caused by infection of pathogenic microorganisms such as Escherichia coli, Streptococcus uberis and Staphylococcus aureus. Alterations in milk composition, reduced milk quality and the production and treatment costs all contribute to the economic impact of this disease. Mastitis causes concern regarding both animal welfare and human health. For the development of effective strategies to control mastitis it is important to gain an in-depth understanding of the molecular mechanisms underlying the host immune response to the infection.

Objective: The objective of this study was to identify the biological pathways affected in mastitis that show most gene expression changes in the bovine mammary gland that has an intramammary infection with E.coli using publicly available transcriptomic data.

Methods: Transcriptomics data from Sipka et al was used in the present study (GSE50685). They measured gene expression in bovine mammary gland infected with E. coli. They compared gene expression after treatment with the antibiotic cefapirin and prednisolone with untreated bovine mammary gland tissue. Gene expression analysis was performed with the Affymetrix Bovine Genome Array. The data quality was evaluated and data normalization was performed using ArrayAnalysis.org. All arrays passed quality control and were analyzed further. Pathway analysis on differentially regulated genes was performed using PathVisio.

Results: The regulation of mastitis is possibly the result of interaction of physiological and immunological processes which affects the bovine mammary tissue, identifying these processes with pathway analysis can help to understand mechanisms of cellular signaling pathways involving in mastitis. Pathway analysis revealed thirty-three pathways that were significantly different (z-score > 1.96), ten of which were known to be involved in immune system regulation and metabolic pathway. Interestingly, IL-3, IL-6, TSLP, B cell receptor and Toll-like receptor signaling pathways which are known to be important in immune response, were enriched in the treated group. In these pathways, transcripts associated with immune response functions were downregulated.

Conclusion: Results of the present study uncover pathways affected in the mammary gland infected E. coli that could help early detection and control measures for the prevention of E. coli mastitis, and thereby improve overall animal health and decrease economic losses to dairy farmers.

Keywords: Pathway analysis, Mastitis, Gene expression

P-23: Study the effect of Silibinin on Cdkn1a (p21) and Bax gene expression in the 4T1 mouse breast cancer cell line

Zarei Golambahri H1, Motamed N2, Nademii NS3

Department of cell and molecular, Science campus, University of Tehran, Tehran, Iran.
hamid.zarei@ut.ac.ir

Introduction: The 4T1 tumor has several characteristics that make it a suitable experimental animal model for human mammary cancer. Silibinin, a flavonolignan, isolated from the fruits of Milk thistle, Silybum marianum, has been developed as a supportive care agent to reduce the toxicity of cancer chemotherapy.

The aim of the present study was to investigate the effect of Silibinin on viability of 4T1 cells and Cdkn1a(p21) and Bax, cell cycle and apoptosis genes, expression in the 4T1 mouse breast cancer cell line.

Materials and Methods: 4T1 cell line obtained from Pasteur institution was cultured in RPMI medium containing FBS 10%. Cells were incubated with 5% CO2 in presence of different concentration of Silibinin in 24, 48 and 72 hours. Cell viability was assessed using MTT assay and IC50 obtained by Excel program. In evaluating the effect of silibinin on expression of Cdkn1a (p21) and Bax genes, RNA extraction, cDNA synthesis and Real Time PCR technique were used.

Results: Silibinin showed significant cytotoxic effects on 4T1 breast cancer cell as well as increased expression of p21 and Bax genes.

Conclusion: According to this study Silibinin had a dose and time dependant cytotoxic effect on 4T1 cells. Furthermore arrested cell cycle in G1/S and induced apoptosis.

P-24: Antidiabetic Effects of Alcoholic extracts of Eryngium Billardieri and Mangifera Indica in Alloxan-Induced Diabetic Rats

Zarif Zargarian Talasaz Y, Montasser Kouhsari, Sh

1. Department of Biology, Kish international campus, University of Tehran, Kish Island, Iran.
2. Department of cellular and molecular, School of Biology, College of science, University of Tehran, Tehran, Iran.
Email: yaldazargarian@yahoo.com

Management of diabetes without side effects is still of great concern to medical practitioner. Medicinal herbs are used for the treatment of Diabetes mellitus worldwide. The present study investigated the anti-diabetic effects of Eryngium billardieri (EB) and Mangifera indica (MI) extracts on biochemical parameters in induced diabetic Wistar rat models. Alloxan monohydrate was used to induce diabetes in rats weighing 200- 250 g. The fasted diabetic rats were divided in to 4 groups of 5 animals each. The positive control group had received normal saline. The negative control group received only 110 mg/
kg of alloxan monohydrate. Diabetic rats in groups 3 and 4 had received EB and MI extracts respectively. This study was conducted over a period of 21 days with oral administration of the plants extracts on the 3rd day after alloxan treatment. All the test groups showed a reduction on blood glucose level, and the antidiabetic effect of EB was less than MI (EB:36%, MI:66.5%). The Insulin gene expression in pancreas was determined in all groups and the results showed that in treated groups with plants extracts the Ins expression was higher than in diabetic group. In conclusion, the results of the current study show that EB and MI extracts significantly reduce the serum glucose levels in alloxan-induced diabetic rats.

Keywords: Type 2 Alloxan monohydrate, Diabetes mellitus, Eryngium billardieri, Mangifera indica

Bioinformatics

P-25: A bioinformatic analysis for identification of therapeutic epitopes in HPV16 and 18 E6 early proteins

A. Panahi H, Bolhassanin A Javadia Gh, Nourmohammadia Z

Department of Biology, School of Basic Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran
Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran
hapanahibo@gmail.com

HPVs are a group of non-enveloped viruses with small, circular double-stranded DNA genomes which have tropism for mucosal tissues. A subset of HPVs is the primary etiologic cause for several human cancer types, causing about 5.2% of all world-wide cancers. HPV16 and 18 take over about 70% HPV-associated cancers. Due to the high prevalence and mortality, HPV-associated cancers have still remained as a major health threat in human society, and thus their effective immunotherapy is urgently needed at present. Previous information has shown that E6 and E7 HPV early proteins are responsible for the initiation and maintenance of HPV-associated cancers. Therefore, the success of HPV-related cancer immunotherapies relies on the recognition of specific and well immunogenic tumor-associated epitopes, inducing a robust cell-mediated immune response. Our goal in this study was the prediction of the best immunogenic MHC I epitopes derived from E6 oncoproteins of HPV types 16 and 18. For this purpose, we carried out a two-step selection protocol. In the first step, selection was made after; MHC I binding predictions, processing prediction and immunogenicity prediction. In the second step, selection was made after performing the following analyses only for the first-step selected peptides; MHC I population coverage prediction, MHC I protein-peptide docking analysis, epitope conservation analysis and cross-reactivity with mouse and human antigens analysis. Finally, we introduced three predicted MHC I epitopes for each genotype oncoprotein which had better scores and no homology >90% in the mouse and human proteomes.

This comprehensive protocol is strongly recommended at the early phase of peptide-based vaccine development, because it greatly reduces time and cost required for experimental studies. In addition, our predicted epitopes are suitable choices for designing novel therapeutic HPV vaccines.

Keywords: Human papillomavirus, cancer, Therapeutic vaccine, Epitope prediction

P-26: Alternative Splicing of EXT1 Gene in Breast Cancer cells

Abbassioun S1, Ghorbanmehr N2, Mowla SJ

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Biotechnology, Alzahra University, Tehran, Iran
saba.abbassioun@gmail.com

Breast cancer is the most commonly diagnosed cancer and the fifth leading cause of cancer-death in women. Since the peak incidence age of Iranian women is a decade younger than that of the word, it is necessary to elucidate the mechanisms involved in the tumorigenesis of breast cancer and discover novel diagnostics markers, specific for Iranian population. The EXT1 gene is located in 8q24.11 and its only protein coding transcript encodes exostosinglycosyltransferase1 which has a vital role in intercellular connections. It has been suggested both tumor promoting and tumor suppressor role for the gene. Due to its 48.13% amplification in breast cancer, investigation of its alternatively spliced variants is important. Here, we are reporting the existence of some alternatively spliced form of the gene, with potential involvement in breast tissue tumorigenesis.

Keywords: Breast cancer, EXT1 gene, alternatively spliced variants

P-27: Isolated Methylmalonic Acidemia: A Systems Biology Approach to a Monogenic Disorder

Abedi M, Fatehi R, Gheisari Y

Department of Genetics and Molecular Biology, Isfahan University of Medical Sciences, Isfahan, Iran
Regenerative Medicine Lab, Isfahan Kidney Diseases Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
abedi777@yahoo.com

Objective and background: Isolated methylmalonic acidemia (MMA) is caused by deficiency of the enzyme methylmalonyl-CoA mutase (MUT). Chronic kidney disease is among the major secondary complications of MMA. In this study, we have re-analysed a microarray dataset to generate a holistic view of MMA-associated renal disease.

Methodology: GSE41044 mRNA microarray dataset deposited by Manoli et al was downloaded from the Gene Expression Omnibus (GEO) database. The quality of microarray data was measured by principle component analysis and hierarchical clustering using ggplot2 package of R and ClusterMaker application of Cytoscape 3.2.1, respectively. Using GEO2R tool of GEO, genes with adjusted p-value<0.05 were assumed as differentially expressed (DE). Using CluePedia plugin version 2.1.7 of Cytoscape, a protein-protein interaction network was

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constructed and analyzed by Cytoscape NetworkAnalyzer tool and nodes with the highest degree and betweenness centrality parameters were identified. Pathway enrichment analysis was performed using Cytoscape ClueGO plugin version 2.1.7 and signaling pathways with adjusted p-value ≤ 0.05 were determined.

**Results:** In this study, we re-analyzed the GSE41044 microarray dataset which explores kidney mRNA expression profiles from Mut +/+ , Mut +/- and Mut +/- mice. In quality analysis steps, the samples were segregated based on their state (homozygous, heterozygous, wild-type), indicating the satisfactory quality of this dataset. The comparison of homozygous and heterozygous with wild-type samples revealed 993 and 10 DE genes, respectively. Moreover, top central genes were identified that they may have critical role in the MMA-associated renal disease. Furthermore, Gene ontology (GO) enrichment analysis resulted in GO terms such as lipid metabolic process and peptide metabolic process which most of them are expected to be associated with MMA pathogenesis.

**Conclusions:** In conclusion, we have here followed a systematic approach to explore the underlying molecular mechanisms of MMA as a monogenic disease. Methods employed in this study may also be used for other monogenic diseases to suggest novel therapies via generation of holistic maps.

**Keywords:** Bioinformatics, Gene ontology, Isolated methylmalonic acidemia, Microarray, Protein interaction network

**P-28: Selection the most appropriate signal peptides for chloroplast targeting of the reporter protein based on bioinformatic tools**

Adigozali Behrouz M, Mousavi A, Salmanian A H

National Institute of Genetic Engineering and Biotechnology, P.O. Box 14195-6343, Tehran, Iran

maaarjaaan.1990@gmail.com

Chloroplasts cannot produce all the proteins they need and most chloroplastic proteins are encoded in the nucleus. These proteins are synthesized in the cytosol as precursors and transferred to chloroplasts. Its targeted approach, due to the existence of a specific sequence of target detection, is at the N-terminal of any chloroplast immature protein that is called signal peptide. In this study, we analyzed 200 signal peptides of different genes and hosts to select the most appropriate signal peptides for chloroplast. Cleavage sites of signal peptide, probability of their transfer to chloroplasts and their hydrophobicity was evaluated by bioinformatics tools such as TargetP, Tppred, PredSL, Predotor and protoscale. Moreover, in order to determine conserved amino acids in the structure, sequences were analyzed by BLAST program. In the next step, we predicted the second structure of the GUS protein after binding of the signal peptide to the N-terminal. The most suitable signal peptides were selected from Spinacia oleracea, Arabidopsis thaliana, Petunia and Zea mays species. It is expected that the sequence of signal peptides can transfer reporter protein into chloroplasts.

**Keywords:** Chloroplast, signal peptide, bioinformatic, gus

**P-29: Devising Multi-Target Systems Biology Procedure to Inhibit Transition from In-Situ Ductal Carcinoma to Invasive Ductal Carcinoma**

Afzali F1, Gardaneh M1, Nayeri Z2

1. Dept. of Stem Cells and Regenerative Medicine, Faculty of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Molecular Medicine Department, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

farzaneafzali@gmail.com

In-situ form of cancer tumor stays at its initial tissue location without spreading around to cause further trouble to the patient but in its invasive form extends to other tissues and become uncontrollable. Breast cancer (BC), the most relevant type of cancer among women, is divided into In-situ Ductal Carcinoma (IDC) and Ductal Invasive Carcinoma (DIC). Transcription Factors (TFs) involved in transition from IDC to DIC play important role so that their targeting inhibits metastasis. In this study, we used microarray dataset in GEO(GSE21422) and normalized it by RMA algorithm to reach differentially expressed genes (DEGs). The expression profile of DIC and IDC groups were compared together by log fold change and adjusted p-Value parameters. The DEGsâ€™ list obtained was given to chEA database to find TFs and then to TargetScan microRNA database to predict miRNAs regulating them. The most affected pathway has been determined by Reactome and cancer cell line mapping has been performed by Cancer Cell Line Encyclopedia. Our results suggested CTNNB1, SMAD3, SOX9, NR3C1 and SMAD4 as involved TFs. Then miR-503 appeared to be the most effective miRNA regulating the DEGs. We have mapped COV504(Ovary), KPNS19S(Autonomic Ganglia) and HS274T(Breast) as top 3 cell lines affected by determined DEGs and NOTCH2 Activation and Transmission of Signal to the Nucleus recognized as the most affected pathway. In conclusion, we identify TFs that can be targeted to prevent metastasis. Also by cell line mapping we propose that by targeting these TFs, other kinds of cancer can also be targeted simultaneously.

**Keywords:** Breast Cancer, systems biology, TF, miRNA, microarray

**P-30: Investigating thermodynamic stability of a molecular beacon for the detection of exosomal miRNA involved in breast cancer**

Aghahosseini M, Tavassoli M, Javadi-Zarnaghi F

Department of Biology, Faculty of Science, University of Isfahan. maeedehhosseini73@hotmail.com

Statistics show breast cancer is the most common cancer worldwide among women. The Percentage of people suffering from breast cancer has drawn attention of researchers to diagnose and prognose this disease. There is a strong correlation
between special miRNAs and cancer. Although there are numerous ways for detection of miRNAs such as northern blotting, bioluminescence and RT-PCR but miRNA detection still remains as a major challenge. Alternatively, miRNAs can be detected on solid phase by Lateral Flow assays (LFA) which is a rapid, cheap and user-friendly assay that takes only five to twenty minutes to reveal the result. There are multiple designs with which different analytes could be detected with a LFA. In this research, a molecular beacon complementary to miRNA is designed for exosomal miRNA detection. The core idea behind this examination is that with the presence of miRNA the molecular beacon would lose its G-quadruplex conformation but in the absence of miRNA it would be in a G-quadruplex conformation. This would produce color with an appropriate substrate. The thermodynamic stability of molecular beacons is calculated in the presence of various conditions.

**Keywords:** miRNA; Breast cancer; Lateral flow assay; Molecular beacon; Thermodynamic stability.

P-31: Promoter analysis of some genes responding to H5N1 virus in chicken

Ahmadi M

Razi vaccine and serum research institute, Karaj, Iran
m.ahmadi@rvsri.ac.ir

Transcriptional regulation is known as a complicated process involving many different proteins, which some of them bind in a specific-DNA sequence harbored within a promoter region of a gene. In this respect, one of the crucial challenges in biotechnology is the recognition of promoter and its regulatory elements. In this study, we isolated the promoter region of several co-expressed genes responding to the H5N1 virus in chicken. Then, cis/trans regulatory elements were identified using TRANSFAC and oPOSSUM databases. According to obtained results, a plethora of overrepresented motifs sharing at most promoter region of these genes were discovered. Among these, however, some ofcis/trans elements were previously studied and confirmed in term of response to influenza virus. MYB and IRF are well-known elements regulating the expression of particular gene so as to strengthen of innate immune system. Moreover, these specified overrepresented motifs were scattered across the promoters. In conclusion, recognition and understanding of these specific motifs may shed some light regarding to transcription factors response related to influenza infection in chickens, and has implications for strategies targeting the innate immune system for boosting resistance to avian influenza.

**Keywords:** Promoter, Cis-regulatory Elements, Influenza, H5N1

P-32: Comparative Phylogenetic Analysis of MADS-box gene family in Rosaceae reveals evolution and functional divergence

Akbari F, Fotovat R

1. Department of Agricultural Biotechnology, Zanjan university, Iran

MADS-box genes encode a family of eukaryotic transcription factors distinguished by the presence of a highly-conserved ~58 amino acid DNA-binding and dimerization domain transcription factors. They play significant roles in plant developmental processes such as floral organ conformation, flowering time, and fruit development. Although the genome wide analysis of this family has been performed in some species, little is known regarding MADS-box genes in rosaceae. 

Prunus persica, Malus Â— domestica and, Pyrus bretschneideri genomes have been fully sequenced recently, and 79, 146, and 95 MADS-box genes in peach, apple and pear were identified respectively. Nevertheless, the comparison of the fully sequenced rosaceae family MADS-box genes has not been accomplished. In this study, the MADS-box transcription factor in fully sequenced Rosaceae were analyzed. Apple and pear, both members of Rosaceae had the closest genetic relationship. Phylogenetic analyses indicated that multiple, independent expansions have taken place in apple. These results provide valuable insights in to the functional analysis of MADS-box proteins in rosaceae family during different biological processes, particularly of floral organ conformation, and flowering time, which may help in devising strategies to improve important traits.

**Keywords:** MADS-box, Rosaceae, Functional divergence

P-33: Comprehensive meta-analysis of several normal and cancerous TGF beta1 treated cell lines reveals different gene signature and significant pathways and suggest possible markers and therapeutic targets

Akbari V, Kallhor M, Mollahash B, Akbari M T

Department of Medical Genetics, Tarbiat Modares University, Tehran, Iran
vahid.akbari1369@yahoo.com

Transforming growth factor beta signaling pathway is involved in various processes of the human body. There is significant evidence that TGF beta activity can change under different conditions, and its use as a therapeutic target has many complications. Accordingly, it is necessary to examine this pathway in different cell types and situations to find its precise function and interaction with the other pathways. Moreover, TGF beta signaling pathway can induce epithelial to mesenchymal transition (EMT) that has a crucial role in cancer metastasis and it has been shown that this pathway can cause both metastasis and suppress it. Here we conducted a comprehensive meta and network analysis on microarray data of 9 cancerous epithelial, 3 normal epithelial, and 8 normal fibroblast TGF beta1 treated cell lines to find most significant genes, gene ontology, and pathways that show change after TGF beta1 treatment. The results indicate most significant genes and pathways and the contradictory role of this pathway in metastasis and suggest alternative therapeutic targets. Finally, we found Focal Adhesion as a significant pathway through TGF beta1 treatment in different conditions. Additionally, by comparing the DEGs of
Epithelial and Fibroblast treated cell lines, we found that JUN transcription family is a crucial TF through TGF beta signaling pathway and this pathway employs c-Jun in epithelial cells through EMT process but in fibroblast cells uses JUNB. We believe that c-Jun can be a significant marker to trace EMT through TGF beta treatment, as well as it can be a possible therapeutic target to halt EMT process and cancer metastasis.

**Keywords:** Epithelial to mesenchymal transition, TGF beta signaling, Metastasis, meta-analysis, network analysis

**P-34:** The role of a novel MirSNPs in autism spectrum disorder

Alizadeh R¹, Tajik M², Bahmanpour Z³, shahram

1. Department of Genetics, school of medicine, Iran University of medical science, Tehran, IR Iran.
2. Department of science cellular and molecular biology faculty of biology Islamic Azad University of damghan
3. Department of Medical Genetics, Iran University of Medical Sciences, Tehran, Iran

A comprehensive systematic search from the searched Medical Literature Databases Analysis and Retrieval System Online (Medline) by PubMed, the web of sciences, Scopus, Excerpta Medical Database (Embase). We used different terms â€œAutismâ€- or â€œAutism Spectrum Disorderâ€- or â€œAutistic Disorderâ€- or â€œASDâ€- and â€œmicroRNAâ€- or â€œMirâ€- or â€œmiRNAâ€-. Title and Abstract of the obtained article were screened and also some full text of articles were studied. Finally, dysregulated microRNAs in Autism Spectrum Disorder were selected. On the other hand, we conducted a bioinformatics approach to recognize the top genes associated with this disease. Recently, the study of single nucleotide polymorphisms (SNPs) has become an attractive issue because of that SNP in the target of the microRNAs may be lead to reduce or increase the tendency of microRNAs to their target. Alteration of the miRNA expression can be a probable disease marker and it may play a role in the pathogenesis of various diseases such as Autism Spectrum Disorder. By apply bioinformatics tools, numbers of SNP in 3UTR of Autism Spectrum Disorder top genes were examined. Hence, in this review, we predicted numbers of novel mirSNPs which researchers can be considered for experimentally and validation studies.

**Keywords:** Autism Spectrum Disorder - MicroRNA – MirSNPs

**P-35:** A Computational Comparative Study of long non-coding RNA genes divergence

Amirmahani F¹, Jamshidi Goharrizi K², Vallian S¹

1. Genetics Division, Department of Biology, Faculty of Science, University of Isfahan, Isfahan, IR Iran
2. Department of Plant Breeding, Yazd Branch, Islamic Azad University, Yazd, Iran farzanemahani@yahoo.com

Now, it is clear that protein is just one of the most functional products produced by the eukaryotic genome. Indeed, major part of the human genome is transcribed to non-coding sequences than to the coding sequence of the protein. However, while we have developed a deep understanding of the relationships between evolutionary limitations and function for the protein coding sequences, there is little information about these relationships for non-coding protein sequences.

Three long non-coding RNAs namely AK082072, AK043754 and AK082467 conserved highly and also, some of the first orthologs present among vertebrates show brain expression conservation. Thus, the conserved sequences of these genes are appropriate for phylogenetic analysis. In the present study the nucleotide sequences of selected long noncoding RNAs from different vertebrates were aligned and the Phylogenetic trees were constructed using Neighbor Joining method with maximum sequence differences of 0.75. Our analysis of nucleotide sequences to find closely evolved organisms with high similarity by NCBI-BLAST tools and MEGA7 showed that the selected sequence of AK082072 in human and M. fascicularis (macaque) were placed into the same cluster and they may originate from a common ancestor. In addition, the human sequence of AK082467 and AK043754 had the closest similarity with cow. The nucleotide conservation patterns for these IncRNA loci demonstrated higher conservation near exon boundaries. Overall, their conservation across different anninotes and in exon structure show that they are functional RNA molecules, that have important roles in brain development of vertebrates.

**Keywords:** AK082072, AK043754, AK082467, Phylogenetic Trees, anninotes

**P-36:** Bioinformatics analysis of the CsACS2 gene in the female flowers in the medicinal plant of Citrullus colocynthis L.

Bagheri.a F, Soltani Howyzeh.a M, Shariati.b V.

1. Department of Plant Breeding, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
2. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

In the cucurbitaceae family, transcriptome profiling data of the flower development are limited to the determination of the flower’s sex, which in the different developmental phases, several genes are expressed differently or specifically. In this family the most species are unisexual florals. Among 800 studied species, 460 are monoecious and 340 are dioecious. Citrullus colocynthis L. is a perennial plant, with male and female flowers on a monoecious. In monoecious, CsACS2 gene increase ethylene production which leads to female flowers and the andromonoecious, in other hands, CmACS-7 gene controls staminal development in female florals. The bioinformatics analysis was carried out using the Sequence Viewer CLC-7 software for the six species plant of the Cucurbitaceae family to study the CsACS2 gene sequences from the obtained data. The alignment of CsACS2 gene showed high similarity in the domains. Also, pattern of the phylogeny tree showed close phylogeny relations between the studied species.

**Keywords:** Citrullus colocynthis L., Inflorescence sexuality,
CsACS2, Bioinformatics analysis

P-37: A Bioinformatic approach to identify novel miR-SNPs in Huntington’s disease

Bahmanpour Z1,2, Tahmasebivand M1,2, Mousavi R1,2, Khameni B1,2, Emamalizadeh B2

1. Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Emamalizadeh_b@yahoo.com

Single nucleotide polymorphism (miR-SNPs) in miRNAs coding genes or target sites are implicated in pathogenesis of various diseases. Such miR-SNPs may have significant role, due to the alteration of miRNA function, and can be a diagnostic marker. However, the role of miR-SNPs has not well understood in the progression of Huntington's disease (HD). Here, we implemented a bioinformatic approach to identify miR-SNPs involved in HD. In a comprehensive study, miRNAs and related genes were examined from Gene Expression Omnibus (GEO) database and previous studies. On the other hand, SNPs located in target site of miRNAs were obtained from PolymiRTS and miRdSNP databases. Supporting the results, miRNA: SNP, SNPs were further evaluated for their involvement in HD. Therefore, by in silico analysis some miR-SNPs such as miR-200a:rs9055: REST, miR-146a:rs2910164: FDX1, miR-141:rs2234975: SIRT1 were identified which may have functional roles in pathogenesis of HD. Results showed that candidate miR-SNPs may affect regulation of target genes by modifying miRNAs function. In this study, a number of novel miR-SNPs introduced which could be considered by researchers for experimentally and validation studies.

Keywords: miR-SNPs, in silico, Huntington's disease

P-38: Bioinformatic exploration for candidate miRNAs as breast cancer biomarkers

Bahonar S, Javanmard A.R, Soltani B.M

1. Department of Genetics, Tarbiat Modares University, Tehran, Iran
s.bahonar.bio@gmail.com

MicroRNAs (miRNAs) are key post-transcriptional regulators that affect protein translation by targeting mRNAs. miRNAs are a relatively new class of non-coding RNAs that have a potential as cancer biomarkers which are a group of molecules that can use for diagnosis and prognosis different types of cancer. To seek this purpose, data of microarray analysis for breast cancer were obtained from the GEO database (GSE103357, GPL6947). Through the usage of this data, two tumors and three adjacent diagnostic normal samples information were used for identification of ten miRNAs with different expression in tumor samples VS control via the usage of R software analyzing tool. These miRNAs are selected as breast cancer biomarker candidates which includes:

- miR-222, miR-221, miR-183, miR-582-3p, miR-376a-3p, miR-582-3p, miR-653, miR-153, miR-216a, miR-139-3p. We further explored the function of candidate miRNAs with DIANA mirpath v.3 and Enrichr database and realized that the previous miRNAs have an important role in controlling PI3K and TGF-β pathways which are correlated with breast cancer tumorigenesis stage.

Keywords: miRNA seq, miRNA, biomarker, PI3K pathway

P-39: Bioinformatics prediction of two putative microRNAs in the frequently amplified genomic region of 3q26 in lung cancer

Biabanaki ZS, Ghanei M, Ahmadi A, Mowla SJ

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
2. Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran
3. Department of Genetics, Arak University of Medical Sciences, Arak, Iran
matin1392@gmail.com

Objective: Approximately 1.6 million new cases of lung cancer are diagnosed each year throughout the world. 5-year survival rate of lung cancer patients is less than 15%. Therefore, one of the most important challenges in lung cancer management is early detection and identification of its specific molecular features to improve patients' treatments. microRNAs (miRNAs) are a group of non-coding regulatory RNAs involved in diverse biological processes, as well as many pathological conditions. One of the methods for studying miRNAs involved in cancer is investigation of chromosomal regions with amplifications or deletions in genomic hot spots. The 3q26 chromosomal region is amplified in ~20% of human tumors, including lung cancer. Herein, the aim of the present study is to investigate a candidate gene located in this region, to identify the presence of putative miRNAs.

Material and methods: in order to predict putative miRNA coding region, we used several software such as RNAfold, PHDcleave, MiPred, and FOM-miR. Afterwards, for experimental verification of this miRNA, predicted pre-miR sequence will be overexpressed in cell line and lung cancer tissue.

Result: bioinformatics analysis indicates two regions with the miRNA coding potential located in the region. Bioinformatics tools recognized these sequences as real miRNAs. Cloning and overexpression of the pre-miR sequence in lung cancer cell lines is being carried out.

Conclusion: The aim of this study is to discover novel miRNAs with potential usefulness in molecular diagnosis of lung cancer. The new predicted miRNAs, had no exact similarities with the reported miRNAs in miRBase site, suggesting their possible existence as real miRNAs, with altered expression in lung cancer.

Keywords: lung cancer, 3q26, miRNA

P-40: Bioinformatic analysis of a halophilic protease from moderately halophilic bacterium Nesterenkonia sp. F.

Boostan Z1,2, Shafiei M1,2

1. Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Biotechnology and bioscience research center, Shahid Chamran
Halophilic enzymes may be useful for harsh condition in industrial processes, especially food industries. Proteases have broad application in industries such as laundry detergents, food processing, and the leather industry. Two third of all industrial enzymes are protease. In this work, one protease from Nestrenkonia sp. strain F has been selected and characterized by some bioinformatic softwares such as SingAlti 4.0, GC plot, 3DLigandsite, ProtParam, and NCBI blast. Based on analysis of the selected protease, it is predicted that it has 1530 base pair, 47.25% GC content, 509 amino acid, 19% alpha-helix, 17% beta-sheet, 3% TM helix, and signal peptides. Prediction research is showed that it is transmembrane, binding site contain serin and valin amino acids, estimated weight is 53kD, and half life in Escherichia coli is less than 20 hours. Homology search revealed homology between protease from Nestrenkonia sp strain F and ßαεδ16Kwάε-ε-. Nucleotide and amino acid sequences of the selected protease were similar to ßαKöcuria flava strain HO-9041â€™ isolated from Micrococcaceaeâ€™ respectively. Accordingly, selected protease from Nestrenkonia sp. F can be used in many harsh industrial processes when concentrated solution would inhibit many enzymatic convergent.

Keywords: protease, halophilic, bioinformatic, Nestrenkonia sp.

P-41: Leiomyosarcoma: Identification of hub genes and pathways based on co-expression network analysis

Darzi M*, Esmaeili R†, Gorgin S‡

1. Advance Information System Research Group for Information and Communication Technology Research Centre, ACECR, Tehran, Iran.  
2. Genetics Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran.  
3. Department of Electrical and Information Technology, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran.

modarzi@yahoo.com

Leiomyosarcoma is one of the rarest and most aggressive soft tissue sarcomas. The currently available systematic treatments for this cancer arenâ€™t still effective. Moreover, treatment options for this cancer are rare due to the insufficient number of patients and clinical trials. In addition, heterogeneous nature of this rare tumor prevents categorization of patients for personalized medicine. Increasing knowledge about molecular characteristics of this type of cancer helps physician for better therapeutic options. Recently, high throughput technologies generate opportunities for discovering new insight into different aspects of the biological system. This opportunity may compensate the rare numbers of clinical trials in finding new treatments in leiomyosarcoma. So, Network constructing and analysis of gene co-expression is one of the advanced approaches for gaining insight via high throughput data set. Some information about gene functions in leiomyosarcoma currently available but it needs more investigation. Here, Weighted Gene Correlation Network Analysis (WGCNA) algorithm is used as a system biology method for constructing leiomyosarcoma co-expression network. The data set of transcriptionics profiling for 80 leiomyosarcoma cases was downloaded via The Cancer Genome Atlas (TCGA) project. We are going to provide functional annotations of genes whose function are unknown. So, clusters (modules) are obtained through the Dynamic Tree Cut method. For exploring hub genes in leiomyosarcoma, Degree centrality analysis of the co-expression network is provided. And for pathway-enrichment analysis, we use the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. During this research, we expect to find some modules that insight us for a better understanding of this cancer. It may help division of patients based on their molecular patterns for personalized medicine and finding probable targets for therapy.

Keywords: Sarcoma, Leiomyosarcoma, Gene Co-expression Network, WGCNA, Clustering


Delshad E*, Shafiee M‡

Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran

mohshafa@gmail.com

Gastric cancer is one of the most important cancers associated with mortality in the world. Clinically, gastric cancer is detected at advanced stages, so it is necessary that be diagnosed at early stages by non-invasive methods, before metastasis. lncRNAs are RNA molecules approximately 200 nucleotides that play a role in regulating of important cellular processes including proliferation, differentiation, apoptosis, and invasion. Thus, changes in their expression levels can lead to diseases such as cancer. With the advancement of new technologies such as next generation sequencing, has facilitated the expression profiling of molecules involved in various diseases, including cancers.

In this research, using bioinformatics databases such as Mi Transcriptome, Cancer RNA-Seq Nexus, OncoLnc, GeneCards, LncRNAWiki, Lnc2Cancer, GCGene, we retrieved a number of novel-predicted lncRNAs that participated in the pathogenesis of gastric cancer. By reviewing these novel lncRNAs in text databases such as PubMed and Octopus, separated lncRNAs that are not yet approved experimentally. The result is two separate lists from lncRNAs, inclusive lncRNAs that have been validated to be involved in gastric cancer such as MALAT1, PVT1, GAS5, TINCR, ZFAS1, TUSC7, MIR22HG, and a list from novel-predicted lncRNAs, such as MF12-AS1, HHIP-AS1, ST7-AS1, CIRL-AS1, USP3-AS1, ZNF790-AS1, MORC2-AS1, OSER1-AS1, RPL34-AS1, DDX11-AS1, ARHGAP5-AS1, RSBN1L-AS1, COX10-AS1, PCBP1-AS1, LIN00883, LOC541471, LIN00920. With experimental validation of the involvement of predicted novel lncRNAs in gastric cancer can be used as non-invasive biomarkers for early diagnosis and targeted treatment of gastric cancer.

Keywords: Gastric Cancer; lncRNAs; Bioinformatics; Next Generation Sequencing

Dianat pour C, Khatami M, Heidari MM
Yazd university
Cimadianatpour@yahoo.com

Introduction: CITED2 is a cardiac transcription factor that plays a critical role in the development of embryonic cardiovascular tissue. CITED2 is a CBP/p300-transactivator and also is a negative regulator of HIF-1α that functions as an important modulator in the development of the heart. Since the structure and dynamics of proteins are an essential part of understanding the biological processes, in this study, the effect of missense mutations on the structure and function of the gene products has been investigated by using 3D molecular visualizing software such as VMD, and computational online databases like SIFT which has been applied to human variant databases. These sequence databases have the high powers for distinguishing between the disease-causing mutations and normal polymorphisms.

Method: We identified three non-synonymous single nucleotide polymorphisms (nsSNPs) in the CITED2 gene (rs766774041, rs751816572, rs201257595) using dbSNP and then analyzed their effect on the protein structure using VMD software, SIFT database.

Result: Our results showed that these missense single nucleotide polymorphisms change the interaction patterns, polar groups, and length of hydrogen bonds. rs766774041 leads T/A amino acid alteration, caused to a hydrogen bond disappearing in protein structure and rs751816572, rs201257595 lead M/T and I/V changes, both just changed the length of hydrogen bonds and protein conformation. The results of the mutation assay in the SIFT database, predict scores more than 0.05 for all three mentioned SNPs.

Conclusions: Based on our results of the pathogenicity prediction and bioinformatics assessments, these nucleotide changes as missense mutations are less important factor risks in heart disease.

Keywords: CITED2, SNP, structure and function prediction, VMD software, SIFT

P-44: Bioinformatics analysis of p-protein genes in the leaf phloem of medicinal plant Citrullus colocynthis L

Dorafshan M1, Soltani Howyzeh M1*, Shariati V2

1. Department of Plant Breeding, Ahvaz Branch, Islamic Azad University, Ahvaz, Iran
2. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
soltani.m@iauahvaz.ac.ir

In higher plants, the phloem as a living tissue functions of transporting the nutrients from leaves to different tissues, such as roots, fruits, flowers, etc., that is composed of micromolecule and macromolecule including amino acids, proteins, RNAs and hormones. The primary phloem have bundles of sieve elements, companion cells, fascicular phloem (FP) and also in the outer part extra fascicular phloem (EFP). Extra fascicular phloem formed an complex network of longitudinal peri-fascicular strands next to the vascular bundles phloem. Called anastomosing that interconnects the vascular bundles to the petiole and stem, effectively known as a strategic defense against pests and diseases, that contain cucurbitacins, terpenoids, alkaloids and proteins. Cucurbitaceae family are a unique models for extra fascicular phloem biology. One of the most common genes identified in the EFP is PP1/PP2 proteins. In this study, the bioinformatics analysis with the Sequence Viewer CLC 7 software examined for PP1/PP2 genes in plant species of the Cucurbitaceae family. The results of PP1/PP2 genes alignment showed that these genes had high preservation in domains, and the results of phylogeny tree showed a close evolutionary relationship among studied species.

Keywords: Citrullus colocynthis, Extrafascicular phloem, PP1/PP2 protein, CLC

P-45: Bioinformatic Analysis of Pathogenic Missense Mutations of MLH1

Ebrahimi Sh, Ebrahimi M
Yazd University
sheida19922991@yahoo.com

MLH1 gene was identified as a locus frequently mutated in hereditary nonpolyposis colon cancer (HNPPC). It is a human mismatch repair gene. The nature of amino acid substitutions in invariant sites will condition the effect on protein structure, while variable positions can be analysed for residues that can be exchanged without detrimental effects.

Here we analyse three SNP to find their pathogenic effect. Each mutant were compared with the corresponding wild-type structures.

1) rs63750792
NM_001258271.1:c.83C>T
XP_005265218.1:p.Pro28Leu
2) rs63750823
NM_000249.3:c.67G>A
XP_005265218.1:p.Glu23Ter
3) rs63751012
NM_000249.3:c.109G>A
XP_005265218.1:p.Glu37Ter

In rs63750792 Proline, changed to Leucine. In the wild type proline has 2 bond with Alanine (A) in position 31, but in the mutated form the bond are different. The replaced amino acid, has a bond to Isoleucine (I) 32 and a bond with Alanine (A) 31. This SNP is related to Lynch syndrome and hereditary cancer-predisposing syndrome.

In rs63750823 Glutamine, changed to Threonine. In the wild type Glutamine has one bond with Glutamine (Q) 26, Isoleucine (I) 19, and Alanine (A) 20. In mutated form, Threonine has a bond with Glutamine (Q) 26 and Alanine (A) 20, but it has two bonds with Isoleucine (I) 19. This SNP is associated with Lynch syndrome and HNPPC.

In rs63751012 amino acid in the wild type, Glutamine (E) has a bond with aspartic acid (D) 41 and two bonds with Lysine (K) 33. The mutated form has same bonds as wild type, but it
causes alteration in protein structure. This alteration maybe a reason of causing HNPCC.

**Keywords:** HNPPC, Mutation, Pathogen, Analyze

**P-46: Affinity enhancement of nanobody: in silico rational and random mutagenesis and molecular dynamics simulation approaches**

Ebrahimi Z, Arezumand R

Department of Medical Biotechnology and Molecular Science, School of Medicine, North Khorasan University of Medical Sciences, Bojnurd, Iran

zahra.ebrahimi6982@gmail.com

Nanobody (Nb)/VHH is an epitope binding domain of Heavy chain Antibody with ~15 kDa in molecular weight. Nb is the smallest antibody domain and because of some properties like small size, high stability and high degree of homology to human VH sequences, they are ideal candidates in therapeutic and diagnosis of different disease especially cancers. The main aim of this study is affinity enhancement of Nb as a model by insilico affinity maturation and molecular dynamics simulation approaches.

For this aim, at first by determining the important residues of Nb which involved in antibody-antigen interaction by docking tools, about 300 single mutant forms were designed, ten thousand models for all of mutants were constructed by modeller 9v8 version software, after evaluation of some bioinformatics parameter such as docking score, VMD visualization tool and Ligplot tool, five out of 300 mutant forms were screened for Molecular Dynamics (MD) simulation approaches. According to MD analysis, free binding energy of all selective variants was higher than native forms. The other MD analysis such as hydrogen bond, secondary structure, RMSD, RG shows that these single mutation forms magnified the stability of Nb in complex with antigen. For next step we should analysis the mutants experimentally.

Keywords: Nanobody, Affinity maturation, In silico, Modeling, Molecular dynamic

**P-47: In Silico analysis of a novel glutaminase from moderately halophilic bacterium Nesterenkonia Sp. strain F**

Eghtedar M.1,2, Shafiei M.1,2*

1. Department of genetics, Faculty of science, Shahid Chamran university of Ahvaz, Ahvaz,Iran.
2. Biotechnology and bioscience reaserch center, Shahid Chamran university of Ahvaz, Ahvaz, Iran.

m-shafiei@scu.ac.ir

L-Glutaminase (L-glutamine amidohydrolase, EC 3.5.1.2) is the important enzyme that catalyzes the deamination of L-glutamine to L-glutamic acid and ammonium ions. Recently, L-glutaminase has received much attention with respect to its therapeutic and industrial applications. It acts as a potent antileukemic agent and shows flavor-enhancing capacity in the production of fermented foods. The present study was an in silico analysis of a novel glutaminase from Nesterenkonia Sp. F (a gram-positive and halophilic bacteria of the Micrococccaceae family) to predict its structure and physicochemical properties

By utilizing bioinformatics tools, such as NCBIblast, SignalP4.1, Genomics %G-C content calculator, ProtParam, and 3DLigandSite. According to the results, nucleotide sequence of the selected glutaminase from Nesterenkonia Sp. F has 1230 bp, 71.79% G-C content, and was similar to Nocardioides dokdonensis FR1436, complete genome(with 65% coverage); its protein sequence has 409 amino acide, doesn't have any signal peptide, and was similar to Glutaminase from Micrococcus luteus K-3 (id%:52). Its secondary structure contain 47% alpha-helix, and 12% beta-sheet. Modeling of its 3-dimension structure revealed that binding site contain: Tyrosine (residue8), Glutamine (residue11), and Argenine (residue297); and presence of ZN as Heterogen in the domain. Moreover, The instability index (II) of the protein is computed to be 28.08, this classifies the protein as stable, and its half-life in mammalian reticulocytes (in vitro) and Escherichia coli (in vivo) has been estimated: 30 hour, >10 hours, respectively. In Conclusion, the selected glutaminase from Nesterenkonia Sp. F was predicted as a salt-tolerant glutaminase which could be employed in fermented food industries.

Keywords: Nesterenkonia Sp.strain F, halophilic bacteria,glutaminase, In Silico analysis, salt-tolerant

**P-48: Transcriptomic and proteomic meta analysis of SOX2 in glioblastoma cancer**

Ghanadpour M, Enteghami M, Ghorbani S.M, Hajjari M.R

Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran.

mahboobeh.enteghami@yahoo.com

The SOX2 gene is located on chromosome 3q26.3 and belongs to the SOXB1 group. The studies on SOX2 have heavily emphasized its crucial role in stem cell maintenance, lineage fate determinant, and reprogramming the somatic cells back towards the pluripotency. The studies on SOX2 have recently switched focus from embryogenesis and development to SOX2s function in disease, particularly in cancer. On the other hand, the importance of regulation of the SOX2 expression by miRNAs has been considered in different studies. The mechanisms underlying the regulation of SOX2 expression and its target genes in cancer remain largely unknown. Our study aimed at investigating the role of SOX2 and its regulation by miRNAs in glioblastoma cancer.

We conducted a meta-analysis on SOX2 in human cancers at transcriptomic, and proteomic levels Through different data sets including Protein Atlas, TCNG, ebioportal, TargetScan and TCGA.

The results demonstrated that it was remarkably overexpressed in some cancer types such as glioma and lung cancers. Moreover, the results on computationally inferred gene interaction networks and gene co-expression network (GCN) in glioma indicated that SOX2 significantly regulates the expression of its target gene â€œNOTCH1â€- in order to regulate different pathways in the glioma. In the next step, we drew the miRNA network, which is responsible for regulating the expression of the SOX2 and the NOTCH1. our studied on miRNA network expression pattern in glioblastoma, as the most severe glioma, demonstrated that mir200c has the highest reduction among all
regulator miRNAs, which can be as An important tumor suppressor for glioblastoma.  

**Keywords:** sox2, glioma, glioblastoma, miRNA network, Transcriptomic and proteomic meta analysis

P-49: Bioinformatics Analysis of Missense Single Nucleotide Polymorphisms (SNPs) in Human NKX2.6 Gene

Ghiasi D\(^1\), Khatami M, Heidari M M

Yazd university

dina.donia41@yahoo.com

Introduction: NKX2-6 gene encodes a homeobox-containing protein that belongs to the NK-2 homeobox family. This gene plays critical roles in regulating tissue-specific gene expression, as well as determining the temporal and spatial patterns of heart development. Several SNPs in the NKX2.6 gene have been identified that associated with congenital heart disease. Determining the protein structure is one of the important issues in the field of bioinformatics assays. PyMOL, a cross-platform molecular graphics tool, has been widely used for 3-D visualization of proteins. The PolyPhen-2 score predicts the possible impact of an amino acid substitution on the structure and function of a human protein. SIFT is able to distinguish mutations involved in disease from neutral polymorphisms.

Method: We identified four non-synonymous single nucleotide polymorphisms in the NKX2.6 gene (rs759945353, rs761239489, rs2676069, rs147172, rs77859360) using dbSNP and then analyzed their effect on the protein structure using PyMOL software and SIFT and polyphen-2 databases.

Results: Our results showed that non-synonymous single nucleotide polymorphisms change the polar groups, and also, number and length of hydrogen bonds. rs77859360 (Leu147Pro), rs759945353 (Thr172Met) and rs761239489 (Arg162Cys) SNPs caused to a hydrogen bond disappearing in protein structure. rs759945353 leads to polar R group into a nonpolar R group conversion. rs761239489 Changes the polar positively charged into a polar uncharged amino acid. Pathogenic rs754763080 (Phe151Leu) SNP changes the length of hydrogen bond. Conclusions: The results of the SNP analysis with the PyMOL software and the SIFT and Polyphen-2 database indicate that all four mentioned SNPs can be deleterious and damaging and change the structure of the protein.

**Keywords:** NKX2.6, Non-synonymous single nucleotid polymorphisms, PyMOL, Polyphen-2, SIFT

P-50: Identification of the best algorithm for horizontal genes study on antibiotic resistance genes in Escherichia coli

Ghiasvand S\(^1\), Vaseghi A\(^2\)

1. Department of Biology, Faculty of Science, Malayer university, Malayer, Iran  
2. SAGENE biotechnology company, science and technology park, Ardabil, Iran

saeedeh2070@yahoo.com

Horizontal gene transfer (HGT) is the most important factor in order to make evolution in prokaryotes, main environmental adaptation, and same bacteria resistant mechanism. There are many algorithms from tools for identification of horizontally transferred genes and genomic islands on the bacteria. The SeqWord Genome Browser (SWGB) based on Oligo-nucleotide usage (OU) statistics to visualize which investigated for sequences of different lengths are comparable provided. The 40 genes sequences, related to antibiotic resistance genes in Escherichia coli in the NCBI database, were analyzed by the following statistical parameters: D for distance two same type, PS for distance two direct and reverse strands of the same DNA sequence; RV and GRV usage variances normalized locally and globally, and reduced to the OU variance expected for a randomly generated sequence, GC-content (GC) and GC-skew (GCS) in DNA. We fund that n1_4mer: RV for the X axis, n1_4mer: GRV for the Y axis and n0_4mer: D for the Z axis is the best program in order to antibiotic resistance genes transfer illusion in the SWGB software. On the dot-plot diagram, each gene is represented by a dot with X and Y parameters chosen from X and Y down-drop lists, respectively.

**Keywords:** horizontal genes, antibiotic resistance genes, Escherichia coli, Bioinformatics

P-51: Comparative Expression of microRNAs in Young-Cardiomyocyte and hESC- Cardiomyocytes by bioinformatics methods

Gholipour A\(^1\), Taheri E\(^2\), Sharifi Zarchi A\(^3\), Irani Sh\(^4\), Shakervarian F\(^5\), Zahedehran A\(^1\), Oveis M\(^6\), Maleki M\(^7\), Mowla SJ\(^8\), Malakootian M\(^9\)

1. 1. Department of Biology, Science and Research branch, Islamic Azad University, Tehran, Iran  
2. 2. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran  
3. 3. Department of Computer Engineering, Sharif University of Technology, Tehran, Iran  
4. 4. Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran

akram.gholipour199069@gmail.com

Introduction: Cardiac development is precisely controlled by complex regulatory networks. Recent reports demonstrated that microRNAs (miRNAs) act as micromanagers of gene expression at all stages of cardiac development. In this study, using different bioinformatics tools, we aimed to determine miRNAs and miRNAs expression profiles in human embryonic stem cell-derived cardiomyocytes (hESC-CMs).

**Methods:** miRNAs and miRNAs were compared between 1-year matured hESC-CMs, and differentiated cardiomyocytes at day 20 (young-CM) samples of GSE62913. Then, differentially expressed miRNAs and miRNAs with padj<0.05 and log2FoldChange?1 were chosen to perform pathway analysis. Pathway enrichment analysis and Regulatory Network were accomplished by Enrichr database and CytoscapeV3.6.0, respectively. In addition, miRWalk database was utilized to analyze the target genes of the highly expressed miRNAs.

**Results:** Our data exhibited 2138 mRNAs and 172 miRNAs with different expression pattern. Pathway analysis and regulatory Network depicted that differentially expressed genes are involved in: complement and coagulation, cardiac progenitor differentiation, dilated cardiomyopathy, hypertrophic, and car...
Heart muscle contraction pathway. In the following, among differentially expressed miRNAs, hsa-miR-98-5p and hsa-miR-122-5p had the highest level of altered expression and were chosen for experimental validation. The upregulated hsa-miR-98-5p and downregulated hsa-miR-122-5p target the 3Â’-UTR of MTUS1 and FUNDC2 genes. The latter genes show high level of expression in artery and heart.

**Conclusion:** All in all, cardiac development and cardiomyocyte-muscle viability are very complicated. Determining the Cardiomyocyte-related miRNAs and their targets would shed more light on molecular processes of cardiac development.

**Keywords:** Differentially expressed miRNAs; Young-Cardiomyocyte; hESC-CMs

**P-52: Modeling and functional assay of SS1P anti-cancer drug in quasi-physiological conditions**

Gonodi Z¹, Farazmand R², Gholampour-Faraji N², Haddad-Mashadrizeh A³, Jalali Ghassam B¹, Rostami F⁴, Abbasi-kalabeh M¹

1. Pharmaceutical Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran
2. Structural biology and Bioinformatics Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran
3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
4. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

zeinab.ganoodi@yahoo.com

**Background:** Mesothelioma tumors with high expression of mesothelin are appropriate target for immunotherapy. In this regard, the SS1P immunotoxin has been developed by combining a variable antibody fragment with a truncated portion of pseudomonas exotoxin A. Accordingly, the purpose of this study is to build the SS1P immunotoxin model in quasi-physiology situation as an approach for its optimization and development.

**Methods:** The Protein Atlas database was used to determine the expression of mesothelin antigen. The NCBI, Uniprot and RCSB provided the sequence and structure of drug and antigens. Modeling and assembling the fragment sequences of drug were performed by using Modeller software by homology modeling method. Determination of protein structures quality was achieved by ERRAT, Verify 3D and RAMPAGE programs. Assessment the stability of drug in quasi-physiological condition was performed with Gromacs at 37 °C. Moreover, HADDOCK and IEDB programs were used to determine the functionality of the drug based on its affinity and immunogenicity.

**Result:** The results of this study led to demonstrate the high expression of mesothelin in ovarian cancer. Moreover, a suitable model of 3D of this antigen was built. Subsequently, dsf, G2S1 and PE were distinctive as parts of sequence context of SS1P with 472 amino acids in the length. On the other hand, assembling of the SS1P result in a model with suitable stability and functionality in quasi-physiology conditions. The affinity assay of this model showed less energy for binding to mesothelin compared to controls. Additionally, we identified immunogenic epitopes in the toxin part of this model.

**Conclusion:** The obtained results revealed range of expression of mesothelin on different tissues, as well as a structural model of SS1P which are consistent with the laboratory and clinical model. Therefore, this model makes the production of this drug justifiable as well as suggests methods for its improvement.

**Keywords:** Mesothelioma, Mesothelin, Immunotoxin, SS1P, thermostability

**P-53: Bioinformatic Analysis of auxin response factor gene family in Arabidopsis**

Hajibarat Z*, Saidi A, Hajibarat Z

Department of Plant Sciences and Biotechnology, Faculty of Life sciences and Biotechnology, Shahid Beheshti University, G.C, Tehran, Iran

zohreh.hajibarat@yahoo.com

Auxin response factors (ARF) are key players in plant growth and development. Arabidopsis thaliana is as a model species for studying plant biology. An extensive bioinformatics analysis including analysis of the protein-protein interaction, conserved motifs, chromosomal map, and phylogenetic relationships were performed for the Arabidopsis ARF gene family. In this study, a set of 22 ARF gene Arabidopsis that identified and categorized into three groups (Class I, II, and III). ARF genes have been studied by molecular methods in several different plant species however to better understand the mechanisms of these proteins more studies are needed. Study of the amino acid composition revealed that totally in all groups, alanine and proline were the most frequent residues while cysteine had the lowest frequency. Chromosomal map showed that the many of genes were distributed on chromosomes. The aims of the present study were to obtain genomic information for the AtARF gene family and to study phylogenetic relationships among genes.

**Keywords:** Arabidopsis, Gene family, Auxin, ARF gene

**P-54: Studying one of the regulating elements of low molecular weight glutenin gene expressions in bread wheat**

Hasrak Sh, Bagheri A, Lohrasebi T, Shariati V, Marashi H.

1. Biotechnology and Plant Breeding Department, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Agricultural Biotechnology Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.

shasrak@yahoo.com

Low molecular weight glutenin subunits (LMW-GS) play important roles in bread making quality particularly in dough strength and viscosity. Although extensive researches have been conducted into the genomic aspects of wheat LMW-GS, there are still very little reports about their regulating expression mechanisms. The purpose of current study was to obtain data which will help to address this research gap. In this study, grain gene expression analyses of two wheat cultivars with contrasting bread making qualities (Pishbaz and Navid) were carried out by RNA-Seq method at 5, 10, 14, 21 and 28 days post anthesis. RNA-Seq analysis revealed there was no significant difference in the case of most LMW-GS gene expression between two cultivars whereas differential gene expression
analysis showed Navid had higher expression in certain LMW-GS genes compared with Pishtaz at all stages. Further bioinformatics analysis indicated there was a specific DNA binding domain of ERF transcription factor in upstream region of certain LMW-GS genes. Comparative analysis showed gene encoded ERF gene had higher expression level in Pishtaz rather than Navid. A complete contrast can be seen in expression pattern of ERF genes and their target genes between two cultivars, as by increasing the ERF gene expressions, the LMW-GS gene expressions were decreased in Pishtaz. These findings and their agreement with previous studies provide further support for the hypothesis that ERF transcription factors have significant roles in negative regulation of LMW-GS gene expressions.

Keywords: ERF, Gluten, LMW-GS, RNA-Seq, Wheat.

P-55: In-silico analysis of miR156a in candidate medicinal plant species in Zanjan province
Hassanlou M, fotovat R, Ashuri N

Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Zanjan, Iran
m.hassanlou2006@gmail.com

MicroRNAs (miRNAs) are critical regulators of gene expression, and exert extensive impacts on development, physiology, and disease of eukaryotes. A high degree of parallelism is found in the molecular basis of miRNA biogenesis and action in plants and animals. Recent studies interestingly suggest a potential cross-kingdom action of plant derived miRNAs, through dietary intake, in regulating mammalian gene expression. Plant miRNAs can be detected in Western human sera and whether these plant miRNAs are able to influence gene expression and cellular processes related to human diseases such as cancer. Functional adhesion molecule A (JAMA) is preferentially concentrated at tight junctions and influences cell morphology and migration. In the total of broccoli conserved miRNAs, miR156a was expressed the most. In addition, synthetic miR156a mimic inhibited the EMT (Epithelial-mesenchymal transition) of NPC (nasopharyngeal Cancer) cells in vitro. Furthermore, it was confirmed that JAMA was the target of miR156a mimic as validated by 3′UTR luciferase reporter assays and western blotting. In this study, we applied miR156a in medicinal plant species of Zanjan province. A number of 212 plant species EST databases were studied and its aims computationally analysed. Results distinguished 12 species that have these ESTs and therefore suggested that miR156a has anti-cancer effects. Moreover, discovering of miR156a targets in native medicinal plant species may have clinical implications for the treatment of patients with NPC and reduce using of chemical drugs with harmful secondary effects.

Keywords: Medicinal plants, Zanjan province, miR156a, Expressed Sequenced Tags, Anticancer

P-56: In-silico evaluation of miR296 polymorphism in colorectal cancer risk
Ilkhani K, Asma Safi, bahmanpour Z, mousavi R, Alivand M

1. Student Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Medical Genetics, Faculty of Medicine, Tabriz

P-57: Investigating the effect of susceptible Transcription factor in progression of type 1 diabetes and autoimmune thyroid disease
Jafari Harandi A¹, Navaderi M², Rahimi Rad S², Roshani F¹
1. Nourdanesh Institute of Higher Education, Iran.
2. Medical Genetic Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.
aysan_jafar@yahoo.com

Background: Genetic studies lead to identify mechanisms for detection of autoimmune diseases. The role of some similar genes has been identified in development of autoimmune diseases. Indeed, clinically distinct autoimmune diseases cluster in families, the existence of joint autoimmune susceptibility genes. This association can be seen in autoimmune thyroid disease (AITD) and type 1 diabetes (T1D). AITD occurs in 10-25% of people with T1D. We aimed to identify the association between similar genes in the related mechanisms that trigger these two diseases.

Method: We searched in PubMed with these keywords; diabetes, autoimmune thyroid disease, gene expression. Resulting was 52 articles from 2010-2018. The gene ontology (GO) of obtained genes figured out from DAVID (https://david.ncifcrf.gov/) (Table.1). The transcription factors (TF) were studied through TRRUST (http://www.grnpedia.org/trrust/) and Reg-NetWork (http://www.regnetworkweb.org/) databases. The association of TFs and genes were assessed. Interactions between genes and their TFs were drawn by GeneMANIA (https://genemania.org/) database.

Result: Among 21 genes (deregulated 8 genes in AITD and 13 in T1D), 5 similar genes (HLA-DR3, HLA-DR4, PTPN22, CTLA4, FOXP3) play pathogenic roles in both diseases. Due to evaluated AITD and T1D process, PTPN22, CTLA4, FOXP3 are effectiveness in more process compare with HLA-DR3 and HLA-DR4. So 3 genes were selected and we obtained 48 TFs for these genes. NFATC2 as TF could regulating the expression...
of FOXP3 and CTLA4 genes.

**Conclusion:** The results of our study showed that FOXP3 and CTLA4 genes promoting inAITD and T1D patients and NFATC2 regulating expression of mentioned genes.

**Keywords:** Type 1 Diabetes, Autoimmune Thyroid Disease, Gene Expression, Bioinformatics Analysis and Transcription Factor

**P-58:** In silico study of anti-CCHFV effect of lactoferrin from different origin

Javadmanesh A, Azghandi M

Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

azghandi.marjan@gmail.com

Crimean-Congo haemorrhagic fever (CCHF) is a fatal viral infection. Human beings become infected through tick bites, or by contact with blood or tissues from viremic livestock. Recent studies suggest that ribavirin is effective against CCHF, although the efficaciousness of ribavirin in the treatment of CCHF has not yet been demonstrated conclusively. The protective effects of lactoferrin (LF) against common viral infections have been demonstrated in several studies. This glycoprotein is one of the innate immune system components with broad range of antimicrobial activates comprising antiviral, antibacterial, antifungal and anti-cancer actions. LF prevents entry of virus in the host cell, either by blocking cellular receptors, or by direct binding to the virus protein. A comprehensive study comparing anti-CCHFV properties of LFs derived from different origins has not yet been conducted. In the current study, in silico evaluation of antiviral effects of LF from different species against CCHFV was evaluated by a protein-protein docking approach. The crystal structures of human, horse, cattle, goat, buffalo and camel LF were retrieved from the uniprot, and the protein structure of sheep and zebo cattle LF were predicted. The crystal structures of CCHF virus envelopment polyprotein Gn and Ge were retrieved from the protein data bank, then the pdb files prepared for docking calculation. Autodock 4.2.6 was used for protein docking. Results showed that the N- and C-lobe fragments and the full-length of LF had effective anti-CCHFV activities. Buffalo LF showed the highest binding energy among studied species and the lowest energy belonged to cattle LF.

**Keywords:** Lactoferrin, antivirus, in silico, CCHF, buffalo

**P-59:** Identification of differentially expressed miRNAs and RNAs by using The Cancer Genome Atlas (TCGA) miRNA set

Javanmard marani A, M. Soltani B

Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran.

amirjavan1372@gmail.com

In order to extraction of the differentially expressed genes (DEGs), several data set of the colorectal polyp and non-polyp sample miRNA expressions were downloaded from TCGA database. In total, 379 of 1883 miRNAs were differentially expressed in 459 Polyp and 5 non-polyp samples compared with normal samples. The raw data were analysis by statistical R software. The data had been normalized by Deseq package and PCA plot had drawn also by Deseq package. Finally by machine learning differentially expressed microRNAs between polyps and non-polyps has been done. After analysis data, top 10 of microRNAs had best p-value were chosen for pathway analysis. Pathway analysis was done by DIANA software. Results from analysis these pathways show that microRNAs up regulated are more involved to progressing of polyps to colorectal cancer and microRNAs down regulated not to progressing of polyps to colorectal cancer.

**Keywords:** bioinformatics, polyp, colorectal cancer, biomarker

**P-60:** The Possible Role of Long Noncoding RNA as Novel Player in Type 2 Diabetes

Javanzad Sh1, Abedi Kichi Z1, Behmanesh M1

1. Department of Genetics, Faculty of Biological Sciences, Tarbait Modares University, Tehran, Iran

sh.javanzad@yahoo.com

Type 2 diabetes mellitus (T2DM) is a chronic disease with increasing rate of prevalence in the world that causes substantial public health and economic burden. Although more than 20 genetic susceptibility loci have been reported for type 2 diabetes (T2D), most reported variants have small to moderate effects and account for only a small proportion of the heritability of T2D, suggesting that the majority of inter-person genetic variation in this disease remains to be determined. So finding genetic variants plays an important role and provides a lot of data. Since non coding region is likely to have a significant effect on pathogenicity and susceptibility of diabetic and cardiovascular diseases, then it is useful to study the involved IncRNAs.

Materials and methods: Analysis of RNA-Seq data has revealed there are three IncRNAs (PVT1, H19, CDKN2B-AS1) that are associated with diabetes. Among these IncRNAs, PVT1 was selected and assessment its functional and regulatory role was done by bioinformatics. Conclusion: PVT1 bioinformatics analysis has shown there is association between variants (rs2720709, A>G) in the plasmacytoma variant translocation 1 gene (PVT1) and end-stage renal disease (ESRD) attributed to both type 1 and type 2 diabetes and the SNP has key role in both pathogenicity and gene regulation. PVT1 fullfills its role by modulating the function of some transcription factors such as c-Myc, P53, YY1.

**Keywords:** Type 2 diabetes mellitus (T2DM), IncRNAs, RNA-Seq

**P-61:** In silico analysis targeting of the genes involved in Notch signaling pathway by hsa-miR-3163

Kabiri F, M. Soltani B

Department of Molecular Genetics, Faculty of Biological Sciences, Tarbait Modares University, Tehran, Iran

farnoushkabiri@gmail.com

In order to extraction of the differentially expressed genes (DEGs), several data set of the colorectal polyp and non-polyp sample miRNA expressions were downloaded from TCGA database. In total, 379 of 1883 miRNAs were differentially expressed in 459 Polyp and 5 non-polyp samples compared with normal samples. The raw data were analysis by statistical R software. The data had been normalized by Deseq package and PCA plot had drawn also by Deseq package. Finally by machine learning differentially expressed microRNAs between polyps and non-polyps has been done. After analysis data, top 10 of microRNAs had best p-value were chosen for pathway analysis. Pathway analysis was done by DIANA software. Results from analysis these pathways show that microRNAs up regulated are more involved to progressing of polyps to colorectal cancer and microRNAs down regulated not to progressing of polyps to colorectal cancer.

**Keywords:** bioinformatics, polyp, colorectal cancer, biomarker
Introduction: Loss of sensory hair cells in inner ear results in two health problems: loss of hearing and balance disorders. In mammals, cochlear hair cells won’t be replaced when lost. One in all promising methods for regeneration of the hair cells is to stop factors preventing the conversion of adjacent non-sensory supporting cells into hair cells. Notch signaling is a highly conserved short-range cell-cell system communication. This pathway plays a prominent role in the formation of wide variety of cell types and regulates several cellular processes. On adult mice, suppression of Notch signaling can turn supporting cells into hair cells via trans-differentiation.

MicroRNAs, small noncoding endogenous RNAs, are one of the most important factors which play post transcriptional regulatory role on genes expression. The aim of this study is bioinformatics analysis of miRNA-mRNA regulatory interactions in Notch signaling pathway.

Methods: here using online bioinformatics tools, we examined the miRNAs which may target the genes involved in Notch signaling. Candidate miRNAs were screened based on free energy, conservation and seed match scores via Diana tools, Targetscan and miRmap. Result: here hsa-miR-3163 was predicted to target 13 genes in Notch signaling such as NOTCH1, DLL1 & JAG1 as the activators of the pathway.

Discussion: hsa-miR-3163 is one of the potential regulators of Notch signaling pathway. However, further experimental analysis is needed for verification of the predicted targets.

Keywords: miRNA, Target prediction, Notch signaling pathway

P-62: Computational genomics of type 2 diabetes: an integrative approach to shed light on genome-wide statistics

Kamali Z1, Vaez A2

1. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences
2. Department of Epidemiology, University of Groningen, University Medical Center Groningen, PO Box 30001, 9700 RB, Groningen, The Netherlands
zkamali1373@gmail.com

Genome-wide association studies (GWAS) have revealed genomic loci for different complex traits; but the biological interpretation of these loci is still elusive. A number of methodologies have been developed to prioritize the most likely causal genes and pathways. Here we follow a post-GWAS pipeline and add an extra step to analyze GWAS results of type 2 diabetes (T2D) and to gain insight about underlying biological knowledge. For this goal, after defining independent associated loci, we prioritized the most likely causal genes using a phenotype- and mechanism-agnostic algorithm, which is predicated on a previously formulated assumption that truly associated genes share functional annotations. Co-expression data with annotated gene sets, are used to predict gene function and gene set enrichment analysis. Mendelian Randomization (MR) analysis is performed to integrate GWAS results with expression data and investigate genes which their expression level is likely causal. 15 genes from associated loci were prioritized (FDR < 0.2) according to the abovementioned functional predictions. MARK1 PPI subnetwork (P = 7.13e-7) and positive regulation of cell cycle (FDR < 0.05) were the most significant pathways enriched for genes from associated loci with P < 5e-8 and P < 1e-5 respectively. MR analysis of gene expression causality showed a robust effect for PLEKHA1 among eQTL studies; this gene is also present among top 10 genes of MARK1 PPI subnetwork. PLEKHA1 is not directly reported for T2D, but regarding its involvement in macular degeneration and eye disease, this result suggests a role for it, maybe via eye related implications, in diabetes.

Keywords: GWAS, post-GWAS, T2D

P-63: Bioinformatics analysis of the most relevant signaling pathways for hsa-mir-513b in breast cancer

Kardanpour M, Ghaedi K, Kardanpour MR

Nourdanesh Institute, Meymeh, Iran
moloud1394@gmail.com

Breast cancer is the most common cause of cancer death in women worldwide. MicroRNAs are small non-coding RNAs that are 20-22 nucleotides long. These molecules participate in vital biological processes such as development, differentiation, apoptosis and cell proliferation by binding to mRNA molecules and transcriptional and transcriptional regulation. hsa-miR-513b, a new subtype encoded from microRNA. This microRNA belongs to the miR506-514 class that is located on the chromosome x. The aim of this study was to investigate the association of rs61959909 gene polymorphisms of hsa-miR-513b gene associated with breast cancer and to determine the genotyping of healthy and breast cancer patients at rs61959909 in ARL11 gene and to investigate the relationship between rs61959909 genotypes with clinical-pathological characteristics. In healthy people with breast cancer, the research findings for the diagnosis and treatment of breast cancer have been completed. By examining the micrograms of these microRNAs in the most relevant pathways, it seems that most of its activities play a role in Tumor suppressor. In this study, hsa-miR-513b target analysis was performed in miRBase, mirwalk2 and DAVID databases, respectively, to find the most relevant signaling pathways for hsa-miR-513b in breast cancer. Given the predictions made by these databases, the most relevant pathways for has-miR-513b in breast cancer are Pathway in cancer and Apoptosis. Therefore, it is suggested that in subsequent practical studies, the role of this microRNA on cancer markers that will play the role of Tumor suppressor will be emphasized.

Keywords: breast cancer, microRNA, hsa-miR-513b

P-64: In silico evaluation of gene and protein structure of 1, 4 alpha glucan branching enzyme in the Neisseria bacteria

Kargar F1, Mortazavi M2, Torkzadeh Mahani M3, Asadi H4, Jamshidi Goharrizi K3
Farzanearam95@gmail.com

Cellulase is an enzyme which degrades cellulose and has the importance of clinical research. Currently, cellulase is used in juicing, animal feed, textiles, pulp, paper and agriculture and considered as an appropriate target in the industry and for scientific research. By using bioinformatics software, comprehensive and accurate information can be obtained from the
MicroRNAs (miRNAs) are 19-25 nucleotide non-coding regulatory RNAs, that participate in a variety of developmental pathways and their deregulation has been implicated in the etiology of human cancers. Association of miR-605 with cancers has been established based on differential gene expression data sets and microRNA polymorphism studies. However, the major targetome of miR-605 has not clearly discovered. In this study target genes of miR-605 and pathways involved in were computationally analyzed. Computational prediction of target genes was done by multiMiR R package in the statistical software R. The multiMiR package finds targetome based on seven external servers including DIANA-microT-CDS, Microcosm, Miranda, miRDB, PicTar, PITA and target scan. The results showed the presence of 3263 unique target genes. In order to find and classify their biological functions and pathways we used DAVID bioinformatics resources and KEGG (Kyoto Encyclopedia of Genes and Genomes) server to classify these genes based on pathways that they were involved. Totally, we found 69 unique pathways for these 3263 genes. Among the pathways, 19 were involved in different signaling pathways in cancer that their dysregulation promote tumorigenesis, metastasis and angiogenesis. Predicted genes and pathways network showed that miR-605 could potentially be a tumor suppressor microRNA.

**Keywords:** MicroRNA, cancer, miR-605, gene network

**P-66: Discovery of Gene Networks contribute in Chronic Lymphoid Leukemia (CLL) through Enrichment Analysis**


1. Department of Molecular Medicine, Faculty of Advanced Technologies in Medicine, Iran University of Medical Sciences, Iran
2. Department of Bioinformatics, Institute of Biochemistry and Biophysics, University of Tehran, Iran
3. Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Iran
4. Department of Genetics and Molecular Biology, School of Medicine, Iran University of Medical Sciences, Iran
5. Department of Gynecology Oncology, Firoozgar Hospital, Iran University of Medical Sciences, Iran
6. Palliative Care center, Firoozgar Hospital, Iran University of Medical Sciences, Iran
7. Cellular and Molecular Research Center, Iran University of Medical Sciences
Mousavik@gmail.com

**Introduction:** According to reduction in time and cost of Massively Parallel Sequencing (MPS), genomic information from malignancies becomes more available. CLL is a hematological malignancy in which genetic alterations in different genes have been suggested using Next Generation Sequencing (NGS) in recent years.**

**Aim:** In this study we have determined involved gene networks in this disorder according to the gene ontologies and molecular pathways.

**Methods:** We reviewed all 36 articles in which whole exome sequencing have been used in CLL patients. We have found 41 important affected genes in this disorder. In order to determine the gene networks that were involved, network based enrichment analysis for gene ontologies and molecular pathways was done using EnrichNet Online tool[1]. Pathways or processes by 3 or more affected genes and significant fisher exact test (p<0.05) are presented.

**Results:** Using GO molecular function, contribution of 14 affected genes in "DNA binding" process was significant. In GO cellular Component, 22 genes with nucleus products, 11 genes which compose nucleoplasm and 4 genes with products in telomeric region showed significant contribution. Cellular response to UV, B cell activation and B cell receptor signaling pathway were involved in GO biological process. In Corum (MIPS) complexes, DDX3X and SF3B1 in spliceosome were affected. Apoptosis and cell cycle genes were involved in KEGG pathways. Apoptosis, miRNAs involved in DDR and TP53 network were involved in wiki pathways. ACD, POT1 and TERF1 in packaging of telomere ends were found in Reactome. In NCI pathway interaction DB also telomerase pathway/Regulation of Telomerase was involved.

**Conclusion:** Involvement of genes in apoptosis (TP53, BIRC3) and telomere structure/function (ACD, POT1, TERF1) were common consensuses in network based enrichment analysis of CLL.

**Keywords:** Enrichment Analysis, Chronic Lymphoid Leukemia
P-67: Searching for novel biomarkers in autoimmune diseases

Khadiemi B1,2, Bahmanpour Z2, Tahmasebivand M2, Mousavi R2, Esmailizadeh B2

1. Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
baharehkhamidi042@gmail.com

MicroRNAs (miRNAs) are noncoding RNA molecules approximately 22 nt in length. It is now clear that miRNAs play a role in regulation of many physiological processes especially immunological functions and the prevention of autoimmunity. Many aspects of miRNAs in modulating these processes in autoimmune diseases are still unknown. In this study, we considered two of the most important diseases in this group, including lupus erythematosus and arteritis rheumatoid. Pathway databases and articles information were applied for specifying common pathways involved genes. Then, based on miRNAs databases including diannahools, miRDB, miRTarbas and miR2disease, miRNAs regulating top genes were identified. As a result, the family of miRNAs 181 regulates a significant number of genes involved in the pathway related to the immune system, which is likely play an important role in these two conditions. Since this study has been conducted on bioinformatics data then more research on experimental level is recommended for researchers.

Keywords: bioinformatics approach, microRNA (miRNA), autoimmunity, lupus erythematosus, arteritis rheumatoid.

P-68: The comparative study of some cyanobacteria genomes

Khani F, Mohammadi P, Zarrabi M

1. Microbiology Department, Faculty of Biological Sciences, Alzahra University
2. Biotechnology Department, Faculty of Biological Sciences, Alzahra University
3. Computational Biology Lab, Faculty of Biological Sciences, Alzahra University
f_khani2000@yahoo.com

Cyanobacteria are the only known prokaryotes that are able to perform oxygenic photosynthesis and considered to be the first colonizer organisms on the earth. Due to their wide-range ecological tolerances, they are almost found in everywhere from the aquatic habitats to the terrestrial niches. This capability can be attributed to their genomes. The aim of this study was to evaluate the similarities and differences of some terrestrial Cyanobacteria strains at the genome level. For this reason, it was selected some Cyanobacteria genome sequences from Gene Bank and Cyanobase. According to the Integrated Microbial Genomes and Microbiomes database, these strains were isolated from terrestrial environments. Clusters of Likely Orthologue Genes (CLOG) were defined based on a pair-wise comparison of all coding sequences using BlastP tool kit. The core and pan genome of these strains were investigated through R programming environment and CD-hit server. Each CLOG was assigned to a strain if at least one member of a CLOG was present in its genome. Core genes were found in all the strains but the unique genes were existed only in one genome.

The results showed that the majority of genes in Cyanobacteria genomes are unique genes and had no likely orthologue in any genomes. Sequencing the other Cyanobacteria genomes will probably result to discover a large number of unknown genes with potential capacities. These capacities can be used for the variety of the biotechnological applications.

Keywords: Cyanobacteria, core gene, unique gene, genome

P-69: Applying data mining algorithms to identify key variants and candidate genes for adaptation to hypoxia in Iranian indigenous chickens


1. Institute of Biotechnology, Shiraz University, Shiraz, Iran.
2. Department of Animal science, School of Agriculture, Shiraz University, Shiraz, Iran.
3. Department of Animal science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.
h.kharrati.ko@gmail.com

This is the first report about identifying key adaptive variants in hypoxia conditions using data mining algorithms in Iranian native chickens. Adaptation to hypoxia, low oxygen condition, is a complex process that includes complex biological pathways. Thereby, understanding the genetic factors underlying adaptation to high-altitude conditions in domestic animals can provide new source of science for finding the adaptation process. We resequenced and analyzed the whole genome of highland (Altitude:2087m) and lowland native chickens (Altitude: 54m) in Iran for identifying differential variants between the two chicken ecotypes. Our results indicated that there were 216 differential SNVs (single nucleotide variations), which can change the amino acid sequences in protein structure. Four attribute weighting algorithms including Uncertainty, Information gain, Gini Index and Relief were run on 216 SNVs by Rapidminer software (7) for discovering the key differential variants between highland and lowland chickens. Our results showed that 23 common variants among the results of attribute weighting analysis have the highest weighting coefficient. The linking variants to protein structure analysis illustrate that key variants have main role in DNA repair process (SLF1, RIF1). A possible explanation for this, might be that high-dose UV radiation in high-altitude condition can lead to DNA damage. Therefore, candidate genes which are involved in DNA repair can be considered. Additionally, our results showed that reproduction (GAS8) and organ developments (NPNT) may contribute in adaptation to hypoxia. High-altitude condition has an extensive effect on the genomic variation, thus our reveals new insight to the adaptive pathways to hypoxia conditions.

Keywords: Hypoxia, Data mining, differential variants, native chickens.

P-70: Using Systems Biology Approaches to Discover DEGs and Regulatory Factors Responsible for Glioblastoma

Khosravifard F1, afzali F2

1. Department Of molecular & cellular, Faculty of Advanced Sci-
In-silico studies with reduced time and expenses has facilitated disease treatment more than before. In the current study, we have made comparisons between expression profile of monocytes obtained from Glioblastoma patients and those of healthy ones provided by GSE77043 dataset in GEO. After normalizing the data by RNA algorithms, differentially expressed genes (DEGs) were identified by adjusted p-Value and logFC parameters. JASPAR PWMs database was then used for finding transcription factors (TFs). Following performing Enrichment analysis, Gene Ontology and the affected pathways revealed by GO and Reactome databases, miRNAs regulating our DEGs were selected by miRtarBase and mapping DEGs on brain performed by Allen Brain Atlas.

Our study suggests E2F1, PCBP1, TCFAP2A, WT1 and SP1 as TFs are involved in Glioblastoma condition. The ontology revealed theses DEGs are mostly involved in negative regulation of transcription from RNA polymerase II promoter and I-kappaB phosphorylation. The top3 pathways affected by them are Hemostasis, Cellular responses to stress and Signaling by Rho GTPases. Our results introduce miR-17-5p, miR-93-5p and miR-20a-5p as important miRNAs regulating gained DEGs. Mapping on the brain showed DEGsâ€™ existence mostly in Anteromedial visual area, layer 6a and layer 3 of PCx.

In conclusion, TFs identified by systems biology approaches are reasonable targets and even shortcuts in research fields. Although miRNAs can be key and new regulators for controlling Glioblastoma condition.

**Keywords:** Glioblastoma, systems biology, TF, miRNA, microarray

**P-71: Evaluation of Pathogenicity Effects of Missense Nucleotide Polymorphisms (SNPs) in NKX2.1 Gene**

**Madani Manshadi SA, Heidari M M, Khatami M**

**Yazd University**

**seyedali.m1993@gmail.com**

**Introduction:** Congenital hypothyroidism (CH) is a most common congenital endocrine disorder, affecting 1 in 3000 to 4000 newborns. Nkx2.1 (thyroid transcription factor-1; also known as TTF-1) is an essential homeodomain-containing transcription factor for the morphogenesis and differentiation of the various tissues such as thyroid, lung and ventral forebrain. The purpose of this study is to examine the pathogenic effects of single nucleotide polymorphisms in the NKX2.1 gene based on bioinformatics analyzes to determine the rule of these mutations in the structure and function of mutated protein.

**Method:** We identified three non-synonymous single nucleotide polymorphisms (nsSNPs) in the NKX2.1 gene: (rs137852693, rs28936671 and rs28936672) using dbSNP and then analyzed their effect on the protein structure using PyMOL software and SIFT and PolyPhen-2 database.

**Results:** Our data showed that non-synonymous single nucleotide polymorphisms change the interaction patterns, polar groups, and length of hydrogen bonds. rs28936671(Arg213Ser), and rs28936672 (Trp208Leu) SNPs caused to reducing the number of hydrogen bonds in the protein structure. In rs28936671(Arg213Ser) the positive-polar amino acid has become non-polar amino acid. rs28936672 (Trp208Leu) converts the aromatic amino acid to aliphatic amino acid. rs137852693 (Glu205Gln) changes the polar positively charged into a polar non-polar amino acid. rs28936671(Arg213Ser), rs28936672 (Trp208Leu) and rs137852693 (Glu205Gln) changes the positive-polar amino acid into a non-polar amino acid. These SNPs can be harmful, deleterious and alter the structure of the protein. Computational biology tools have advantages and disadvantages, and their results are predictions that require confirmation.

**Keywords:** NKX2.1, non-synonymous single nucleotide polymorphisms, structure prediction, PyMOL, SIFT

**P-72: A method to study 5’ untranslated region of genes**

**Mahnadiasser M, Najafi A, Salehipour P, Modarressi MH**

**Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran**

**majdehmahdian@yahoo.com**

**Introduction:** The 5’ untranslated region includes exons located upstream of the coding sequence. They are transcribed into mRNA, but not translated into protein. Genes encoding transcription factors, proto-oncogenes, tumor suppressors, growth factors and their receptors tend to produce transcripts with a longer and more complicated 5’UTR region, containing various elements such as the upstream open reading frame (uORF) and the internal ribosome entry site (IRES). Such genes are highly regulated at the translational level.

**Aim:** Regarding the significance of the 5’UTR region in the regulation of gene expression, we report a method to identify different regulatory elements in the 5’UTR region.

**Method and Result:** In order to locate the regulatory elements including uORF and IRES in the 5’UTR region, the sequence of the mRNA is screened using UTRdb, UTRscan, and RgRNA. Additionally, the sequences of the 5’UTR in different species are obtained from NCBI and aligned using the MAFFT multiple sequence alignment program to identify conserved regulatory elements. Finally, the RNAfold web server from the Vienna RNA and MFOLD program are used to determine secondary structures as well as the corresponding free energy changes (AG) related to the folding of the 5’UTR.

**Conclusion:** There is massive evidence that the deregulation of gene-specific translation plays a critical role in oncogenic transformation and tumor progression. Further studies are necessary to ascertain the function and the specific roles of uORF and IRES in translational regulation, considering various environmental conditions, such as hypoxia.

**Keywords:** 5’ untranslated regions, IRES, uORF, Translational regulation

**P-73: Bioinformatics analysis of P1 protein of Mycoplasma pneumonia to design a model of recombinant vaccine**
Mahmoodi M, Esmaeilzad M, Saffarian P

1. Central laboratory department, Razi vaccine and serum Research institute, Agricultural Research, Education and Extension Organization(Areeo), karaj, Alborz, Iran.
2. Department of Microbiology, Islamic Azad University, Science and Research Branch, Tehran, Iran.
mahdihmahoodi66@gmail.com

Mycoplasma is a bacterium that has no cell wall. Mycoplasma pneumoniae is the causative agent of primary atypical pneumonia in humans and also responsible for other respiratory tract infections such as tracheobronchitis, bronchiolitis, croup, and less severe upper respiratory tract infections in older children and young adults. P1 protein is an surface protein on the M. pneumoniae which are functional in receptor recognition, as the probable adhesion protein. Full length of P1 protein (1628aa) were analysed by bioinformatics tools. All of 94 P1 protein sequences available in NCBI data base were compared by multiple alignment tools. Consensus and hypervariable regions of P1 proteins were identified. B-cells and T-cells epitopes were predicted by IEDB online software based on different MHC I and MHC II alleles. 4.6 % divergency were observed in P1 protein sequences. 44 complete protein sequences were divided to five distinct groups. Bioinformatics analysis identified four a to d conserved and three hypervariable regions in 1628aa of P1 protein. All of important B-cell and T-cell epitopes were located only in two conserved b and d regions. This study recommended two P1b and P1d conserves regions of P1 protein as a target for designing of new generation vaccine against mycoplasma pneumonia.

Keywords: Bioinformatics, P1 protein, Mycoplasma pneumonia, recombinant vaccine

P-74: Computer-Based Genetic Manipulation of the Emitter Site of Renilla Luciferase: An Attempt to Improve a Gene Sensor

Mazidi Y1, Emamzadeh R2, Nazari M3
1. Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
2. Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
3. Monoclonal Antibody Research center, Avicenna Research Institute, ACECR, Tehran, Iran
ya3.milim@gmail.com

Luciferases are oxidoreductases widely used as gene reporters during both cellular and cytogenetic studies. Additionally, luminescent molecules that are converted to luciferin upon the activity of a particular enzyme can detect enzyme activity in coupled or two-step luciferase assays. Among luciferases, Renilla luciferase (RLuc) is a blue light emitter luciferase which can be applied as a valuable tool in medical diagnosis. However, the binding properties of its emitter site remain largely unknown. In the present study, active site and the ligand-receptor interactions of RLuc were investigated. Molecular docking simulations with the coelenterazine substrate against a recently determined RLuc crystal structure were used to build hypotheses to identify functionally-related residues. The previous data highlighted two triads of residues that are critical for catalysis. The putative catalytic triad residues D120, E144, and H285 and also three other residues implicated in product binding (N53, W121, and P220). We obtained the needed structural data from RCSB database (http://www.rcsb.org) with 2PSF PDB ID for RLuc and the substrate from https://pubchem.ncbi.nlm.nih.gov with 2830 PubChem ID.

Comparison between the binding energies before and after modification using PyRX v0.8 and docking package Molegro Virtual Docker v6.0 showed that substitution of Trp121 to Phe121 is completely favorable in all of its orientations. So it can be concluded that this mutation may improve the light-producing reaction. These kinds of studies may lead the process to make more sensitive and reliable biomarkers from available systems by the use of genetic engineering.

Keywords: Renilla Luciferase; Coelenterazine; Gene reporter; in-silico Analysis; Docking

P-75: In Silico Pocket and Binding Sites Detection of Acinetobacter Baumannii Outer Membrane Protein, FilF, As a Potential Vaccine Candidate

Mirzaeian F, sefid F
1. Department of Biology, Yazd University, Yazd, Iran
2. Department of Medical Genetics, Shahid Sadoughi University, Yazd, Iran
f.m136719@yahoo.com

Acinetobacter baumannii has emerged as an important pathogen causing a variety of infections. Due to the prevalence of infections and outbreaks caused by multi-drug resistant baumannii, few antibiotics are effective for treating infections caused by this pathogen. Therefore treatment of A. baumannii infections has become a considerable health care challenge and new strategies against its infections are needed. Because of outer membrane proteins importance as vaccine candidate, we exploited bioinformatics tools to detection of pocket and binding sites of baumannii fimbrial protein, FilF that recently was predicted as a potential vaccine candidate. Methods: multi-scale pockets on protein surfaces were finding by GHECOM server that used mathematical morphology. castP is another server we used to identification and measurements of surface accessible pockets as well as interior inaccessible cavities for query protein. MetaPocket 2.0 (MPK2) server employs eight element predictors: LIGSITEcs, PASS, Q-SiteFinder, SURFNET, Pocket, GHECOM, ConCavity and POCASA to predict protein pockets and potential binding sites. Furthermore we used SiteHound-web, PLB_SAVE web system, eF-seek and Depth servers for potential binding sites prediction. Results: Ligand binding sites determined using these softwares, indicate involvement of conserved residues from 477 to 590. Pockets and graph of residue-based pocketness confirm the ligand binding site as the largest and the most important pocket.

P-76: Bioinformatics Analysis of Expressed Simple Sequence Repeats (EST-SSR) in Dunaliella salina

Mohammadi R*, Mohammadzadeh Jalaly H, Amin Hejazi M
Branch for Northwest & West region, Agricultural Biotechnology
Research Institute of Iran (ABRIJ), Agricultural Research, Education and Extension Organization (AREEO), Tabriz, Iran.

m_riza51@yahoo.com

Nowadays microalga Dunaliella is under increasing attention for its beta-carotene production, its applications in nutritional, pharmaceutical industries, cosmetics and in research field such as genetic engineering. Hence identification of this genus and high productive species of it would be very useful. However, the current taxonomy of the genus Dunaliella has done by morphological and physiological traits, on one hand due to environmental adaptation and lacking of a cell wall, this genus may exhibit different morphological and physiological behavior in different conditions. So morphological and physiological studies were not sufficient and cause the confusion in the systematics of this genus. Therefore, molecular markers have been successfully used to evaluate the genetic diversity of various organisms. In this study, to investigate of EST-SSRs in Dunaliella, EST libraries were downloaded from NCBI site and EST-SSRs were identified using BatchPrimers3 software. In this species 643 SSRs were taken from 6811 ESTs were identified. The results showed that among the studied SSRs, the most frequent repetition was related to trinucleotide motifs with 377 (58.63%) frequencies and the lowest repetition was related to hexanucleotide motifs with 12 (1.86%) repetition. In general, EST-based SSRs are useful to study of genetic diversity in related species and they have a high potential in comparison to genomic SSR markers.

Keywords: Genetic diversity, EST-SSR, Dunaliella salina

P-77: Bioinformatics Identification of the Binding Site of Transcription Factors in some Candidate Genes in cow

Moradi shahrbabak H, Ghafoori F, Nejati Javaremi A, Bakhhtiari Zadeh M R, Nikbakhsh A

Department of Animal Science, University of Tehran, College of Agriculture & Natural Resources, Department of Animal Sciences

farzadgafouri@gmail.com

The aim of the present study was to investigate the expression of genes and transcription factors associated with the replacement stage and before replacing the embryo in the endometrium of the cattle. The expression of the identified genes in the endometrial tissue of the cow species was investigated and the transcriptional regulation network of these genes in the endometrium tissue was examined by promoter analysis. Finally, 13 genes with high expression and known function in two stages of pregnancy (embryo replacement and before it) were selected as candidate genes in the immunization response process stages for promoter analysis. The binding sites of transcription factors on the promoters of these genes are used to better understand the regulatory network of the genes in question. Investigation of the regulatory network involved in the intended stages has led to the identification of several transcription factors (TFs), including TFs that were previously not known as involved factors in these stages. In order to analyze the changes between the expression of the microarray data used the R software and the Affy package, also the GO analysis to identify the biological stages associated with each gene. To identify the binding sites were used the GENOMATIX package. The results show that new known factors may have a regulatory role in the early stages of conception. Also, identification of these factors on the promoter regions of both expression and function genes, and along with the factors confirmed in this section, reinforces their probability of being functional.

Keywords: Promoter analysis, endometrium, immune response, embryonic abortion, transcription factors

P-78: A Bioinformatics approach to identify overlapped miRNAs in some motor neuron diseases

Mousavi R1, 2, Bahmanpour Z2, Tahmasebivand M3, Khademí B4, Emamalizadeh B4
1Tabriz University of Medical Sciences, Tabriz, Iran
2Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
reza.mousavi9057@gmail.com

Recently, the importance of microRNAs in the pathogenesis of various diseases has been intensely studied. These molecules control the expression of genes after transcription by inhibiting gene expression or inducing its degradation. So, the identification of miRNAs in neurological disorders, including ALS and related diseases, can make new advances in new molecular diagnostic markers. On the other hand, Progresses in bioinformatics have helped better understanding the role of these regulatory factors. Here, ALS and diseases with similar phenotypic symptoms including Progressive muscular atrophy (PMA) and dementia are examined. Based on DisGenet database, common genes among ALS and these two similar diseases identified. Then miRNAs databases like Mirtarbase, MirDB, and mirtargetlinkhuman were used to recognize the miRNAs which regulate these genes. As a result, a number of miRNAs including hsa-miR-142, hsa-miR-204, hsa-miR-211 and hsa-miR-302e have been reported which can play a significant role in these three diseases. Therefore, in this study, some novel miRNAs have been introduced bioinformatically, which can be used by researchers for further experimental studies.

Keywords: bioinformatics approach, miRNAs, motor neuron diseases

P-79: Evaluation of hsa-mir-29c-3p impact on inhibition of triggering EMT process in gastric adenocarcinoma

Mozafari Nahavandi P, Naderi M, Irani sh

Department of biology, science and research branch, Islamic Azad University, Tehran, Iran

Cell-Based Therapies Research Center, Digestive Disease Research Institute, Tehran University of Medical Sciences, Tehran, Iran

prs_mozafari@yahoo.com

Gastric cancer is the fourth most common cancer worldwide and the second reason of cancer-related mortality. The disease â€” especially in its diffuse type â€” is poorly cohesive representing an invasive phenotype. Epithelial to Mesenchymal Transition (EMT) is known to profoundly contribute to invasion and subsequent metastasis of the disease. Therefore, EMT interruption may be an effective prospect in metastasis suppression. Several factors are involved in EMT process one of
which is miRNAs, noncoding RNAs which regulate as much as 30% of the human genes. According to The Cancer Genome Atlas (TCGA) report, mir-29c-3p is a well-established miRNA in Tumor suppression and is remarkably decreased in human gastric adenocarcinoma. Therefore, in this study, we have tried to provide a shortlist of genes that simultaneously 1) have positive role in EMT process 2) escape the inhibitory function of hsa-miR-29c-3p. Due to the existence of thousands of putative target genes for every single miRNA and the need to extract genes with a positive role in EMT process, different bioinformatics tools were used including miRBase, target scan, miRwalk, Quick GO, DAVID, and Aura through which we could achieve a shortlist of target genes for hsa-miR-29c-3p comprising AKT3, CDK6, COL15A1, COL1A1, COL1A2, COL3A1, COL4A1, COL4A2, GNB4, LAMC1, which most probably contribute to EMT and invasion occurrence in gastric tumor cells. The results of our study may be worthwhile to further investigation via a functional study to fully understand the underlying regulatory role of mir-29c-3p in gastric cancer metastasis.

Keywords: gastric adenocarcinoma, hsa-miR-29c-3p, EMT

P-80: Immunogenicity assessment of high-risk human papillomavirus L1 capsid proteins: A bioinformatics approach

Namvar A1, Bolhassani A2, Javadi Gh1, Noormohammadi Z1
1. Department of Biology, School of Basic Science, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran
alinamvar@outlook.com

Background and aim: Cervical cancer, the second most common malignancy in women worldwide, is regularly associated with high-risk human papillomavirus (hrHPV) infections. The HPV genome is packaged within a non-enveloped, icosahedral capsid composed of 72 pentamers of the major capsid late protein (L1) and an unknown number of the minor capsid proteins L2. Each HPV type has unique specificity for infection of skin or mucosa. At present, two prophylactic vaccines have been designed to prevent HPV infections. The licensed HPV vaccines focus only the two types most frequently found in cervical cancer, HPV16 and HPV18 that cause 70% of cases. The aim of this study is to find immunodominant and conserved epitopes of L1 protein among four common HPV types in Iranian populations using bioinformatics tools.

Material and methods: The full sequences of four hrHPV L1 proteins were obtained from PaVE database. The protein sequence sets were aligned using MUSCLE. The MHCI/II binding and processing (Proteasomal cleavage and TAP transport) scores of conserved peptide regions were analyzed by IEDB, NetMHC 4.0 and Rankpep online servers. Finally, MHCI/II-Peptide flexible Molecular docking was performed in selected regions using CABS-dock server.

Results: Immunogenicity, MHCI/II affinity levels, proteasomal cleavage, TAP transport and MHCI/II-Peptide flexible molecular docking of conserved domains were calculated. We found the conserved immunogenic epitope of hrHPV L1 proteins can be used in next generation prophylactic vaccines.

Discussion: Immunogenicity analysis of four hrHPV L1 proteins could provide information for understanding the immunogenic domains and their roles in interactions of viruses with the immune systems. Moreover, the conserved peptide epitopes can be used for development of vaccines targeting several HPV types in future.

Keywords: HPV, Cervical cancer, Bioinformatic analysis, Immunodominant epitopes

P-81: Integrating In-silico and In-vitro studies to Investigate Thymoquinone effect in Parkinson Disease

Nayeri Z, Sabouni F, Gardaneh M, Aفزali F
1. Molecular Medicine Department, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.
2. Department of Stem Cells and Regenerative Medicine, Faculty of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.
zahra.z.nayeri@gmail.com

Parkinson disease (PD) is a progressive neurodegenerative disorder caused upon demise of dopaminergic neurons in the midbrain. Thymoquinone (TQ) has been suggested to have an effect on PD. Protein interaction network provides an effective approach to investigate the molecular mechanism of PD. To identify differentially expressed genes (DEGs) in PD, GSE7621 dataset downloaded from GEO and DEGs were identified by affyLinGUI and using a moderated t-test. Protein-protein interaction (PPI) network of DEGs was constructed using STRING database. Next, Target Proteins (TPs) were identified following two constraints: (i) hub nodes were discarded as potential TPs; (ii) bridging nodes were elected as TPs. STITCH databases were also used to detect protein-drug interactions network for an up-regulated form of TPs. Molecular docking was used to study inhibitory effects of TQ on TPs by AutoDock tools. Finally, we determined the IC50 of 6-OHDA and the highest dose of TQ tolerated by dopaminergic cell line SH-SY5Y using separate MTT assays. We identified 133 hub nodes and 225 bridging nodes in PD network. Bridging nodes were selected as TPs. The Drug-Protein network showed 104 nodes and 1723 edges. Molecular docking showed that TQ could be an inhibitor in this network. In vitro experiments showed SH-SY5Y cells are more resistant to toxicity when they pretreated with TQ. In our study, molecular docking shows TQ can be a potent inhibitor in Drug-Protein network of PD so it can be proposed as a potential drug to be targeted. Finally, our in silico findings were confirmed by in vitro experiments.

Keywords: Parkinson disease, Thymoquinone, Protein-protein interaction (PPI) network, protein-drug interactions network.

P-82: Biological network exploration with Cytoscape from SIGLEC to MMP-9 pathway for finding microRNA involved in this pathway

Noorbakhsh N1, Zamani M1, Galehdari H1
1. Department of Genetics, faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
negar_noorvakhs@yahoo.com

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MMPs and Sialic acids play an important role in inflammatory neurodegenerative diseases. Sialic acid has interaction with receptor such as Siglecs which leads phosphorylation of ITIM and downstream inhibitory signaling through the recruitment of SHP-1/2 phosphatases.

SHP1 positively regulated TNF-α. SHP-1, by controlling the extent of the production of TNF-α, could play an important role in inflammation. TNF-β is involved in the signaling pathways leading to MMP-9 gene expression.

MMP-9 is one of the members of metalloprotease family with different biological function in tissue-damaging and inflammatory diseases. MMP-9 is a recognized therapeutic target in autoimmune diseases, vascular pathologies, and cancer. One of the most important regulations of genetic processes is through the mechanisms of micro-RNA. It is estimated that these molecules regulate the expression of one-third of all genes. The purpose of the present insilico study is to find the most effective micro-RNAs to control the signaling pathway from Sialic acids to MMP9. For this purpose, based on review articles and information from KEGG, 19 genes involved in siglec to MMP-9 pathway have been indicated. In the next step DIANA-TarBase, TARGET SCAN and miRDB database were used to predict the miRNAs which can target these genes. Finally, using bio-studying software such as Cytoscape, three microRNA-miRNA networks were created for existing banks. With statistical studies conducted on 491 micro-RNAs that potentially intervened in this pathway. We find eighteen micro-RNAs (hsa-miR-320c, hsa-miR-15a-5p, hsa-miR-195-5p, hsa-miR-424-5p, hsa-miR-218-5p, hsa-miR-16-5p, hsa-miR-497-5p, hsa-miR-15b-5p, hsa-miR-28-5p, hsa-miR-22-5p, hsa-miR-18a-5p, hsa-miR-548d-3p, hsa-miR-23b-3p, hsa-miR-29a-3p, hsa-miR-766-3p, hsa-miR-29c-3p, hsa-miR-29b-3p, hsa-miR-28-5p) were shared in the three networks. Eventually, microRNAs that were linked to more genes or more sites on a gene in this path have been assigned a higher privilege; the selected micro-RNAs can be the proper options for subsequent experimental studies on the path from Sialic Acid Receptors to MMP-9.

Keywords: inflammatory neurodegenerative diseases, micro-RNA-miRNA network, insilico

P-83: Identification of molecular pathways involved in Intellectual disability in family with mutation in CDK9 gene using RNA-seq

Peymani F1, InanlooRahatloo K1, Kahrizi K1, Najmabadi H1

Genetic Research Center, University of Social Welfare and Rehabilitation Sciences, Iran
fatemehpeymani@yahoo.com

Around 1-3% of world population have been affected by Intellectual disability (ID), a neurodevelopmental disorder with high level of genetic and clinical heterogeneity. Although intellectual disability is a clinically important disorder, the etiology and pathogenesis are poorly understood. Transcriptome profiling is a very important tool for understanding how genetic variants alter cell expression. Detecting differentially expressed genes and isoforms, specifying the functional consequence of known and novel variants and linking between genetic changes and phenotype are some of the countless applications of RNA-seq. In the current study, we applied whole blood transcriptome analysis using RNA-seq to identify biological and molecular pathways underlying ID disease in consanguineous family with mutation in CDK9 transcription factor. Overall, 972 differentially expressed genes (q value <0.01 and fold change > 1.5) were identified between CDK9 mutant patients and controls, of these 972 genes, 459 genes downregulated and 513 genes upregulated in patients. Upstream regulator analysis revealed GABPA, YY1, and EHF as upstream regulators for down-regulated genes and SP1, KLF7, and EGR1 as upstream regulators for upregulated genes in patients. Based on detailed gene ontology analysis, the most significant pathways dysregulated in patients were gene expression, structural constituent of ribosome and RNA binding, histone modification, tyrosine protein kinase, small GTPase signal transduction, and actin cytoskeleton. The findings reported here provides new insights into the molecular pathways underlying intellectual disability and highlight the importance of these transcription factor in neuronal functions.

Keywords: Intellectual disability (ID), blood transcriptome profiling, gene expression, RNA-seq, pathway analysis

P-84: Negative regulation of anti-apoptotic Mcl-1 by miRNA-363 in breast cancer

Pourmoshir N, Vailian S

Division of Genetics, Department of Biology, Faculty of Science, University of Isfahan, Isfahan, IR Iran
pourmoshir.nadia@gmail.com

Introduction: Myeloid cell leukemia-1 (Mcl-1) is an anti-apoptotic Bcl-2 family member that is often overexpressed in breast tumors, and has been reported to have an important role in regulating drug resistance in various types of cancers including breast cancer. However, the mechanisms underlying the aberrant expression of Mcl-1 are still unclear. The aim of this study was to identify the role of miR-363 which leads to the activation of oncogenic pathways by regulation of Mcl-1 in human breast cancer (BC).

Methods: The predicted targets of miR-363 were obtained from "target scan human database" and validated by "miRTarBase database". The expression patterns of targetable genes in mammary gland and breast (mammary gland) tumors were investigated by "UniGene". Then, breast cancer specific pathways were classified into molecular pathways by "DAVID database". Finally, molecular interaction networks of miR-363 were visualized by "Cytoscape software".

Result: The suggested that miR-363 could directly targete Mcl-1 3'-UTR (3'-untranslated regions) and cause downregulation of Mcl-1 in breast cancer, suggesting that miR-363 could be considered as a negative regulator of Mcl-1 expression.

Keywords: Breast cancer, mir-363, Mcl-1, UniGene

P-85: In silico studies of rs1799971 (A118G) OPRM1 gene structural polymorphism binding to buprenorphine as an opioid addiction substrate
According to the previous studies, A118G has showed a critical role in the association studies of mu-opioid receptor 1 (OPRM1, MOR). We aimed to investigate homology modeling and docking analyses of rs1799971 (A118) in binding to buprenorphine as an opioid addiction substrate for the first time. Methods: The tertiary structures of human MOR protein in wild-type (Asn40) and mutant (Asp40) alleles of rs1799971 (A118G) were modeled by the chosen template (PDB ID: 4DJH) through Swiss Model, PS2, and Phyre2 online softwares. Then, best models (from Phyre2) were built after energy minimization by Swiss-PdbViewer ver. 4.1.0 software. The structures of designed models were then validated using RAM-PAGE and ProSA softwares. Final models were visualized by Autodock ver. 1.5.6. To prepare the ligand for docking, energy minimization of ligands was performed using Hyperchem professional tool ver. 8.0.8. Active site of OPRM1 was predicted by COACH. Best conformation among 10 conformations was opted based on the lowest binding energy and H-Bonds in cluster. Finally, dominant and recessive complexes with same ligand were compared to each other. Results: In silico analyses of OPRM1 protein with buprenorphine as ligand showed that the best conformation of buprenorphine had more binding affinity to Asp40 (binding Energy=−8.46 kcal/mol with 2 Hydrogen bonds formation) compared to Asn40 model (binding Energy=−5.26 kcal/mol with lack of H-bond formation). Conclusion: Consequently, genotyping of A118G as a remarkable marker of opioid addiction may be helpful in buprenorphine administration and treatment among populations which have shown significant susceptibility to opioid addiction.

Keywords: A118G, homology modeling, docking, buprenor- phine, addiction

P-86: In silico analysis of correlation between HOTAIR and miR-34a expression profile in CRC

Rafieian M, Kazeminezhad SR, Hajjari MR, Tahmasbi Birgani M
Chamran University, Ahvaz, Iran

Expression profile of these two key genes in prostate, breast, gastric, pancreatic cancers. Therefor we investigated alteration expression of them in CRC samples. We did this study by bioinformatic tools and in silico analysis. Data for this study were collected using several datasets in GEO database. In next step data were analysed by two softwares. The aim of this study is to investigate the correlation between HOTAIR and miR34a expression level. To confirm the results, we also used experimental tumor tissue analysis in molecular laboratory. With due to attention the result of statistical analysis on changing the expression profile and correlation of these genes, they can be considere as potential as biomarker in CRC.

Keywords: HOTAIR- miR-34a- colorectal cancer- bioinformatic tool

P-87: Investigating the effect of microRNA-6895 in resistance of breast cancer cells to doxorubicin chemotherapy: Bioinformatics analysis

Rahimi Rad s1, Rahimi rad Sh2, Navadere M1, Jafari Harandi A3, khorami ruz Sh4
1 Medical Genetic Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran.
2 Genetic Department, Faculty of Science, University of Shahre-kord, Shahrekord, Iran.
3 Nourdanesh Institute of Higher Education, Iran.
4 Microbiologist in American Hospital Dubi_oud metha-po BOX566

Resistance of breast cancer cells to chemotherapy is a major barrier of successful treatment. Evidence have indicated the impact roles of gene function mediated by short noncoding RNA in chemotherapy resistance. The MDR1 gene also known as P-Glycoprotein is a member of the superfamily of ATP-binding cassette (ABC) transporters. This gene is responsible for decreased drug accumulation in multidrug-resistant cells by affecting the susceptibility to Doxorubicin (DOX). The present study, we investigated the MDR1 gene expression modulators which affects the resistance of the cancer cells to chemotherapeutic drug doxorubicin.

Method: Using CTD database (http://ctdbase.org/) the interaction of MDR1 gene with DOX were confirmed. To identify the miRNA based regulators of MDR1 gene we constructed the miRNA-mRNA interaction network using Cytoscape version 3.4. Next, DIANA tools microRNA target databases (http://diana.imis.athena-innovation.gr/DianaTools/index.php) and single nucleotide polymorphism (SNP) searching tools (https://www.ncbi.nlm.nih.gov/projects/SNP/) were used for studying gene expression regulation by the effect of SNP in miRNA seed region associated with MDR1 gene.

Result: Our microRNA target analysis of MDR1 gene showed that has-miR-6895-5p regulates this gene expression (Fig.1). In a complementary analysis, we identified deletion of bases -/ ACAGAGAG (rs56794014) located within miR-6895-5p target site in 3′UTR of MDR1 gene(Fig.2). This variant may result in a blockade of miRNA â“ Based downregulation of MDR1 gene which is results in the increased chemoresistance property of cancer cells to DOX.

Discussion: Our results indicating that MDR1 gene deletion
in miR-6895-5p target site may have significant implications for identifying the therapeutic strategies aiming to overcome breast cancer cell resistance.

**Keywords:** Breast Cancer Cell, Drug Resistance, Bioinformatics, microRNA.

**P-88: The prediction of miR-429 and miR-557 role in azoospermia**

**Rajabi Dehnavi F, motevalli-Bashi M, javadirad S M**

Genetic Division, Department of Biology, Faculty of Science, University of Isfahan, Isfahan, I.R. Iran.

**saiedabadak@yahoo.com**

**Objective:** Infertility in couples is a multifactorial complex problem, which has affected about 10-15% of the couples and half of cases due to male factors. Azoospermia or lack of sperm in each ejaculation is one of the infertility etiology in men. Azoospermia has many reasons. One of its causes is a disorder of spermatogenesis. Spermatogenesis is a phenomenon in which male sex cells are produced. It must be precisely regulated both in transcriptional and post-transcriptional stages. One of the major regulators of spermatogenesis are microRNAs. miRNAs are one class of noncoding RNAs that have about 21-25 nucleotides long and their function is gene silencing and regulation post-transcriptional and generally adjust the genes.

**Method:** This is a theoretical study of bioinformatics. Significant genes that are essential for spermatogenesis were identified by using KEGG pathway database and corresponding literature mining and then by using databases such as miRwalk2.0, TargetScan, picTar and miRanda, interaction between the selected genes with miRNAs that their expression in azoospermia have been proven, were investigated.

**Results:** According to the finding, miR-429 and miR-557 could inhibit spermatogenesis by inhibiting NANOS1 and DAZL genes respectively and probably causing disorders including azoospermia.

**Conclusion:** Role of the miR-429 and miR-557 in inhibiting NANOS1 and DAZL genes and their prevention of spermatogenesis, maybe used as a therapeutic and pharmaceutical potential as well as azoospermic identification biomarkers.

**Keywords:** Azoospermia, Spermatogenesis, miR-429, miR-557, NANOS1, DAZL.

**P-89: In silico identification of miRNAs and their target genes in Sugarcane (Saccharum officinarum)**

**Rajabi M1, Taheri Azam A2, Amiri S3**

1. Seed and Plant Improvement Research Department, Hamedan Agricultural and Natural Resources Research and Education Center, AREEO, Hamedan, Iran.
2. Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Tabriz, Iran.
3. Department of Tissue Culture, Branch for Northwest and West Region, Agricultural Biotechnology Research Institute of Iran, Agricultural Research Education and Extension Organization (AREEO), Tabriz, Iran.

mhsnrababi11278@gmail.com

Sugarcane is one of Germaine family. Sugarcane is the most important sugar crops which had long history of cultivation. MicroRNAs regulate gene silencing and have an important role in transcriptional and post-transcriptional levels. miRNAs play controlling role in Physiological and biological processes and response to environmental stresses in plants. Since the discovery of the miRNA identified in other cereals has been reported compared to the number of miRNA in sugarcane, but these numbers are much less than rice and corn that will study in future. High conservation of these molecules in plants makes identification of new molecules in other plant species and provides through sequence alignment. In this study, four miRNA in sugarcane including miRNA156e, miR166f, miR2936 and miR395a were identified by analyzing of miRNA and using EST information in miRBase databases and the target genes and molecular activity of them evaluated, respectively. Results showed that miRNA156e identified the most target genes. Also miR395a determined group of enzymes involved in sulphate handling.

**Keywords:** In Silico, MicroRNA, Sugarcane, EST

**P-90: In Silico Identification of Housekeeping Genes by Expressed Sequence Tags**

**Ramezani M1, Oladnabi M1,2, Karimian A3**

1. 1. Department of Medical Genetics, School of Advanced Technologies in Medicine, Golestan University of Medical Sciences, Gorgan, Iran.
2. 2. Congenital Malformations Research Center, Golestan University of Medical Sciences, Gorgan, Iran.
3. 3. Deputy Of Research and Technology, Golestan University of Medical Sciences, Gorgan, Iran.

oladnabidizin@yahoo.com

**Introduction:** In gene expression studies, for validation and obtain reliable results, normalization of qRT-PCR data by Housekeeping Genes (HKGs) is required. Failure to select an appropriate HKGs may lead to biased gene expression data. HKGs are critical for maintenance of basic cellular functions and existence of a cell. They are constantly expressed in different types of cells, tissues and in different experimental conditions. However, several reports have indicated that they are not expressed in all cells/tissues and experimental treatment or have variable expressions. Expressed Sequence Tags (ESTs) are a high-throughput method providing a new dimension to transcriptome analysis and discovering genes. ESTs represent the amount of mRNA expressed in the cell or tissue.

**Methods:** In this study, the EST profile of 17242 protein-coding genes was randomly selected from the UniGene database. Based on the definition of HKGs mentioned above, only 100 genes were expressed in 7 developmental stages, 45 normal and 25 normal tumor tissues. In order to normalize EST counts across normal and tumor tissues, we used Log2 (TPM + 2) scale. Then fold changes in 15 normal and tumor tissues were analyzed.

**Results:** Our results showed that 57 genes not only have no expression changes between the normal and tumor tissues but also show overlapping expression in some tissues.

**Discussion:** Our findings demonstrate that some of the common HKGs are not suitable for gene expression normalization and may suggest novel genes as potential HKGs but its proof
requires further investigations and another high-throughput data.

**Keywords:** Housekeeping gene, EST, In Silico.

**P-91: Bioinformatics analysis of missense single nucleotide polymorphisms in human GATA4 Gene**

Rezaei Adriani M’, Khatami M, Heidari M M

Department of Biology, Faculty of science, Yazd University, Yazd, Iran

serin.adrian@gmail.com

**Introduction:** GATA4 gene encodes a member of the GATA family of zinc-finger transcription factors. This protein is thought to regulate genes involved in embryogenesis, myocardial differentiation and function, and normal testicular development. Mutations in this gene have been associated with cardiac septal defects. Protein structure and dynamics are an important part of understanding molecular bases of complex biological processes and play an important role in computational biology. There are many mutations in coding regions. All of these mutations were associated with Atrial Septal Defect (ASD). The functional significance of observed GATA4 mutations was analyzed using PROVEAN, PMut, and PYMOL software.

**Method:** We identified three missense single nucleotide polymorphisms in the GATA4 gene (rs387906771, rs387906772, and rs56298569) using Ensembl database and then analyzed their effect on the protein structure using PyMOL software and PROVEAN and PMut databases.

**Results:** Validation results showed that missense single nucleotide polymorphisms change the polar groups, interaction patterns, and length of hydrogen bonds. rs387906771, rs387906772, and rs56298569 lead to Thr280Met, Met310Val, and Gln316Glu. According to PyMOL, all these SNPs caused to change hydrogen bonds length in protein structure. The results of the variants in the PROVEAN predict scores below -2.5. PMut showed all of these SNPs with a high percentage of 85% pathogenic.

**Conclusions:** According to ROVEAN database, all scores less than -2.5 were considered deleterious. PMut expresses the degree of SNP’s pathogenicity by percentage. Although bioinformatics studies have contributed greatly to the progress of studies, it needs experimental researches to confirm the results.

**Keywords:** GATA4; missense single nucleotide polymorphisms; PyMOL software; PMut database; PROVEAN database

**P-92: long non-coding RNA NONHSAG071305.2 SNPs are associated with Autism Spectrum Disorder**

Rezaei Z, Hakimi naini S

saghihakimi@yahoo.com

Autism spectrum disorder (ASD) includes a range of complex neurodevelopmental diseases with childhood onset before 3 years old. It refers to a range of conditions characterized by challenges with social skills, repetitive behaviors, speech and nonverbal communication, as well as by unique strength and differences. In the past ten years, ASD have been the most prevailing pediatric disorders and its prevalence is increasing throughout the world, with an estimated 1 in 68 eight-year-old American children affected by ASD. Long non-coding RNAs (lncRNAs) are large class of transcribed RNA molecules with a length of more than 200 nucleotides that do not encode proteins. Bioinformatics and statistical data have shown correlation between some SNPs (Single Nucleotide Polymorphisms) and increase the risk of ASD. In the present study, we have checked the most important associated genes with ASD and among them we candidate NTRK3 (Neurotrophic Receptor Tyrosine Kinase 3) gene (p-value<0.001). This kinase is a membrane bound receptor that, upon neurotrophic binding, phosphorylates itself and participate in the MAPK pathway. In the NCBI database, it was shown that this gene has the highest expression in the brain. NTRK3 has four known lncRNAs based on NONCODE database, between all of them NONHSAG071305.2 has shown association with ASD. We have found the most associated SNPs, include rs8031996, rs3870431, rs10459693 and rs10459694 based on the bioinformatics analysis. The results suggest that the SNPs in NONHSAG071305.2 gene play an important role in ASD pathogenesis and can be useful for personalized medicine applications.

**Keywords:** ASD, Long non-coding RNA, NTRK3, SNP

**P-93: Bioinformatics analysis of long non-coding RNA NONHSAG099313.2 with coronary artery disease**

Rezaei Z, Abedi Z, Hakimi naini S

rezaizeynab7@gmail.com

Coronary artery disease (CAD) is the most common type of heart disease. CAD happens when the arteries that supply blood to heart muscle become hardened and narrowed. This disease is the most important cause of death in the world. The prevalence of mortality from cardiovascular diseases in Iran is about 33-38%, which is significant. Studies of twin have shown that genetics play a very strong role in the development of CAD. One of genetics factors that showed association with CAD are IncRNAs. IncRNAs or long non-coding RNAs are type of non-coding RNAs that exceed 200 nucleotides in length. IncRNAs have been implicated in several cellular functions. Some evidences of statistical and bioinformatics analysis have shown a noticeable association between IncRNAs and increase the risk of CAD.

Since the importance of CAD, a lot of researches have been done on probable associated genes. MSR1 gene encodes the class A macrophage receptor has implicated in many macrophage-associated physiological and pathological disorder including CAD (p-value: 0.002). MSR1 has almost 18 IncRNAs (bases on NONCODE database) between all of them NONHSAG099313.2 gene have shown most association with type 2 diabetes. Tag SNPs of it include rs735861, rs352774, rs2604308, rs1178521 (Lnc2Meth database). In conclusion our studies indicate that there is association between IncRNAs and CAD. Specially tag SNPs of NONHSAG099313.2 may play role in pathogenesis of CAD and these are important for clinical aspects particularly in personalized medicine.

**Keywords:** CAD, MSR1, IncRNAs, SNP
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P-94: Polymorphisms in miRNA binding sites may contribute to the pathobiology of colorectal cancer: evidence based on in-silico analysis

Safi A, Ilkhani K, Bahmanpour Z, Mousavi R, Bastami M
1. Student Research Committee, Tabriz University of Medical Science, Tabriz, Iran
2. Department of Medical Genetics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
safi.asma@gmail.com

Single nucleotide polymorphisms (SNP) located in miRNA coding genes or their target sites have emerged as a new class of variants conferring susceptibility to complex diseases such as colorectal cancer (CRC). Here, we employed an in-silico pipeline to identify SNPs in target site of miRNA involved in CRC. CRC-related genes were obtained from the Gene Expression Omnibus (GEO) database and SNPs resided in target site of miRNA were obtained from PolymiRTs database. The resulting miRNA: mRNA: SNPs were further evaluated for the functional evidence supporting their involvement in CRC. In-silico analysis revealed that miR-503: CHGA: rs7610 may be a functional interaction pertaining to the CRC pathogenesis. Results showed that rs7610 may potentially disrupt a functional interaction between miR-503 and CHGA, leading to dysregulation of CHGA and may potentially contribute to the pathogenesis of CRC.

P-95: Bioinformatic analysis of Cas9 gene

Saidi A, Sarvmeili J
Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C, Tehran, Iran
javadsarvmeili@yahoo.com

Crisper/cas9 technology is a bacterial immune system that enables bacteria to kill viruses. Cas9 gene derived from Streptococcus pyogenes is used in gene therapy to treat genetic diseases. Using bioinformatic softwares, Cas9 gene was evaluated in different strains of the bacterium, namely Streptococcus pyogenes. Only 30 genes in BLASTn analysis with homologous genes were selected. In the first step, these genes were matched with the Bioedite software. Then, Mega software was used to perform phylogenetic analyses. FgenesB program found that the Cas9 gene is located on positive strand with a predicted protein of 1384 amino acids. The isoelectric point (pI) of the target protein was 9.09. The study of indices of instability showed that this protein has a high half-life because it requires a bacterial defense mechanism. The molecular weight of the Cas9 protein is 160 kDa, and gene ontology of Cas9 gene is located in the cell. Cas9 plays a role in bacterial defense mechanisms and protects them against viral attacks.

Keywords: Cas9 gene Crisper, cas9 technology, Bioinformatics analysis

P-96: CBAF: An automated, easy-to-use R package for comparing genetics data across multiple cancers/ a cancer’s subgroups

Shahrissa A

Bioconductor is a repository of open-source packages written in R programming language to fulfill the needs of biologists. CBAF is a Bioconductor package that enables the researchers to compare at least one group of genes across several cancers /cancerâ€™s subgroups that are available at cbioportal.org. So far, it is compatible with RNA sequencing (RNA-Seq), microRNA sequencing (microRNA-Seq), microarray, and methylation data.

There is a function that scans all the cancers in cbioportal.org to identify which of the mentioned high-throughput technique exists for every cancer. Two other functions automatically obtain, process and store the results. They require at least one group of gene names, any desired name to title the created database, name of cancer(s), and the desired technique. Firstly, they obtain the required data from cbioportal.org. Then, they compute the required statistics such as frequency percentage and mean value of samples having a value greater than a specific number. Moreover, they determine the genes that occupy the first to fifth rank in every section.

The output is prepared as one heatmap for every statistical measurement and a multi-section excel file that contains the result of validating genes on the cbioportal, numerical values of requested statistics and the genes that occupy the first to fifth rank in every category. If more than one groups of genes are requested, the output for every gene group is stored in a separate folder. To sum up, this easy-to-use package is recommended to all biologists.

Keywords: R, Bioconductor, RNA-Seq, microRNA-Seq, Microarray, Methylation

P-97: A novel in silico synthesized analog of AM-251 as a candidate antagonist of mu-opioid receptor

Sharafshah A, Keshavarz P
Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran.
alierezascharafschah@gmail.com

Previous studies demonstrated that the AM-251 directly bind to Mu-Opioid Receptors (MORs). Here, we developed an analog of AM-251 through in silico procedures to find a more effective compound with better affinity for future studies of MOR inhibitors in analgesia.

Methods: The tertiary structures of human MOR protein were modeled by Swiss Model, PS2, and Phyre2 online softwares (template PDB ID: 4DJH). Then, best models (from Phyre2) were built after energy minimization by Swiss-PdbViewer DeepView ver. 4.1.0 software. The structures of designed models were then validated using RAMPAGE (95.7% in favored and allowed regions) and ProSA softwares (z-score=-2.33). 45 new analogs of AM-251 were created by adding OH and CH3 substituents to various atoms of AM-251. To prepare the ligand for docking, energy minimization of ligands was performed us-
ing Hyperchem ver. 8.0.8. Active site of OPRM1 was predicted by COACH. First, all 45 molecules were docked through Autodock vina in PyRx tool, and then best molecule with lowest energy binding docked again by Autodock vina ver.4.2.

Results: We found that C27 had potential role to be changed by substituents like CH3 and OH. The best conformation was CH3 substituent added to the C27 of AM-251 with binding energy of -9.05 kcal/mol and 2 hydrogen bonds with ASN371 compared to AM-251 Energy binding of -7.9 kcal/mol and no H-bond formation.

Conclusion: Suggested in silico synthesized (1(2,4dichloro-3-methyl[phenyl]-5-(4-iodophenyl)-4-methyl-N-(piperidin-1-yl)-1H-pyrazole-3-carboxamide) can be an analog candidate of AM-251 as a specific inhibitor of MOR but this needs both chemical and clinical validations.

Keywords: MOR, AM-251, Homology Modeling, Docking

P-98: Tree-based machine learning algorithms for identifying minimal set of miRNA biomarkers for cancer diagnosis and molecular subtyping

Sherafatian M
Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
mashishrftn@gmail.com

Cancer is a complex disease and its effective treatment needs affordable diagnosis and subtyping signatures. While the use of machine learning approach in clinical computation biology is still in its infancy, the prevalent approach in identifying molecular biomarkers remains to be screening of all biomarkers by differential expression analysis.

Availability of diverse and vast amount of cancer datasets like The Cancer Genome Atlas facilitated the molecular profiling of patients’ tumors and introduced new challenges like clinical grade interpretations from big data. In this study, miRNA expression datasets of cancer patients from TCGA database was used to develop prediction models from which miRNA biomarkers were identified for diagnosis and molecular subtyping of this cancer. I took the advantage of interpretability of tree-based classification models to extract their rules and identify minimal set of biomarkers.

P-99: Genome-Wide Identification and Functional Analysis of Genes Expressed in salt tolerance of Barley

Sohrabi E, Tohidfar M, Javadi M, Ahmadikhah A
Department of Plant Science and Biotechnology, Faculty Of Life Science And Biotechnology, Shahid Beheshti University, Tehran, Iran
gtohidfar@yahoo.com

In recent years, an explosion has been made in advancing high tech techniques to achieve and demonstrate different aspects of the gene. Use of these new technologies has made it possible to identify new connections between genes with higher resolution. The microarray is a tool for measuring and gaining information from the expression of genes. Barley is a plant that is resistant to non-biological stresses. So it can be used as a robust model plant for studies. In this study, salinity-tolerant genes were collected through two methods including microarray and resource review. Then, for each category, the gene network was reconstructed by the string software. By drawing on protein-protein interactions with Cytoscape software and using computational algorithms defined candidate genes. For each of the identified genes, promoter analysis was performed with plant care. It was found that most genes were candidates for the heat shock proteins such as HSP70, HSP70-15, and functional proteins such as SBPASE, P5CS1. The promoter analysis of the dominant commonality between these genes was mostly responsive to methyl jasmonate and temperature and photosynthesis. Which indicates that jasmonic acid plays a special role in non-biological stresses, especially salinity.

Keywords: Gene network, Barley, salt tolerance, promoter analysis

P-100: The bioinformatic evaluation of the interaction between APC gene in rs 3733961 and microRNA 155 in gastric cancer

Soufastaei F1, Houshmand M2, Ghaedi K3, Azadeh M4, Ashrafinia B
1. Division of cellular and molecular biology, NourDanesh Institute of Higher Education, Meymeh, Iran
2. Medical genetic dep. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
3. Department of Biology Faculty of sciences university of Isfahan, Iran
4. Biotechnology Institute of Novin, Isfahan, Iran
farinaz.soufastaei72@gmail.com

Introduction: Gastric cancer is one of the most common cancer around the world [1]. Adenomatous polyposis coli or APC, has been known as a tumor suppressor gene. Inactivation of APC causes the development of some gastric cancers, and mutations of the APC gene, occur during the early stages of gastric adenoma development. The APC gene inhibits the members of Wnt signalling pathway that promotes β-catenin expression as a stimulator of cell division within the intestinal. MicroRNAs contribute to gastric carcinogenesis by altering the expression of oncogenes and tumor suppressors, affecting cell proliferation, apoptosis, motility, and invasion.

material and method: miRNASENP2 database was used to identify the miRNAs with the ability to bind to the 3’UTR of APC transcripts. Then, miRTarbase and David were used to investigate the function and related pathways of obtained miRNA and relationship with APC. We, by kegg database, observed pathways in which miR-155 and APC are involved in cancers. Altogether, these stages helped us to find relationship between pathways of APC and miR-155.

Results: In silico investigation of SNPs in the 3’UTR of APC gene showed that rs3733961 could alter the binding properties of miR-155. due to rs3733961, the binding activity of miR-155 (as an tumor suppressor) undergoes loss; consequently, this SNP could act as a poor-prognostic factor. On the other hand, the binding affinity of miR-155 alleviates as a result of rs-3733961; therefore, rs-3733961 could also act as good-prognostic factor.

Conclusion: Bioinformatically, rs3733961 could have associ-
P-101: Transcriptomics-based Identification of Echinacea purpurea Transcription Factors Involved in Biosynthesis of Secondary Metabolites

Tafarroj Norouz H, Razi H, Ebrahimie E

Institute of Biotechnology, School of Agriculture, Shiraz University, Shiraz, Iran
Department of Crop Production & Plant Breeding, School of Agriculture, Shiraz University, Shiraz, Iran
tafarrojh@gmail.com

Echinacea purpurea is a medicinal plant that produces a large variety of secondary metabolites including alkaloids, terpenoids, flavonoids, and phenylpropanoids. These compounds have pharmacological activities to strengthen the immune system by stimulating the production of T cells against tumor cells. Methyl jasmonate (MeJA), one of the derivatives of jasmonic acid, is a plant hormone which induces biosynthesis of many secondary metabolites in Echinacea purpurea. Transcription factors are known as key regulatory proteins involved in various plant processes including biosynthesis of secondary metabolites. Here, we give an overview of various families of Echinacea purpurea transcription factors that were identified by analyzing RNA-seq transcriptomics data and metabolic pathways in MeJA-induced secondary metabolite biosynthesis. The results showed that MeJA activated expression of a number of transcription factors and downstream enzyme-coding genes involved in secondary metabolite biosynthesis. 101 transcription factors were known to be responsive to MeJA treatment using BLASTX against NCBI non-redundant protein database. The functional interaction networks of TFs were also generated by STRING and GENEMANIA databases. Among the transcription factors, the members of the following families were overrepresented: AP2/ERF family members which modulate biosynthesis of terpenoid indol alkaloids; R2R3-MYB family members which are involved in flavonoids, isoprenoids and phenylpropanoids pathways; bHLH-MYC family members which regulate terpenoid indole alkaloids, and anthocyanins pathways and finally GRAS family members which contribute to diterpenoid biosynthesis.

Keywords: RNA-seq, Transcription factor, Secondary metabolite, Methyl jasmonate.

P-102: Promoter Analysis of the gene related to beta-amyrin synthase enzyme in Eutrema salsugineum

Taheri- Azam A, Amiri S', Rajabi M

plant biotechnology, Tabriz, Iran
Amiri.A.taheri2013@yahoo.com

A group of important enzymes that catalyze biosynthesis of triterpene saponins are oxidosqualene cyclases. Beta amyrin synthase is one of including these enzymes. In this study, genome sequence of the beta-amyrin synthase enzyme using data bank Phytozone related to Eutrema salsugineum were obtained. The promoter analysis in 1500 bp of upstream showed that there were different regulatory elements with various roles in the promoter region of this gene. They have role in different physiological pathways in plants. The most important element in this plant was TATA-box element in 55 numbers that is activoregulatory promoter. Then CAAT-box motif was in 27 numbers that were as gene regulatory elements for gene enhancement and multiplication and after that Skn-1 motif was in 4 numbers that is cis-active regulatory elements requiring for endosperm expression. According to the results, the promoter region analysis of this gene showed that the most identified motifs in this plant act as responding elements to light reactions. Therefore, it is clear majority of transcription factors that bind to this motifs, are involved in light and photosynthesis reactions. Isolation of the promoter of this gene and its use in production of resistant transgenic plants to biotic and abiotic stresses can be used.

Keywords: Promoter Analysis, Bioinformatics, β- amyrin synthetase, Eutrema salsugineum

P-103: Long Non-coding RNAs PICSAR as a Novel Biomarker for Triple-Negative Breast cancer

Tahmasebi Birgani M¹, Sharisa A², Savari B³, Moradzade-gan H⁴

1. Department of Medical Genetics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Department of Molecular Genetics, Faculty of Biosciences, Tarbiat Modares University, Tehran, Iran
3. Department of Biology, Faculty of Basic Science, Gonbadkavoous University, Gonbadkavous, Golestan, Iran
4. Pasteur medical laboratory, Ahvaz, Iran
batoolsavari67@gmail.com

Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer. This type of breast cancer comprise 15%-20% of all breast cancers. Due to absence of receptors (ER, PR and HER2), treatments like hormone therapy are ineffective. So the only treatment for patients with TNBC is chemotherapy. To overcome limitations of TNBC, new approaches are needed. Recent studies demonstrated that long noncoding RNAs (IncRNAs) have a critical role in the regulation of cancer biology. However, little is known about their role in TNBC pathogenesis. To address this issue 287 approved IncRNAs were extracted from HGNC. All genes were imported into the cbioPortal database for genomic and transcriptomic analyses. We queried all the samples of TNBC (TCGA, provisional) with RNA-seq v2 data in our study (n = 82). TCGA RNA-Seq raw data was extracted in R using the cbaf extension package with Z-score = 3. The data was then presented as Heatmap plot. Notably, We found 40 out of the 287 IncRNAs with altered expression patterns at least in 1% of patients. We also found IncRNAs including SNHG6, PVT1, PICSAR, SNHG1 and TUG1 were the IncRNAs which were altered in more than 10% of the patients. However, the highest transcript level was related to PICSAR among the 82 samples of TNBC. This is the first in silico study showing the probable role of the novel IncRNA PICSAR in TNBC malignancy although further experimental role is needed to introduce it as
Recent advances in bioinformatics have helped better understanding the role of these regulatory factors. Here, ALS and diseases with similar phenotypic symptoms including Progressive muscular atrophy (PMA) and dementia are examined. Based on DisGenet database, common genes among ALS and these two similar diseases identified. Then miRNAs databases like Mirtarbase, MirDB, and mirtarlinkhuman were used to recognize the miRNAs which regulate these genes. As a result, a number of miRNAs including hsa-miR-142, hsa-miR-204, hsa-miR-211 and hsa-miR-302e have been reported which can play a significant role in these three diseases. Therefore, in this study, some novel miRNAs have been introduced bioinformatically, which can be used by researchers for further experimental studies.

**Keywords:** bioinformatics approach, miRNAs, motor neuron diseases

**P-105: A Comprehensive Analysis on Coexpressed Genes related to Flowering in Rice (Oryza Sativa L.)**

Tavakol E. and Khahani B,
Department of Agronomy and Plant Breeding, Shiraz University  
E-mail:elahetavackol@gmail.com

Rice is the world’s most important staple food and it is one of the utmost species in plant genetic studies as a model crop. Floral transition is a critical stage of the physiological processes that thoroughly changes vegetative stage to reproductive stage. Moreover, flowering is predominantly associated with grain yield in rice and substantially affects the final grain production. Some genes such as OsGI, Hd1, Ehd1, Hd3a and RFT1 are known to regulate the main part of flowering pathways in rice. Co-expression analysis unravels group of genes simultaneously expressed under specific condition that therefore they tend to be involve in similar biological processes. Here we identified co-expressed genes including transcription factors (TFs) related to transition stage in rice using ExPath 2.0 database. Thereafter, Singular Enrichment Analysis (SEA) and pathways survey on co-expressed genes were carried out using AgriGo tools (http://bioinfo.cau.edu.cn/agriGO/) and ExPath 2.0 database, respectively. Moreover, co-expressed TF families were further studied in more details. Coexpressed genes were mainly involved in metabolic process, catalytic activity and binding. Moreover, ubiquitin and endocytosis were illustrated to have immense roles in this developmental stage and plant growth. The major TF families identified in our analysis were AP2, bZIP, GATA, Homeodomain, MADS box and WRKY that could be considered as the most important transcriptional regulators controlling flowering in cereals. These results help to uncover genetic mechanism of rice flowering.

**Keywords:** Flowering, Ontology, Pathway, Transcription factors

**P-106: Effect of the Sequence Identity and Similarity in Homology Modeling**

Yazdan Najaf Abadia M, Abbasi Baharanich A, Khezric A, Karkhane AA
Department of Industrial and Environmental Biotechnology (IIEB), National Institute of Genetic Engineering and Biotechnology, Tehran, 14965/161, Iran  
mohsenyazdan1321@yahoo.com

By studying the 3D structure of proteins, valuable information of their performances can be obtained[1]. In most cases the proteins structure is not specified in contrast with the recent advances in the crystallography and NMR methods[2]. Homology modeling is an alternative way for studying the structure of proteins that are not determined by laboratory methods[3]. Modeller and SWISS-MODEL are two advanced bioinformatic ways that are widely used for homology modeling [1,4]. In this study, the crystallographic structure of hyperthermophile, thermophile, mesophile, sachrophile, halophile, acidophile and alkalophile that belong to cellulase families was first taken from PDB database. Template structures were taken from PDB database in the sequence identity ranges from 10 to 100 and protein similarity from 25 to 100 percentage. Subsequently, using the SWISS-MODEL and Modeller tools, assuming that there is no crystallographic structure, the structure of this enzymes was predicted. Finally, using the SuperPose tool, the RMSD for alpha carbon, backbone and sidechain between crystallographic structures and models were calculated. The results indicated that with the increasing in the sequence identity, the models and crystallographic structures are more similar. Also, the two SWISS-MODEL and Modeller tools were compared in different identity and similarity ranges.

**Keywords:** Homology Modelling; Sequence Identity; Protein Similarity; SWISS-MODEL; Modeller

**P-107: Differentially expressed spliced variants of FOXF1 in Lung Cancer**

Zareei M1, Ghanei M2, Ahmadi A3, Mowla SJ1

1. Department of Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran  
2. Chemical Injuries Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran  
3. Department of Genetics, Arak University of Medical Sciences, Arak, Iran

Rice is the world’s most important staple food and it is one of the utmost species in plant genetic studies as a model crop. Floral transition is a critical stage of the physiological processes that thoroughly changes vegetative stage to reproductive stage. Moreover, flowering is predominantly associated with grain yield in rice and substantially affects the final grain production. Some genes such as OsGI, Hd1, Ehd1, Hd3a and RFT1 are known to regulate the main part of flowering pathways in rice. Co-expression analysis unravels group of genes simultaneously expressed under specific condition that therefore they tend to be involve in similar biological processes. Here we identified co-expressed genes including transcription factors (TFs) related to transition stage in rice using ExPath 2.0 database. Thereafter, Singular Enrichment Analysis (SEA) and pathways survey on co-expressed genes were carried out using AgriGo tools (http://bioinfo.cau.edu.cn/agriGO/) and ExPath 2.0 database, respectively. Moreover, co-expressed TF families were further studied in more details. Coexpressed genes were mainly involved in metabolic process, catalytic activity and binding. Moreover, ubiquitin and endocytosis were illustrated to have immense roles in this developmental stage and plant growth. The major TF families identified in our analysis were AP2, bZIP, GATA, Homeodomain, MADS box and WRKY that could be considered as the most important transcriptional regulators controlling flowering in cereals. These results help to uncover genetic mechanism of rice flowering.

**Keywords:** Flowering, Ontology, Pathway, Transcription factors
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P-108: Erythropoietin (EPO) and granulocyte-colony stimulating factor (G-CSF) fusion protein: A therapy in multiple myeloma

Zarghamian P1, Mardani A2, Ghorbani nia M3

1. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
2. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
3. Department of Biology, University of Esfahan, Esfahan, Iran
Pzarghamiyah@gmail.com

Background and aims: Multiple myeloma is a blood cancer related to lymphoma and leukemia. It also can cause anemia and other blood problems. Designing a fusion protein of erythropoietin (EPO) and granulocyte-colony stimulating factor (G-CSF) can be a possible approach to treat the cancer besides the chemotherapy.

Materials and methods: The optimized genetic construct encoding EPO/G-CSF with AGSGGGGS linker was multiplied by polymerase chain reaction (PCR). Cloning was carried out in Baculovirus transfer plasmid called pAB-GSTα-ε then expressed in S2 expression system. For purify, the chromatography was used with glutathione attached to the resin. Ram page and I-tasser software were used to predict the structure and stability of protein.

Results: I-tasser result showed five top models. The first model was submitted in ram page and according to Ramachandran plot this fusion protein is stable. Conclusion: Based on the results discussed erythropoietin (EPO) and granulocyte-colony stimulating factor (G-CSF) with Baculovirus transfer plasmid and S2 expression system can be used as a possible therapy in Multiple myeloma.

Keywords: Erythropoietin, Granulocyte -colony stimulating factors, Fusion protein, Multiple myeloma

P-109: Inhibitors for treatment of Gaucher’s disease

Zolfaghari N
National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
nargeszolfaghari90@yahoo.com

Gaucher’s disease is a genetic disorder in which a glucocerebroside named glucosylceramide accumulates in cells and certain organs. The disease is caused by deficiency in the gene related to beta-glucosidase, EC 3.2.1.45, which is located in 1q22. Gal?1-4Glc?1-Cer is catalyzed by beta galactosidase and glucosylceramide would be produced. In the next step, ceramide galactosyl transferase acts and produce ceramide. To prevent accumulation of glucosylceramide, beta galactosidase should be weakened to produce less glucosylceramide.

For gaining this purpose, we have used high throughput virtual screening by MolDock scoring function using a library containing 200,000 drug likes derived from Zinc database. Top 20 successive hits were then analyzed regarding MADE properties by AdmetSar and finally, top hits were filtered by Lipinski and MolSoft rules and one ligand with the IUPAC name: 7-amino-5-{3-ethoxy-4-[2-(4-morpholinyl)ethoxy]phenyl}-4-hydroxy-5H-pyrano[2,3-d]pyrimidine-6-carbonitrile could pass all pharmacological filters and predicted to be in the drug like category. We introduce this ligand as a potential drug candidate for treatment of Gaucher’s disease.

Keywords: AdmetSar, Gaucher’s disease, beta-glucosidase, MolDock

Human Gentsics and Medicine

P-110: The Frequency of PLA2 Gene among Iranian Population

Abbasi Sh1, Rabbani B2, Javadi Gh1
1 Department of Biology, College of Basic Sciences, Tehran Sciences and Research Branch, Islamic Azad University, Iran
2 Genetic research Center, Rajaie Cardiovascular Medical and Research Center, Iran university of Medical Sciences, Tehran, Iran
shv.abbasi@gmail.com

Background: It is demonstrated that there is a relation between rs5918 on Integrin GPIIIa gene (integrin beta 3) and the possibility of platelet aggregation, coronary artery occlusion, stent thrombosis or lower reactivity to antiplatelet drugs â€“ aspirin and clopidogrel or inhibitors of GPIIb/IIa. The aim of present study was to determine the frequency of the polymorphism on the ITGB3 gene in a sample population of Iranian patients with atherosclerosis.

Method: fifty-two referred patients to â€œRajaie Cardiovascular Hospitalâ€- with the average age of 59 were included in this study. The GPIIa PlA1/A2 polymorphism (rs5918) determined in all samples using PCR based restriction fragment length polymorphisms (RFLP) for genotyping.

Results: according to the obtained results, the frequency of T allele was higher in comparison with other allele. The frequency of TT genotype, heterozygote CT and CC genotype have been calculated around 81%, 17.3% and 0.9%, respectively.

Conclusion: We demonstrated that these genotypes for the mentioned SNP are in Hardy-Weinberg Equilibrium (HWE).
Likewise, similar to previous studies among other populations including Turkish, Slovenian, Chinese and Austrian populations, the T allele is the most common specified allele for this polymorphism. Further studies would be helpful for clinical management the patients with atherosclerosis who receiving Plavix (clopidogrel).

**Keywords:** GPIIIa PlA1/A2 polymorphism, Clopidogrel

**P-111: Report of a Complex Chromosome Abnormality in an Infertile male with Azospermia**

Abdi A1, Bagherizadeh I1, Vahedi R1, Hadipour Z1, Shafaghati Y1,2, Behjati F1,2

1. Sarem Cell Research Center & Department of Medical Genetics, Sarem Hospital, Tehran, Iran
2. Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

**Correspondent:** Prof. Farkhondeh Behjati: Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

**Ak.abdi1362@gmail.com**

**Introduction:** Infertility affects approximately 1 in 6 couples worldwide, and male factor infertility accounts for an estimated half of all infertility cases. Male infertility occurs because of various factors, including those of environmental and genetic origin. The most common genetic cause of male infertility is chromosomal abnormality.

The frequency of chromosomal abnormalities including both aneuploidies and structural rearrangements in infertile men is ten times higher compared with the normal population. In translocation carriers, reduced fertility is mediated by the fact that the rearranged chromosomes need to synapse through a pairing cross, in order to progress through meiosis. This pachytene cross can interfere with XY sex vesicle causing spermatogenesis arrest.

**Case:** The Patient was a 40 -years-old man who was referred for azoospermia and primary infertility. Chromosomal analysis was carried out using T lymphocytes and standard cytogenetics techniques. 15 Chromosome spreads were studied using high resolution GTG banding technique, with light microscope.

**Results and discussion:** Conventional cytogenetic investigation showed complex chromosomal abnormality and heterochromatin variation: 46,XY,t[der(1)inv(1)(p32q23);9;5] [?p32;?p22;?q33.3] Karyotype. Structural chromosomal abnormalities are an important cause of male infertility.

**Conclusion:** Chromosomal rearrangement may interrupt an important gene or alternatively may exert a position effect. These rearrangements may alter the functionality of genes at specific breakpoints such as those with a specific role in spermatogenesis. However, the pachytene cross of abnormal chromosomes could activate the XY sex vesicle, arresting spermatogenesis, hence male infertility.

**Keywords:** Chromosomal Abnormality, Azoospermia, Primary Infertility

**P-112: Evaluation of Association between DAT1 3’UTR-VNTR polymorphism and Attention-Deficit Hyperactivity Disorder susceptibility among children from Northwest of Iran**

Abdi A1, Mehdizadeh Fanid L1, Zeinalzadeh N1, Nourazar Gh2

1. Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.
2. Psychiatry and Behavioral Sciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

**adel_abdi94@ms.tabrizu.ac.ir**

Attention deficit hyperactivity disorder is one of the most common psychiatric disorders in childhood. It affects 3%–6% of school age children worldwide and it is characterized by age inappropriate levels of inattention, hyperactivity and impulsivity. Dopaminergic pathways genes are among the different genes which are involved in the incidence of ADHD, DAT1 or SLC6A3 is one of the dopaminergic pathways genes which is located on the chromosome 5p15.3.

The aim of the present study was to evaluate association of DAT1 3’UTR-VNTR with attention deficit hyperactivity disorder susceptibility among children from Northwest of Iran. In this case-control study 200 patient with attention deficit hyperactivity disorder and 150 healthy individuals as case and control groups were chosen and their peripheral blood were provided. DNA was extracted from peripheral blood using salting out method and 3’UTR-VNTR polymorphism was genotyped by the use of PCR and agarose gel electrophoresis. Collected data were analyzed using SPSS software.

Genotype frequency was 9R-9R (10.5%), 9R-10R (25%), 9R-8R (0.1%), 10R-10R (38%), 10R-11R (10.5%), 11R-11R (13.5%), 11R-12R (1%), 8R-8R (0.5%), 11R-8R (0%) among ADHD patients and 9R-9R (16.5%), 9R-10R (24%), 9R-8R (3.97%), 10R-10R (35.09%), 10R-11R (10.95%), 11R-11R (6.62%), 11R-12R (0.0%), 8R-8R (1.32%), 11R-8R (1.32%) among controls. The statistical analysis showed no significant association between DAT1 3’UTR-VNTR polymorphism and susceptibility to ADHD in our study (P value=0.07).

**KEYWORDS:** ADHD, DAT1, 3’UTR-VNTR, polymorphism, Northwest of Iran, PCR

**P-113: Gene Expression Study in Radiation Workers Occupationally Exposed to Low Levels of Ionizing Radiation**

Abdulsahib K. Ali ; Amel, J. Muttar ; Abbas Ouda Z*

Ministry of Science and Technology/ Central Laboratories Directorate/Iraq
*Al- Mustansiriya University/College of Science/Iraq

**sahib1966@yahoo.com**

The present study aims to use of the gene expression as biomarker for investigation of exposed to low ionizing radiation in radiation workers occupationally in Al-Tuwaitha site, this study including 30 male blood samples, aged (30 - 55 year), as well as 30 male blood samples, aged (29 - 55 year) which are not smokers and alcohol as control. Total RNA was isolated using Trizol method from blood for the study groups men and genetic origin. The most common genetic cause of male infertility occurs because of various factors, including those of environmental and genetic origin. The most common genetic cause of male infertility is chromosomal abnormality. The frequency of chromosomal abnormalities including both aneuploidies and structural rearrangements in infertile men is ten times higher compared with the normal population. In translocation carriers, reduced fertility is mediated by the fact that the rearranged chromosomes need to synapse through a pairing cross, in order to progress through meiosis. This pachytene cross can interfere with XY sex vesicle causing spermatogenesis arrest.

**Case:** The Patient was a 40 -years-old man who was referred for azoospermia and primary infertility. Chromosomal analysis was carried out using T lymphocytes and standard cytogenetics techniques. 15 Chromosome spreads were studied using high resolution GTG banding technique, with light microscope.

**Results and discussion:** Conventional cytogenetic investigation showed complex chromosomal abnormality and heterochromatin variation: 46,XY,t[der(1)inv(1)(p32q23);9;5] [?p32;?p22;?q33.3] Karyotype. Structural chromosomal abnormalities are an important cause of male infertility.

**Conclusion:** Chromosomal rearrangement may interrupt an important gene or alternatively may exert a position effect. These rearrangements may alter the functionality of genes at specific breakpoints such as those with a specific role in spermatogenesis. However, the pachytene cross of abnormal chromosomes could activate the XY sex vesicle, arresting spermatogenesis, hence male infertility.

**Keywords:** Chromosomal Abnormality, Azoospermia, Primary Infertility

**P-112: Evaluation of Association between DAT1 3’UTR-VNTR polymorphism and Attention-Deficit Hyperactivity Disorder susceptibility among children from Northwest of Iran**

Abdi A1, Mehdizadeh Fanid L1, Zeinalzadeh N1, Nourazar Gh2

1. Department of Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.
2. Psychiatry and Behavioral Sciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

**adel_abdi94@ms.tabrizu.ac.ir**

Attention deficit hyperactivity disorder is one of the most common psychiatric disorders in childhood. It affects 3%–6% of school age children worldwide and it is characterized by age inappropriate levels of inattention, hyperactivity and impulsivity. Dopaminergic pathways genes are among the different genes which are involved in the incidence of ADHD, DAT1 or SLC6A3 is one of the dopaminergic pathways genes which is located on the chromosome 5p15.3.

The aim of the present study was to evaluate association of DAT1 3’UTR_VNTR with attention deficit hyperactivity disorder susceptibility among children from Northwest of Iran. In this case-control study 200 patient with attention deficit hyperactivity disorder and 150 healthy individuals as case and control groups were chosen and their peripheral blood were provided. DNA was extracted from peripheral blood using salting out method and 3’UTR_VNTR polymorphism was genotyped by the use of PCR and agarose gel electrophoresis. Collected data were analyzed using SPSS software.

Genotype frequency was 9R-9R (10.5%), 9R-10R (25%), 9R-8R (0%), 10R-10R (38%), 10R-11R (10.5%), 11R-11R (13.5%), 11R-12R (1%), 8R-8R (0.5%), 11R-8R (0%) among ADHD patients and 9R-9R (16.5%), 9R-10R (24%), 9R-8R (3.97%), 10R-10R (35.09%), 10R-11R (10.95%), 11R-11R (6.62%), 11R-12R (0.0%), 8R-8R (1.32%), 11R-8R (1.32%) among controls. The statistical analysis showed no significant association between DAT1 3’UTR-VNTR polymorphism and susceptibility to ADHD in our study (P value=0.07).

**KEYWORDS:** ADHD, DAT1, 3’UTR_VNTR, polymorphism, Northwest of Iran, PCR
addition to the primers for internal control (?-actin) gene. All of these genes play an important role in the organization of the Cell cycle/proliferation DNA repair and apoptosis. Therefore, the study was contributed to the possibility of using it as a biological evidence for the detection of radiation exposure or contamination and thus may contribute to understand some of unknown mechanisms that may occur during the process of cancer formation perhaps caused by radiation.

The products of replicated specialized primers for the genes concerned and the cDNA for the studied samples were electrophoretically separated in agarose gels. The banding profiles were visualized by ethidium bromide staining, as the molecular weight were 135 bp, 165 bp, 185 bp and 470 bp, (nitrogen base pair) for RHOA, CDKN1A, GADD45A and RAD52 genes, respectively. Gene expression analysis revealed statistically significant transcriptional changes in a 4 genes (RHOA, GADD45A, CDKN1A up-regulated and RAD52 down-regulated). This study raises the possibility of using these genes as biomarkers for assessment of low radiation exposure in humans.

**Keywords:** Ionizing radiation, Radiation workers, Occupational exposure, Gene expression profiles

**P-114:** Rivastigmine and aqueous extract of Olibanum may boost the expression of FMR1 gene in AlCl3-induced Alzheimer's disease model rats

abedinzadeh gheshlaghi A, Khalaj-kondori M, Shaghaghi Z, Zafarpiran M

Department of Genetics, Animal biology Group, Faculty of Natural Science, University of Tabriz, Tabriz, Iran.

abedinzadeh71717@gmail.com

Alzheimer's disease (AD) is a multifactorial disorder that it’s progression is associated with many genetic and environmental factors. Many studies showed that FMR1 gene is important in AD because FMR1 protein is participated in regulating of APP production. So, we investigated the effects of aqueous extract of Olibanum and Rivastigmin on the expression of FMR1 gene in AlCl3-induced Alzheimer’s disease model rats. 28 adult male rats were treated with AlCl3 (20 mg / kg) for 60 days and then randomly divided into 4 groups (n=7). Group 1 was treated with Rivastigmine (0.3 mg / kg), group 2 with aqueous extract of Olibanum (200 mg / kg) group 3 with AlCl3 (20 mg / kg) and group 4 with distilled water (1 ml) for 60 days. Animals were tested with Morris-Water-Maze and their hippocampus was isolated and used for FMR1 gene expression analysis by real-time PCR. Morris-Water-Maze test showed that AlCl3 impairs memory performance in rats, while treatment with Rivastigmine or aqueous extract of Olibanum improves learning in the AlCl3-treated rats. Moreover, real-time PCR data analysis indicated that while AlCl3 led to significant reduction in FMR1 gene expression, Rivastigmine and aqueous extract of Olibanum increased its expression.

**Keywords:** Rivastigmin, Olibanum, FMR1, Alzheimer’s disease

**P-115:** NQO1 gene rs1800566 polymorphism is not associated with the risk of multiple sclerosis in kerman population

Abolhasani M1, Amirkhosravi A2, Asadikaram Gh1, Ebrahimi Gh1, Mandegary A2, Mehrabani M3, Esmeei Tarzi M3, sheikholeslami M3, Nematollahi M H1*

1. Department of Biochemistry, School of Medicine, Kerman University of Medical Sciences, Kerman, Iran
2. Department of Pharmacology & Toxicology, Kerman University of Medical Sciences, Kerman, Iran
3. Physiology Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

mh.nematollahi@yahoo.com

Background: Oxidative stress plays a pivotal role in the pathogenesis of multiple sclerosis (MS). NAD(P)H:quinone oxidoreductase 1 (NQO1) is a phase II detoxification enzyme that catalyzes the two electron reduction of endogenous and exogenous quinones, preventing their participation in redox cycling and subsequent generation of reactive oxygen species (ROS). NQO1 C609T polymorphism results in the substitution of serine amino acid instead of proline at 187. This mutation reduces the activity of the NQO1 enzyme. The aim of this study was to evaluate the possible influence of the C609T polymorphism in the NQO1 gene in the risk for MS in Kerman population.

Methods: We analyzed allelic and genotypic frequencies of NQO1 C609T in 126 patients with MS and 80 healthy controls, using PCR-RFLP assay.

Results: NQO1 rs1800566 allelic and genotypic frequencies did not differ significantly between MS patients and controls.

Conclusion: Our results indicate that NQO1 rs1800566 does not have an effect on MS disease risk.

**Keywords:** Multiple sclerosis, NQO1, polymorphism

**P-116:** Downregulation of miR-21 and miR-155 through oleuropein, a new aperture for breast cancer prevention and therapy

Abtin M1, 2, Alivand MR2, Shekari Khaniani M2, Bastami M2, Zaeifizadeh M2, Mansoori Derakhshan S1, 2*

m.abtin.genetics@gmail.com

Breast cancer (BC) is the leading cause of cancer mortality in women worldwide. It recently was proven that miRNAs play a critical role in BC development. The use of natural agents for control of cancer by modulating miRNAs is promising. Oleuropein is a natural polyphenolic agent with anti-neoplastic properties and is well tolerated by humans. This study was undertaken to determine the therapeutic effects of oleuropein through modulation of master oncomiRs (miR-21 and miR-155) in BC cells. The present study provides the first link between miRNA and oleuropein as a mechanism in BC. MCF-7 cells were tested with and without oleuropein and the cell viability, apoptosis and migration were examined. The effect of oleuropein on miR-21 and miR-155 expression was assessed through qRT-PCR. It was found that oleuropein induced apoptosis and retarded cell migration and invasion in a dose-dependent manner in the human MCF7 BC cell line. It was observed that oleuropein significantly decreased expression of both miR-21 and miR-155 over time in a dose-dependent manner. These results demonstrate that oleuropein is a potential therapeutic and preventive agent for BC. Oleuropein exhibits an anti-cancer effect by modulation of tumor suppressor gene expression, which is targeted by oncomiRs.

**Keywords:** Breast cancer, miRNA, oleuropein, q-real-time PCR, anti-cancer effect, miR-21, miR-155

**P-117:** Evaluation of cytotoxicity effects of synthetic com-
Cancer, the second leading cause of death worldwide, is a heterogeneous disease which leads to uncontrolled cell growth. More than 100 types of cancer are known, and due to the progression of cancer, drug resistance, and side effects of current medications, researchers are looking for new synthetic drugs with fewer side effects to treat cancer. In this study, a series of synthetic drugs with anticancer activity previously prepared were tested. Since breast cancer is the most commonly reported cancers and is the leading cause of cancer death among women, the aim of current study was to identify and evaluate the cytotoxic activity of the synthetic drug of imidazopyrimidin-3-amines derivatives as anticancer agents and its effect on apoptosis in the breast cancer cell line, MCF-7, which ultimately reduces the growth of these cell lines and can contribute to cancer treatment. So far, the effects of 12 samples of imidazopyrimidin-3-amines derivatives in different concentrations of 50µM to 1000µM with a three-time repeat count on the cells of the breast cancer cell line have been investigated and Etoposide as positive control and DMSO as negative control have been used to determine the accuracy of the work. MTT assay was used for assay to measure the toxicity of the drugs. Our results showed that in MCF-7 cells, among the 12 available samples, IC50 were found in samples B9, B4, B2, B10, B6 at concentrations of 200µM, 300µM, 400µM, 500µM, 600µM.

Keywords: Apoptosis, Cancer, Cytotoxicity, Drug

P-118: Association between genotype of miR-4270 binding site within 3′-UTR of ERCC1 gene and some clinicopathological features of breast cancer patients


1. Infectious Diseases Research Center (IDRC), Arak University of Medical Sciences, Arak, Iran
2. Khansari Hospital and Department of Internal Medicine, School of Medicine, Arak University of Medical Sciences, Arak, Iran
ahmadia22@yahoo.com

Introduction: One of the Effective factors in management of breast cancer (BC) is early diagnosis. The involvement of deregulated miRNA networks in cancer progression is validated in several studies. MicroRNAs control expression of genes by binding to their regulatory regions (UTR). A SNP within a miRNA binding site or recognition element (MRE) could change mRNA level and protein. ERCC1 (gene ID: 2067) is involved in the Nucleotide excision repair (NER). This signaling pathway is associated with BC. In present study, we genotyped 3′-UTR ERCC1 and evaluated staging in BC patients in Markazi province.

Method: The 3′-UTR of ERCC1 were analyzed for MRE sites using bioinformatics software. In present case-control study, 45 BC samples were evaluated by digestion method. Two selected miRSNPs were genotyped with MboII and AvAI.

Results: Bioinformatics analysis showed that the restriction sites of aforementioned enzymes in the 2342bp 3′-UTR of ERCC1 are related to MRE of miR-4270 and miR-2355. These bindings have negative ΔG (-0.07& -0.002). Electrophoresis indicated accuracy of PCR-RFLP reaction. Also, the positive correlation of MRE change of miR-4270 (homozygote TG) with metastasis and grade of the tumour was confirmed statistically (95% CI, P=0.03).

Conclusion: Several SNPs have been implicated in genetic susceptibility of BC. Studies have shown that deregulation of ERCC1 is associated with resistance to chemotherapy and ionizing radiation. We confirmed association between MRE nucleotide changes of miR-4270 within 3′-UTR ERCC1 with tumor-staging. This change could be used as a diagnostic biomarker for risk and progression of BC and response to treatment probably.

Keywords: Breast Cancer, ERCC1, 3′-UTR, miRSNP, Biomarker
P-120: A rapid, Simple Genomic DNA Extraction from Yeasts for Large Scale Genetic Analyses  
Aajorloo F, Vaez M, Hemmat J  
1. Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST), P.O. Box 33535111, Tehran, Iran  
vaez_m@yahoo.com  
DNA extraction methods usually rely on time-consuming procedures that may involve handling hazardous compounds or using expensive kit. Here, we have developed a rapid genomic DNA extraction technique from yeasts and applied it for amplification of genomic DNA using gene specific primers. DNA extraction was evaluated at different stages from initial step to final pure DNA. In addition to final pure DNA of each sample which runs on an agarose gel to check the quantity and efficiency of whole yield, the extracts at different stages of extraction procedure were qualitatively examined and evaluated using gene amplification in a PCR reaction for minimal time consumption and lack of PCR inhibitors. The results showed that the earliest step for successful gene amplification was immediate after one hour incubation in 65°C with extraction buffer. The obtained DNA was validated with different sets of primers for DNA amplification. The PCR products from each sliced bands were purified and sequenced. This evaluation showed that the presented method is a reliable, rapid and simple procedure with minimal equipment and resources requirements which is also environmentally friendly in absence of toxic chemicals for a cost efficient DNA extraction especially for large number of samples in less than one hour for sufficient genomic DNA for different purposes from yeasts for large scale genetic analyses including downstream PCR-based genetic analysis. methods usually rely on time-consuming procedures that may involve handling hazardous compounds or using expensive kit. Here, we have developed a rapid genomic DNA extraction technique from yeasts and applied it for amplification of genomic DNA using gene specific primers. DNA extraction was evaluated at different stages from initial step to final pure DNA. In addition to final pure DNA of each sample which runs on an agarose gel to check the quantity and efficiency of whole yield, the extracts at different stages of extraction procedure were qualitatively examined and evaluated using gene amplification in a PCR reaction for minimal time consumption and lack of PCR inhibitors. The results showed that the earliest step for successful gene amplification was immediate after one hour incubation in 65°C with extraction buffer. The obtained DNA was validated with different sets of primers for DNA amplification. The PCR products from each sliced bands were purified and sequenced. This evaluation showed that the presented method is a reliable, rapid and simple procedure with minimal equipment and resources requirements which is also environmentally friendly in absence of toxic chemicals for a cost efficient DNA extraction especially for large number of samples in less than one hour for sufficient genomic DNA for different purposes from yeasts for large scale genetic analyses including downstream PCR-based genetic analysis.  
Keywords: DNA extraction , PCR, Yeast

P-121: A whole-exome sequencing study of polymorphic teratozoospermia in multiple consanguineous families  
Akbari A1,2, Carrera P3,4, Almadani N5, Mohseni Meybodi A6, Gourabi H7, Anvar Z8, Zeighami Sh9, Jafarinia M1,2, F Maurizio5,6, Totonchi M9  
1. Department of Biology, Faculty of Science, Fars Science and Research Branch, Islamic Azad University, Marvdasht, Iran  
2. Department of Biology, Faculty of Science, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran  
3. IRCCS San Raffaele Scientific Institute, Division of Genetics and Cell Biology, Unit of Genomics for Human Disease Diagnosis, Milan, Italy  
4. Laboratory of Clinical Molecular Biology, Ospedale San Raffaele, Milan, Italy  
5. Department of Genetics at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran  
6. Infertility Research Center, Department of Obstetrics and Gynecology, School of Medicine, Shiraz University of Medical Sciences, Shiraz, Iran  
7. Vita-Salute San Raffaele University, chair of Clinical Pathology, Milan, Italy  
m.totonchi@royaninstitute.org  
Introduction: Almost half of the male infertility cases remain idiopathic indicating that despite careful physical and molecular examinations, no definitive cause can be identified. Due to its heterogeneous nature, examination of various genes used to be an obstacle but with the advent of next-generation sequencing technology it is now a feasible option. We have identified a large family with a host of consanguineous marriages in which seven people have been diagnosed with polymorphic teratozoospermia whom share relatively similar phenotypes of sperm cells. Our objective is to determine a novel pathogenic variant by performing a family-based exome sequencing study.  
Methods: Whole-exome sequencing (WES) was performed on six people including four affected and two unaffected who are parents to affected. Sequencing run was performed on Illumina nextseq 500 platform. Resulting FASTQ files were checked for quality via FASTQC software. Burrows-Wheeler Aligner (BWA) was used to map the files against the hg19 reference genome provided by the UCSC Genome Browser. Output files were validated and sorted using the Picard tools package. Next steps of the analyses were performed in accordance with GATK best practices. Annotation of finalized VCF files was done via ANNOVAR.  
Results: Candidate variants that co-segregate with the disease phenotype were double-checked by Sanger Sequencing and will be published very soon.  
Conclusion: Since infertility involves various proteins working in concordance with each other, we hope our data can provide a new insight into its pathogenesis  
Keywords: Male infertility, teratozoospermia, Whole Exome Sequencing, family-based study, Next-generation Sequencing

P-122: Investigation of IL-17RA and IL-23R gene expression in patients with bullous pemphigoid  
Akbari M1, Tabatabaei Panah PS1, Montazer Haghighi M1  
Biology Department, Islamic Azad University-East Tehran Branch, Tehran, Iran  
akbari_maryam54@yahoo.com  
Bullous pemphigoid (BP) is the most frequently occurring entity among autoimmune bullous skin diseases. Although the genetic determinants of BP have not been precisely elucidated, some studies have shown an association between the IL-17RA and IL-23R expression and BP disease susceptibility. Yet, these findings had so far not been independently replicated, and no
data on a possible association of expression and BP in Iranian population were available.

**Methods:** This study contains 20 BP patients and 20 healthy controls. cDNA was synthesized after RNA isolation. IL-17RA and IL-23R expression levels were measured by Real-time PCR. Several relevant information such as demographic data (age, gender â€” or clinical characteristics were analyzed for a possible effect of these factors on susceptibility to BP in patients.

**Results:** IL-17RA gene expression levels were significantly higher in patients with BP (1.39±0.13) in comparison with control individuals (1.02±0.23) (P=0.002) and IL-23R gene expression levels were significantly lower in patients with BP (0.41±0.14) in comparison with control individuals (1.44±0.11) (P=0.001).

**Conclusion:** Evaluation of IL-17RA gene shows that the expression of this gene is significantly upper in patients than that of controls and Evaluation of IL-23R gene shows that the expression of this gene is significantly lower in patients than that of controls.

**Keywords:** Bullous pemphigoid, Autoimmune disease, IL-17RA, IL-23R

**P-123: RPS6KB expression analysis in multiple sclerosis patients compared to healthy controls**

**Akbarian F, Tabatabaiefar MA,1, Rahimi A2, Dabiri A, Shayannejad V2, Shahpouri MM2, Jalilian N, Noori-Daloii MR**

1. Department of Medical Genetics, School of Medicine, Tehran University of medical sciences, Tehran, Iran
2. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Pediatric Inherited Diseases Research Center, Research Institute for Primordial Prevalent of Noncommunicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran
4. Department of Immunology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
5. Isfahan Neuroscience Research Center, Isfahan University of Medical Sciences, Isfahan, Iran
6. Department of clinical biochemistry, school of medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran

akbarian0001@gmail.com

**Introduction:** Multiple sclerosis (MS) is an autoimmune disease of the CNS. mTOR signaling pathway regulates the function of a wide range of immune cells and CNS myelination during development. Immunosuppressive effect of Rapamycin confirms the role of this pathway in the immune system. RPS6KB1, one of the main downstream targets of mTOR, has been identified as a susceptibility locus for MS in association studies. Here we analyzed the expression of RPS6KB1 gene in MS patients in comparison with healthy controls.

**Methods:** Blood samples were collected from 30 MS new case-patients and 30 age and gender-matched healthy controls. Total RNA was extracted and was turned into cDNA. Then mRNA expression was measured by Real-time PCR and final data was analyzed by LinRegPCR and REST software.

**Results:** Fold Change expression of RPS6KB1 gene in MS patient was 1.701 ± 0.170 (P=0.009) regardless of gender, 1.588 ± 0.702 (P=0.198) in men and 1.654 ± 0.16 (P=0.001) in women.

**Conclusion:** mTOR signaling pathway is interconnected with MS and RPS6KB1 is one of the main genes in this pathway. Increased expression of RPS6KB1 could be caused by rs180515 polymorphism, located at the 3â€™UTR of RPS6KB1 gene.

**Keywords:** Multiple Sclerosis, mTOR pathway, RPS6KB1, Real-Time PCR

**P-124: Comparing the expression level of circulating miR-93 and miRNA-21 in PCOS and healthy women**

Akbari-Moghadam M1,2, Hajipour F1,2, Montazeri F1,2, Miresmaeili SM1, Kalantar SM1

1. Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Biology, Yazd university of Science and Art, Yazd, Iran

smkalantar@yahoo.com

**Background:** Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine and metabolic disorders in premenopausal women from Iran. PCOS is currently defined as a mainly hyper androgenic disorder with a constellation of symptoms include clinical and/or biochemical hyper androgenism together with ovulatory dysfunction and/or polycystic ovarian morphology. PCOS has been associated with metabolic disorders, including insulin resistance, obesity and diabetes. MicroRNAs (miRNA) are a novel class of small noncoding single-stranded RNA molecules that regulate gene expression. There is increasing evidence of their importance in polycystic ovary syndrome (PCOS).

**Objective:** The objective was to determine if miRNA-93 and miRNA-21 are differentially expressed in the circulation of women with PCOS compared to age matched healthy women.

**Material and Methods:** A caseâ€“control study was performed between women with PCOS (n = 10) and age matched controls (n = 10). Total RNA extraction from plasma and cDNA synthesis done using commercial kits. The expression of miRNA-93 and miRNA-21 and Snord mRNA as reference gene was evaluated by Real Time PCR.

**Result and Conclusion:** The expression level of both miRNA-93 and miRNA-21 were significantly increased in PCOS relative to the control group. Despite significant relation between miRNA and serum level of AMH, there was no correlation of understudied miRNA with serum level of other reproductive hormone include FSH and estradiol. The result suggesting that miR-93 and miRNA-21 could be an efficient biomarkers for diagnosis of PCOS.

**Keywords:** Real Time PCR, polycystic ovary syndrome (PCOS), microRNA, biomarker, reproductive hormone

**P-125: Analysis of the SLC26A4 and CDH23 mutations in Iranian hearing loss patients**

Alimardani M1,2,4, Farjami M3,4, Mojarrad M, Shekari Khani M1,4, Mansoori Derakhshian S1,2,4

1. Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Medical Genetics, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Medical Genetics, Mashhad University of Medical Sciences, Mashhad, Iran
4. Ebne sina Medical Genetic Diagnostic Laboratory, Tabriz University of Medical Sciences, Tabriz, Iran
5. Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

alimardanim942@mums.ac.ir

**Abstract:** There is increasing evidence of their importance in polycystic ovary syndrome (PCOS).

**Introduction:** Polycystic ovary syndrome (PCOS) is one of the most frequent endocrine and metabolic disorders in premenopausal women from Iran. PCOS is currently defined as a mainly hyper androgenic disorder with a constellation of symptoms include clinical and/or biochemical hyper androgenism together with ovulatory dysfunction and/or polycystic ovarian morphology. PCOS has been associated with metabolic disorders, including insulin resistance, obesity and diabetes. MicroRNAs (miRNA) are a novel class of small noncoding single-stranded RNA molecules that regulate gene expression. There is increasing evidence of their importance in polycystic ovary syndrome (PCOS).

**Keywords:** Real Time PCR, polycystic ovary syndrome (PCOS), microRNA, biomarker, reproductive hormone
In this case-control study, blood samples were collected from 50 patients and 50 controls. The polymerase chain reaction (PCR) for detection of five mutations include c.719C>T and c.6085C>T in CDH23 gene, and IVS7-2A>G, c.1975G>C, and c.2168A>G in SLC26A4 gene in 100 cases with autosomal-recessive NSHL, originating from Eastern-Azerbaijan and Khorasan province, were screened.

Results: In all samples, none of mentioned mutations could be identified. The results were checked by sequencing in 15 samples and the absence of these mutations was validated.

Conclusion: It seems that although the precise portion made by such mutations needs to be defined using a larger patient cohort, the present data show that these mutations in the CDH23 and SLC26A4 genes are not important causes of autosomal-recessive NSHL in Iranian population.

Keywords: hearing loss, CDH23, SLC26A4, Iranian population.

P-126: The investigation of rs174547 in FADS1 gene, rs1260326 in GCKR gene variation, for identification of related to high triglyceride

Alipanah daryavarsari K<br>, Houshmand M<br>, Farhadi langaroudi F<br>, Akbarzadeh A<br>

1. NourDanesh Institute of Higher Education, Meymeh, Isfahan, Iran
2. Faculty member of Genetic Engineering of National Research Institute of Iran, Scientific Collection, Research, Laboratory, Tehran, Iran
3. Graduated from Zanjan University of medical science, Working at shahrara specialized laboratory

Evaluation of blood fat because of the association with certain diseases in the medical community has always been important. Blood fat including cholesterol, LDL (low-density lipoprotein), HDL (high-density lipoprotein), triglycerides. Triglycerides are an important source of energy is generally more people on calorie intake from protein used. High triglycerides can block blood vessels and damage the pancreas. Additional triglyceride tissue also has effect on metabolic diseases, including fatty liver, type 2 diabetes and some cardiomyopathies. Among the factor involved in the regulation of triglyceride metabolism can be pointed to genetic factors and environmental factors. Both of genetic mutation and environmental factor (diet, physical activity, having a high-carbohydrate diet and more) might effects on Increase of triglyceride level. The objective of this study was to evaluate RS174547 in FADS1 gene and RS1260326 in GCKR gene in tow groups Patient and control for identification of related to high triglyceride. The blood samples were collected from 50 patients and 50 controls. The polymerase chain reaction was carried out using genomic DNA. Consequently, PCR production were sequence. The results of this study indicate the relationship between FADS1 and GCKR mutations in patients with high triglycerides. In this study, we investigated the T/C allele in Hyper triglycerides, some of which were pure and some of them gross. In fact, the initial results suggest that this polymorphism has been seen in some control samples, and this is not a symptom of a person's disease, but suggests that the underlying disease.

Keywords: FADS1, GCKR, Triglyceride, type 2 diabetes, cardiovascular disease

P-127: Study of glutathione peroxidase (GPX1 pro198Leu) and catalase (CATC262T) genes polymorphism in pregnant women with preeclampsia

Alipour M<br>, Naeimi S<br>, Moghanibashi MM<br>

1. Department of Genetics, collegue of science, kazerun branch, Islamic Azad University, kazerun, Iran.
marzyehalipour1982@gmail.com

Preeclampsia is a multifactorial disease, specifically for pregnant women. It is more prevalent in both reproductive ages. Glutathione peroxidase and catalase are enzymatic antioxidant against oxidative damage, functioning as preventing damage from reactive oxygen species. The aim of this research is to survey the effect of two CAT-262C (rs1001179) and GPX1 Pro198Leu (rs1050450) polymorphisms in patients with preeclampsia in southern of Iran.

Materials and Methods: In this case-control study, blood samples of 150 patients with preeclampsia and of 150 healthy pregnant women, having no illness in the third trimester, have been collected. Genomic DNA has been extracted from peripheral blood. By PCR-RFLP method, genotypes of the mentioned polymorphisms have been checked. Data has been analyzed by SPSS software.

Results: Results show that there is meaningful relationship between control and patient groups, with polymorphism of CAT-262C at (rs1011779 C/T) position (p=0.032). TT genotype has significant correlation with preeclampsia at this position (OR=0.323, CI= (0.797-0.130), p=0.01). It was determined that there is significant relationship between C allele and preeclampsia (p=0.028). There is meaningful relationship between control and patient groups, with polymorphism of GPX1 Pro198Leu at (rs1050450 C/T) position (p=0.047); C/T genotype has meaningful relationship with preeclampsia at this position (OR=0.336, CI= (0.685-0.164), p=0.002).

Discussion and conclusions: Due to achieved results, it might be direct relationship among TT genotype at the position of (rs1001179 C/T) and C allele and C/T genotype at the position of (rs1050450 C/T) with developing preeclampsia.

Keywords: CAT, GPX1, preeclampsia, eclampsia

P-128: Association between epidermal growth factor gene +61A/G polymorphism and EGF serum level with the risk of idiopathic male infertility

Amin Malek M<br>, Mashayekhi F<br>

Department of Biology,Faculty of Biological Science,Tonekabon Branch,Islamic Azad University,Tonekabon, Iran
Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran
mashayekhi@guilan.ac.ir

Infertility is defined as the inability to achieve pregnancy after one year of regular, unprotected intercourse. Cytokines including interleukins and Epidermal growth factor (EGF) were shown to be implicated as regulator of spermatogenesis. EGF gene is located in the long arm of chromosome 4. EGF can stimulate human sperm capacitation and also regulates the germ...
cell development. The purpose of this study was to investigate the association of A61G (rs:4444903) polymorphism of EGF gene and its serum concentration with the susceptibility to idiopathic male infertility in Iranian population. Genomic DNA was extracted from 120 men with idiopathic infertility and 140 healthy men and was genotyped by PCR-RFLP. EGF level was measured in serum by ELISA. Genotype frequencies for AA, AG and GG in patient group were 77%, 23%, 0%, and in controls were 93%, 7%, 0%, respectively. The A and G allele frequencies in patients were 88.3%, 11.7% and in controls were 96%, 4% respectively. There was significant difference in allele (P=0.0008) and genotype (P=0.0005) distribution between two groups. Moreover the concentration of serum EGF in patient group (314.68 ± 45.16 pg/ml) were significantly lower than those in controls (464.28 ± 66.87 pg/ml, P<0.00001). The results of this study indicate that +61A/G polymorphism of EGF gene be associated with idiopathic male infertility. It is also suggested that EGF serum concentration is involved in the pathophysiology of male infertility.

**Keywords:** EPIDERMAL GROWTH FACTOR, Idiopathic INFERTILITY, POLYMORPHISM, SERUM, rs4444903

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**P-129: Association of the -31C>T polymorphism in interleukin-1B gene and the risk of gastric inflammation and peptic ulcer from Helicobacter pylori Infection**

Amini A1, Foroughmand AM1, Nejatizadeh A2, Shekari M2, Motamed Rad N2, Rezaei M2

1. genetic Department, Faculty of Sciences, Shahid Chamran University of Ahwaz
2. Molecular Medical Research Center, Hormozgan University of medical sciences, Bandar Abbas, Iran

athar.amini65@yahoo.com

Inflammation cytokines in gastric mucosa are produced in response to H. pylori infection by inflammatory cells. Mononucleotide variation in the promoter of these cytokine genes may affect the gene expression level. It can also cause different inflammations in gastric mucosa and secretion of acid in response to the infection. Therefore, the genetic background of people and their individual differences can play a role in the development and final outcome of such diseases as peptic ulcer. Interleukin-1b (IL-1b) is a cytokine that plays a key role in moderating inflammatory response in gastric-intestinal ulcer. In the present research which is of a case-control study, the PCR-RFLP technique was used to determine the allelic and genotypic frequency of the -31C>T polymorphism in the control and case groups and the data were analyzed by SPSS software by logistic Regression test. The results showed no significant association between the target polymorphism and gastric inflammation or peptic ulcer disease. However, environmental factors and life habits such as smoking can affect the risk of affliction can increase this risk several times as much. There seems to be a complicated association between mononucleotide variation that can account for individual differences and one's potential of affliction with diseases. That is due to the fact that other factors can also affect the potential for a disease. These include the interaction between poly-morphisms as well as life style.

**Keywords:** interleukin-1 beta, H.pylori, peptic ulcer disease, gastric inflammation

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**P-130: The Study of valproic acid effects on H19 noncoding RNA expression and apoptosis in cisplatin-resistant ovarian cancer cells**

Amini Farsani Z12, Sajadpoor Z1, Teimori H2, Hashemi S1, Shamsara M1, Hosseini E1, Sangtarash MH1, Asgari M1, Khazaie H1, Khosravi N1, Yadollahi F2, Yadollahi F2, Teimori T1

1. Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran.
2. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Rahmatiyeh, Shahrekord, Iran.
3. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
4. National Research Center for Transgenic Mouse, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
5. IVF center, Mousavi Hospital, Zanjan University of Medical Sciences, Zanjan, Iran.
6. Zahedan University of Medical Sciences, Zahedan, Iran.
7. Department of Biology, Kharazmi University, Tehran, Iran.
8. Department of Anesthesiology, Clinical Research Development Unit, Kashani Hospital, Shahrekord, Iran
9. Kashani Hospital, Shahrekord, Iran

zeinabaminifarsani@yahoo.com

Objective: Resistance to chemotherapy drugs, including cisplatin, is a major obstacle to effective treatment of ovarian cancer. Recent evidence suggests that IncRNA H19 correlates with drug resistance in different cancers. Valproic acid (VPA) is a histone deacetylase inhibitor that has anti-cancer effects. The objectives of this study were to investigate VPA effects on H19 expression and also the relation of the H19 levels with apoptosis and cisplatin-resistance in ovarian cancer cells.

**Methods:** A2780/CP cells were treated with cisplatin and/or VPA for 48 hours. The cell viability was evaluated using MTT assay and the apoptosis was measured using flow cytometry. The expression of genes and proteins were determined by qRT-PCR and western blotting, respectively. Also, the involvement of H19 in VPA-induced apoptosis and cisplatin-sensitivity, was investigated by H19 inhibition using specific siRNA.

**Results:** Treatment with VPA, not only led to significant increase in apoptosis rate, but also increased the cisplatin-sensitivity of A2780/CP cells. Our results showed that following VPA treatment, the expression of H19 and EZH2 decreased, but the expression of p21 and PTEN increased significantly. Moreover, knockdown of H19 by siRNA induced apoptosis and sensitized the A2780/CP cells to cisplatin-induced cytotoxicity.

**Conclusion:** These data indicated that VPA suppresses H19 expression in ovarian cancer cells, which subsequently leads to apoptosis induction, cell proliferation inhibition and overwhelming to cisplatin-resistance. The implication of H19/EZH2/p21/PTEN pathway by VPA treatment suggests that we could repurpose an old drug, valproic acid, as an effective drug for treatment of ovarian cancer in the future.

**Keywords:** Cisplatin; EZH2; H19; Ovarian cancer; Valproic acid

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**P-131: Evaluation of the expression of miR-4270 gene in plasma of breast cancer patients in northwest of Iran**

Aminisepehr F1, Babaei E1, Hosseinpourfeizi MA1, Montazem V1

1. Department of Animal Biology, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran
2. Department of Pathology, Imam Khomeini Hospital, Tabriz University of Medical Sciences, Tabriz, Iran

objective: The miR-4270 is a member of the miR-203 family. In this family, members that share 100% identity will have the same expression pattern, but members that have less than 100% identity will have different expression patterns. The miR-203 is a tumor suppressor in various kinds of cancer. The objective of this study was to evaluate the expression of miR-4270 gene in plasma of breast cancer patients in northwest of Iran.
Introduction & aim: MiRNAs are a principle class of short non-coding RNAs which play major roles in the initiation, progression, and metastasis of all types of cancers especially breast cancer. The aim of this study was to quantitatively evaluate the expression pattern of miR-4270 in the plasma of patients with invasive ductal carcinoma (IDC), the most common type of aggressive breast cancer in North West of Iran.

Materials and methods: Fresh blood specimens from 40 women with invasive ductal carcinoma (IDC) and 28 healthy women, were obtained. Total RNAs were extracted from plasmas and treated with DNase I. Then polyA tail was added by an enzymatic reaction and the RNAs were reverse transcribed to cDNA with miRNA cDNA synthesis kit. The expression of miR-4270 and 5s rRNA as endogenous control was studied by real-time PCR.

Results: miR-4270 expression level was significantly upregulated in breast cancer plasma compared with healthy control women (P value<0.00), but the expression of miR-4270 decrease in patients with larger tumor, lymph node invasion, and higher grades (P value<0.05). However, this reduction is not significant. The results of the plasma specimens yielded an area of 0.5 (AUC) under the ROC curve for the target gene. This implicated that low sensitivity and specificity of miR-4270 could be used as a diagnostic and prognostic biomarker for breast cancer.

Conclusion: In conclusion, upregulation of mir-4270 in breast cancer plasma versus normal healthy plasma and its relation with the progression of breast cancer adds a new dimension to our more research to help the diagnosis and treatment of breast cancer.

Keywords: Breast Cancer - miR-4270 - Invasive Ductal Carcinoma - Real-Time PCR

P-132: Expression analysis of Long non-coding RNA SNHG17 in breast cancer

Amirian-ghatar S1, Khalaj-kondori M1, Zafarpiran M1, Hosseinpour Feizi MA1

1. Department of Genetics, Animal Biology Group, Faculty of Natural Science, University of Tabriz, Tabriz, Iran. amirian.saeed888@gmail.com

Breast cancer is a common type of cancer among women worldwide. In recent decades despite of impressive advances in diagnostic and therapeutic strategies, mortality of breast cancer is still remaining high. Studies demonstrated that Long non-coding RNAs (lncRNAs) play key roles in development and progression of different cancers, including breast cancer. This study aimed to evaluate the expression of SNHG17 in breast cancer. Breast tumor tissues and their non-tumoral margin samples were obtained from 30 patients with breast cancer. Total RNA was purified with RNX-Plus and expression of lncRNA SNHG17 was quantified using qRT-PCR. The qRT-PCR results indicated that SNHG17 expression in tumor tissues was rather lower than to margin tissues. However, SNHG17 expression did not show correlation with demographics of patients.

Keywords: breast cancer, lncRNA, SNHG17, qRT-PCR

P-133: Association of rs6983267 polymorphism in lncRNA CCAT2 with gastric cancer susceptibility in Iranian population

Amiri-Moghaddam SM1, Goshayeshi L2,3, Nohtani M1, Bahrami A1,4, M. Matin M1,4,5, Karimi F1

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
2. Department of Gastroenterology and Hepatology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Gastroenterology and Hepatology Research Center, Mashhad University of Medical Sciences, Mashhad Iran.
4. Novel Diagnostics and Therapeutics Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran
5. Stem Cell and Regenerative Medicine Research Group, Iranian Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi Branch, Mashhad, Iran

s.m.amiril1372@gmail.com

Background: Globally, gastric cancer as one of the leading cause of death, can be affected by both genetic and environmental factors. According to the Iranian Ministry of Health and Medical Educations reports, 50% of all types of cancers in Iran are related to gastrointestinal and liver cancers with gastric cancer contributing to approximately 30% of them. Accumulating evidence suggests that non-coding regions of genome play a key role in cancer susceptibility and there are strong associations between many of (SNPs) single-nucleotide polymorphisms located on these regions with different types of cancers, while these SNPs may have association in one ethnic population the outcome is not extendable globally. Colon cancer-associated transcription 2 (CCAT2), a long non-coding RNA within a gene-free region on 8q24, encompasses the SNP rs6983267 which has been implicated in predisposition to many cancers. Therefore, we studied the role of rs6983267 in gastric cancer predisposition in Iranian population.

Method: In present case-control study, the rs6983267 SNP of the genomic DNA samples extracted from blood of 48 patients diagnosed with gastric cancer and 54 healthy controls, was investigated. Genotyping was performed using Taqman method.

Result: Hardy-Weinberg equilibrium was conducted with chi-square test in control group, it showed that the genotype distribution of rs6983267 was in equilibrium, (p<0.05, X2 = 0.96), but the genotype frequencies of rs6983267 in healthy controls were not different from those in patients (p>0.05).

Conclusion: No association was observed between rs6983267 and risk of gastric cancer, however, more studies are required in a larger population.

Keywords: SNP, gastric cancer, rs6983267

P-134: The association between the plasma levels of miR-211, miR-219-5p and miR-298 and early detection of colorectal cancer

Arabsorkhi Z, gharib E, Nazemalhosseini Mojarrad E

Gastroenterology and Liver Disease Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

elmira.arab92@gmail.com

Introduction: Colorectal cancer (CRC) is the second leading cause of cancer death in the world. Many environmental factors and genetic alterations are involved in colorectal carcinogenesis. Of them, miRNAs can regulate variety of biological conditions and their expression were changed in pathological conditions including tumorogenesis. Studies show that mir-211, miR-219-5p and miR-298 have critical role during cancer progression. Considering the stability and abnormally of miRNAs in body fluids, we examined the diagnostic values of
these molecules within the plasma samples of CRC patients.

Material and Methods: plasma samples consisted of 39 CRC, 32 polyps and 26 normal controls were obtained from the Taleghani Hospital, Tehran, Iran. Samples were subjected to miRNA isolation and then reverse transcribed. The obtained cDNAs were amplified by Realtime-PCR method. Relative expression abundances of miRNAs were measured by normalizing to miR-1228 using the 2-ΔΔCT method.

Result: The data showed that the expression level of miR-211 and miR-298 were significantly increased in plasma samples of poly and caner groups compared to healthy controls. In contrast, miR-219-5p was markedly lowered during malignancy. These observations were statistically associated with patient TNM stage, tumor location and differentiation.

Conclusion: Taking together, changing the expression pattern of miR-211, miR-219-5p and miR-298 provokes tumorigenesis in CRC patients. So, these miRNAs could be applied as diagnosis biomarker for cancer detection.

Keywords: Colorectal cancer, miR-211, miR-219-5p, miR-298, plasma samples

P-135: MicroRNAs investigation and their roles on KD-M3A gene silencing in azoospermic man

Arefnia M, motovali-Bashi M, Javadirad SM

Biology Department, Faculty of Sciences, University of Isfahan, Isfahan, Iran

mohammad.aref9171@gmail.com

Infertility is the inability to achieve pregnancy at least a year after a non-contracepting couple attempting to conceive. In general, 8-12 percent of couples are infertile, 35% of whom are caused by male infertility. Male infertility can be developed by multiple factors such as environment and genetic changes that are believed to account for more than 30% of all male infertility. Knock out studies show that KDM3A histone demethylase gene act as a hormone-dependent transcriptional activator with important roles in spermatogenesis. Therefore, silencing or lower expression of KDM3A may lead to the infertility. The main function of microRNAs is in RNA silencing and post-transcriptional regulation of genes. In this study we first used bioinformatics tools such as targetscan, mirwalk, Diana tool, mirtarbase and mirbase to predict microRNAs that can hit KDM3A gene transcript. Our primary in-silico analysis identified three candidates has-mir-30a-5p, has-mir-30d-5p, has-mir-30b-5p being able to potentially bind to KDM3A 3'UTR and downregulate its expression. Our experiments are ongoing to investigate the expression of these miRNAs via qPCR in testis tissue obtained from obstructive and non-obstructive azoospermic men. Our final goal is to study a plausible link between the expression of our candidate miRNAs and KDM3A that may induce azoospermia. In conclusion, our in-silico analysis suggests that several miRNAs of the same family may affect the expression of KDM3A. Therefore, we propose that the differential expression of such miRNAs could be associated with azoospermia and our future experiments are directed to test this hypothesis.

Keywords: infertility, MicroRNA, azoospermia, KDM3A, epigenetic

P-136: Genetic polymorphism in XRCC1 and breast cancer risk in relationship with TP53 mutation status

Arghavanian Y
Abstracts of the 3rd International & 15th Iranian Genetics Congress

**P-138: Inheritance Modes of congenital heart defects among Iranian patients**

Ashrafi N, Omidi S, Soveizi M, Rabbani B, Maleki M, Mahdieh N

Genetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
nushin.ashrafi@gmail.com

**Introduction:** Congenital heart defects (CHD) are the most common cause of life-threatening in human CHDs have a heterogeneous etiology. Genetic factors have been known to be involved in these types of defects. In this study, we present the frequency of different patterns of inheritance among Iranian families with CHDs.

**Materials and methods:** This study was conducted on 100 affected families referred to Rajaie hospital. Pedigree drawing was performed for each family. Other information such as age of onset, gender, were also documented. The pattern was considered as autosomal recessive, autosomal dominant mitochondrial, sporadic, and familial.

**Results:** 55.76% had consanguineous marriages. Familial heritage was major mode of inheritance which 29.13% of families showed this type of inheritance

**Discussion and conclusion:** CHDs have a strong genetic liability in Iran. Further studies are required to determine genetic basis of CHD in Iran.

**Keywords:** Congenital heart defects, hereditary pattern, CHD, Iranian population

**P-139: Differential Expression of TUG1 LncRNA in The Inflammatory Condition in 1321N-1 Cell Line**

Askari Sh’, Khani-Habibabadi F, Behmanesh M

Department of Genetics, Faculty of Biological Sciences, University of Tarbiat Modares, Tehran, Iran
behmanesh@modares.ac.ir

**Introduction:** Multiple Sclerosis (MS), afflicting about 2.5 million people worldwide, is an inflammatory neurodegenerative disease of the central nervous system (CNS) and imposes a major personal, social and economic burden. Astrocytes are the most abundant type of glial cells in the CNS. There is an observed dichotomy in astrocyte’s roles contributing to the pathophysiology of MS. Better understanding about molecular and functional characteristics of astrocytes, could pave the way to light up the specific roles of this cell in disease progression. Long non-coding RNA (lncRNA) TUG1 is up-regulated in the serum of MS patients, and also in neurodegenerative disorders such as Huntington’s disease. As a component of p53-regulatory pathways, this gene is involved in development and regulation of cell-cycle and apoptotic pathways. Methods: 1321N-1 cell line was treated with H2O2 in three sequential time series. Doing MTT assay, the appropriate concentration of H2O2 was chosen. Induction of inflammation was examined by detecting the expression level of IL-1B gene. The expression level of TUG1 was evaluated using qRT-PCR. Result: Induction of Inflammatory condition with H2O2 treatment, was proved base on differential expression of IL-1B, as an inflammatory gene. Consistent with IL-1B, TUG1 showed differential expression under induced inflammation. Conclusion: In the CNS milieu of MS patients, there is an inflammatory condition, which is mimicked in this study using H2O2. Considering the pathways that TUG1 plays a role, and its differential expression under the inflammatory condition, TUG1 could be marked for further analysis, and detecting molecular mechanisms which may be contributed to astrocyte role in MS pathophysiology.

**Keywords:** multiple sclerosis, inflammation, IncRNA, TUG1

**P-140: Promoter Methylation, Polymorphism and Expression status of Cytotoxic T-Lymphocyte-Associated Antigen-4 in Patients with lupus**

Atighi S, Nosrat zehi SH, Kordi Tamandani D.M

Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran
Department of internal medicine, Zahean university of medical science, Iran
E-mail: s.atighi2302@gmail.com

Systemic lupus erythematosus (SLE) is an autoimmune disease with unknown etiology, in which autoantibodies directly contribute to the destruction of organs such as kidneys, joints, and skin. The CTLA4 play an important role in inhibition the activity of T cells and thus preventing autoimmune disorders like lupus.

**Material and methods:** We isolated genomic DNA from peripheral blood of 50 individuals with SLE and 50 control subjects. Analysis of CTLA4 gene polymorphisms in polymorphic sites -318(CT) and +49(AG) was done by Tetra-ARMS-PCR. Methylation-specific polymerase chain reaction (MS-PCR) used to estimate promoter hyper methylation of the CTLA4 gene. Also we analyzed CTLA4 mRNA levels in 30 blood samples from cases and healthy controls using real-time PCR.

**Results:** Promoter methylation changes of CTLA4 gene was remarkably different in patients with lupus in contrast with healthy controls (OR= 0.48; 95% CI= 0.1859, 1.202; P-value= 0.005). However, gene expression level of CTLA4 were not statistically different in patients and healthy controls

**Conclusion:** This epigenetic study may give us an overview of the role of promoter methylation in the pathogenesis of SLE. However, further studies with larger sample sizes on more populations require to be made to prove this theory in the future.

**Keywords:** Systemic lupus erythematosus, Promoter Methylation, Polymorphism, Gene Expression

**P-141: Correlation analysis between SNHG1 and miR-195 in colorectal tumor tissues**

Avazpour N, Hajjari M.
Colorectal cancer (CRC) is one of the most common types of cancer worldwide [1]. However, the molecular mechanisms involved in CRC initiation and progression is remained to be unknown. Recent studies have shown that up to 98% of the genomic DNA is transcribed to a complicated network of non-coding transcripts such as miRNA, LncRNA [2]. Also, it has been revealed that IncRNAs, as the major regulators of gene transcriptional system, are involved in different biological processes such as cell cycle regulation and cytoplasmic transport [3]. In this report, we studied the correlation expression level of SNHG1 IncRNA and miR-195 in colorectal cancer tissues. Method: We performed an in silico analysis on Starbase database and confirmed the results by experimental analysis of 20 pairs of CRC tissue samples through real time PCR. The correlation analysis between miR195 and SNHG1 genes was performed using SPSS v22. A P-value> 0.05 was considered as significant. Result: Our findings indicate that overexpression of IncRNA SNHG1 could contribute to a decreased miR-195 expression in tumor tissues in comparison with adjacent normal tissues. A Pearson score of 0.074 indicates that these two genes could have a clinically and statistically value. The deviation of 0.05 is probably due to the small sample size of the statistical population and further investigation is needed on the large sample sizes to find a stronger correlation. Discussion: Zhang et al. demonstrated miR-195 might act as a potential target of SNHG1 IncRNA [4]. As suggested by the Starbase database, miR-195 may interact with SNHG1. Since both SNHG1 and miR-195 expressions levels are altered in CRC, we suspected that SNHG1 may regulate miR-195 expressions, thereby affecting the carcinogenesis process of CRC. Our result indicated that SNHG1 and miR195 may act as potential biomarker in colorectal cancer.

Keywords: COLORECTAL CANCER, biomarker, SNHG1, miR-195

P-142: Optimization of the saffron (Crocus sativus) extract concentration on HER-2 gene reduction in adenocarcinoma gastric cancer cells

azari khanghah SH1, dellami khiabani Z2

Faculty of Basic Sciences, Zanjan Branch, Islamic Azad University, Zanjan, Iran
E-mail: Hamideh.azari.1368@gmail.com

The study of HER-2 gene in cancers, especially gastric cancer, is of great importance. This gene has been shown to increased expression rate in gastric cancer. The aim of this study was to investigate level of HER-2 gene expression in adenocarcinoma gastric cells(AGS) after treatment of the cells with Iranian saffron extract to choose the best concentration. The advantage of this extract is its anti-cancer properties and the absence of side effects.

105 AGS cell were cultured in 12 wells plate. The cells were treated with concentrations of 800, 1200, 2000 μg / ml of Iranian saffron extract for 72 hours and then RNA extraction and cDNA synthesis was performed using special kits. Finally, the HER-2 expression rate examined with specific primers using Real time PCR. GAPDH gene was used as an internal control. Data analysis was performed with 2 - ΔΔCT . Analysis of the results showed that the most reduction of HER-2 gene was in 2000 mg / ml concentration of saffron extract. There was almost 2 fold reduction of HER-2 gene. Herbal extract seem to be a good alternative to chemical drugs, given the effectiveness of preventing tumor growth and less side effects. In this study, Iranian saffron extract, have reduced HER-2 gene expression in AGS cell line effectively in higher concentration.

Keywords: HER-2 gene, Gastric cancer, AGS, Iranian saffron

P-143: Identification of the mutation in the ASL gene in patients with Argininosuccinic aciduria disorder in Southwest Iran

Azarshin SZ1,2, Zamani M1,2, Yadegari T1, Jahangirnezhad E1, Sarvari M1, Zeighami J1, Sharaitai Gh1,3, Sedaghat A1,4, Galehdari H1,2

1. Narges Genetics Diagnostic Laboratory, Ahvaz, Iran
2. Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Department of Genetics, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran
4. Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran

Keywords: Argininosuccinic aciduria, mutation, ASL gene

Introduction: Argininosuccinic aciduria is an autosomal recessive genetic disorder of the urea cycle. It caused by mutations in the ASL gene (OMIM 608310). This gene encodes Argininosuccinate lyase enzyme. This enzyme catalyzes the reversible breakdown of argininosuccinate to arginine and fumarate that leads to hyperammonemia, accumulation of argininosuccinic acid in body fluids. Methods: In the present study, Sanger sequencing of the whole ASL gene was performed in the patient with hyperammonemia and Parents. Results: A homozygous mutation c.706C>T (p.R236W) has been identified in this patient. Parents were heterozygous for the same mis-sense mutation. Therefore, 50 normal individuals were analyzed for this change with negative results. Conclusions: The mutation was found to be reported for the first time in Argininosuccinic aciduria patients in Southwest Iran. This mutation was predicted disease-causing by bioinformatics analysis and it was previously reported to affect the active site of the enzyme. Therefore, p.R236W may be useful in the clinical detection of Argininosuccinic aciduria in Iranian patients.

Keywords: Argininosuccinate lyase deficiency, ASL gene, mutation, Southwest Iran

P-144: The Importance of VEGF-KDR Signaling Pathway Genes should Not Be Ignored When the Risk of Developing Multiple Sclerosis is Taken into Consideration

Azimi Gh, Taheri M

Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
E-mail: ghazalehazimi.1990@yahoo.com

Abstracts of the 3rd International & 15th Iranian Genetics Congress

Keywords: Vascular endothelial growth factor (VEGF) and its receptor kinase insert domain-containing receptor (KDR) pathway trigger the process of angiogenesis as well as inflammation, which contributes to the development and progression of demyelinating lesions in multiple sclerosis. This work is a case-control study comprising of a total of 400 subjects with multiple sclerosis and 400 healthy controls. Participants were subjected to neurological examination and peripheral blood sampling for genotyping. Polymorphisms in the VEGF and KDR genes were
assessed using the restriction fragment length polymorphism (RFLP-PCR) method. A significantly higher frequency of the T allele and TT genotype of the VEGF 936C > T (rs3025039) polymorphism was found in the multiple sclerosis group than in the healthy control group ($P = 0.01$ 1.41 and $P = 0.013.12$, respectively). In addition, VEGF 936C > T showed an association with patients in a recessive model. However, the KDR -604T > C (rs2071559) polymorphism showed no significant difference in either allelic or genotype frequency between the two groups. Taken together, the results of the present study suggests that the T allele of the rs3025039 in VEGF gene could be considered a risk factor for developing multiple sclerosis in the Iranian population.

**Keywords:** Multiple sclerosis, VEGF, KDR, Polymorphism

**P-145: Association of Neuro D1 and leptin genes polymorphism in healthy and diabetes patients**

Azimnasab Sorkhabi P', Javanmard A', soltaniai1 M', Shahoori Z

1. Islamic Azad University of Ahar
2. Tabriz University

parviz.sorkhabi@yahoo.com

Diabetes is an increasingly common disease. Currently over 400 types of genes that are effective in the process of developing diabetes have been reported. Neuro D1 and Leptin Genes are associated with the development of diabetes. 25 patients selected regardless of sex from Hakim clinical laboratory and 10 cc blood samples were taken for biochemical parameters analysis and DNA extraction. The biochemical parameters were measured by the alpha auto analyzer and DNA quality and quantity were extracted determined by spectrophotometry and electrophoresis on agarose gel and DNA sequences determined by PCR technique. Our studies showed that Neuro D1 gene polymorphism is not associated with the risk of diabetes however This relationship exists in the leptin gene and leptin gene is associated with diabetes. A positive effect of Neuro D1 gene has been reported in some populations in the world, including Japan. In Iran population polymorphism of Neuro D1 gene just can be as a risk factor for Diabetes. Finally, by Chi-Square test we donâ€™t recognize any relationship between NeuroD1 and Leptin genes in formation of diabetes in fact These two genes are independent of each other. For data analysis, the statistical program R was used.

**Keywords:** Neuro D1, Leptin, Diabetes, PCR.

**P-146: Evaluation of BANCR level in CRC tumors and its association with clinical features**

Azizi E, Nazemolhosseini Mojarad E, Arbabian S

Department of cellular and molecular, North Tehran Branch, Islamic Azad University
elina.tirdad@gmail.com

**Introduction:** Colorectal cancer (CRC) is the third known cancer worldwide that has been mostly diagnosed at advanced stages. Therefore, finding new biomarkers with marked sensitivity and specificity is matter of urgent. Studies show that alteration of BANCR expression pattern is effective in the initiation and progression of tumorigenesis. So, we evaluated the level of this lncRNA within the CRC patients.

**Materials and Methods:** 52 tumor tissue samples and 10 normal controlswere collected along with from the cases that had been diagnosed and approved by the Taleghani Hospital, Tehran, Iran. Samples were subjected to RNA isolation and then reverse transcripted. The obtained cDNAs were amplified by Realtime-PCR method. Relative expression abundances of BANCR were measured by normalizing to Ribosomal 18S RNA using the 2-????CT method.

**Results:** The data showed a marked upregulation in the expression pattern of BANCR within the tumor samples compared to the controls. These observations were statistically associated with patient TNM stage, tumor location and differentiation.

**Conclusion:** Taking together, increasing of the BANCR provokes tumorigenesis in CRC patients. So, this lncRNA could be applied as diagnosis biomarker for cancer detection.

**Keywords:** BANCR, Colorectal cancer, Diagnosis biomarker, Tumorigenesis

**P-147: Investigation of the relationship between ZNF804A and DISC1 polymorphisms in RS61886494 and RS12133766 positions in schizophrenic patients**

Azizi P

Bsc of Cell and Molecular biology, Central Tehran Branch, Islamic Azad University
parisaazizi2017@gmail.com

**Introduction:** Schizophrenia is a mental disorder characterized by abnormal social behavior and failure to understand reality. The aim of this study was to determine the relation between FOLH1 and DISC1 genes polymorphism in patients with schizophrenia in Iran.

**Materials and Methods:** In this study, 50 patients with schizophrenia and 50 healthy controls were evaluated. PCR-RFLP was used for FOLH1 gene and Tetra-ARMS for the DISC1 gene for evaluation of single nucleotide polymorphism in both groups of patients and control. For enzymatic digestion of PCR products, the positions of RS61886494 and RS12133766 were used for enzyme MseI and BseLI and incubated for 37 hours at 16°C.

**Results:** The frequency of CC, CT and TT genotypes for FOLH1 gene in RS61886494 region was 92%, 8% and 0%. In the DISC1 gene, the frequency of GG, GA and AA genotypes in the RS12133766 region was 84%, 8% and 8%.

**Discussion and Conclusion:** For RS61886494 region, the frequency of CC and CT genotypes were 2% and 8% higher in healthy people, while TT genotype was 6% higher than patients in healthy people. Interestingly, TT genotype was not observed in patients and healthy genotype CT was not observed. Regarding the DISC1 gene, the results showed that the frequency of GG and AA homozygote genotypes in the patients was higher in the RS12133766 region when the heterozygote GA was high in healthy people and was not observed in patients with this heterozygote.

**Keywords:** Schizophrenia, Polymorphism, DISC1, FOLH1

**P-148: Identification of novel variants in sixteen Iranian patients with intellectual disability by targeted next-generation sequencing**

Babasalari M', Saleh-Gohari N1,2, YariA1,3

Department of Medical Genetics, Kerman University of Medical Science
m.salar1994@gmail.com

**Abstracts of the 3rd International & 15th Iranian Genetics Congress**

**P-148:** Identification of novel variants in sixteen Iranian patients with intellectual disability by targeted next-generation sequencing

Babasalari M', Saleh-Gohari N1,2, Yari A1,3

Department of Medical Genetics, Kerman University of Medical Science
m.salar1994@gmail.com
Background: Intellectual disability (ID) is one of the most common cognitive disorders affecting 1-3% of the general population. Both of environment and genetic factors is involved in etiology of ID. Diagnosis of ID by cytogenetic and molecular routine methods is difficult because of its extensive genetic heterogeneity. However, the advent of NGS has simplified the identification of mutations in ID-related genes.

Methods: In this study we performed NGS for 16 Iranian patients with ID. Via using a custom NimbleGen chip capturing, samples were screened for mutations in 514 genes that are associated with pathogenesis of ID according to NGS approach.

Result: By using the targeted sequencing, we identified one causative gene in 1 of 16 patients. The variant of this gene (PAH: c.727C>T) was previously determined pathogenic. Furthermore, 11 novel variants were identified that CDK5RAP2, C5orf42 and CDH15 genes were the most common ID-related genes. These variants were CDK5RAP2-c.171A>G, CDK5RAP2-c.868C>T, CDK5RAP2-c.1136T>A, CDK5RAP2-c.1946C>G, C5orf42-c.2911A>G, C5orf42-c.3358A>G, C5orf42-c.9367C>T, C5orf42-c.7979G>A, C5orf42-c.7400+7C>G, CDH15-c.1211C>T and CDH15-c.1251C>T. Also, all of the identified candidate causing-disease variants have been confirmed by direct sequencing. More analysis of this variants demonstrated that NLRP3-c.1695T>A and DPAGT1-c.85A>T are likely pathogenic.

Conclusion: The present study showed that target ID as a novel, highly-sensitive and rapid screening approach is facilitated the diagnosis of ID. Targeted sequencing due to selectable genes, affordable costs and high sequencing coverage has the advantage to better diagnosis of this heterogeneous disorder. Finally, we suggest that functional studies be conducted for these variants to determine whether these variants are cause of ID.

Keywords: Intellectual disability, Targeted sequencing, Next generation sequencing, NGS, ID, ID-related genes, Mental retardation, Novel variants

P-149: Investigating the Presence of Putative MicroRNA located in the E-Cadherin Human Gene

Badakhshian R1, Dabiri Sh2, Dokanehfard S3, Gharbi S4

Department of Biology, Faculty of Basic Sciences, Shahid Bahonar University, Kerman, Iran
Department of Pathology, Pathology and Stem cell Research Center, Afzalipour Medical School, Kerman, Iran
Department of Molecular Genetics, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran
Department of Biology, Faculty of Basic Sciences, Shahid Bahonar University, Kerman, Iran

The expression of many genes is regulated by microRNAs (miRNAs), which are an important class of small non-coding RNAs. Of over 55,000 estimated miRNAs in the human genome, about 2,600 miRNAs have already been identified. E-cadherin is a major cell adhesion molecule that is expressed in the surface of the epithelial cells, and its loss of expression plays a role in the metastasis of many cancers. Experimental evidences suggest the possibility of a functional relationship between the intragenic miRNAs and their host gene. Therefore, this study examined the presence of a putative miRNA in human E-cadherin gene. To predict miRNA-like structures, bioinformatics programs were used. From 27 predicted miRNAs, one of the most prominent miRNAs was selected. In order to facilitate the experimental identification of miRNA, its over expression was performed in HEK293 cell line. At first, the genomic region of putative miRNA precursor was PCR amplified and cloned into the pTG19-T vector. Then, this fragment sub cloned into the pEGFP-C1 expression vector. The recombinant vector was transfected into the HEK293 cell line and expression of miRNA was investigated by RT-PCR with specific primers designed for probable mature miRNA sequence. Finally, the exact sequence of this predicted miRNA was determined by sequencing. In subsequent studies, the target genes and functional analyses of this novel miRNA will be examined.

Keywords: novel microRNA, E-cadherin gene, bioinformatics programs

P-150: Overexpression of pvt1 in CML patients positive for t(9; 22) translocation

Bagherieh A, Galehdari H, Zamani M

Genetic laboratory, College of science, Shahid Chamran University, Ahwaz, Iran
atefeh.bagherieh@gmail.com

Introduction: The ENCODE project results revealed that only <3% of the human genome encodes proteins, while 87.3% of it, is transcribed. These results cause scientists to be familiar with new regulatory factors beyond protein functions in cells. Noncoding transcripts include two general categories: short noncoding RNAs and long noncoding RNAs. Progressing researches have uncovered the roles of lncRNAs in different cellular processes such as stress response, alternative splicing and cell cycle, but many questions about precise molecular mechanisms of them are remained to be answered. The lncRNA pvt1 gene -located in downstream of myc oncogene- shows dysregulation in many cancers. It is reported that pvt1 binds to oncoproteins, stabilizes them and causes to uncontrolled proliferation of cells. For example, pvt1 binds to myc protein and protects it from degradation by phosphorylation.

Material and methods: We have studied the expression of pvt1 and myc in CML. By using qRT-PCR. This study includes 35 blood samples positive for t(9; 22) translocation with MRD>1% and 43 blood samples negative for the Philadelphia chromosome.

Results: Our results show almost 2.8-fold overexpression of lncRNA pvt1 levels in positive samples compared to negative ones (p-value = 0.067), while there is no significant difference of myc expression between positive and negative groups.

Discussion: These results suggest that overexpression of pvt1 without an increase in oncoprotein levels can operate its own oncogenic function in cells. To our best knowledge, this is the first time that the pvt1 expression in CML is studied, but the mechanism of its function in this cancer needs more researches.

Keywords: CML, LncRNApvt1, long noncoding RNA, myc oncogene

P-151: Association of Vascular Endothelial Growth Factor, (VEGF), expression with tumor grade in esophageal cancer

Bahramian Sh1, Shafiee M2,3, Sahebi R4,5, roohinejad Z6

1. Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran
2. Department of Medical Genetics,Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran
P-153: Evaluation of the expression level of PINT Long non-coding RNA in tissue samples of patients with colorectal cancer

Bakhtiyari nezhad S1, Moradi A2, Nazemalhosseini-Mojarad E3, Tajadod G1

1. Department of cellular and molecular North Tehran Branch, Islamic Azad University, Tehran ,Iran
2. Department of Pathology, Shohada Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Gastroenterology and Liver Disease Research center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

Introduction: Colorectal cancer (CRC) is commonly diagnosed in advanced stages with poor prognosis so development and using of biomarkers such as long non coding RNA to detect at early stages and reduce cancer-related mortality is recommended. Long non-coding RNAs (lncRNAs) are non-protein coding transcripts longer than 200 nucleotides that have a main role in regulation of gene expression. In this study we evaluated the expression level of PINT Lnc RNA, main regulator of the P53 gene expression, in the tissue samples of CRC patients and compared with clinical characteristics.

Materials and Methods: In this study, 50 CRC tissue samples with pair normal were collected from surgery department of Taleghani hospital, Tehran, Iran. Lnc RNA PINT expression was evaluated using Real Time PCR method. Fold change of gene expression was evaluated by (2-∆∆ Ct) method.

Results: Down regulation of mRNA expression was found in PINT level in tissue samples of CRC patients comparing to pair normal. These changes were linked with patient's TNM stage (p<0.05).

Conclusion: The results of this study indicate that evaluation of PINT LncRNA expression in the tissue sample can be used as a biomarker for early diagnosis of colorectal cancer.

Keywords: PINT Lnc RNA, Colorectal cancer, early diagnosis, TNM

P-154: Study of effect of anticancer) metformin( drug on cell viability assay in T47D breast cancer cell lines on cell culture

Balakheyli H1, Asadi J2, Roshandel Gh3

1. Golestan Rheumatology research center, Golestan University of Medical Sciences, Gorgan, Iran.
2. Golestan metabolic disorders research center, Golestan University of Medical Sciences, Gorgan, Iran.
P-155: A Novel Mutation in Neurofibromatosis 1 (Case Report)

Bazireh H, Morovati S
1. Department of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Department of Human Genetics Research Centre, Baqiyatallah University of Medical Science, Tehran, Iran
Homabazireh@yahoo.com

Objective: Neurofibromatosis 1 (NF1) is the most common inheritable disease with estimated birth incidence of 1:3,000. Neurofibromatosis 1 is an autosomal dominant inherited disorder caused by germ line mutations in the NF1 tumor suppressor gene located on chromosome 17q11.2. NF1 (with a high frequency of spontaneous mutations) that features developmental changes in the nervous system, muscles, bones, and skin, most notably in tissue derived from the embryonic neural crest. Further, this disorder is characterized by cafe-au-lait spots, Lisch nodules in the eye, and fibromatosus tumors of the skin.

Methods: A 31-year-old lady with clinical presentation of Neurofibromatosis referred to Biogene Clinical and Genetics Laboratory in 2016. She had a positive family history of the disease including her father and some other members in the family. Blood sample was obtained then Nimblegen chip capturing the genes of NF1 and NF2 followed by Next Generation Sequencing was conducted.

Results: New likely pathogenic mutation (c.1261-2A>G, Het) on NF1 gene of the sample has been detected.

Conclusions: Although c.1261-2A>G mutation has not been reported previously, the frequencies of it in normal population are very low, and the splice mutation is expected to affect the mRNA’s splicing. The splice mutation c.1261-2A>G on NF1 gene, is possible to be the pathogenic mutation of the sample which is consistent with the clinical diagnosis. Another variant identified in NF1 gene of this patient was c.289-7A>G mutation in a heterozygous state.

Keywords: Neurofibromatosis 1; NF1 Mutation; next generation sequencing (NGS)
Introduction: One of the most common malignancies in women is breast cancer. Thrombin is a multifunctional serine protease involved in inflammation, coagulation and hemostasis. In this study the first aim is considering the molecular and signaling mechanism of thrombin tumorigenesis in breast cancer.

Materials and Methods: In this research the effect of thrombin on expression of Cyclin D1 protein is studied at mRNA and protein levels using Real Time and Western blot methods in human breast cancer cell line, MCF-7. Then to find out the signaling pathway responsible for tumorigenesis in breast cancer cells, some pharmaceutical inhibitors including PNU and Rapamycin, were used to inhibit the signaling pathways of Wnt and mTOR respectively.

Results: The results of this research showed that thrombin could play an important role in the proliferation of cancer cells by increasing the expression of Cyclin D1 in post-translational level. Further studies showed that the effect of thrombin-induced cyclin D1 overexpression is mediated by the activation of oncogenic mTOR/Wnt ?-catenin signaling axis, as the pre-treatment of breast cancer cells with pharmacological inhibitor of any of these two signaling pathways inhibits the effects of thrombin on increasing the expression level of Cyclin D1.

Conclusion: The results of this study show that thrombin enzyme would increase the Cyclin D1 protein through the Wnt and mTOR signaling pathways which might be a novel molecular mechanism for tumorigenesis property of thrombin. Moreover, inhibition of the involved signaling pathways could be a candidate to inhibit the effect of other simulator molecules for cell proliferation.

Keywords: Thrombin, Wnt signaling, mTOR Signaling, Cyclin D1

P-158: Digenic inheritance of non-syndromic hearing loss caused by novel mutations at MYO3A and DIABLO genes

Behroozi S1, Parvin F2, Fahimi H3, Jamali P4

1. Pharmaceutical Sciences Research Center, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
2. Department of Cell and Molecular Biology, Faculty of Science, Semnan University, Semnan, Iran
3. Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences and Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran
4. Shahrood Genetic Counseling Center, Welfare Office, Semnan, Iran
behroozi.samira@yahoo.com

Hearing loss (HL) is known as a most common sensorineural disorder affecting approximately 2 in 1000 neonates. Genetic factors are involved 50% of the all HL cases. The aim of this study was to explore the genetic factors of HL in two affected sisters derived from consanguineous marriage throughout an Iranian family from Semnan province. Targeted sequencing using Next Generation Illumina Sequencing was used to enrich all exons of 154 genes causing deafness. Subsequently, Sanger sequencing was used for confirmation of mutations found. The obtained results showed a novel stopgain mutation (c.C3154T:p.R1052X) in the MYO3A gene and a novel missense mutation (c.G235A:p.A79T) in the DIABLO gene of proband studied. Mutations in MYO3A and DIABLO genes are associated with nonsyndromic deafness-30 and young-adult onset of nonsyndromic deafness-64, respectively. A detailed analysis of the phenotypes and haplotypes shared by the affected sisters supported the notion that two genes segregated together with hearing impairment in the family suggesting the digenic inheritance pattern of HL. In addition, her consanguineous parents were heterozygote for either MYO3A or DIABLO genes. In overall, this study uncovered two rare novel stopgain and missense mutations causing HL of affected siblings in a digenic mode of inheritance. Such studies may help to conduct genetic counseling and prenatal diagnosis more accurately for individuals at the high risk of this type of HL.

Keywords: Hearing loss, Digenic inheritance, Mutation, MYO3A, DIABLO

P-159: Study of c-Src gene expression in response to an anti-angiogenesis peptide in breast cancer mice

Bejary M, Talesh Sasani S, Asghari SM
maede9371@gmail.com

Breast cancer is the main cause of cancer death in women. Human p60c-Src (or c-Src) is a 60 kDa non-receptor tyrosine kinase encoded by the Rous sarcoma gene (Src). C-Src functions in several signal transduction cascades that affect cellular proliferation, motility, differentiation, survival and regulate blood vessels growth. Tyrosine phosphorylation of VEGF receptor1 (VEGFR1) facilitates binding to the Src homologue domain. Inhibition of vascular endothelial growth factor (VEGF) binding to its receptor increases the efficiency of chemotherapy. In this study we will assess c-Src expression level in Balb/c mice (pasteur institute, Iran) having 4T1 cell-line induced and were treated with a VEGFB antagonist peptide. Breast tissue samples were obtained from treated and untreated mice (as control). Total RNA was isolated using Trizol reagent(Invitrogen) followed by cDNA synthesis. Real time polymerase chain reaction was carried out using c-Src specific primers and relative expression of c-Src gene was assessed. We used U6 gene as the reference. Our results showed the expression level c-Src gene was different between treated group and control. The results suggest that Src inhibition may be a useful drug target for angiogenesis suppression.

Keywords: Angiogenesis; VEGFR1; c-Src; Cancer

P-160: Genetic study of the NOTCH3 gene in CADASIL patients

Chavoshi Tarzjani SP1, Shahzadeh Fazeli SA2,3, Sanati MH4, Mirzayee Z5

1. Department of Biological Sciences, Tehran North Branch, Islamic Azad University, Tehran, Iran
2. Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran
3. Departments of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran
4. National institute for Genetic Engineering and Biotechnology, Tehran, Iran
5. Fazeli-Sanati Genetics Laboratory, Tehran, Iran
Romisa_magic@yahoo.com

Background: Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is a monogenic, hereditary, neurological syndrome characterized
Peripheral blood samples were collected from 10 CADASIL patients to extract genomic DNA. DNA sequences of exons 2-8, 11-12 and 18-19, where NOTCH3 mutations are typically located; were amplified by using PCR and analyzed by direct sequencing.

**Result:** 11 NOTCH3 exons were analyzed. Homozygous IVS7+15A>G mutation in 5 number of patients, Homozygous IVS7+16A>G mutation in 1 number of patient, Heterozygous for the Pro109Thr and Pro203His mutation in 1 number of patient, were found; which were not reported previously. Heterozygous for the C395R and R153C mutation in 2 number of patients were found. One of the total numbers of patients has no mutation in 11 analyzed NOTCH3 exons.

**Conclusion:** We found four novel mutations (P109T, P203H, IVS7+15A>G and IVS7+16A>G) and 2 reported NOTCH3 mutations. Exon 4 and Intron 7 are hotspots in the patients we examined with NOTCH3 mutation. These findings broaden the mutational spectrum of CADASIL.

**Keywords:** CADASIL, NOTCH3, mutation, Exon

P-161: Evaluation of Combined Status of Fas-P53 SNPs in Relation to Breast Cancer Risk

Dastmalchi N¹, Safaralizadeh R¹, HosseinipourFeizi MA², Pouladi N²

1. Animal Biology Department, University of Tabriz, Tabriz, Iran
2. Cellular and Molecular Biology Department, Azarbaijan Shahid Madani University, Tabriz, Iran

A single nucleotide polymorphism (SNP) in the proline-rich domain of P53, codon 72 polymorphism (a G-C exchange) can alter the structure and apoptotic role of P53 protein thereby may be causing a deficiency of FAS expression level and its tumor suppressor action. A common -1377 G-A exchange is found in FAS promoter, which can reduce the expression level of FAS. Previously, these polymorphisms have been shown to be associated with various tumor types, independently. It is probable that the investigation of combined genotypes may be more valuable than individual SNPs. In the present study, we have investigated whether the combination of the P53 and FAS polymorphisms could be responsible for genetic susceptibility to breast neoplasms. 90 breast cancer patients and 120 healthy controls entered the investigation. The javastat online statistics package software was used for statistical analysis. There was no significant discrepancy for various FAS-P53 combined genotypes between cases and control subjects in this study (p>0.05). Thereby, the current study showed no significant correlation between FAS-P53 polymorphisms combinations and breast cancer possibility in the examined population.

Keywords: FAS; P53; combined genotype; breast cancer

P-162: miR-142-3p Expression in Different Breast Cancer Cell Lines

Dehghan R², Mansoori B¹, Moradi M¹, Baradaran B¹

1. Faculty of Veterinary medicine, University of Tabriz, Tabriz, Iran
2. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

raziyeh_dehghan71@yahoo.com

**Introduction:** Breast cancer is the most common malignancy diagnosed in women, and the incidence of breast cancer is increasing every year. MicroRNAs are 22â€“25 nucleotide RNA segments that engage in post-transcriptional regulation by targeting messenger RNA sequences. These endogenous small non-coding RNAs play significant roles in tumorigenesis and tumor progression. The miR-142-3p expression is dysregulated in several breast cancer subtypes. One of the aims of breast cancer treatment is to restore the expression of tumor suppressor miRNAs to normal levels in breast cancer cells by miRNA replacement therapy. Expression of Mir 142-3p decreases in breast cancer. This study aimed to evaluate the expression of Mir 142-3p in different breast cancer cell lines.

**Methods:** Four main cell-lines including Mcf-7, MDA-MB231, MDA-468, and SkBR3 were chosen. After culture, their total RNA was extracted and cDNA was performed by cDNA synthesis kit. The micro RNA expression was determined using qRT-PCR.

**Results:** miR 142-3p expression in MDA-MB-231 cell line was the highest among the others in the vicinity of 0.014. Whereas, its expression was approximately the same in cell lines MCF-7 and SKBR3 with around 0.007. The least expression levels belonged to cell line MDA-MB-468 which was roughly reached 0.004.

**Conclusion:** In this study, we found that expression of miR-142-3p in the MDA-MB468 cell line is less than the MDA-MB-231, SkBR3 and MCF-7 cell lines.

Keywords: miR-142-3p, Breast Cancer, MCF-7, MDA-MB231, MDA-468, and SkBR3

P-163: Report of an abnormal chromosome 10 in a patient with Dysmorphism and Congenital Heart Defect(CHD)

Dokhanchi A¹, Bagherizadel H¹, Hadipour F¹, Vahedi R¹, Behtjati F²

1.Sarem Cell Research Center & Department of Medical Genetics, Sarem Hospital, Tehran, Iran
2.Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
f_behtjati@uswr.ac.ir

**INTRODUCTION:** Chromosomal abnormalities can contribute to dysmorphism and congenital heart defects. Both numerical and unbalanced structural chromosome abnormalities can lead to these anomalies. The unbalanced structural abnormalities can be generated due to recombinant product of pericentric inversion in one of the parents during gametogenesis.

**PATIENT AND METHODS:** The current study presents the case of a three- month- old female child with dysmorphic features and congenital heart defects(CHD). The clinical features of the patient included Congenital Heart Defect, Hirsutism, Small Nose, Simple Ear, Snaddle gap, Micrognathia, Long Filtrum and IUGR. Chromosomal analysis was carried out using T lymphocytes and standard cytogenetics techniques. 20 metaphase spread were studied using high resolution GTG banding technique.
RESULTS AND DISCUSSION: Cytogenetic analysis revealed a karyotype of 46,XX,add(10)(q26.1) in the child. Parental karyotyping was requested in order to determine the origin of this abnormality. Chromosomal analysis revealed a karyotype of 46,XY,inv(10)(p11.2q26.3) in the father and a karyotype of 46,XX in the mother. Pericentric inversion of chromosome 10 can give rise to recombinant chromosomes by duplication and deletion of 10p and 10q. Chromosomal analysis in this child revealed a karyotype of 46,XX,rec(10)dup(10p)inv(10)(p11.2q26.3)pat. Several defects had been reported to be associated with gene mutations/deletions within 10q, such as ectrodactyly (split hand/split foot malformation) at locus 10q24-10q25. A multiple gene locus study of 10q reported multiple defects.

CONCLUSION: Cytogenetic investigation for patients with dysmorphism and CHD is strongly recommended. Use of Array-CGH is further recommended if the Karyotype is normal.

KEYWORDS: Chromosome 10, pericentric inversion, Recombinant product, Congenital heart defect, Dysmorphism

P-164: Frequency of IVS II-1 ?-thalassemia mutation in Baloch population of Chabahar city

Dolatabadi F, Soheili F, Naseri F

Department of Marine Biology, Faculty of Marine Sciences, Chabahar Maritime University, Chabahar, IR, Iran
f.a.dolatabadi71@gmail.com

?-thalassemia is an inherited autosomal disorder which reduces the production of hemoglobin. Considering the multi-ethnic population in Iran, there is expected to be a various range of mutations in this region. The aim of this study was to define the molecular Frequency of IVS II-1 ?-thalassemia mutation in in Baloch population of Chabahar city. After sampling from 101 patients with major ?-thalassemia from the Baloch population of Chabahar, DNA was extracted and IVS II-1 mutation was performed using Amplification refractory mutation system (ARMS) technique. Age, gender, history, and consanguinity between the parents were recorded by studying the patients archives.

The results showed that about 30 of these patients had IVS II-1 mutations. The results of this study show the widespread distribution and variation of ?-thalassemia mutations due to the high rate of consanguineous marriage in this population, as a result of which these findings can be useful in prenatal programs.

KEYWORDS: ?-thalassemia - Baloch population - Chabahar city

P-165: Expression of long non-coding RNAs (UCA1 and CCAT2) in the blood of multiple sclerosis patients

Dust mohammadi Zehi N, Dastmalchi R

Shahidbeheshti university of medical sciences , medical genetics department
mohamadzehinasim@gmail.com

Multiple sclerosis (ms) is an autoimmune and multifactorial disease ØE and its pathogenesis is associated with many genetic and environmental factors.incRNAs are a group of genes that have recently been identified as predisposing genetic factors for the development of many cancers. objectives: to study the expression of two incRNAs including urothelial carcinoma associated 1 (UCA1) and cancer-associated transcript 2 (CCAT2) in relapsing-remitting multiple sclerosis (RRMS) patients.

Method: in the expression of UCA1 and CCAT2 was evaluated in 50 RRMS patients compared to 50 healthy controls ,using the taqman real-time PCR technique. results: there was no significant difference between the overall expression of these two incRNAs between the case and control groups. however, there was a significant difference in the expression of UCA1 in female patients older than 40 years in comparison with healthy age-matched females. in addition, there was a significant correlation between the expression of UCA1 and CCAT2. conclusion: these results suggest the synergistic effects of UCA1 and CCAT2 on pathogenic aspects of MS , by affecting cellular signaling pathways such as WNT and NF-kB.

KEYWORDS: CCAT2, UCA1, multiple sclerosis

P-166: Investigation of IL-17A gene expression and its polymorphism (rs3819025) in patients with Bullous pemphigoid

Ebrahimi E', Tabatabaei Panah PS', Kavosi M'

1. Biology Department, Islamic Azad University-East Tehran Branch, Tehran, Iran
Elaheh.ebrahimi086@gmail.com

more appropriately known as bullae, at the space between the epidermis and dermis skin layers., some studies have shown an association between a IL-17A polymorphism (rs3819025) a mutation and BP disease susceptibility. Yet, these findings had so far not been independently replicated, and no data on a possible association of these mutations and BP in Iranian population were available.

METHODS: This study contains 20 AA patients and 20 healthy controls. Genomic DNA was isolated using DNG-plus and PCR-RFLP analysis was performed to detect IL-17A rs3819025, polymorphism. Thereafter, cDNA was synthesized after RNA isolation. IL-17A expression levels were measured by Real-time PCR. Several relevant information such as demographic data (age, gender,..) or clinical characteristics were analyzed for a possible effect of these factors on susceptibility to BP in patients.

RESULTS: There was no significant difference in genotypes of IL-17A(rs3819025) polymorphism, in both patients and control groups(P>0.05). IL-17A gene expression levels were significantly lower in patients with BP (0.709±0.49) in comparison with control individuals (2.14±0.36) (P=0.001).

CONCLUSION: This study did not showed an association between IL-17A(rs3819025) polymorphism and BP disease in Iranian population. Evaluation of IL-17A gene shows that the expression of this gene is significantly lower in patients than that of controls. This results shows that the genetic predisposition to develop BP can greatly varies among different ethnic groups.

KEYWORDS: Bullous pemphigoid, Autoimmune disease, IL-17-A rs3819025,PCR-RFLP,Real-time PCR


Ebrahimis NS1, Nazemalhosseini-Mojarad E2

1. Tehran shomal University,Tehran, Iran
2. Department of Pathology, Shohada Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3.Gastroenterology and Liver Disease Research center, Research Institute for Gastroenterology and Liver Diseases, Shahid Behesh-
**Introduction:** colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide. Long non-coding RNAs (lncRNAs) are defined as transcripts longer than 200 nucleotides that are not translated into protein. lnc RNA HOTAIR helps the invasion and metastasis of tumor cells. This molecule can reverse the process of converting epithelial to mesenchymal, and suppresses the invasion by inhibiting MMP1 and MMP3. Many studies emphasize the high expression of HOTAIR with the stage. Advanced tumor, lymph node metastasis, and poor survival.

**Materials and Methods:** In this study, 50 CRC tissue samples with pair normal were collected from surgery department of Taleghani hospital, Tehran, Iran. Lnc RNA HOTAIR expression was evaluated using Real Time PCR method. Fold change of gene expression was evaluated by (2-ΔΔ Ct) method.

**Results:** The data showed a marked upregulation in the expression pattern of HOTAIR within the tumor samples compared to the controls. These observations were statistically associated with patient TNM stage, tumor location and differentiation.

**Conclusion:** The alteration in the expression pattern of HOTAIR is assessable during CRC development, and therefore could be used as diagnosis biomarker for cancer detection.

**Keywords:** Colorectal cancer, Long none coding RNAs, Lnc RNA HOTAIR

**P-169:** Upregulation of hsa-miR-498 enhances tumorigenesis and metastasis and can be a putative biomarker in breast cancer

**Ebrahimi S O, Reiisi S**

Department of Genetics, Faculty of Basic Sciences, Shahrekord University, Shahrekord, Iran

**Ebrahimi.s.omar@gmail.com**

**Introduction:** MicroRNAs (miRNAs) are short non-coding RNAs that regulate the function of target genes at the post-transcriptional phase. miRNAs are considered to have roles in the development, progression and metastasis of cancer. The aim of this study is to compare expression level of miR-498 between tumoral and non-tumoral adjacent tissues and its association with metastasis.

**Methods:** In this study, 40 tumoral tissues of breast cancer and 40 non-tumoral adjacent tissues were enrolled. Total RNA was extracted and microRNA cDNA was synthesized, subsequently the relative gene expression was determined using quantitative real-time RT PCR (qRT-PCR) and evaluated by 2-ΔΔCt method. Finally, the expression pattern was analyzed by statistical analysis.

**Results:** The results showed that, the average of miR-498 relative expression was significantly higher in tumor tissues compared to adjacent healthy tissues (P = 0.0002) and its expression in metastatic tumor compared to non-metastatic sample was also significantly higher (P = 0.02). The receiver operating characteristic (ROC) curve was analyzed and The area under curve (AUC) for miR-498 was 69% (p < 0.001).

**Conclusions:** The results of recent study have revealed that miR-498 may promotes tumorigenesis and metastasis. The ROC curve analysis suggested that this miRNA can be a good tumor marker for breast cancer.

**Keywords:** miR-498, metastasis, breast cancer

**P-169:** Non-invasive screening for miR-20a and miR92a can explain pathophysiological variations between COPD and Mustard Lung: Genetic or epigenetic involvement

**Edalat H*, Ghorbani Alaveneh A, Tavallaee M**

Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran

**h597783@yahoo.com**

**Background:** Chronic obstructive pulmonary disease (COPD) and Mustard Lung (an illness resulting from Mustard gas used against Iranian victims by Iraqi forces) have many clinicopathological features in common making them difficult to distinguish. While COPD symptoms are gone after cessation of smoking, Mustard Lung symptoms progress by time throughout the decades after one-time exposure. While 20-30% of smokers develop COPD, only 1:50 of chemical patients progress severe stages of the disease demonstrating a stronger role of epigenetics in COPD compared to that of Mustard Lung.

**Objectives:** Thus, to achieve a precise understanding of the molecular pathogenesis of these respiratory diseases and to bypass the problem of obtaining lung tissue samples, a non-invasive screening method of bio-molecules [micro-RNAs (miRNAs)] in bio-fluids (serum) was performed. We compared the relative expression of miR-20a and miR-92a (as representatives of the miR-17/92 cluster) in COPD and Mustard Lung patients.

**Methods:** Stem-loop real-time quantitative polymerase chain reaction was employed for evaluation of miRNA expression. Student t-test and Mann-Whitney test were used for statistical analysis after performing one sample Kolmogorov-Smirnov (KS) test to check the normal distribution of samples (p<0.05).

**Results:** A 8.6 and 97 folds of reduction was obtained, respectively for miR-20a and miR-92a in COPD patients relative to Mustard lung victims.

**Conclusions:** Researchers confirmed the more intense role of epigenetics in COPD relative to Mustard lung observed at the bedside. This introduces innovative effective epigenetic strategies for treatment of respiratory diseases through regulating mir17/92 cluster as a target.

**Keywords:** Sulfur Mustard; Chronic Obstructive Pulmonary Disease (COPD); miR-20a; miR-92a

**P-170:** Augmented expression of long non-coding RNA ZEB1-AS1 associates with developed histopathological grade and promotes tumorigenesis in ovarian cancer

**Eftekhar Ki M, Malek ZK, Moraghebi M**

Student Research Committee, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

E-mail: maryam.e.k@gmail.com

The Ovarian Cancer is one of the most lethal diseases. Initial studies exhibited that long noncoding RNAs (IncRNAs) have fundamental regulatory roles in cancer biology. In current study, it has been tried to find a relation between the over expression of a new long non-coding RNA (IncRNA) Zinc finger E-box-binding homeobox1 (ZEB1) antisense RNA (ZEB1-AS1) in ovarian cancer tissues comparing to paired noncancerous tissues. Furthermore, the over expression of ZEB1-AS1 observed in ovarian cancer lead to increased cell growth and...
survive and also ZEB1-AS1 not only can suppress cell growth but also can inhibit migration and induce apoptosis in ovarian cancer cell lines. In conclusion, these findings designated that ZEB1-AS1 plays onco-lncRNA roles in ovarian cancer and it may become a novel molecular biomarker of prognosis and therapy in ovarian cancer.

P-171: Effect of 6-week interval training on Insulin receptor substrate 1 (IRS-1) expression in gastrocnemius muscle and insulin resistance in Wistar obese rats

Eizadi M1, Mirakhorli Z2

1. Department of Exercise Physiology, Saveh Branch, Islamic Azad University, Saveh, Iran.
2. Department of Physical Education and Sport Sciences, Amirkabir University of Technology, Iran
izadimojtahabi2006@yahoo.com

The purpose: Apart from the metabolic and hormonal components, genetic factors disorder play an important role in insulin function in target tissues. The aim of this study was to determine the effect of 6 week interval training on type 1 insulin receptors (IRS1) expression, fasting glucose levels and insulin resistance in obese male rats.

Methods: In present experimental-practical study, 14 male Wistar rats that fattened by a high-fat diet (6 weeks), were randomly assigned to control (without training) and exercise (interval training) groups. The exercise group performed 5 sessions per week for 6-weeks interval training. 48 hours after the last exercise session, fasting glucose levels, serum insulin, insulin resistance, and expression of IRS1 in the gastrocnemius muscle were measured in both groups. Data analysis was performed by independent t-test. Changes were less than 5% Was considered significant.

Results: Interval training were associated with fasting glucose (p <0.001) and insulin resistance (p <0.001) reduction in the exercise group compared with control group. Furthermore, interval training induced significantly increased in IRS1 expression in gastrocnemius muscle of exercise group compared to control group (p = 0.016). There was also a significant reverse relationship between change in relative expression and insulin resistance in response to exercise program (p = 0.019).

Conclusion: Improvements in Glycemic profiles and insulin resistance in obese rats following long-term interval training can be attributed to increased in expression of IRS1 in muscle tissue of the trained muscle.

Keywords: Interval training, obesity, gen expression, insulin resistance

P-172: Association between mir-145 rs41291957 gene polymorphism and idiopathic male infertility

Eskandari M1, Kohan L1,2*

1. Department of Biology, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran
2. Young Researchers and Elite Club, Islamic Azad University, Arsanjan Branch, Arsanjan, Iran
mohsen.eskandari93@gmail.com

Male infertility is any health issue in a man that lowers the chances of his female partner getting pregnant. This phenomenon, may be a result of genetic or environmental factors or both. Recent research has shown that miRNAs and their target mRNAs are differentially expressed in male infertility. The aim of this study, was to investigate association between mir-145 rs41291957 gene polymorphism and idiopathic male infertility.

Methods: This case-control study was done on 339 men with infertility and 289 healthy man as a control group. After DNA extraction, rs41291657 genotypes was determined using restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) method. The association between genotypes and the risk of male infertility was examined by odds ratios (OR) and 95% of confidence intervals (CIs).

Results: Our results showed that there were no significant differences in AG genotype frequencies between case and control groups regarding mir-145 rs41291957 polymorphism (OR = 0.71, CI = 0.46-Â–Aâ–½1.11, P = 0.13). In a dominant model for the A allele (AA + AG genotypes), there were no associated with male infertility risk (OR = 4.07, CI = 0.47-35.09, P = 0.2).

Conclusion: The finding of this study indicate that there was no significant association between mir-145 rs41291957 gene polymorphism and risk of idiopathic male infertility. This is the first study about association of mir-145 rs41291957 gene polymorphism and idiopathic male infertility risk. Further studies in other populations with larger samples are needed to confirm these findings.

Keywords: Male infertility, miR-145, Polymorphism, rs41291957

P-173: Newly modified ciprofloxacin causes G0/G1 cell cycle arrest and apoptosis in human leukemia K562 cells via down-regulation of Bcl2 and surviving

Esfandi F1, Mahdavi M2, Babaei E3, Mostafavi H1

1. Department of Biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran.
2. Department of Organic Chemistry & Biochemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran.
majid.mahdavi@tabrizu.ac.ir

INTRODUCTION: Ciprofloxacin (CP) is an antibiotic with low side effects, which have anti-proliferative and apoptotic activities in numerous cancer cell lines. Prior studies indicated several CP derivatives, displayed higher in vitro anticancer activity than parent CP anticancer activity. Here, the anti-proliferative and apoptosis-inducing effects of a new modified CP, 1-isopropyl 4-methyl ciprofloxacin (IMC), was examined in human chronic myeloid leukemia (CML) K562 cell line.

METHODS: Chronic myeloid leukemia K562 cells were treated with different concentration (10-120 Â–M) of IMC and cell viability determined by MTT assay. Induction of apoptosis evaluated morphologically by fluorescence microscope, as well as cell cycle analysis. Expression levels of some anti-apoptotic genes including Bcl-2 and Survivin were analyzed by qRT-PCR.

RESULTS: IMC mediated growth inhibition in a dose- and time-dependent in the K562 cells. G0/G1 cell cycle arrest was showed after treatment of the cells with IC50 value (20 Â–M) of the compound. Furthermore, the qRT-PCR analysis revealed that treatment of the K562 cells with IMC down-regulates the expression of Bcl-2 and Survivin.

CONCLUSION: According to the present data, it looks that this new modified CP is a good candidate for further evaluation as an effective chemotherapeutic drug in CML.

Keywords: Ciprofloxacin, Apoptosis, Chronic myeloid leukemia, Bcl-2, Survivin.

P-174: Pediatric Cancer and Li-Fraumeni Syndrome in
North West of Iran

Esmaeilzadeh Aghjeh M, Hosseinpour feizi M A, Safaralizadeh R, Hosseinpour feizi A A, Pouladi N

University of Tabriz
es_maryam70@yahoo.com

Introduction: In 1969, Li-fraumeni syndrome (LFS) which is a rare and cancer predisposition syndrome, has been reported by Frederick LI and Joseph F.Fraumeni for the first time. In 1990, Malkin, et al. have represented that, the main problem in LFS is mutation in TP53 gene that is a crucial tumor suppressor gene in cell cycle. Therefore, any alternation or mutation in the TP53 gene will cause some abnormalities in genome which leads to cell overgrowth and eventually cancers.

Material and Methods: In this study, 45 children with cancer in North West of Iranian population were investigated. Patients DNA have been extracted using high salt method, then the region within exons 5 to 8 have been replicated via PCR method then sequenced the products and finally analyzed the results.

Results: In 12 cases (26.67%) we detected polymorphisms in Exon6 and Introns 6 and 7. In the examined probands, no mutation was observed in exons 5 to 8 of the TP53 gene to indicate the possibility of Li-Fraumeni syndrome in these families.

Conclusion: Our results show that, there was no mutation in exons 5 to 8 of the TP53 gene to indicate the possibility of LFS in these families, further studies need to be done in bigger population and can complete our data.

Keywords: Li-Fraumeni syndrome, cancer, TP53 gene, mutation

P-175: Complexity of beta thalassemia diagnosis: experience of a case study

Esmaili Kordar P, Taghavi Basmenj M, Mojtaban M, Bagheerian H, Zeinali S

1. Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
2. Kawsar Human Genetics Research Center, Tehran, Iran
3. Cancer Research Center, Golestan University of Medical Sciences, Gorgan, Iran
4. Cancer Research Center, Golestan University of Medical Sciences, Gorgan, Iran
5. Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran

Introduction: Beta thalassemia is an autosomal recessive disorder that is caused by reduced synthesis of beta-globin chains. Mutations in the HBB gene are responsible for the disease. Most of the common genetic defects in -thalassemia occur due to point mutations and small deletions in the -globin gene.

Method: Proband and his family were referred to Kawsar Human Genetics Research Center (KHGRC, Tehran, Iran). The proband was a 10 year old boy who underwent blood transfusion for three times during his life. Blood samples were collected, and genomic DNA was extracted using salting out procedure. DNA analyses were performed using ARMS PCR (Amplification Refractory Mutation System), MLPA (Multiplex Ligation-dependent Probe Amplification) and Sanger sequencing.

Results: According to the CBC electrophoresis result, the proband’s mother was normal (MCV: 96.4, MCH: 31.6, Hb: 13.1, HbA2: 3, HbF: 0.4); the proband’s father was carrier of beta thalassemia (MCV: 63.6, MCH: 19.7, Hb: 14, HbA2: 4.9, HbF: 0). Using ARMS-PCR showed homozygote mutation in the proband and heterozygote mutation in probands’ father. Both mutations were the same (frameshift (–AA)) but no mutation were found in the mother. This could be four possibilities for it: 1. Simultaneous occurrence of Alpha triplication with heterozygote frameshift (–AA). 2. Denovo deletion or duplication in HBB 3. Non-Parental Event 4. Denovo frameshift (–AA) mutation. 5. Maternal gonadal mosaicism. Possibility of 1-3 was rule out using MLPA and Identifier kit. The only explanation could be denovo frameshift mutation in mother or gonadal mosaicism which needs more investigation.

Conclusion: The result of this case study suggested that complete molecular testing must be performed in complex cases like what we have in this study which can help the family in prenatal diagnosis of their future embryos.

Keywords: Beta thalassemia, Beta globin gene, Iran, ARMS-PCR, MLPA

P-176: MEST intronic transcript 1 gene expression level is associated with Breast carcinoma tumorigenesis

Fakhari R1,2, Sattari A1, Bahramian Sh1, Saghaeian Jazi M1, Fazel A4, Shafiee M2,4,5

1. Department of Biology, Gorgan Branch, Islamic Azad University, Gorgan, Iran
2. Stem Cell Research Center, Golestan University of Medical Sciences, Gorgan, Iran
3. Biochemistry & Metabolic Disorders Research Center, Golestan University of Medical Sciences, Gorgan, Iran
4. Cancer Research Center, Golestan University of Medical Sciences, Gorgan, Iran
5. Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran

Esmailzadeh Aghjeh M, Hosseinpour feizi A A, Pouladi N

Introduction & aims: MEST intronic transcript 1 (MESTIT1) is a long non-coding RNA located in one of introns of Mesoderm Specific Transcript (MEST) gene, which supposed to be involved in the regulation of MEST expression. Both MEST and MESTIT1 genes are paternally imprinted in fetal tissues. MESTIT1 is composed of two exons separated by an intron which is transcribed in antisense direction from a shared promoter region (P2) with MEST gene. Both genes are located in chromosome 7q31–34 that has been associated with different types of cancers including breast and prostate carcinoma. We aimed to investigate the expression level of MESTIT1 gene in breast tumor tissue and matched marginal non-tumor tissue of 38 individuals.

Methods: for this purpose total RNA was extracted from the fresh frozen tissues and then was reverse transcribed to the cDNA, following the DNaseI treatment. The MESTIT1 gene expression level was assessed using the specific primer and RT-PCR or qRT-PCR Sybr green techniques. The gene expression was normalized to GAPDH housekeeping gene (2-dct). Finally data was analyzed by SPSSv.16.

Results: our finding showed that MESTIT1 gene is expressed mainly in normal tissues (39.4%) however its expression was undetectable (86.8%, p value=0.009) or was down-regulated (mean of normalized gene expression T/N: 0.08, p value=0.1) in breast tumor tissues.

Conclusion: altogether our results indicated that MESTIT1 gene, as an antisense non-coding RNA might have a function in breast cancer tumorogenesis or progression and it can be considered as a potential molecular candidate for future experiments.

Keywords: Breast tumor, MESTIT1 gene expression, RT-PCR, down-regulation

P-177: The p22phox gene -930A/G and A640G polymorphisms are not associated with essential hypertension in...
Iranian hearing loss patients

Alimardani M1,2,4*, Farjami M1-4, Mojarrad M1, Shekari Khani-ani M1,4, Mansoori Derakhshani S1.2,4*

1. Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Department of Medical Genetics, Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of Medical Genetics, Mashhad University of Medical Sciences, Mashhad, Iran
4. Ebne sina Medical Genetic Diagnostic Laboratory, Tabriz University of Medical Sciences, Tabriz, Iran
5. Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Introduction: non-syndromic hearing loss (NSHL) is an autosomal recessive disease with incidence at least 1.9 per 1000 infants at birth. Efficient molecular diagnosis needs a detailed knowledge about genes and mutations involved in NSHL patients for individual populations. Today, several genes have been studied in Iranian population; whereas some other genes such as SLC26A4 and CDH23 genes are not important causes of autosomal-recessive NSHL in Iranian population.

Methods and materials: we are used allele-specific polymerase chain reaction (PCR) for detection of five mutations include c.719C>T and c.6085C>T in CDH23 gene, and IVS7-2A>G, c.1975G>C, and c.2168A>G in SLC26A4 gene in 100 patients with autosomal-recessive NSHL, originating from Eastern-Azerbaijan and Khurasan province, were screened.

Results: In all samples, none of mentioned mutations could be identified. The results were checked by sequencing in 15 samples and the absence of these mutations was validated.

Conclusion: It seems that although the precise portion made by such mutations needs to be defined using a larger patient cohort, the present data show that these mutations in the CDH23 and SLC26A4 genes are not important causes of autosomal-recessive NSHL in Iranian population.

Keywords: hearing loss, CDH23, SLC26A4, Iranian population.

P-180: Down-regulation of SNF-related kinase (SNRK), in patients tissue samples with breast cancer

Fattai Sh1, Shafiee M.1-2, Bahramian Sh1, F T. Shamsabadi F1, Fazel A4

1. Golestan Research Center of Gastroenterology and Hepatology, Golestan University of Medical Sciences, Gorgan, Iran
2. Department of Medical Genetics, Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran
3. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Golestan University of Medical Sciences, Gorgan, Iran
4. Cancer Research Center, Golestan University of Medical Sciences, Gorgan, Iran

mohshafa@gmail.com

Introduction: Breast cancer is known as one of the most salient and prevalent cancers among women and responsible for death in people, affected with this cancer. This study aims to investigate a coding protein called SNRK. Whose function is not well characterized. According to studies conducted, we have come to the conclusion that SNRK is a member of the sucrose non-fermenting (SNF)-related kinase family of serine/threonine kinases, a ubiquitous expression in the bone marrow. As a matter of fact, this gene is generally known as cellular
metabolism and adipocyte inflammation regulatory. SNRK has been studied in thin body of cancers, while it has Down-regulated in colon cancer.

In this study, we have investigated expression changes of SNRK gene in clinical tissue samples of breast cancers for the first time.

Methods: In the present study, clinical samples of breast cancer tissues were separately collected and SNRK expression levels were measured through total RNA isolation, cDNA synthesis and quantitative Real-Time PCR, in tumor tissue cells and their normal tissue margins. Data was statistically analyzed.

Results: Our data analyses indicate that the expression level of SNRK gene is down-regulated in tumor cells compared to their normal tissue margins.

Conclusion: In overall, this is the first report of SNRK in a cancer, especially in breast cancer. Based on the outcomes of the current research, SNRK may potentially considered as a tumor suppressor, but it corroboratation requires further investigations.

Keywords: SNRK gene, breast cancer, SNRK expression

P-181: Identification Of miR-24 and miR-27 as CFIm25 regulator in glioblastoma multiforme

Foroutan M, kouhkan F, hashemi M

1. Islamic Azad University, medical branch, Tehran, Iran
2. Stem Cell Technology Research Center, Tehran, Iran
3. Islamic Azad University, medical branch, Tehran, Iran
mozhgan.forootan@yahoo.com

Identification of miR-24 and miR-27 as CFIm25 regulator in glioblastoma multiforme

Introduction: Glioblastoma Multiforme (GBM) is one of the most malignant types of central nervous system tumors. Cleavage Factor Im (CFIm) is an essential component of the pre-mRNA 3' processing complex that act through the regulation of poly(A) site selection. CFIm25 is a tumor suppressor gene that when decreased increases cell proliferation and tumor growth via stimulation of proximal poly A site usage. Recent data shown different roles of miRNAs in various tumors including GBM. MicroRNAs (miRNA) are small non-coding RNAs that function in regulation of gene expression acting at the post-transcriptional level. According to significance of miRNAs in tumors the aim of this study is investigation of miR-24 and miR-27 roles in regulation of CFIm25 in glioblastoma.

Material and Methods: miR-24 and miR-27 were cloned to pCDH vector. U251 cells were transduced with (pCDH- miR-24) and (pCDH- miR-27) viruses then the expression level of miRNAs and CFIm25 were estimated by QRT-PCR in normal and normal cells. Also luciferase assays were performed for confirmation of miRNAs binding to CFIm25.

Result: Real-time PCR showed that the overexpression of these miRNAs decreased CFIm25 in U251 cell line.

Conclusion: Our data indicated that miR-24 and miR-27 could play important roles in inhibition of tumor growth through regulation of CFIm25 in GBM.

Keywords: GBM, CFIm25, miRNA

P-182: Analysis of HLA haplotypes association in Multiple Sclerosis patients from Khuzestan province

Galehdari H1, Mohaghegh M*, Madjinasab N2, Khatami SR1, Shafiee M1, delfan N1, latifi T1, Zabihi R1, Ghanbari Mardasi F1, Ghanavati R1, Shariati Gh1, Hosseini Behbahani M4

1. Department of Genetic, Faculty of science, Shahid Chamran University, Ahvaz, Iran
2. Department of neurology of Ahvaz jundishapur university of medical sciences, musculoskeletal rehabilitation research center, Ahvaz, Iran
3. Narges Medical Genetic Laboratory. Mihan east 18, kianpars, Ahwaz, Iran
4. Department of Biochemistry, Payame Noor University, Tehran, Iran
m_mohaghegh87@yahoo.com

Introduction: Multiple sclerosis is neurodegenerative disease results from the interaction between both genetic and environmental factors. HLA genes have been identified to be involved in MS susceptibility. The HLA gene variants are associated with some autoimmune disease. Our aim was to study the association of this haplotypes in MS patients from Khuzestan province.

Methods: In this case-control study, the association of HLA alleles was investigated in 200 multiple sclerosis patients from Khuzestan province (168 female and 32 male), with female-male ratio about 4:1. HLA-typing was performed by polymerase chain reaction with sequence-specific primers (PCR-SSP).

Results: we find that some haplotype of HLA have significant association in MS patients such as HLA-A*03(46.5% patients vs. 29.5%controls, p-value:0.000 ), &HLA-DRB1*1501(41.5% vs. 22.8%, p-value:0.001); while two haplotypes have no significant association: HLA-DQB1*0602 (61.5% vs. 64%, p-value:0.605) and HLA-DRB5*01(27.72% vs. 21.39%, p-value:0.148), and two haplotypes have negative association in MS patients: HLA-A*02 (29.5% vs.54%, p-value:0.000) & HLA-DQA1*0102 (60% vs. 73%, p-value:0.006).

furthermore, we analyzed the haplotype map of this patients and find that 20 % of patients have two significant haplotypes (HLA-A*03, and HLA-DRB1*1501)together.

Conclusion: confirmed other studies, HLA-A*03, and HLA-DRB1*1501 alleles can increase the risk of MS in khuzestan population, and HLA-A*02 and HLA-DQA1*0102 alleles probably have protection function and can reduce the risk of MS. We think that HLA haplotypes can use as a primary marker for distinguish of MS susceptibiltiy.

Keywords: Multiple sclerosis, genes, HLA-typing

P-183: Expression Analysis of GSTT1-AS1 Long Noncoding RNA and Its Coding Target Gene, TNFA, in Iranian Multiple Sclerosis Patients

Ganji M1, Taheri M1,2, Omrani MD1,2, Sayad A1

1. Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
2. Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
ar.sayad@sbmu.ac.ir

Introduction: Multiple sclerosis (MS) is a complex autoimmune disorder and the most common cause of nontraumatic disability in young people. Long noncoding RNAs (IncRNAs) have been recently reported to participate in immune responses adjustment. Likewise, epigenetic regulation of gene expression is substantial in immunopathology of various autoimmune diseases.

Methods: The aim of this experimental study was to investigate the expression levels of GSTT1-AS1 IncRNA and TNFA gene, as its target, in the blood of 50 relapsing-remitting MS (RR-MS) patients and 50 healthy controls through SYBR
P-184: Genetic study of 21 Iranian patients affected with hemophilia B

Ghadyani F¹, Morovvati S²

1. Department of Cellular and Molecular, Faculty of Biology Sciences, Islamic Azad University of Tehran-North, Tehran, Iran.
2. Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.

Fatemeh.ghadyani210@yahoo.com

Introduction: Hemophilia B is a recessively inherited X-linked bleeding disorder which results from deficiency of procoagulant factor IX (FIX). This disorder is caused by various defects in the factor IX gene, which is, being about 34 kb long and consisting of eight exons, localized in the Xq27 locus. Exon 8 is the largest exon of F9 gene and representing almost half of the F9 coding region. Approximately half of all F9 mutations are found in this exon. The birth prevalence of hemophilia B is approximately one in 30,000 live male births worldwide. To date, more than one thousand pathogenic mutations have been found in the F9 gene, most of them are missense mutations.

Materials and Methods: Blood samples from 21 Iranian patients affected by F9 deficiency were collected and mutation analysis of all exons and their intron-flanking regions of F9 gene were performed using PCR and sequencing methods.

Results: 21 mutations were identified in Factor IX gene including seven mutations in exon8, five mutations in exon2, two mutations in exon5, one mutation in exons 3, 4, 6 and 7, two mutations in promoter region and one mutation in intron4. The mutations found in this study included 10 missense mutations, 7 nonsense mutations and 1 deletion.

Conclusion: In this study, as in previous studies, missense mutations were the most common type of mutations and most mutations were found in exon8. We found five novel mutations including, p.C97Y, p.N138T, p.G253EsX11, p.S369V, and p.T381K in our patients.

Keywords: F9, gene, mutation, Hemophilia B, novel

P-185: Discovery of a Novel microRNA Located in Human ERBB4 Gene

Ghaemi Z, M.Soltani B, Mowla SJ

Molecular Genetics Department, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
zghaemi@gmail.com

Introduction: The HER signaling pathway is one of the major pathways associated with various cancers, including breast cancer. The pathway contains four cell surface receptors: ErbB1/EGFR, ErbB2/HER2, ErbB3, and ErbB4. ErbB4, as one of the HER receptors, activates several intracellular pathways via their capability to interact with numerous signal transducers and influences the variety of cellular processes. Some of the varied functions of ERBB4 could be mediated by a microRNA embedded within the gene. MicroRNAs act as post-transcriptional regulators of gene expression and their dysregulation affect signaling pathways in carcinogenesis. The objective of this study was to introduce a novel miRNA as a possible regulator of HER signaling pathway.

Methods: RNAfold and Mfold online tools were employed to search for possible microRNA hairpin structures within the gene. Conservation status of the ERBB4-miR1 and its precursor sequence was examined using blast search for human genome and other organisms in UCSC database. For miRNA expression analysis, Sequence Read Archive (SRA) was evaluated in different human cell lines and tissue. CID-miRNA was used for prediction of Drosha processing sites. The genomic fragment containing ERBB4-premir1 was cloned into PEGFP-C1 vector and the human cell lines were transfected with the vector. To evaluate endogenous and exogenous expression level of ERBB4-miR1 in human cell lines and breast tumors, RT-qPCR method was used.

Results and Discussion: Predicted miRNA expression was evaluated in different breast cell lines and tumors to verify its endogenous expression. Furthermore, Overexpression of ERBB4-premir1 vector in human cell lines resulted in production of mature exogenous ERBB4-miR1 that represents the successful processing of predicted miRNA precursor. The results along with bioinformatics data introduce ERBB4-miR1 as a novel miRNA encoded within the ERBB4 gene. Hence, deregulation of ERBB4-miR1 might play a pivotal role in malignancies through its effect on HER signaling pathway but more experimental validation of methods are needed to uncover ERBB4-miR1 role in HER signaling pathway regulation and breast cancer.

Keywords: ERBB4, microRNA discovery, HER signaling, Breast Cancer

P-186: Association of the miR-365b Polymorphism with Colon cancer in Mashhad

Ghanaatgar Kasbi S

Department of Biology, Khorasan Razavi Science and Research Branch, Islamic Azad University, Neyshabur, Iran.
ghanaatgar.sadaf@yahoo.com

As one of the most commonly diagnosed cancers worldwide, colorectal adenocarcinoma often occurs sporadically in individuals aged 50 or above and there is an increase among younger patients under 50. Routine screenings are recommended for this age group to improve early detection. The multifactorial etiology of colorectal cancer consists of both genetic and epigenetic factors. Recently, studies have shown that the develop-
P-187: Mll2 gene expression changes and related clinicopathological features in breast cancer

Ghanbari M, Hosseinpour Feizi M.A, Safar Alizadeh R
Natural science faculty, university of Tabriz
E-mail: mohammad.ghanbari3232@gmail.com

Breast cancer as a second cause of cancerous death among women, is one of the most prevalent cancer. In addition of variable genetic factors that cause breast cancer, epigenetic has an important role in initiation and development stages. Mll2 gene as a member of KMT2 family, methylates lysine 4 of histone H3 in promoters of target genes. According to studies MLL2 gene expression changes correlates with initiation and developmental stages of tumors, so to achieve this aim, tumors and clinicopathological features compared with MLL2 gene expression change levels in patient with breast cancer.

Methods and materials: 43 samples from fresh tumors and 43 marginal breast tissues were collected and kept in liquid nitrogen. RNA was extracted from tissues, CDNA synthesized and in order to gene expression changes study, real time PCR technic was carried out , for analyzing of data REST and SPSS softwares were used.

Conclusion: our study reports that MLL2 gene expression in tumor tissues shows significantly downregulation in compare with marginal tissues. Also, this study shows that MLL2 genes expression alters with clinicopathological features but, these changes are not significant.

Keywords: breast cancer, Epigenetic, MLL2, gene expression, real time PCR

P-188: Genome-wide analysis of long non-coding RNAs in Alzheimer's disease reveals the causal variant at chr17q22 susceptibility locus within the miR-142 promoter region

Ghanbari M1,2, Ma B3, Lendemeijer B4, Munshi Sh1, Bansal S1, Wang W3, J. Erkeland S1, Pan Q1, de Vrij F5, Kushner S1, Ikram MA1,2
1. Department of Epidemiology, Erasmus University Medical Center, Rotterdam, the Netherlands;
2. Department of Genetics, School of Medicine, Mashhad University of Medical Science, Mashhad, Iran
3. Department of Gastroenterology, Erasmus University Medical Center, Rotterdam, the Netherlands;
4. Department of Psychiatry, Erasmus University Medical Center, Rotterdam, the Netherlands;
5. Department of Neurology, Erasmus University Medical Center, Rotterdam, the Netherlands.

Genome-wide association studies (GWAS) have identified multiple loci associated with Alzheimer's disease (AD). However, the majority of AD-associated variants map to non-coding regions of the genome in which the biological consequences of candidate variants remain poorly understood. Here, we aimed to investigate the extent to which genetic associations with AD may act through long non-coding RNAs (lncRNAs). We retrieved genetic variants in lncRNAs from online databases and examined their association with AD risk by leveraging data from the available GWAS of AD. Genetic variants in five lncRNAs passed the significance threshold of p-value < 9.02E-10. For the associated variants, we performed various in silico studies (e.g. functional mapping and annotation to regulatory features and eQTL analysis) to prioritize the potential functional variants. Of these, the leading candidate variant localized to chr17q22, a recently-identified susceptibility locus for AD, that overlaps with two lncRNAs (BZRAP1-A51 and MIR142), was selected for further investigations. Our in vitro experiments (e.g. testing the impact of either alleles on the ncRNA expression level) provided evidence that the most likely casual variant in the locus occurs within the promoter region of mir-R-142. Furthermore, we used human induced pluripotent stem (iPS)-derived neural progenitor cells and miR-142 knock-out mice and found multiple putative mir-R-142 targets that may function to mediate the miRNA effect in relation to AD. Collectively, we confirm chr17q22 as a susceptibility locus for AD and suggest that the casual variant at this locus is located within the mir-142 promoter region, which functions by altering the mir-142 expression.

Keywords: Alzheimer's disease, Non-coding RNAs, GWAS, MIR142

P-189: Association between miR-34b/c rs4938723 and the recurrence risk in patients with breast cancer

Ghanbarpanah E1, Kohan L2, Mohammadianpanah M3, Tahmasebi S4
1. Department of Biology, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran
2. Colorectal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
3. Breast Diseases Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
4. elham.ghanbarpanah@gmail.com

Introduction & Aim: The recurrence of breast cancer (BC) is a major cause of cancer death in females. Mir-34b/c negatively regulates many cancers, by P53 gene targeting. The aim of our study was to examine association of the miR-34b/c rs4938723, with the recurrence of BC.

Methods: The present study was done on 424 females with BC (102 patients with the recurrence of BC, as the case group, and 322 women without any recurrence of BC, as the control group). After DNA extraction from peripheral blood, genotype
determination was done using Tetra ARMS PCR Technique. Thereafter, the genotype and allele frequency of miR-34b/c rs4938723 was compared between the two groups.

**Results:** The results were shown that TC (OR=3.777; P=0.001) and CC (OR=7.094; P=0.001) genotypes and C (OR=2.605; P=0.001) allele of miR-34b/c rs4938723 are associated with BC recurrence.

**Conclusion:** Thus, miR-34b/c rs4938723 polymorphism is associated with the susceptibility of BC recurrence.

**Keywords:** Breast Cancer Recurrence, miR-34b/c, Polymorphism, micro RNA

P-190: Investigation of TBX5, GATA4, BMP4, CRELD1, NKX2-5 genes and 22q11.2 deletion using multiplex ligation-dependent probe amplification test in patients with congenital heart disease

Ghassemi S, Kalayinia S, Salahshourifar I, Mahdavi M, Maleki M, Mahdieh N

**Department of Biology, College of Basic Sciences, Tehran Science and Research Branch, Islamic Azad University, Tehran, Iran**

Carcinogenetic Lab, Shaheed Rajaei Cardiovascular Medical and Research Center, Valiasr Ave, Niayesh Intersection, Tehran, Iran

srva.ghasmi20@gmail.com

**Introduction:** Congenital heart defects (CHD) are a group of heart structural disorders and blood vessels that are present at birth. CHD is one of the leading cause of childhood mortality that occurring in 1% of newborns, worldwide. Recent studies have demonstrated the role of copy number variations (CNVs) in CHD etiology. Regarding to the incomplete knowledge about the CHD pathogenicity in Iranian CHD patients, therefore, in this study we have surveyed the frequency of CNVs in CHD-related regions in 40 patients with non-syndromic CHD.

**Methods:** Sampling was performed of 40 CHD children who referred to the Rajaei Cardiovascular, Medical and Research Center between the period 2015-2016 and their diseases were diagnosed by heart specialists. The Syndromic CHDs were excluded from our study. Multiplex ligation-dependent probe amplification (MLPA) SALSA MLPA P311-B1Á kit was used for CNVs evaluation in GATA4, BMP4, TBX5, CRELD1, NKX2-5 genes and detection of 22q11.2 deletion syndrome.

**Result and Discussion:** We failed to found any deletion/duplication CNVs in CHD index members from 40 families. These results may have some explanations; small studied sample size so the absence of CNVs should be confirmed in larger numbers of patients, unknown CNVs which can't be detected by MLPA kit with known regions probes, and maybe genes other than GATA4, BMP4, TBX5, CRELD1, NKX2-5 genes involved in our CHD patients etiology.

**Keywords:** Congenital heart defect, Multiplex ligation-dependent probe amplification, Copy number variation

P-191: Synergistic Effects of Lauryl Gallate and Tamoxifen on Human breast Cancer Cell.

Ghatreh Samani K, Farrokhi E, Jafari M, Tabatabaea E, Jalilian N.

1. Clinical Biochemistry Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, IR Iran
2. Cellular and Molecular Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, IR Iran

3. Student Research committee, Shahrekord University of Medical Sciences, Shahrekord, IR Iran

E-mail: Mahbube71jafari@gmail.com

Tamoxifen (TAM) is widely used for adjuvant therapy in breast cancer patients. However tamoxifen therapy may lead to serious side effects. Anti-apoptotic substances in combination with chemotherapy drugs can result in additive or synergistic effects. Lauryl gallate (LG), a Gallic acid derivative, has been proven to inhibit tumor growth, without affecting normal cells. The aim of this study was to investigate the synergistic effect of TAM and LG in a breast cancer cell line (MCF-7).

**Methods:** Cultured MCF-7 cells were treated by final concentrations of 10 μM TAM alone, and in combination with 200 μM of LG. We also used EX-527, as SIRT-1 inhibitor in order to examine the role of SIRT1 in cell apoptosis. BCL-2 and SIRT1 gene expression were measured by real-time PCR method, and cell apoptosis was investigated by flow cytometry. Results: Tamoxifen alone and in combination with LG decreased BCL-2 expression 2.64 ± 0.75 and 6.38 ± 1.9 fold, respectively, after 48 h (p<0.05). SIRT1 expression was increased 1.67 ± 0.22 and 2.47 ± 0.34 - fold by TAM alone and in combination with LG, respectively (p<0.05). TAM alone and in combination with LG increased the percentage of apoptotic cells 15.79 ± 2.81 and 60.67 ± 6.23 percent, respectively after 48 h (p<0.001). Conclusion: Our results showed that the combination of LG and TAM is more effective for the induction of apoptosis in breast cancer cells, compared to individual use of each. Thus, our data pave the way for new therapeutic options for suppressing breast cancer growth.

**Keywords:** Tamoxifen, Lauryl gallate, Apoptosis, Breast Cancer

P-192: Causative gene discovery of rare Mendelian disorders: A 3-year cohort study

Ghayoor Karimiani E1,2, Beiraghi Toosi M3, Ashrafzadeh F4, Boostani R5

1. Next Generation Genetic Polyclinic, Mashhad, Iran
2. Honorary Research Associate, University of Manchester, UK
3. Pediatric Neurology Department, Ghaem hospital, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Pediatrics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
5. Department of Neurology, Ghaem Medical Center, Mashhad University of Medical Sciences, Mashhad, Iran

Eghayoor@gmail.com

**Backgrounds:** Whole exome sequencing (WES) is rapidly becoming a common molecular diagnostic test for individuals with rare genetic disorders. The possibility of a highly penetrant mutation in a single gene enters the differential diagnosis of rare genetic disorders. We set out to determine the molecular basis of different genetic disorders through whole exome sequencing technique.

**Methods:** One hundred families with at least two affected individuals and mendelian inheritance pattern compatible with genetic disorders such as intellectual disability, cardiomyopathy, seizures, epilepsy, paralysis, speech difficulties, vision and hearing problem have been conducted and entered into the rare mendelian genetic disorders cohort. A complete clinical and paraclinical examination have been done by expert specialists and clinical geneticist. Genomic DNA was evaluated through whole exome sequencing followed by bioinformatic analysis of data. According to segregation analysis parents and healthy children were examined for the candidate gene variants.
Results: Our data resulted in three gene discoveries followed by functional studies comprising KHLH24, IARS2, and RNFL170 genes in families with cardiomyopathy, mitochondrial and hereditary spastic paraplegia (HSP), respectively. Moreover, different rare pathogenic variants in several genes such as COQ4, TOE1, ALG1, ALDH5A1, MRPS35 and other genes have been found and confirmed through sanger sequencing in all family members.

Conclusion: Our data demonstrates that in this sample of patients with undiagnosed and suspected genetic conditions, WES was associated with high molecular diagnostic yield. Whole exome sequencing has rapidly become a component of the clinical approach that require a broad search for causal variants across the spectrum of genetically heterogeneous Mendelian disorders.

Keywords: Whole exome sequencing, Genetics, Mendelian disorders

P-193: Detection Of BRCA1 Exon 2 & 15 Mutations Among High risk Breast Cancer Patients In Azerbaijan

Ghobari B, Sadi N, Hosseinpour-feizi M.A, Safaralizadeh R, Pouладی N.

Department of Animal Science, University Of Tabriz.
E.mail: babak.ghobari.1990@gmail.com

Breast cancer is the most common cancer in women worldwide and one-third of cancers in women studying on patients with this cancer represents the lower age for breast cancer patients undergoing surgery in the region. BRCA1 (Breast Cancer Susceptibility Gene1) is the subject of study in this article. An important Tumor Suppressor Gene, and it is involved in DNA Damage Repair, Using BRCT Domain, and it also has a significant role in DNA mismatch Repair according to the studies, changes in expression levels of BRCA1 gene could lead to vulnerability breast cells to function as oncogenes. BRCA1 gene has 24 exons, coding a protein with the same name, having 1863 amino acids. BRCA1 gene is located on the long (q) arm of chromosome 17 at region 2 band 1. It was cloned and identified in 1996 by Miki et al. Methods and materials: In this research project, 50 DNA samples extracted from the blood of women with breast cancer were used. Exon 2 and 15 of BRCA1 genes were investigated using PCR and direct sequencing methods to detect mutations. Information about each patient entered Excel software and statistical analysis was performed by this software. Conclusion: In the present study, no new mutations in exons 2 and 15 were observed. However, exon 15 had a high prevalence rate of polymorphism. Respectively, The 4812A>G, 4956G>A, 4837A>G polymorphisms in the exon 15 had a frequency of 12.3, 63.58, and 81.85 in the population studied. The highest frequency was related to 4837A>G (Ser1613Gly). This study examined the first data on exons 2 and 15 of this gene in the northwestern Iranian population.

Keywords: Breast cancer, BRCA1 gene, Exon 2 and 15 mutations

P-194: Study correlation between inversion chromosome 9 and infertility in 348 cases

Ghomipoor Z, Ebrahimi A
Yas medical genetics lab, Tehran, Iran
Zahra.ghomipoor@yahoo.com

Background: Diagnosis of chromosomal abnormalities is important before birth, especially in the early stages of pregnancy. One of the most common structural balanced chromosome rearrangements is pericentric inversion of chromosome 9; inv(9) (p11q12), which is considered to be normal variant has been found in general population. However, this chromosomal abnormality is not limited to normal phenotype even it may lead to abnormal clinical conditions such as infertility and recurrent abortions. The incidence is occurred approximately 1% - 3% in the general population. We examined correlation between inversion of chromosome 9 with FBHCG and PAPP-A.

Material and Method: We investigated the karyotypes of 348 pregnant women with high risk first trimester screening being referred to our hospital using standard GTG banding for karyotype preparation. Result: The chromosomal analysis revealed a total of 8 (2.24%) inversions, among these, 4 male patients were inversion 9 carriers (2.40%) and 4 female patients was affected (2.20%). The incidence of inversion 9 in male and female patients is as same as together. Correlation between inv(9) with PAPP-A and FBHCG was 0.78 and 0.13 respectively that indicated no significant relation (P>0.05).

Conclusion: This result suggests some previous reports have indicated that inv(9) is related to clinical problems. In order to achieve significant correlation between chromosomal abnormality we should consider more information, for instance investigation carriage history from the point of view inv(9) in parents of cases.

Keyword: inv(9), infertility, abortion, FBHCG, PAPP-A

P-195: MicroRNA-572 expression level in multiple sclerosis patients in Zanjan

Golbon P1, Nazari A2, Ghoreishi A3, Mahmazi S4

1. Department of Genetic, Science and Research Branch, Islamic Azad University, Zanjan, Iran.
2. Department of genetics, Faculty of basic Sciences, Islamic Azad University, Zanjan Branch, Zanjan, Iran.
3. Assistant professor of Neurology, Zanjan University of Medical Science.
4. Department of genetics, Faculty of basic Sciences, Islamic Azad University, Zanjan Branch, Zanjan, Iran
purandokht_golbon@yahoo.com

Background and Objective: Multiple Sclerosis (MS) is a progressive neurodegenerative disease in the central nervous system. Many epidemiologic and genetic studies are revealed that both genetic and environment factors can influence the susceptibility of MS. Demyelination are caused disturbance in signal transduction in the length of nerve fibers. Investigation of substantial biomarkers could be important to early diagnosis of disease and its progress. Micro-RNAs (miRNAs) are regulatory agents for many genes, and some of them are detectable in body fluids including serum and plasma, which can be studied as biomarkers. Mir-572 is one of the most important micro-RNAs in the pathways of immune regulation. In this study, serum expression level Mir-572 in patients with MS has been investigated.

Materials and Methods: miRNAs were extracted from blood plasma of 24 MS and 24 healthy subjects. After cDNA synthesis, the expression of mir-572 was evaluated by qPCR and using the mir-16 gene as a reference gene. Results by t-test were statistically analyzed.

Results: A significant increase in the expression of mir-572 was observed in patients with MS.

Conclusion: Due to the difference in plasma levels of miR-572 in MS patients, it may be used as a biomarker for screening...
and controlling the progression of the disease. Mir-572 is an inhibitor of NCAM protein mRNAs or CD56 whose presence in the nervous system can protect the nerve cells membrane and repairs the demethylated cells. Therefore, suppression of this miRNA as a therapeutic target in MS disease might be considered.

**Keywords:** mir-572, MultipleSclerosis, NCAM, Biomarkers, qPCR.

**P-196:** Incidence of Maple Syrup Urine Disease in Infants 2007-2017, Babol, Mazandaran. Do we have founder effect

Gorjizadeh N1,2, Alijanpour M3, Jazayeri O2

1. Department of Molecular and Cell Biology, Azarbaijan Shahid Madani University, Tabriz, Iran
2. Department of Molecular and Cell Biology, Faculty of Basic Science, University of Mazandaran, Babolsar, Iran
3. Non-communicable pediatric Disease Research Center, Health Research Institute, Babol University of Medical Science, Babol, I.R.Iran.
o.jazayeri@umz.ac.ir

**Background:** Maple syrup urine disease (MSUD) is a rare autosomal recessive genetic disorder that is caused by deficiency in branched chain -ketoacid dehydrogenase complex activity (BCKD). MSUD can be caused by mutation within any of the BCKDHA, BCKDHB, DBT, DLD genes encoding the branched chain -ketoacid dehydrogenase complex. BCKD is a mitochondrial complex and is participated in metabolism of branched-chain amino acids (BCAAs) in the energy production pathway. BCKD defects results in accumulation of BCAAs and their -ketoacids in the plasma and urine to toxic levels. Patients with MSUD present neurological dysfunctions and cognitive impairments such as ketoacidosis, failure to thrive, poor feeding, ataxia, seizure, coma and psychomotor delay. The aim of the present study was to determine the incidence of MSUD in Babol city (Mazandaran province).

**Method and material:** Medical record of patients were investigated in Apr 2007- January 2017. MSUD suspected patients underwent metabolic tests to confirm the disease. All patient came from Babol. Total number of infants during this period of time was obtained from organization of civil registration in Babol.

**Conclusion:** During 10 years, 13 patients were diagnosed. Based on total number of infants in Babol, The incidence of MSUD is estimated to be 1 in 5,795 live births. Some of the patients were the result of consanguineous marriage. In our study, the rate of incidence was much higher than worldwide (1:185000). Our finding suggests that founder effect may be responsible for the high incidence of MSUD in this population.

**Keywords:** Metabolic disease, MSUD, BCKDHA, BCKDHB, BCAAs

**P-197:** Drosophila model to study APOE4 Neuropathogenesis

Haddadi M

Department of Biology, Faculty of Science, University of Zabol, Zabol, Iran

hadadimohamad@gmail.com

The 4 isoform of APOE gene has been demonstrated as the main genetic risk factor for late-onset Alzheimer's disease (AD). APOE (apolipoprotein E) mainly functions in brain lipoprotein metabolism and homeostasis. It has been proposed that APOE4 isoform slows down A42 clearance, increases Tau hyperphosphorylation, and mitochondrial dysfunction. However, the exact mechanism of ApoE4-mediated neurodegeneration remains uncertain. For the first time, we created a Drosophila transgenic in vivo model of AD expressing human 3 and 4 isoforms of APOE. This genetic model showed progressive neuronal degeneration, shortened lifespan and memory deficits in adult flies when APOE expressed in the neurons of the central nervous system (CNS). Behavioral assays have been made at the larval stage which demonstrates the potential neurotoxicity of APOE4 during development in terms of memory impairments. Moreover, human APOE over expression in glial cells of fly CNS was undertaken. The latter experimental design approved neuroglial cells involvement in apeo-mediated neurodegeneration. In addition to behavioral tests, biochemical assays are carried out to determine the lipid profile of brain tissues in APOE expressing flies and compare the same parameters to that of control flies. The results show an unbalanced level of lipid structures i.e. triglycerides, cholesterol, HDL, LDL in AD model flies. Taken together, the constructed Drosophila model of APOE4-mediated neurodegenerative disorder facilitates systemic analysis of molecular mechanisms implicated in APOE4 neurotoxicity.

**Keywords:** Human apolipoprotein E, Neurodegeneration, Transgenic Drosophila model, Lipid profile

**P-198:** HOTAIR, a long non coding RNA with potential role in cancer progression

Hajjari M

Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Mohamad.hajjari@gmail.com

One of the well-known long non-coding RNAs is HOTAIR (HOX transcript antisense RNA) which is known to effect on the chromatin structure. This RNA is a long non coding RNA with the potential role in different cancers. However, there is limited knowledge of genetic and epigenetic elements and interactions between them for the gene encoding this IncRNA. Also, there is no comprehensive transcriptomic meta-analysis of this RNA in different cancers. Therefore, understanding of mechanisms of the HOTAIR regulation is remained to be challenging.

We used different in silico analyses tools to find and consider genetic and epigenetic elements of the HOTAIR gene. We reported different elements such as canonical promoters, TSSs, CpGIs, genetic regulatory elements and epigenetic marks that are involved in HOTAIR gene expression regulation. We also used different datasets to find the potential role of HOTAIR in different cancers such as gastric cancer, breast cancer, and colorectal cancer etc. We compared the expression level of HOTAIR between tumors and normal tissues. We found significant up-regulation of HOTAIR in tumors compared with normal tissues. We highlighted the genetic and epigenetic features as well as significant expression of HOTAIR in tumor tissues. The current study provides a better biological knowledge of HOTAIR regulation and helps the researchers for further studies.

**Keywords:** HOTAIR, Long non coding RNA, Cancer
P-199: Induction of apoptosis in MCF-7 cell line using curcumin-loaded Graphene derivatives

Hashemi MS1, Gharbi S1, Shahrorkhi M2, Ansari Asl Z1, Shiralizadeh-Dezfuli A1, Jafarinejad-Farsangi S2

1. Department of Biology, Faculty of Science, Shahid Bahonar University of Kerman, Kerman, Iran
2. Physiology Research Center, Institute of Basic and Clinical Physiology Sciences, Kerman University of Medical sciences, Kerman, Iran

Abstract: Curcumin is the major phenolic component of turmeric derived from the plant Curcuma longa. Curcumin as an anti-inflammatory factor that causes apoptosis have attracted much attention in the treatment of diseases including cancer. One of the major challenges of using curcumin in medical therapy is its low solubility and plasma clearance. It has been suggested that Graphene derivative nanoparticles such as Graphene Oxide (GO) and Graphene Quantum Dot (GQD) improves systemic bioavailability of hydrophobic drugs such as curcumin. Here, we investigated induction of apoptosis in MCF-7 cells by curcumin loaded on GQD and GO nanoparticles.

Method: Curcumin loading was performed by adding curcumin to GQD and GO in PBS solution in the ratio of 4:1 under stirring for 2 h at room temperature. Binding of Curcumin was characterized by UV-Vis spectroscopy and FTIR. Then cells were incubated with GQD-Cur and GO-Cur and were stained with PI and Hoechst 33324 and analyzed by fluorescence microscopy.

Result: In the UV, Vis and FTIR spectra, a new peak appeared after conjugation, confirming loading of Curcumin on GQD and GO. We observed more apoptosis in MCF-7 cells treated with GQD-Cur and GO-Cur compared to treated cells with naked curcumin-treated cells.

Conclusion: Our results suggested GQD and GO as suitable carriers for curcumin in the treatment of MCF-7 Cells and possibly other types of cancers.

Keywords: Apoptosis, MCF-7 Cells, Curcumin, GQD, GO

Cisplatin is a chemotherapy drug that is widely used against several types of cancers, including ovarian cancer. Because treatment with this drug produces serious side effects such as severe toxicity and drug resistance, clinical use of the drug is limited. Valproic acid (VPA) induces apoptosis in various cells and overcomes the drug resistance of cisplatin via changing in the expression profiles. This study examined the anticancer effect of VPA alone and in combination with cisplatin on ovarian cancer, and eventually the effect of VPA on the expression of Bim and Fas, two pro apoptotic gens, in A2780 S and A2780/CP ovarian cancer cell lines.

METHOD: A2780 cells were treated with cisplatin, VPA, and combination of them for 48 hours. The toxicity and occurrence of apoptosis were evaluated by MTT and Flow cytometry, respectively. The expression of apoptotic genes including Bim and Fas were determined by qRT-PCR.

RESULTS: This study showed that VPA exerted anti-proliferative effects of cisplatin on ovarian cancer cells. We demonstrated that VPA, alone and combined with cisplatin led to considerable increase in apoptotic rate of A2780 cells through up-regulation of intrinsic and extrinsic apoptotic pathway genes, Bim and Fas, and it tendererized ovarian cancer cells to cisplatin therapy.

CONCLUSIONS: this result revealed that exposure of ovarian cells to VPA increased expression of Fas and Bim and leading to promote the cisplatin-induced apoptosis that can restore cisplatin chemosensitivity in the ovarian cancer cells. Therefore, VPA can be used to treat ovarian cancer in the future.

Keywords: Ovarian cancer, Cisplatin, Valproic acid, Fas, Bim, apoptosis

P-201: Investigating of CXCL12 polymorphism (rs1801157) in patients with RPL

Hashemian Z, Vafaii M, Nikkhah H, Farashahi E

1. Genetic Engineering and Genome Editing Laboratory, Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran
3. Department of Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

E-mail: somayehhashemi86@gmail.com

Abstract: Repeated abortions or Recurrent Pregnancy Loss (RPL) include a failure in continuously implantation that results in the end of pregnancy before 20 weeks of gestation. This is seen in 2-5 percent of the pregnant women. It may be caused by lack of proper blood relationship between mother and fetus occur. Many chemokines play in the development of angiogenesis, one of the most important of which is CXCL12. This protein is a polytropic chemokine that interacts with its receptor with various biochemical pathways. This ligand controls the proliferation and differentiation fate. The polymorphism, rs1801157, is located in the 3’UTR region of the CXCL12 gene, which has been identified as a risk factor for many cancers with a similar biological pathway.

Materials and Methods: In this study, DNA was extracted from peripheral blood of 80 persons of RPL patients and control. This SNP (rs1801157) was evaluated by using PCR and RFLP method. The results were confirmed by sequencing. The
statistical analysis was done by Chi-Square (P<0.05).

Results: AA, AG and GG genotypes were observed 29%, 32% and 39% in patients respectively, whereas they were 18%, 44% and 38% in control group respectively.

Conclusion: According to our results, this polymorphism (rs1801157) was not significantly correlated with susceptibility of RPL in our studied population. It seems to require more samples to express the certainty of the results.

Keywords: Recurrent Pregnancy Loss (RPL), C-X-C Motif CXCL12 Chemokine Ligand 12, Polymorphism

P-202: A novel study of polyamides therapeutic potential as transcription factor inhibitors in CML

Hayatigolkhatmi K1, Padroni G2, Su- W3, Fang L1, Gmez-Castaeda E1, Hsieh YC3, Jackson L1, L. Holyoake T1, Pellicano F1, A. Burley G2, G. Jrgensen H3

1. Paul O’Gorman Leukaemia Research Centre, Institute of Cancer Sciences, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK.
2. Department of Pure and Applied Chemistry University of Strathclyde, Thomas Graham Building, Glasgow, UK.
3. Guangdong Key Laboratory of Nanomedicine, Institute of Bio-medicine and Biotechnology, Shenzhen Institutes of Advanced Technology, Chinese Academy of Sciences, Shenzhen, Guangdong, P. R. China.
4. Drug Discovery Program, Cancer Research UK Beatson Institute, Garscube Estate, Glasgow, UK.

Introduction: Despite clinical success of tyrosine kinase inhibitors (TKI) in chronic phase (CP) CML patients, nearly all have minimal residual disease owing to persistent, primitive, leukaemia stem cells (LSC). E2F transcription factor 1 (E2F1) is a critical survival factor for TKI resistant LSC. Polyamides (PA) that target specific DNA sequences have a successful history of inhibiting transcription factors (TFs).

Purpose: To investigate synthetic programmed PA’s ability to inhibit E2F1 transcriptional activity so causing CML cell death.

Methods: Blast crisis (BC) KCL22 cells and CP CML CD34+ cells were assessed after treatment with PA, TKI or their combination. Assessment methods included: cell density by dye exclusion; flow cytometry for apoptosis, proliferation and cell cycle status; gene expression by RT-qPCR; and colony forming cell (CFC) assay with clonogenic primary CP CML CD34+ cells.

Results: Our global gene expression analysis revealed significant down-regulation of TRIM45 and RARS2 with PA treatment. TRIM45 has an established role in proliferation, development, oncogenesis, and apoptosis. In addition, our PA successfully inhibited CP/BC CML cell growth based on cell density and flow cytometry analysis compared to untreated control. Indeed the combination of PA with nilotinib had the potential of increasing nilotinib efficacy in killing CP CML cells. CFC assay showed reduction in colony formation of CP CML cells.

Conclusion: Going forward, this technology could enable a systematic “DNA-binding scan” providing a detailed map of genes under E2F1 control in CML LSCs that could assist in the development of TF inhibitors as novel therapeutic alternatives in the disease.

Keywords: Polyamide, Chronic Myeloid Leukaemia, CML, E2F1, TKI

P-203: Structural modeling and functionality assessment of IMTOX immunotoxin family in quasi-physiological circumstance

Hazrati MH, Gholampour-Faroji N, Haddad-Mashadrizeh A, Rastegar-Moghadam M

1. Pharmaceutical Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran
2. Structural biology and Bioinformatics Research Group, Khayyam Bioeconomy Institute, Mashhad, Iran
3. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran

Introduction: Commensurate with the spread of technology various therapies in the field of cancer such as immunotoxin has been developed. Hence, recognizing the features of these drugs could be provide opportunity for development, which are considered in this study in association with IMTOX family.

Material and methods: In this study, sequences of IMTOX were achieved based on patents. NCBI and PDB databanks were used to achievement the protein sequences and 3D structures. Modeller were employed to modeling and assembling via homology modeling method. ERRAT, Verify 3D and RAMPAGE programs were used to determine the quality of the models. Evaluation the thermostability in quasi-physiological condition were carried out by GROMACS. Functionality features were assay by affinity to corresponding antigens and immunogenicity properties which evaluated by HADDOCK and IEDB programs, respectively.

Result: Literature review has led to obvious 3 isoforms of IMTOX family, including IMTOX19, IMTOX22 and IMTOX25 with the ability to target CD19, CD22 and CD25 for lymphoma treatment, respectively. Chain A of the Ricin and various antibodies fragments were discovery in the sequence context of these drugs. Structural modeling of the components of drugs and corresponding antigens showed suitable quality. Fragments assembling resulted in the production of several isoforms from each drug, which one of them showed the suitable quality at 37Á°C. Meanwhile, appropriate affinity to corresponding antigens and immunogenicity feature of these drugs were obvious.

Discussion and conclusion: Generally, the results of this study has led to introduced functionally models of IMTOX family which can used in lab and opportunity for drug optimization.

Keywords: Cancer, immunotoxin, IMTOX, Modeling, Simulation

P-204: Effect of DNA methylation and expression of CDK5 gene in patients with Oral Squamous Cell Carcinoma

Hejazi H1, Kordi Tandumani DM1

Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran
dor_kordi@yahoo.com

Background: OSCC or oral squamous cell carcinoma is the sixth common type of cancer in the world and is the common form of malignancy of head & neck cancers. CDK5 has emerged an essential kinase in CNS and includes the process of neuronal maturation, migration, axon plasticity, cytoskeleton organization, endocytosis, exocytosis and apoptosis. Recently, CDK5 has been concerned in the development of a difference of cancers

Materials and Methods: Samples are including 50 paraffin
blokes of oral tissue caseO€E and 50 biopsy of oral tissue for DNA methylation. The analysis of promoter hyper-methylation of CDK5 gene has been done by MSP-PCR technique. Furthermore, the expression level of CDK5 gene assessed in 15 patients and 15 controls by real time PCR.

Results: Promoter methylation of CDK5 gene was statistically different in OSCC patient in comparison to control. (OR=3.5, 95% CI=1.15-2.38, Pvalue=0.021). However, gene expression level of CDK5 gene was different in patient and healthy control (Mean SD, 1.55 5.99 respectively, Pvalue=0.001).

Conclusion: This is a credible evidence of CDK5 gene methylation and expression in patient s with OSCC. This first hand attempt of epigenetic changes of the CDK5 gene in pathogenesis of OSCC.

Keywords: CDK5, OSCC, MSP, Methylation, Expression.

P-205: Relationship between Epstein-Barr virus and breast cancer in Isfahan province

Hejazi SH1*, Salahshournia Z2, Hadi F1, Saeedi Z1

Department of Biology, Faculty of Science, Lorestan University, Khoramabad, Iran

hejazi_h32@yahoo.com

Background and aim: Breast cancer is one of the most common tumors in women. Although the etiology of breast cancer is not completely understood, but exposure to Epstein-Barr virus is suggested as a risk factor for breast cancer. Studies have reported since 1995 that EBV is involved in the development of breast cancer. The aim of this study was to assess the presence of EBV in patients with breast cancer from Isfahan province.

Materials and methods: This study was performed for 40 tumor and 40 free tumor paraffin embedded tissues from women in Isfahan province with breast cancer. After extraction of DNA with salting out method and amplification of housekeeping gene (beta-actin), all samples were examined to detection of DNA-EBV virus applying PCR (polymerase chain reaction) method. All data statistically were analyzed were by Cramer test using SPSS 16 software.

Results: EBV was detected by PCR in 20 out of 40 (50%) cases of breast cancer samples and 5 out of 40 (12.5%) control samples. Statistically, crammer indicator analysis for EBV infection in tumor samples and normal samples was 0/405 which indicates a significant relationship between breast cancer and EBV infection in Isfahan province.

Discussion: The presence of EBV gene in a significant subset of women with breast cancer in Isfahan province shows that Epstein-Barr virus can be one of the reasons for breast cancer, but the studies are needed to demonstrate the relationship between virus and breast cancer.

Keywords: Breast cancer, Epstein-Barr virus, PCR, Isfahan province.

P-206: ApoA5 variants could affect the triglyceride levels among cardiovascular patients

Hejazipoor L1, Mahdieh N2

1. Department of Genetic Tabriz Branch, Islamic Azad University, Tabriz, Iran
2. Genetics Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran

leila.hegzi2@gmail.com

Background: one of the important factors for regulating plasma triglyceride levels is ApolipoproteinA5 (APOA5) which could acts as a risk factor for coronary artery disease. The APOA5 gene mutation affects the metabolism of plasma triglycerides. Here, mutations of the APOA5 gene are studied in patients with triglyceridemia.

Methods: Patients with cardiovascular disease and TG>150mg/dl were selected from individuals referred to Shahid Rajaei Hospital. Exclusion criteria were as follows being overweight, diabetes, thyroidism, kidney disease and alcohol abuse. DNA was extracted from the blood sample. APOA5 gene coding region was amplified and sequenced.

Results: 8 APOA5 gene variants (rs2266788, rs2072560, rs651821, rs12287066, rs3135506, rs3135507, rs34089864 and rs619054) were observed in the studied individuals. The frequency of rs2266788 and rs2072560 were 95.5% and 91.1%, respectively.

Conclusion: The polymorphisms of the APOA5 gene may have an important role in regulating triglyceride level among cardiovascular patients.

Keywords: cardiovascular disease, APOA5 mutations, triglyceride level.

P-207: Production of the hurler syndrome cellular model using CRISPR-Cas system

Heydari M1, Hatami bardar A2, Eslahi A1, Bozorg Ghami S1, Jafari Y1, Alizadeh F1, Mojarrad M1

1. Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Molecular Genetics, Faculty of Sciences, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran

ali.net1@yahoo.com

Background: The Hurler syndrome has clinical manifestations including coarse faces, corneal opacity, mental retardation, and enlargement of the liver and spleen. The bone marrow transplantation (HCST) and enzymatic treatment (ERT) are two common treatments for this disorder. Efforts to carry out gene therapy for the genetic modification of the disease continue on the basis of viral carriers while the results are not very promising. The mentioned treatments have limitations in the field of feasibility and implementation, and the treatment of this disease requires alternative methods to overcome these constraints. With the CRISPR-Cas technique discovery as a genome editing technique, hopes for treatment of the monocytes in metabolic disorders have increased. In this study, we optimized the CRISPR-Cas technique to provide the defective gene modification to a healthy and active version in the patients.

Method: The sgRNA sequences were designed with an online software tool (http://crispr.mit.edu/). The sgRNAs were cut off by the bbsI enzyme and sub-cloned into pX335 CRISPR-Cas vector. The pX335/sgRNA vector was transfected into the NIH3T3 cell line to knock out the IDUA gene; then, the modified cells were selected using the High Resolution Melting (HRM) technique and enzymatic activity level was evaluated by the ELISA.

Results: Recombinant pX335/sgRNA vector was confirmed by bbsI and small digestion. Accuracy of the transfection into the NIH3T3 cell line was confirmed by the sequencing, and enzymatic activity level was evaluated by ELISA.

Keyword: IDUA, Hurler syndrome, CRISPR-Cas, High Resolu-
Background and objectives: Etiology of preterm delivery in humans includes many genetic and environmental factors. C677T polymorphism in exon 4 of the MTHFR (Methylene-tetrahydrofolate Reductase) gene is one of the genetic factors in women with preterm delivery. This study aimed to investigate the association between the methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and preterm delivery susceptibility.

Materials and Methods: In this case-control study, 40 preterm delivery pregnant women as cases and 40 healthy pregnant women as controls were analyzed for MTHFR C677T polymorphism by the PCR and sequencing methods. The differences for frequencies of gene type and alleles in cases and controls were tested by SPSS, Chi-square test and logistic regression. The relevant risk of preterm delivery was represented by odds ratios (ORs) with 95% confidence intervals (95% cIs).

Results: Based on the results of this study, there was no significant difference in the frequency of T allele (p-value=0.069) and TT gene type (p-value=0.27) in the cases and controls. But, there was a significant correlation between heterozygote CT gene type and preterm delivery (p-value=0.003).

Conclusion: The results of this study demonstrated that gene type CT of MTHFR C677T polymorphism might make preterm delivery risk rise in Iranian women.

Keywords: methylenetetrahydrofolate Reductase; preterm delivery; C677T polymorphism

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P-209: The association study of IGFBP3 -202 C>A polymorphism with breast cancer prevalence in Iranian females referred to Yazd Reproductive Sciences Institute

Hoseini SA, Ashrafzadeh H, Nazari T, Kalantar SM

Yazd Reproductive Sciences Institute, Recurrent Abortion Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Amirhosein.hb12@gmail.com

Background: Breast cancer included 33% of all cancers and 20% of cancer deaths in women. In Iran the fifth leading cause of death from cancer, is breast cancer. Several factors, including genetic factors involved in breast cancer.In recent years role of the Insulin-like Growth Factor gene family (IGF) in breast cancer is considered. Insulin-Like Growth Factor binding protein3 (IGFBP-3) is one of the most important members of IGF family. IGFBP-3 gene has various polymorphisms that affected increase and decrease of its serum level. One of its most common polymorphism, is rs2854744 that its association with breast cancer has studied in different populations, But so far has not been studied in Iranian women with breast cancer.

Methods: 85 patients with breast cancer and 76 healthy female blood samples were collected and genomic DNA was extracted. The polymorphism was analyzed by PCR-RFLP method.

Results showed no significant correlation between genotype and allele frequency of rs2854744 polymorphism with increased risk of breast cancer in the study population.

Conclusion: Although this study showed no correlation between the rs2854744 polymorphism and breast cancer risk but its association with disease in some other populations has been established. The difference may be due to the influence of other factors such as diet, physical activity, age and weight on circulating levels of IGFBP-3 and therefore risk of breast cancer.

Keywords: breast cancer, rs2854744, IGFBP-3, PCR-RFLP

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P-210: Association of vaspin rs2236242 gene polymorphism with insulin resistance and BMI in patients with type 2 diabetes

Hosseini M1, Nezhadali M2, Hedayati M2

1. Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran
2. Cellular and Molecular Endocrine Research Center, Research institute for Endocrine Sciences, Shahid Behesht university of Medical Science, Tehran, Iran.

mariahosseini87@gmail.com

Introduction: Vaspin was originally identified as an adipokine, which is predominantly secreted from visceral adipose tissue. We have recently shown that vaspin mRNA expression in adipose tissue is related to parameters of obesity and glucose metabolism. Vaspin is a serine protease inhibitor with insulin-sensitizing effects. The regulation of vaspin serum concentrations in type 2 diabetes is unknown. The present study aimed to investigate the impact of vaspin rs2236242 gene polymorphism on serum vaspin levels and type 2 diabetes.

Materials and Methods: This case-control study was run on 75 patients with type 2 diabetes and 80 healthy subjects. Determine polymorphism rs2236242 of vaspin gene was performed by Tetra-ARMS PCR method and electrophoresis technique. The weight, height and Fasting Blood sugar were measured using standard methods. Their fasting plasma vistatin and insulin were measured using the Merckodia ELISA kit (Merckodia Company, Sweden). Statistical analysis performed by SPSS version 19.

Results: In the present study genotypes of rs rs2236242 had not association with T2DM. The vaspin levels of type 2 diabetics were significantly different from the non-diabetics studied. There was not a correlation between vistatin and BMI, circulating vaspin significantly correlated with FBS (p>0.05).

Conclusions: Our findings showed that there is no association between vaspin level and vaspin rs2236242 gene polymorphism. The genotypes of rs2236242 were not associated with the risk of T2DM but vaspin serum concentrations are associated with type 2 diabetes.

Keywords: vaspin, Type 2 diabetes, rs2236242..

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P-211: A mutation in 5' untranslated region of PCSK9 is related to drug response

Hosseini moghadam M1, moradi A2, Boudagh Sh1, Ghaemmaghami Z2, Maleki M1, Mowla SJ1, Malakootian M1

1. Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
2. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

m.hosseinimoghadam@gmail.com

Introduction: PCSK9 or Proprotein Convertase Subtilisin Kexin type 9, is a protein that in humans is encoded by the PCSK9 gene. It is a serine protease that is involved in regulating levels of Low-Density Lipoprotein (LDL) and is a drug target for lowering LDL-cholesterol. It was recently discovered that a PCSK9 mutation was associated with statin resistance. Here we aimed to study the effect of a PCSK9 mutation, observed in some of patients with statin resistance, on the efficacy of statin treatment.

Materials and Methods: In this study, we performed a case-control study on patients with statin resistance who were referred to the Cardiogenetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran. The mutation was screened by sequencing the PCSK9 gene. The efficacy of statin treatment was evaluated by measuring the level of LDL-cholesterol before and after the treatment.

Results: The mutation was observed in 10% of patients with statin resistance. Interestingly, patients with the mutation showed a significant increase in the level of LDL-cholesterol after the treatment, while patients without the mutation showed a significant decrease in the level of LDL-cholesterol.

Conclusions: Our findings suggest that the PCSK9 mutation observed in patients with statin resistance is associated with statin resistance. Further studies are needed to confirm these findings and to understand the mechanism by which the mutation affects the efficacy of statin treatment.

Keywords: PCSK9, statin resistance, drug response.
**Introduction:** Familial hypercholesterolemia (FH) is a genetic disorder characterized by elevated low-density lipoprotein (LDL) cholesterol (LDL-C) in blood, leading to an increased risk of premature cardiovascular diseases. Gain and loss of function mutations of PCSK9 have been associated to hypercholesterolemia and hypcholesterolemia, respectively. Currently, two FDA approved drugs, Repata and Praluent, inhibitors of PCSK9, administrated in some patients suffering from dyslipidemia.

**Material and methods:** In this study, we investigated probable nucleotide changes in PCSK9 gene of 10 patients who referred to Rajaei Cardiovascular Medical and Research center because of familial hypercholesterolemia. The genomic DNA of all patients was extracted, using salting out method, and PCR amplification and Sanger sequencing was applied by specific designed oligonucleotides.

**Results:** Our data revealed a probable pathogenic nucleotide change in 5‘UTR of one patient. Although other patients have nucleotide changes in exons and introns of PCSK9 gene, they are almost belong to benign or likely benign variations.

**Conclusion:** It has been exhibited that some dyslipidemia patients who are resistant to statin drugs have mutation in PCSK9 gene. After evaluating this variant in other family members of the patient, we conclude that nucleotide variation in 5‘UTR of the PCSK9 gene may cause hypercholesterolemia in this patient who responds to Repata drug.

**Keywords:** PCSK9, hypercholesterolemia, Pathogenic mutation

**P-212: Comparison of the effect of silibinin's freshness on its fatality impact on A549 cells using MTT Assay**

**Hosseini S**, Foroughmand AM*, Hajjari M, Mirzaaghai S

1. Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran

somayehhosseini67@gmail.com

Lung cancer remains the leading cause of cancer death worldwide and its incidence is increasing. Non-small cell lung cancer (NSCLC), including adenocarcinoma and squamous cell carcinoma is the predominant form of lung cancer and accounts for ~85% of all lung cancer cases despite improvement in staging and the chemotherapy 5-years survival rate for individuals with lung cancer is only about 15%. Anti-cancer properties of herbal compounds have attracted widespread interest in new researches. Silibinin as a herbal substance has an antitumor property and the chemotherapy 5-years survival rate for individuals with lung cancer is only about 15%. Anti-cancer properties of herbal compounds have attracted widespread interest in new researches. Silibinin as a herbal substance has an antitumor property and can be used as a chemo preventive agent in treating strategies in lung cancer patients.

**Keywords:** NSCLC, A549, Silibinin, MTT

**P-213: Association of XRCC1 and hOGG1 polymorphisms with colorectal cancer risk in an Iranian population**

Hosseini SM*, Mohammadi asl J', Alidadi R', Talaeizadeh A², Bijanazzadeh M²

1. Department of Medical Genetics, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
2. Cancer, Environmental and Petroleum Pollutants Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

s.mohammadhosseini95@yahoo.com

Introduction: The DNA repair gene X-ray cross-complementing group 1 (XRCC1) and 8 Oxo guanine DNA-glycosylase 1 (hOGG1) genes are implicated in the base excision repair (BER) mechanism of DNA. Polymorphisms in DNA repair genes are supposed to cause genetic instability and carcinogenesis. This study was designed to investigate association between XRCC1 Arg399Gln (rs25487) and hOGG1 Ser326Cys (rs1052133) polymorphisms with susceptibility to colorectal cancer in an Iranian population.

**Materials and methods:** This case control study comprised 150 controls and 150 patients. Patients and controls which selected from 2 educational hospitals in Ahvaz, southwest of Iran were matched for age and gender. Environmental risk factors were calculated and genotyping was carried out by PCR*r*/RFLP method.

**Results:** Our results indicated that the frequencies of the Gln allele of XRCC1 Arg399Gln were significantly higher in CRC patients (p=0.01, OR1.54, 95% CI 1.1-2.1) and significantly increased of cancer risk was observed in XRCC1 Arg399Gln homozygous Gln / Gln genotypes (p<0.001 OR: 5.3, 95% CI 1.9-14.2), While no association was found between hOGG1 Ser326Cys and colorectal cancer risk (p =0.06).

**Conclusion:** Our research suggests an increased risk for colorectal cancer in individuals with XRCC1 Arg399Gln polymorphism. Among all risk factors evaluated, smoking had significant association with colorectal cancer in Ahvaz population, southwest of Iran.

**Keywords:** Polymorphism, colorectal cancer, polymerase chain reaction.

**P-214: The effect of antiangiogenic peptides on eIF4E gene expression in a Balb/c mouse model with 4T1 induced breast cancer**

Irvani GhR, Salehi Z, Asghari SM, Talesh Sasani S

Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran
s.sasani@guilan.ac.ir

Angiogenesis, the process of new blood vessel formation, plays a central role in both local tumor growth and distant metastasis in breast cancer. Inhibiting angiogenesis is a promising strategy for treatment of cancer. VEGF is now recognized to play an essential role in physiological as well as pathological...
angiogenesis. eIF4E level affects transformation, tumorigenesis, metastasis, and drug resistance in both experimental cancer models and human cancer tissues. Peptides are being used to generate therapeutics for enhancing cellular uptake, drug targeting and vaccination. Peptides have other advantages over proteins and antibodies as drug candidates, including lower manufacturing costs, higher activity per unit mass, lower royalty stack than antibodies, greater stability, reduced potential for interaction with the immune system and better organ or tumor penetration. Vascular endothelial growth factor (VEGF) is a potent vascular endothelial cell (EC) specific mitogen that stimulates EC proliferation, microvascular permeability, vasodilation, and angiogenesis. The purpose of this study was to investigate the effects of the antagonist peptides on expression levels of eIF4E in 4T1 xenograft mouse. The eIF4E expression level was investigated by real time PCR method. Treatment with antiangiogenic peptides significantly decreased the tumor size and inhibited tumor growth in a concentration-dependent manner. Moreover, the expression level of eIF4E was significantly reduced in peptide-treated mice group comparing to the control. In conclusion, the antiangiogenic peptides inhibitory effect on the VEGFR mediated signaling pathway could be targeted for the development of pharmaceutical agents that inhibit tumor angiogenesis via eIF4E.

**Keywords:** Angiogenesis; VEGF1; eIF4E; Cancer

P-215: Comparison of the gene expression of Nox5 and sperm parameters in oligospermic men

izadi raenin M1, allameh zadeh Z2, amini A3, fallahi S4, malekzadeh K

1. Medical biotechnology research center, ashkezar branch, Islamic azad university, ashkezar, yazd, iran
2. Department of biology, Islamic azad university, arsanjan, iran
3. Department of molecular genetic, university of ahvaz shahid chamran, ahvaz, iran
4. 5. Fertility and infertility research center, hormozgan university of medical sciences, bandarabasas, iran

**Background:** Infertility is a global issue which has affected 15-20% of couples. Within the past two decades, understanding of reproduction system and its related factors has grown dramatically, especially the role of male factor in infertility. Previous research has shown that 20% of infertilities are caused by male infertility. Oxidative stress which leads to the production of Active oxygen types (ROS) is recognized as a key factor involved in male infertility. However, its physiological values are estimated by the enzymes in NADPH family. In the case of disrupted spermatogenesis and maintenance of extracytoplasm, there is an increase of ROS in unnatural sperm. Hence, it is believed that ROS is responsible for interaction with the immune system and better organ or tumor penetration. Vascular endothelial growth factor (VEGF) is a potent vascular endothelial cell (EC) specific mitogen that stimulates EC proliferation, microvascular permeability, vasodilation, and angiogenesis. The purpose of this study was to investigate the effects of the antagonist peptides on expression levels of eIF4E in 4T1 xenograft mouse. The eIF4E expression level was investigated by real time PCR method. Treatment with antiangiogenic peptides significantly decreased the tumor size and inhibited tumor growth in a concentration-dependent manner. Moreover, the expression level of eIF4E was significantly reduced in peptide-treated mice group comparing to the control. In conclusion, the antiangiogenic peptides inhibitory effect on the VEGFR mediated signaling pathway could be targeted for the development of pharmaceutical agents that inhibit tumor angiogenesis via eIF4E.

**Keywords:** Angiogenesis; VEGF1; eIF4E; Cancer

P-217: Bioinformatic evaluation of the miR-3613-3p effect on TGF-beta/SMAD signaling pathway in colorectal cancer

Jafarian Kaikanlou M1, Mohammad Soltani B2

1. Department of Genetics, Faculty of Biological sciences, Tarbiat Modares University, Tehran, Iran

**Introduction:** Colorectal cancer (CRC) is the second common cancer and the fourth major reason of cancer death in the world. Changes in the DNA inside our cells that turn on oncogenes or turn off tumor suppressor genes can cause cancers, they resulting in cells growing out of control. Accumulation of genetic changes in the epithelial cells and different intracellular signaling pathways such as TGF-beta/SMAD and WNT pathways can cause CRC. Transforming growth factor-betas (TGF-betas), play main function as tumor promoters and tumor suppressors through colorectal carcinogenesis. MicroRNAs are post-transcriptional regulators of gene expression, dysregulation of miRNAs expression has been demonstrated in most cancers. Here, the ability of miR-3613-3p (located on chromosome 13) to target TGF-beta/SMAD signaling pathways was evaluated.
Methods: DIANA, Mirlmap, TargetScan, and miRWalk algorithms was used to find out whether miR-3613-3p have the potential to target TGF-beta pathway key genes. Present study focused on the number of MRE in 3\`eUTR region, score and conservation of seed region.

Results and Discussion: Bioinformatic analysis showed miR-3613-3p might be a regulator of key genes in TGF-beta pathway including SMAD2, SMAD4, TGFBR1. It is estimated that miR-3613-3p might play important role in CRC through interaction with TGF-beta/SMAD pathway and might be considered as a new candidate for experimental evaluation for further researches.

Keywords: Colorectal cancer, TGF-beta/SMAD pathway, Bioinformatics, MicroRNA

P-218: NONHSAT028579 lncRNA Acts as a Tumor Suppressor gene by Titrating MicroRNA-133

Jafarzadeh M, Mohammad Soltani B
Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

Introduction: In recent decade, several studies have shown that long noncoding RNAs (lncRNAs) are involved in numerous physiological and pathological processes through regulating gene expression at the transcriptional, post-transcriptional, and epigenetic levels, especially in the development and progression of different carcinomas. Thus, the investigation of the role of lncRNAs could help in the understanding of oncogenesis and identify novel potential target treatments. Recently, a role of competing endogenous RNAs, or natural microRNA sponges, has been proposed. In this model, lncRNA can post-transcriptionally regulate other genes expression by competitively binding to a microRNA. The aim of this study was to investigate NONHSAT028579 gene role as a competing RNA.

Methods and Materials: Bioinformatics
To find the lncRNAs that may play important role in gastric cancer, TCGA RNA-seq data was analyzed. Assuming that the selected lncRNA may act by sponging mechanism, the whole length of NONHSAT028579 gene was scanned in searching the microRNAs that have predicted MREs on the lncRNA (RNA Hybrid, Target scan and miRWalk online tools were used for this purpose).

Experimental Procedures: To investigate possible direct interaction of the lncRNA with miR-133, NONHSAT028579 transcript was PCR-amplified and cloned into Psi-check2 dual luciferase vector in downstream of Renila luciferase gene. Then, psi-check2-NONHSAT028579 and PEGFPC1-Mir-133 vectors were co-transfected to Hek293 cell line and after 48 hours cell lysates were extracted and emission is measured in a counting Luminesmeter. A fragment with no MRE for miR-133 was used as a negative control. Normalization was performed and data is presented as Mean +/- SEM.

Results and Discussion: The result of TCGA RNA-seq data analysis introduced NONHSAT028579 as differentially expressed lncRNA in gastric cancer compared with adjacent normal tissues that may have a crucial role in the cancer. To investigate possible molecular mechanism of function of this lncRNA, the whole transcript was scanned in order to find domains that by means of them; NONHSAT028579 may exert its function. The online tools predicted miR-133 to interact with NONHSAT028579. Hence, direct interaction of miR-133 and NONHSAT028579 was surveyed by Dual luciferase assay. The normalized data showed that NONHSAT028579 directly interacts with miR-133 and therefor by titrating miR-133 off the target genes, may attenuate oncogenic function of the microRNA. But more experiments are needed to elucidate NONHSAT028579 function and other partners (microRNAs, lncRNAs and proteins) that may cooperate with this lncRNA.

Keywords: lncRNA, NONHSAT028579, miR-133

P-219: Unveiling of the mechanism by which Cancer-associated fibroblasts affect colorectal cancer cell

Jahangiri B, Khalaj-kondori M, Asadollah E, Sadeghizadeh M
1. Department of Genetics, Faculty of Natural Science, University of Tabriz, Tabriz, Iran
2. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares
3. University, Tehran, Iran Department of Biochemistry, Protein Research Center, University of Shahid Beheshti, GC, Tehran, Iran

jahangiri.babak@gmail.com

Cancer cells are in a close communication with the fibroblast cells in cancer microenvironment, Cancer-associated fibroblasts (CAFs). The CAFs play an important role in malignant behaviors of colorectal cancer (CRC) cells. To gain insight into the underlying mechanism, we treated SW480 cells with conditioned medium from CRC CAFs (CAF-CM) and evaluated its effects on EMT, invasion, and migration characteristics of the treated CRC cell. Moreover, the expression pattern of UCA1/mTOR/miR-143/KRAS signaling pathway was studied by qRT-PCR and western blotting. Our study indicated that CAFs dramatically stimulated invasion and migration of CRC cell. Furthermore, CAFs induced the EMT phenotype in CRC cell, with an associated change in the expression of EMT markers including vimentin, E-cadherin, N-cadherin, and induced metastasis-related genes (MMPs). The following mechanism investigation revealed that CAFs induced overexpression of UCA1, which leads to upregulation of mTOR. Overexpression of UCA1/mTOR axis suppressed miR-143 while KRAS was significantly upregulated in mRNA and protein level compared with control group. Moreover, UCA1 silencing in treated CRC cell suggested that overexpression of UCA1, which was induced by CAFs, regulates the expression of downstream key effectors. Taken together, these findings provide a better discernment of intercellular communication whereby CAFs incite the UCA1/mTOR axis to direct CRC cell invasive manner. These study support the hypothesis that CAFs may be a prominent therapeutic target of stroma-based therapy in CRC treatment, besides the critical role of cooperation between UCA1 and mTOR in cancer metastasis.

Keywords: Cancer-associated fibroblasts (CAFs), UCA1, Metastasis, mTOR, miR-143

Frequency of eNOS gene polymorphism(-786T>C) in women with recurrent pregnancy loss

Jalili MS1, Ghasemi N2, Seyfati SM1, Ashrafzadeh HR3
1. Medical Biotechnology Research Center, Ashkezar Branch, Islamic Azad University, Ashkezar, Yazd, Iran
2. Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
maryamsadatjalili5577@gmail.com
Background: Recurrent pregnancy loss is an important reproductive health issue, affecting 2%-5% of couples. Common established causes include uterine anomalies, antiphospholipid syndrome, hormonal and metabolic disorders, and cytogenetic abnormalities. However, in 50% of cases the cause of abortions is not clear. eNOS gene polymorphisms have been proposed as an important factor causing RPL. Many studies have investigated the different polymorphisms of this gene in RPL. Studies which examined -786 T>C polymorphism in RPL are very limited. Therefore illustrating -786 T>C polymorphism roles in RPL would bring useful information in this term.

Materials and Methods: peripheral blood samples of 200 women (100 normal women and 100 affected women) were obtained. After DNA extraction from blood cells, PCR-RFLP method was used to determine the genotypes of -786 T>C polymorphism.

Results: The investigation showed that genotypes of -786 T>C polymorphism in affected women were as follows: homozygous TT, 40%; heterozygous TC, 6% and homozygous CC, 54%. While genotypes of control group were as these: homozygous TT, 46%; heterozygous TC, 5% and homozygous CC, 49%. The frequency of the genotypes in cases and controls were not significantly different.

Conclusion: The present study did not find association between eNOS gene polymorphism of -786T>C and the risk of RPL in these women.

Keywords: recurrent pregnancy loss, Polymorphism -786 T>C, eNOS, PCR-RFLP

P-220: Estimated of Chromosome Structural abnormalities inpatients passes on Genetic laboratory of Qazvin University of medical sciences

Jalilvand M1, Ansari J1, Najafipour R2, Moghbelinejad S3, Ramezani M1, Alimohammadi M1

1. Cellular and Molecular research center, Qazvin University of medical Sciences, Qazvin, Iran
2. Department of Biochemistry and Genetic, Cellular and Molecular research center, Qazvin, University of medical Sciences, Qazvin, Iran
rahamj7@yahoo.com

Introduction: Structural chromosomal abnormalities are estimated to occur in around 0.5% of newborn infants. Chromosomes are the structures that hold our genes. If a chromosome or piece of a chromosome is missing or duplicated, there are missing or extra genes respectively. Many children with a chromosomal abnormality have mental and/or physical birth defects, ranging from mild to severe. In addition, some chromosomal abnormalities result in miscarriage or stillbirth. Chromosome disorders are of conditions, caused by constitutional numerical or structural abnormalities of chromosomes. Structural changes occur within the chromosomes themselves, not necessarily accompanied by any numerical change. There are varieties of chromosomal rearrangements that occur caus- ing changes in the structure or components of a chromosome including: Translocations, Inversions, Ring chromosomes and Deletions.

Materials and Methods: After blood sampling, culture, harvesting and preparation of metaphase spreads, we analyzed karyotype according to standard protocol in 3378 referred patients to the Genetic section of Reference Lab of Qazvin University of medical sciences during 2010-2017.

Results: Our result shown 70 affected (23 translocations, 22 inversion, 4 deletion, 5 insertion, 2 ring chromosome, 14 inversion 9 (normal polymorphism) cases have chromosomal rearrangement.

Discussions: Our result has shown chromosomal rearrangement were present in 2% of 3378 patients. In our results, the most frequent chromosome rearrangement observed is translocation similar other studies.

Keywords: inversions; insertions; deletions; ring chromosomes; karyotype

P-221: LncRNA Pnky is upregulated in breast cancer and promotes cell proliferation and EMT in breast cancer cells

Jannat Alipoor F, Asadi MH

Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran.

firoozjannat@gmail.com

Cancer stem cells (CSCs) are sub-population of the cells in the heterogeneous context of tumor that have characteristics same stem cells. In addition to the stem cell factors including oct4, Nanog and KLF4, non-coding RNA, espacially, Long non coding RNAs (LncRNAs) can affect the functions of CSCs. Expression of these RNAs in cancer as an important and common cause of death in the world may be helpful for curing this disease. In present study, the expression of Pnky lncRNA was assessed in 4 tumor, including brain, breast, prostate and colorectal cancer. 5 stam/cancer cell lines and 33 breast tumor with their marginal samples analysed by qRT-PCR method. As a first study in cancer, these results were shown that Pnky lncRNA expressed in 4 cell lines. Also the significant upregulation of Pnky was observed in high grades of breast cancer. Also, our data revealed that Pnky was upregulated in ER, PR and HER2 negative breast cancer cell line and tumor. Knock down of Pnky in MDA-MB-231 cell line was triggered apoptosis and to some extent lead to cell cycle arrest. Upregulation of mir-150 and downregulation of Zeb1 and snail after knockdown of Pnky, leads to EMT suppression. This primary study showed that the Pnky lncRNA expression maybe is related to ER, PR and HER2 receptors in breast cancer, and upregulation of Pnky in breast cancer can promote invation and metastasis of breast cancer by promoting EMT and inhibiting of apoptosis.

Keywords: Pnky; LncRNAs; Breast cancer, cell cycle, apoptosis, EMT

P-222: Identification of causative genes (three known and one novel) in four unrelated Iranian families affected to hereditary spastic paraplegia (HSP) using whole exome sequencing

Javan Parast Sheikhani L1, Alavi A1, Rahimi Bidgoli MM1, Pashaei M1, Mohamm2, Fatehi F1, Nafissi Sh3, Kahrizi K1, Najmabadi H1

Genetic Research Center, University of Social Welfare and Rehabilitation Sciences
Leila.Javanparast@gmail.com

Hereditary spastic paraplegia (HSPs) is a group of inherited and incurable neurodegenerative disorders characterized by progressive spasticity and weakness in lower limbs. The mode
of inheritance in HSP can be autosomal-dominant, autosomal-recessive, X-linked, or mitochondrial. There is significant genetic heterogeneity in HSP, with at least 60 genes and 80 loci identified thus far. Whole-exome sequencing (WES) has been used for gene discovery in HSP since 2011, resulting in a marked increase in the rate of new genes being identified. Despite the use of WES, genetic analysis has failed in finding of causative genes in 45%-67% in the autosomal dominant-HSP (AD-HSP) and 71%-80% in the autosomal recessive-HSP (AR-HSP) groups, indicating that, the majority of HSP-genes especially AR-HSPs have remained unknown. So, in order to identification of novel HSP-disease causing genes, we investig-ated the cause of AR-HSP in four unrelated Iranian HSP-family using WES. This approach led us to identify the mutations in three known disease-causing genes including SPG7, CYP7B1, and ZFYVE26 and one novel candidate HSP gene. Functional analyses to evaluate of the biological implication of the novel gene and the GAL4-UAS method for targeted gene expression in Drosophila are ongoing. The precise mechanisms underlying the HSPs are unknown and the rapid and affordable methods like NGS methods are useful for rapid acceleration of novel genes discovery. Identification of novel genes and molecular pathways will greatly enhance our understanding of the cellular pathways that are critical for axonal health and our knowledge about pathogen-esis of the disease.

Keywords: Hereditary spastic paraplegia, HSP, Whole exome sequencing, WES, Candidate novel HSP-genes

P-223: A report of a novel mutation in AURKC gene in ab-nornal fetus amniotic fluid samples

Javid M1, Ebrahimi A2*, Fahimi H3

1. Department of Genetics, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran -Iran (IAUPS ).
2. Kowsar Human Genetic Research Center, Tehran, Iran.
3. Head of Department, Department of Genetics, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran -Iran (IAUPS ).

javid_marzie@yahoo.com

Background: Aneuploidy is the most common chromosomal abnormality in human that causes congenital anomalies and abortions. Aneuploidy errors during the meiosis division include: adhesion of Kinetekor-microtubules, spindle assembly checkpoint and cytokinesis abnormalities. The Aurora kinase gene families play an essential role in cell division, including control of the centrosome, function of spindle, kinetochor-microtubule interactions and cytokinesis. A member of this gene family is named AURKC which is responsible for meiosis spindles and it seems some chromosomal disorder mutations related to the exon 6 of this gene.

Methods: in this study, we extracted DNA of abnormal fetus amniotic fluid samples with AmpliSens Kit. The exon 6 of AURKC gene were amplified by Polymerase Chain Reaction (PCR) technique and finally the Sanger Sequencing System was used to analyze and identify the mutation point.

Results: a heterozygous c.704G>A (p.Gly235Glu) mutation in the exon 6 of AURKC gene detected and this mutation was not found in normal population. It is predicted to be damaging by SIFT.

P-224: A new mutation in CLCN1 gene in an Iranian fam-ily with Myotonia Congenita

Jazayeri R1*, Saberi SH2, 3

1. Non-Communicable Diseases Research Center, Alborz University of Medical Sciences, Karaj, Iran.
2. Medical-Genetic Counseling Center, Alborz Welfare Organization, Karaj, Iran.
3. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

roshanakjazayeri@gmail.com

Myotonia congenita is an inherited myopathy, characterized by the inability of the skeletal muscles to quickly relax after voluntary movements, begins in early to late childhood causes problems with the tone and contraction of skeletal muscles can cause muscle enlargement. There are two types: Becker-type is the most common form autosomal recessive, while Thomsen’s is a very rare, relatively mild, autosomal dominant. Both are caused by loss of function mutations in the gene encoding the chloride channel (CLCN1) plays a role in muscle cell repolari- zation. This study was designed to find the genetic defect in a 35 years-old affected male, with lordosis, myotonia, difficulties in walking, and muscles stiffness, having a brother with the same symptoms, a healthy sister and unaffected consanguineous parents, who was married with an apparently normal non-consanguineous female, and they wanted to know about recurrence of this disorder in their children. Sanger Sequencing of the CLCN1 gene was performed for the affected individual. He was homozygote for a probably pathogenic variant in CLCN1 gene (c.10645 C>A). Co-segregation analysis was performed in other family members. His mother was died, his father determined heterozygote, the affected sibling was homozygote, the unaffected one was negative for this variant. So, it seems the disease has an autosomal recessive hereditary pattern in this family. Knowing this probably mutation, they can use preimplantation genetic diagnosis (PGD) to assure that the embryos implanted are not affected, or use prenatal diagnosis. Genetic counseling and carrier detection is recommended for all high-risk individuals in this family.

Keywords: Myotonia Congenita, genetic counseling, CLCN1 gene, sanger sequencing, Iran

P-225: Association of TRAILR1(rs20576) with Response to Interferon ? Therapy in Multiple Sclerosis Patients in Iran

Jazireian P1, Talesh Sasani S2, Assarzadegan F3

1. University of Guilan, University Campus 2, Rasht, Iran
2. Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran.
3. Department of Neurology, Imam Hossein Hospital, Shahid Beheshti University of Medical Sciences, Tehran, Iran

sasani@guilan.ac.ir

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) with unknown ethiology that leads to significant neurologic disability in young adults. There are strong evidences that it arises from complex interactions between environmental and genetic factors. Recombinant interferon beta (IFN-?) is one of the most widely used first line therapy in MS. It reduces the number of relapses and delays disability progression in relapsing/remitting (RR) MS. Nevertheless, up to 50% of patients treated with IFN-? continue experiencing relapses and/or worsening disability. Several studies have shown association between gene allelic variations and...
response to IFN-? treatment. In the present work, we examined the potential role of TRAILR1 polymorphism (rs20576) on response to IFN beta therapy in Iranian MS patients. This study was carried out with 30 responder and 30 non-responder patients. Responders had neither relapses nor increase in expanded disability status scale (EDSS) over the 2-year follow-up period, whereas nonresponders had at least two relapses or an increase in EDSS of at least 1 point. Genomic DNA was isolated from peripheral blood of both group individuals. TRAILR1(rs20576) genotyping was performed by PCR-RFLP method. In conclusion, this study provides more information for identification of a biomarker associated with the response to IFN beta therapy in Iran.

Keywords: Multiple sclerosis, Interferon-?, TRAILR1, Single nucleotide polymorphism

P-226: Investigation of miR-210 expression in response to a VEGFB antagonist peptide in breast cancer mice

Kaboudan F, Talesh Sasani S, Asghari M

1. Department of Genetics, Faculty of Basic Sciences, Islamic Azad University, Tonekabon, Iran.
2. Assistant Professor, Department of Biology, University of Guilan, Rasht, Iran.
3. Associated Professor, Department of Biology, University of Guilan, Rasht, Iran.
E-mail: sasani@guilan.ac.ir

Breast cancer is the most common cancer in women worldwide. MicroRNAs, small non-coding RNAs, are pivotal regulators of cancer metastasis and progression through angiogenesis inhibition. MiR-210 is an important gene regulator induced under hypoxia conditions and acts as an oncogenic or tumor suppressor miRNA in different cancer types. VEGF signaling pathway plays a serious role in cell proliferation and angiogenesis. MiR210 regulate VEGF and VEGFR (angiogenesis key factors) expression and can be a main regulator of cancer progression. In this study we evaluated miR-210 expression level in Balb/c mice having 4T1 cell-line and treated with a VEGFB antagonist peptide. Breast samples were obtained from treated and untreated mice as case and control subjects, respectively. We extracted microRNAs by High Pure miRNA Isolation Kit (Roche), after poly adenylation process cDNA was synthesized using anchored Oligo-dT. RT-PCR and Real-Time PCR were performed in order to investigation of miR-210 expression level. MiR-210 and U6 (as reference gene) specific primers were used. Our results showed a significant difference of miR-210 expression level between the case and control subjects. The results show that miR-210 may be a useful target for tumor metastasis and progression suppression strategies.

Keywords: miR-210, Breast Cancer, Angiogenesis, VEGFB

P-227: Leishmania major alters apoptotic genes on human infected macrophages to survive and replicate intracellularly

Kalavi Kh1,2, Jorjani ON1, Faghihi MA3,4, Mowla SJ3

Golestan University of Med. Sciences
Tarbiat Modares University
Shiraz University of Medical Sciences
4University of Miami
kalavi25@yahoo.com

Background: Parasites of the genus leishmania cause leishmaniasis, a group of infective diseases that range from cutaneous lesions to lethal visceral forms. As macrophages phagocytose to monitor pathogens, while inside the host cell, leishmania species adapt and modulate to make a hostile environment of its pleasure to survive and replicate. In this study we performed a transcriptome analysis using RNA-seq on human monocyte derived macrophages infected with L major.

Methods: High purity human monocytes isolated using Magnetic Activated Cell Sorting(MACS) method and cultured in presence of CSF-1(MCS-F) to derive macrophages. MDMs then co cultured with metacyclic promastigotes of L. major for 4 hours. RNA isolation performed on lysed and homogenized cells using Trizol reagents(qiagen). RNA-sequencing was performed using ILLUMINA platforms after RNA quality controlling. Data analysis performed using Dseq2(Bioconductor) packages.

Results: We used RNA-seq generated data sets to assess transcriptome changes in human macrophages infected with L.major in a 4 hour post infection time point. Differential gene expression, pathway and gene ontology analyses showed us upregulations of inflammatory (pro & post) immune responses, metabolic pathways such as glycolysis, and downregulations of some others as Fc gamma receptor genes. Apart some critical transcriptome modulations in direction with parasite’s pleasure as reprogramming of apoptosis, critical cell proliferation regulations and ECM remodeling, there is a persisted efforts performed by the parasite to prevent host macrophages’ apoptosis; while as a rule infected cells have to be get to apoptosis in a programmed manner.

Conclusion: As the parasite uses its tools to modulate host cell to survive and replicate intracellularly; there is evident due to this study that preventing infected macrophages from apoptosis is a major strategy to infect and deepening the disease.

Keywords: RNA-seq, Macrophages, L.major, apoptotic genes

P-228: Association between Q7R Saitohin polymorphism and susceptibility to Alzheimer disease; a meta-analysis study

Kamali K, Taravati A, Sayyadi S, Tohidi F, Mahghani A

Department of Molecular and Cell Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran
E-mail: kamalikasra@yahoo.com

Background and Objectives: The association of Q7R Saitohin polymorphism with Alzheimer disease has been investigated in several studies of different populations and conflicting results were found among them. For this reason, this meta-analysis performed to determine whether Q7Rsaitohin polymorphism is associated with susceptibility to Alzheimer disease in Caucasian population. Methods: A literature search was conducted in electronic databases including PubMed, Scopus, Elsevier, Springer and Google Scholar to find eligible studies in Caucasian population. The pooled odds ratios (ORs) with 95% confidence intervals were calculated to evaluate the association of Q7R polymorphism with Alzheimer disease. Association of Q7Rsaitohin polymorphism evaluated under dominant, recessive, co-dominant, and allelic models. Results: The ORs for the Q7R saitohin polymorphism and Alzheimer disease were indicative of positive association under recessive genetic model. The results indicated that Q7Rsaitohin polymorphism was significantly associated with the increased risk of Alzheimer disease in recessive model (RR vs. QR+QQ: OR=1.555; p=0.05).

Conclusions: In summary, Q7R saitohin polymorphism
is positively associated with the increased risk of Alzheimer disease in Caucasian population, especially the homozygous carriers. It could be of value to investigate its association with Alzheimer disease in combination with additional risk factors. However, very large studies with different ethnic population are required to accurately demonstrate the role of this candidate gene in development of Alzheimer disease.

**Keywords**: Alzheimer disease; satoihin polymorphism; meta-analysis

**P-229**: The association of rs266729 and rs17300539 of adiponectin gene with cholesterol and triglyceride level in a Iranian population

Karimi H1, Nezhdadi M 1, Hedayati M

1- Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran

2- Cellular and Molecular Endocrine Research Center, Research institute for Endocrine Sciences, Shahid Beheshti university of Medical Science, Tehran, Iran.

helma.k4455@yahoo.com

Introduction: Adiponectin is a hormone mostly secreted from adipose tissue and studies shows that it has association with metabolic syndrome. The genetic contribution of polymorphism of adiponectin gene, located in the promoter of gene has not been completely clarified and there are contradictions in Iranian population. We aimed to evaluate single-nucleotide polymorphism (SNP) association with obesity factors of cholesterol and triglyceride since having more information on the subject helps to integrate the studies.

Material and method: a case-control study was performed on 80 participant with fasting blood sugar >100 mg/dl as diabetic group and 80 participant with fasting blood sugar 70-100 mg/dl as non-diabetic (healthy) group, which referred to a laboratory of hospital. The samples of HBA1C of last 3 mounth was also gathered. all cases had no drug history in diabetes and no other special disease (there were first line). DNA was extracted from blood samples and genotyping method using PCR-RFLP and digested enzymes of HhaI and MspI was performed.

Result: There was statistically significant association between rs17300539 and levels of triglyceride and total cholesterol level (p=0.039 and p=0.032) respectively. However there were no significant association between rs266729 of adiponectin gene and two factors.

Keywords: cholesterol, rs266729, rs17300539, adiponectin gene

**P-230**: TOR1A variants cause a severe arthrogryposis with developmental delay, strabismus and tremor

Kariminejad A1, Dahl-Halvarsson M2, Ravenscroft G1, Afroozan F3, Keshavarz E1, Faraji Zonooz M1, Najmabadi H1, Goulia H3, R Davis M3, G Laing N3, Tajsharghi H1,4

1. Kariminejad-Najmabadi Pathology & Genetics Center, Tehran, Iran
2. Department of Pathology, University of Gothenburg, Sahlgrenska University Hospital, Sweden
3. Centre for Medical Research, The University of Western Australia and the Harry Perkins Institute for Medical Research, Nedlands, Western Australia, Australia
4. Department of Radiology, Mahdieh Hospital, Shahid Beheshti University of Medical Science, Tehran, Iran.
5. Department of Diagnostic Genomics, Pathwest, QEII Medical Centre, Nedlands, Western Australia, Australia
6. School of Health and Education, Division Biomedicine and Pub-

lic Health, University of Skovde, SE-541 28, Skovde, Sweden
arianakariminejad@yahoo.com

**Background**: Autosomal dominant torsion dystonia-1 is a disease with incomplete penetrance most often caused by an in-frame GAG deletion (p.Glu303del) in the endoplasmic reticulum luminal protein torsinA encoded by TOR1A.

Findings: We report an association of the homozygous dominant disease-causing TOR1A p.Glu303del mutation, and a novel homozygous missense variant (p.Gly318Ser) with a severe arthrogryposis phenotype with developmental delay, strabismus and tremor in three unrelated families. All parents who were carriers of the TOR1A variant showed no evidence of neurological symptoms or signs, indicating decreased penetrance similar to families with autosomal dominant torsion dystonia-1. The results from cell assays demonstrate that the p.Gly318Ser substitution causes a redistribution of torsinA from the endoplasmic reticulum to the nuclear envelope, similar to the hallmark of the p.Glu303del mutation.

Conclusion: Our study highlights that TOR1A mutations should be considered in patients with severe arthrogryposis and further expands the phenotypic spectrum associated with TOR1A.

**Keywords**: TOR1A; Endoplasmic reticulum luminal protein torsinA; DYT1 dystonia; TOR1A p.Glu303del; Severe arthrogryposis

**P-231**: Investigation of NOD1 and NOD2 gene expression in coronary artery disease patients in Yazd province

Keikha T, seifati S.M (Corresponding author)

Department of Biology, Islamic Azad University Ashkezar Branch
E-mail: keikkhatahere6894@gmail.com

Coronary artery disease (CAD) is the most common type of heart disease associated with the activation of innate immune TLRs and NOD-like receptor pathways. The NOD1 gene, expressed in cardiac and vascular smooth muscles, is a cytosolic receptor that plays an important role in inflammatory responses by activating the NF-kB pathway. It is involved in many human diseases, but its role and its relationship with the cardiovascular system are less understood. The NOD2 gene is expressed in endothelial cells of the vascular wall and play important roles in promoting vascular inflammation and linking cardiac injury to inflammation. The aim of this study was to investigate NOD1 and NOD2 expression in 13 patients with CAD in Yazd province.

**Materials and methods**: In this Study, we recruited 13 patient and 10 normal control blood samples from Afsar Hospital in Yazd. RNA samples were isolated using Trizol reagent. The mRNA levels of NOD1 and NOD2 genes were determined by Real time PCR. Statistical analysis was performed using t-test.

**Results**: It was revealed that the expression of NOD2 gene in the patients was significantly increased compared to the control group (p<0.05). Moreover, our results demonstrated that although NOD1 expression in patients was more than control group, this difference was not statistically significant (p>0.05).

**Conclusion**: It can be concluded that NOD2 gene plays an important role in CAD disease. Pharmacological targeting of NOD2-mediated signaling pathways may provide a novel approach to treatment of cardiovascular diseases.

**Keywords**: Coronary artery disease, NOD1, NOD2, inflammation
P-232: Fingolimod treatment induces expression of FENDRR lncRNA
Khani-Habibabadi F1, Javan M2, Sahraian MA3, Naser Moghadasi A4, Behmanesh M1

1. Genetics Department, Faculty of biological science, Tarbiat Modares University, Tehran, Iran
2. Department of Physiology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3. Multiple Sclerosis Research Center, Neuroscience Institute, Tehran University of Medical Sciences and Sina Hospital, Tehran, Iran/Iranian Center of Neurological Research, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran

Introduction: Fingolimod has gotten the FDA approval for Multiple Sclerosis (MS) treatment due to its capacity in trapping the lymphocytes at lymph nodes and decline the disease progression rate. For activation, Fingolimod would be transported actively to the cytosol and phosphorylated to play the role of sphingosine phosphate 1 (S1P) in triggering the downstream signaling pathways of S1PR1-5. In this study, we focused on the signaling pathways located downstream of S1PRs to find the possible targets of Fingolimod which could be involved in the drug’s action mechanism. We specifically considered lncRNAs altering the chromatin structure by binding to chromatin remodeler complexes. Methods: To find the possible target of Fingolimod, we analyzed the promoters of chromatin remodeler lncRNAs by JASPAR database to find the transcription factors binding sites for those factors located downstream of the S1PRs signaling pathway. Jurkat cell line was treated by 10nM and 100nM Fingolimod concentration in 24 and 48 hours and qRT-PCR was performed on cDNA samples. Results: Several lncRNAs including HOTAIR, ANRIL, and FENDRR were chosen by bioinformatics analysis and among them, FENDRR was dramatically upregulated in response to Fingolimod treatments in a dose and time-dependent manner. Conclusion: Fingolimod could alter the chromatin structures by influences the expression of FENDRR and may play some aspects of its role in this way.

Keywords: Fingolimod, FENDRR, lncRNA, S1P

P-233: TLR2 and TLR4 Genes Expression Analysis : A Hopeful Approach To Recognition Causes of Essential Hypertension

Kharaei F1, Shekari M2, Nejatizadeh A3, Eghbal Eftekhari T4, Farahbakhsh E5, Nabizadeh F1

1. Department of medical Genetics, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran; 2Molecular medicine research Center, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran

Background: Cardiovascular diseases (CVD) refer to a group of life threatening disorders that affect heart and circulatory system. As a main risk factor, essential hypertension is the most common cause of damages. Previous studies showed different pathways contribute to pathogenesis of hypertension. Among them, inflammatory pathways, particularly immune receptors, due to their important role, need more attention to research. In the other hand, the exact cause of hypertension is not clear. Therefore, in this study we aimed to evaluate TLR2 and TLR4 genes expression in primary hypertensive patients compared with healthy controls.

Methods: 50 hypertensive patients and 50 healthy controls (35-65 years old) without any drug consumption and inflammatory disorders participated in this study. peripheral blood obtained and TLR2 and TLR4 genes expression measured by Real-time PCR.

Results: TLR2 gene expression up regulated in 36% of patients compared with healthy controls but it was not statistically significant. About TLR4, statistically significant up regulation was observed (P< 0.001).

Discussion: Up regulation of innate immunity receptors indicates undeniable role of this pathway in pathogenesis of hypertension and CVD. Our findings suggest that, these pathways can be a suitable candidate for control and treatment of hypertension.

Keywords: Essential Hypertension; TLR2; TLR4; Inflammation

P-234: Whole Exome Sequencing revealed a novel mutation in a case with SOFT syndrome

Khoshevan A1, Hajjar M2, Mohammadladi J2

1. Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Department of Medical Genetics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences

SOFT syndrome (MIM614813) is a rare primordial dwarfism characterized by short stature, onychodysplasia, facial dysmorphism and hypotrichosis, which is caused by mutations in the POC1A gene. SOFT syndrome is characterized by severely short long bones, peculiar facies associated with paucity of hair, and nail anomalies. Growth retardation is evident on prenatal ultrasound as early as the second trimester of pregnancy, and affected individuals reach a final stature consistent with a height age of 6 years to 8 years. The facial dysmorphism includes a triangular face with a pointed chin, relative macrocephaly with frontal bossing, frontal balding and midface hypoplasia. Only a few patients with mutation-confirmed SOFT syndrome have been reported to date, most of whom carried homozygous variants that were strongly associated with consanguineous marriages.

We report a 5 years old girl with SOFT syndrome showing short stature. Karyotyping did not show any chromosomal abnormality. After DNA extraction, Whole Exome sequencing was used to reveal if pathogenic variants exist. In silico analyses were applied to see if the variants are pathogenic. PCR and Sanger sequencing was performed to check the variant in the patients. Segregation analysis was also done to check the family members.

The results showed a novel homozygote mutation in exon5 of POC1A gene. The mutation was confirmed by sanger sequencing in family members. POC1A encodes the POC1 centriolar protein A, which plays a role in centrosome-mediated cell mitosis control via mitotic spindle organization and cilia formation. Therefore, SOFT syndrome could be classified as a type of ciliopathy. The novel mutation found in this study can provide another evidence for the role of POC1A in SOFT syndrome. This variant can help to identify mutations in families with SOFT syndrome. This is the first study presents the mutation in Iranian patients with SOFT syndrome.

Keywords: SOFT syndrome, Whole Exome Sequencing, Novel mutation
**P-235: Novel mutation in PNPLA1 gene, the causative defect in Lamellar Ichthyosis (LI)**

Koochakkhani Sh1, Shekari M2, Nejatizadeh A2, Nabizadeh F1, Allamehdezeh Z1, Ahmadi B2, Shams Sh2

1. Student Research Committee, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
2. Bandar Abbas Medical Genetics Laboratory (BMGL), Bandar Abbas, Iran

shab.koochak@gmail.com

**Introduction:** Lamellar Ichthyosis (LI) is an autosomal recessive and heterogeneous group of congenital disorders. Mutations in one of the TGM1, ALOXE3, ALOX12B, ABCA1, NIPAL4, CYP4F22, PNPLA1, LIPN, CERS3 genes can cause LI. Infants with LI disorder are generally born with waxy skin that is typically shed within the first two weeks of life and then affected babies have scaly skin. Other signs and symptoms of the condition include lips that turn outwards, infections, dehydration, respiratory problems, frequent skin itching and dark stain on the skin.

**Methods:** We analyzed a five-year-old girl with LI that was born of a consanguineous marriage in Hormozgan province of Iran with no history of LI in her family. Blood sample was prepared for targeted NGS (Next Generation Sequencing). To validate the mutations, sanger sequencing was performed in family members and the data were analyzed.

**Results:** A novel missense homozygote mutation (c.939-952 Del 13 Nt ins T) was identified in PNPLA1 gene. Sanger sequencing showed that her parents carry one copy of the mutated gene, but typically do not have any symptoms of the LI.

**Conclusions:** This is the first case in the literature describing a novel c.939-952 Del 13 Nt Ins T mutation in PNPLA1 gene. NGS and complimentary investigations were done for the patient and sequencing results showed that she is homozygous for the noted mutation in PNPLA1 gene. Regarding the identification of the parents’™ heterozygosity, it is possible to prevent the occurrence of this rare disease in Hormozgan province during the next pregnancy.

**Keywords:** Lamellar Ichthyosis, PNPLA1, NGS

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**P-236: The effect of herbal Urtica extract on claudin-3 gene expression in gastric adenocarcinoma cell line (AGS)**

Mahmoodi F1, Deilami Khiabani Z2

Department of molecular genetics, Zanjan Azad University, Zanj, Iran

mahmoodi.fatemeh.72@gmail.com

Gastric cancer is the most common cancer in the world and the altered expression of differÃ¬ent genes such as claudins have been demonstrated in this cancer. Claudins are the main proteins of tight junctions in epithelial cells. It has been reported that claudin-3 is overexpressed in gastric adenocarcinoma. Considering the least side effect of herbal extracts, in this study we have evaluated claudin-3 gene expression in AGS cells treated with Urtica extract. The AGS cells were incubated at 37°C containing 5% CO2 with 85% humidity DMEM with 10% FBS. The cells were treated with concentrations of 800, 1200, 2000 ?g /ml of Urtica extract for 48 hours. Extraction of RNA, synthesis of cDNA has been done using kits. The study of claudin-3 gene expression was performed by Real time PCR and also GAPDH gene was used as the internal control. The results have shown significant decrease in expression rate of claudin-3 gene in all concentrations of Urtica extract. The most significant reduction have shown with concentrations of 2000 ?g /ml of Urtica. Claudin-3 may become therapeutic targets for cancer treatment and Urtica as herbal extract decreased the expression rate of claudin-3 significantly.

**Keywords:** Gastric cancer, Claudin-3, Urtica, AGS

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**P-237: RNA Sequencing Indicated Circular RNAs With Regulatory Potential and Abundant Expression in Human Dorsolateral Prefrontal Cortex**

Mahmoudi E, Fitzsimmons C, Cairns M

1. School of Biomedical Sciences, Faculty of Health and Medicine, University of Newcastle, Callaghan, NSW 2308, Australia.
2. Schizophrenia Research Institute, Sydney, Australia

Mahmoodi E, Fitzsimmons C, Cairns M

mahmoudi.e@uon.edu.au

**Introduction:** Only recently, a new class of non-coding RNA, known as circular RNA (circRNA), was discovered in several animal species including humans with an abundant expression in the brain. CircRNAs are constructed by the back-splicing of two RNA ends, generating a circle structure with a length ranging from one to many exons. CircRNAs act as transcription regulators, microRNA regulators, host gene expression modulators and template for translation. In this study, we investigated whole-transcriptome profiling of circRNA in the post-mortem brain using Circ-Seq analysis to better understand their function in the brain.

**Methods:** Following enrichment for circRNA species by RNase R treatment, sequencing libraries were prepared from cerebral cortex (BA46) of 23 individuals using Illumina TruSeq (150 cycles) and sequenced by an Illumina NexSeq500. Sequencing data were analysed by CIRCExplorer2 pipeline to identify circRNA transcripts.

**Results:** The results revealed, surprisingly, a large number (52,000) of circRNAs, many of which were highly expressed across the samples. Interestingly, a large proportion of the identified circRNAs were rare or not previously reported. Furthermore, de novo assembly for circRNAs showed many of them are alternatively spliced, suggesting complexity of these molecules. We also discovered 2,440 novel circRNA that are spliced out of unannotated exons. Gene pathway analysis showed many of the circRNAs are transcribed from the genes implicated in important neurological activities, including synaptic function. Moreover, subsequent bioinformatics analysis indicated that many of the circRNAs potentially interact with miRNAs, supporting the miRNA sponging function for these circRNA. To validate the sequencing findings, real-time PCR was performed using outward primers sets designed to uniquely amplify circular transcripts, with the results confirming the observations in the Circ-Seq.

**Conclusions:** These findings indicated the abundance of circRNA as well as the complexity of these transcripts in the human brain. Furthermore, our results support the hypothesis that circRNA are potentially functional, acting as a sponge, through binding to target miRNAs.

**Keywords:** Circular RNA (circRNA), Expression analysis, RNA-Seq, Alternative splicing

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**P-238: Association of miR-217 upregulation and breast cancer in Iranian breast cancer patients**

Majidi F2, Salimi M1, Mozdarani H3

1. 2. Bandar Abbas Medical Genetics Laboratory (BMGL), Bandar Abbas, Iran
2. Department of molecular genetics, Zanjan Azad University, Zanjan, Iran

ebrahim.mahmoudi@uon.edu.au

**Introduction:** Breast cancer is the most common cancer in Iranian breast cancer patients and also GAPDH gene was used as the internal control. The results have shown significant decrease in expression rate of claudin-3 gene in all concentrations of Urtica extract. The most significant reduction have shown with concentrations of 2000 ?g /ml of Urtica. Claudin-3 may become therapeutic targets for cancer treatment and Urtica as herbal extract decreased the expression rate of claudin-3 significantly.

**Keywords:** Gastric cancer, Claudin-3, Urtica, AGS

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**Abstracts of the 3rd International & 15th Iranian Genetics Congress**
1. Department of Medical Genetics, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
2. Research and technology department, Islamic Azad University
3. Department of Medical Genetics, Tarbiat Modares University, Tehran, Iran
faezechmajid159@gmail.com

Despite the advances in diagnosis and new treatments such as targeted therapies, breast cancer is still the most common cause of cancer death in women. Finding biomarkers related to breast cancer in different aspects such as early detection, prognosis, treatment response, etc. has huge importance. microRNAs play variety of significant roles in tumorigenesis, tumor progression and metastasis in breast cancer. In the present study the miR-217 expression was investigated as a potential breast cancer related biomarker in 45 tumor and normal adjacent breast tissues using micro RNA extraction and cDNA synthesis followed by Real-time RT-PCR. The data was statically analyzed by student t-test using SPSS software. Our data showed that the expression of miR-217 was significantly upregulated in breast cancer tissues compared with normal adjacent breast tissues. In conclusion miR-217 up regulation was associated with breast cancer. Further analysis are needed to distinguish its association with pathological and clinical characteristics.

Keywords: breast cancer, miR-217, biomarker, epigenetics

P-239: Poly(A)-tail shortening Length: an Alternative Epigenetic Mechanism for Translational Control of Oncogenes
Malek Zadeh K
Hormozgan University Medical of Science
dkeyanoosh@gmail.com

Introduction: mRNA poly(A) tails in 3’UTR is important for its stability and translation. t is observed Poly(A) tails is dynamic and impact the stability and translation of most eukaryotic messenger RNAs and affected on microRNAs in repression. In this study, it is hypothesized that this mechanism could epigenetically explain the reason of overexpressing of oncoproteins proteins without any genetic alterations in proto-oncogenes in many cancers.

Material & Method: Proliferating non-transformed cell lines were cultured. The breast, lung and colon cancers cell lines were selected and compared to immortalized non-transformed normal epithelial cell lines and normal corresponding tissues. The genes included CyclinD1, and RAB10, which were expressed in all samples. Cell lines were also treated with Actinomycin-D for inhibition the transcription. 3’-RACE and sequencing was done to analyses 3’-UTR length of oncopenes.

Results: It is revealed that most of the cell lines expressed a higher amount of the shorter mRNA isoform (P<0.001). The shorter mRNA was 1.9 times more stable than was the longer mRNA. Shorter mRNA isoforms was more stable and typically producing 4.3-fold more protein.

Conclusion: It is found that cancer cell lines often expressed mRNA isoforms with shorter 3’-UTR. These shorter isoforms usually resulted from alternative cleavage and polyadenylation (APA). The APA had functional consequences in such a way that shorter mRNA isoforms showed loss of microRNA-mediated repression.

Keywords: Poly(A)-tail shortening Length; alternative cleavage and polyadenylation (APA); Oncogenes; Epigenetic

P-240: Lack of association between variant of miR-125a-3p and Neuregulin1 gene among Iranian patients with schizophrenia

Malekzadeh A1, Mirzadeh Azad F2, Torkashvand S1, Matin M1, Nafissi N1, Mowla SJ2
Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
afz_malek@yahoo.com

Schizophrenia is a mental disorder with 1% lifetime prevalence. Both genetic and environmental risk factors are playing a role in the disease etiology. Hence, we have conducted a study to indicate that variation in the miR-125a-3p would reduce possibility of reaction mRNA of NRG1 gene. Blood samples were collected from 102 patients with schizophrenia. The diagnosis of schizophrenia was based on clinical interview according to DSM-IV-TR. Blood samples of 113 healthy individuals were collected in a coordinated manner with patients. The genomic DNA was extracted by Salting-out method and PCR-RFLP was used to examine SNP polymorphism. In demography survey the significant differences in the occupational and educational status, alcohol abuse as well as smoking (P = 0.000, P = 0.034 and P=0.000 respectively) were observed between case and control groups. In genetic study, All 223 samples showed ancestral genotype in rs143525573 of miR-125a-3p (GG). Our results indicate that rs143525573 of miR-125a-3p have not involved in the risk of schizophrenia.

Keywords: Neuregulin-1, miR-125a-3p, SNP, schizophrenia

P-241: A novel variant of C1orf-neRNA is upregulated in breast cancer cell lines and tissues
Malekzadeh A1, Mirzadeh Azad F2, Torkashvand S1, Matin M1, Nafissi N1, Mowla SJ2
Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran
afz_malek@yahoo.com

Introduction: long non-coding RNAs (lncRNAs) play an important role in regulating gene expression at various levels, including alternative splicing, regulation of protein activity, localization, post-transcriptional processing, as well as chromatin modification and transcription. lncRNAs are aberrantly expressed in various cancers, including breast cancer, where they play vital roles in tumor initiation and progression. Recently, a clinical potential for them as a new class of biomarkers and therapeutic targets, has been claimed. C1orf is a ~70kb long IncRNA with 5 exons, located on chromosome 1.

Method: We used several bioinformatics tools to find out the lncRNAs with significant expression alteration in breast cancer. We designed specific PCR primers to detect any potential alternatively spliced variants of the IncRNA. Then, we used real-time PCR approach to compare the expression of the gene in breast cancer vs. apparently normal tissues of the same person.

Result: Using specifically designed primer pairs, we amplified an additional band with different amplicon size to the main variants. Sequencing data of the RT-PCR product led to the discovery of a novel alternative expressed variant of C1orf, which is expressed in MCF7 breast cancer cell line, as well as breast cancer tissues. Real-time PCR revealed a significant upregulation of the variant in breast cancer tissues, compared to the apparently normal tissues, obtained from the same patients.
Conclusion: Here, we are reporting the discovery of a novel alternatively-spliced variant of C1orf, with a potential role in breast tissue carcinogenesis. Further functional analyses is needed to confirm a causative role of the variant in breast carcinogenesis.

Keywords: lncRNA, alternative splicing, breast cancer

P-242: Decreasing of viability in Sodium Nitrite treated of ITPA down-regulated Human Umbilical Vein Endothelial Cells

Marashi SM1, Abedi kichi Z1, Ahmadi AH2, Behmanesh M1
1. Department of Genetics, Faculty of Biological sciences, Tarbiat Modares University, Tehran, Iran
2. Department of Biology, Faculty of Basic Sciences, Persian Gulf University, Bushehr, Iran
maral.marashi@yahoo.com

ITPA gene has been identified as a DNA repair gene that maintains stability of the genome. DNA repair systems are essential for the maintenance of genome integrity. When repair proteins function is impaired due to mutation, the genome may become unstable. Defects in ITPA can result in inosine triphosphatase deficiency, so inosine triphosphates (ITP) accumulate in the living cells. Sodium nitrite treatment induce Oxidative deamination that causes membrane damage, protein oxidation, lipid peroxidation and DNA damage. This study was focused on investigating the survival of ITPA down-regulated HUVEC compared to normal HUVEC in the presence of Sodium Nitrite. To evaluate the cell viability assay, we used the 3-(4,5-di-methylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Briefly, 2x104 cells were incubated in a 96-well plate in the presence of various concentrations of sodium nitrite solution for 24-48 hours to determine the effect on endothelial cell proliferation. Sodium nitrite decreases proliferative activity in ITPA down-regulated compared to normal HUVEC cells. The proliferation of treated cells was significantly lower than in the control wells (p<0.05).

Keywords: inosine triphosphatase(ITPA), Sodium Nitrite , MTT, Cell Viability

P-243: Correlation between P21 and miR-605 expression in the colorectal tumor tissues; A pilot study on 10 Iranian patients

Mashayehk Z, Kazemi Nezhad S R, Hajjari MR
Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
ze87.mashayehk@gmail.com

Considering the high prevalence and high mortality rate of colorectal cancer and also the importance of the functional p53 network in this cancer, it is important to examine the factors affecting the P53 functional network. According to studies, increasing the p53 transcriptional activity by miR-605 was further evidenced by elevated mRNA levels of p53 target gene p21/CDKN1A.

Considering the fact that no studies have been done on correlation between P21 and miR-605 expression in colorectal tumor tissues, We decided to investigate the correlation between fold changes in the expression of p21 and miR-605 genes in the tumor tissue of colorectal cancer. According to our findings, there is a positive correlation between fold changes in the expression of these two genes in the tissues of the subjects (p=0.00001).

Material and Method:
In this study, colorectal tumor tissue of 10 subjects were investigated. Total RNA was isolated by RNX-Plus from tumor tissues and the correlation between fold changes in the expression of p21 gene and miR-605 was performed by quantitative analysis of Real-Time PCR.

Result:
The result of this study, showed there is a positive correlation between fold changes in the expression of the miR-605 and P21 genes in the tumor tissues.

Discussion and conclusion:
The results of this study, showed there is positive correlation between fold changes in the expression of the miR-605 and P21 genes in colorectal cancer tissues (p=0.00001), but this requires an examine more of the number of sample

Keywords: miR-605, p21gene, correlation, colorectal cancer.

P-244: Evaluation of relationship between hypermethylation of DKK1 gene promoter with Laryngeal Squamous Cell Carcinoma

Marshhadi Nezhad A, Assadi Tehranri G , Hajmanouchehri F
Department of Genetics, Zanjan Branch, Islamic Azad University, Zanjan, Iran
Arefc.m.n@gmail.com

Laryngeal squamous cell carcinoma is the second most common malignancy among Head and Neck cancers. Several factors are involved in this cancer but epigenetic mechanisms are the most important factor in carcinogenesis. The most important epigenetic change known is methylation, which is divided into two types of hypermethylation and hypomethylation. The aim of this study was to investigate the status of methylation of promoter of DKK1 gene and its relationship with the prevalence and progression of laryngeal squamous cell carcinoma.

Materials and Methods: Genomic DNA was extracted from tissue samples of 29 patients. Also, 30 healthy tissues were used as controls. Extracted DNA was treated by bisulphite and tested by Methylation Specific PCR. The results were analyzed by electrophoresis on agarose gel and SPSS software.

Results: Methylation, hem-methylation and non-methylated were found to be 13.79%, 75.86% and 10.34% respectively in cancerous tissue. Methylation, hemi methylated and non-methylated were also found to be 0, 53.33% and 46.66% in normal tissue. Also, there was a significant relationship between the status of methylation of patients and the degree of tumor differentiation (p = 0.04). Overall, this study showed that methylation of promoter of DKK1 gene with laryngeal squamous cell carcinoma has a significant relation (P = 0.004)

Conclusion: The results suggest the role of the genetic factors in the incidence of laryngeal cancer and the methylation of the promoter of the DKK1 gene could be used as a biomarker in the prognosis and development of laryngeal squamous cell carcinoma.

Keywords: Squamous Cell Carcinoma, DKK1 gene , Methylation

P-245: Ovarian tumor derived-exosomes augment the expression of the nuclear factor NF-kB in human umbilical vein endothelial cells
Masoumi S, Sadeghizadeh M, Babashah S

Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran

sajadmansoumi352@gmail.com

Purpose: Ovarian cancer is one of the most common gynecologic malignancies and the fifth most common cause of cancer death in women. Since the development of cancer cells is a complex process, one of the major concerns is the existence of intercellular communication especially by cancer cells and their non-cancerous cells (e.g., endothelial cells). Exosomes are lipid-bilayer-enclosed extracellular nano-sized (<100 nm) vesicles released by most types of cells and function in intercellular communication. As activation of the nuclear factor NF-κB has been found to control cellular process in cancer, we aim to identify the effects of ovarian tumor-derived exosomes on NF-κB expression in human umbilical vein endothelial cells (HUVECs).

Materials and methods: Exosomes derived from ovarian tumor cells (SKOV3) were purified by ExoSpin kit and characterized by scanning electron microscopy analyses and dynamic light scattering measurements. HUVECs were treated with exosomes (100 µg/ml) or vehicle control (PBS). The effect of tumor-derived exosomes on NF-κB expression was assessed by western blot analysis.

Results and Discussion: Scanning electron microscopic examination revealed that all exosomes had a spherical shape with a diameter of ~50-200 nm. Exosome size measurements by DLS indicated a single bell-shaped size distribution with a peak at ~90 nm. We found that ovarian tumor-derived exosomes augment the protein expression levels of NF-κB in exosome-treated HUVECs compared with that of cells treated with vehicle control (PBS). This data is consistent with the previously suggested role for NF-κB signaling in tumor angiogenesis.

Keywords: Ovarian cancer, NFκB

P-246: Association between Eomes gene expression and clinical morphological characteristics in breast cancer

Matinzadeh M1, Saffari M1, Shirkoohi R2

1. Group of Genetics, Cancer Research Center, Cancer Institute, Imam Khomeini Hospital Complex, Tehran, Islamic Republic of Iran.
2. Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Poursina Ave, Keshavarz Blvd, Tehran, Iran.
3. Cancer Biology Research Center, Cancer Institute, Imam Khomeini Hospital Complex, Tehran, Islamic Republic of Iran.

minamatinzadeh@yahoo.com

Breast cancer is the most common cancer in the world after lung cancer and is the fifth cause of cancer mortality, about 90 percent of cancer mortality is virtue of metastasis. According to our previous study, we know eomes that are involved in type1 EMT, downregulat the E-cadherin so eomes has important role in cell adherence and therefore in metastasis. This study sought to investigate the eomes gene expression in tumor tissues versus pair-match non-tumor tissues that find probably associated with stage, grade, age, size and metastasis in breast cancer tissues. This retrospective study includes 72 breast cancer tumor and normal tissues that obtained from tumor bank of cancer Institute Imam Khomeini Hospital according ethical principles. Eomes gene expression was evaluated by Real-time PCR. Statistical analysis was accomplished with SPSS. Our results have shown that the expression level of Eomes gene was increased in breast cancer tumors, additionally it has negatively associated with high staged and grade. According to our results, it can be said that Eomes (regarding to its potential) can play an effective role as a predictive biomarker.

Keywords: eomes, breast cancer, EMT

P-247: Novel mutation in gene SLC6A8 causes cerebral creatine deficiency syndrome-1; a NGS clinical report

Maydanchi M, Jamshidabadi Sh, Ebrahimi A

Parse Clinic of the Genetics, Second floor, The building of doctors 75, Royan alley, Keshavarz boulevard, Tehran, Iran

melika.maydanchi@gmail.com

Introduction: Cerebral creatine deficiency syndrome-1 is an X-linked disorder of creatine (Cr) transport characterized by mental retardation, severe speech delay, behavioral abnormalities, and seizures. It has a prevalence of 0.3 to 3.5% in males. Carrier females may show mild neuropsychologic impairment. We report a male patient with developmental delay and hypotonia clinically evaluated and confirmed by NGS panels.

Case report: A couple with consanguine marriage whom had two pregnancy including a dysmorphic child and a positive history for abortion in the second delivery. A child with severe neurologic disturbances including seizures, behavioral problems, speech delay, and inability to engage in structured play, as well as creatine deficiency. H-MRSI showed absence of creatine in the whole brain, which was not corrected by creatine supplementation. However there was not a definite clinical description but the preclinical findings and genetic counselling shows an XLR pattern of inheritance.

Results: Chromosomal abnormalities was ruled out in parents and affected child so the Whole Exome Sequencing (WES) of the proband was done and the results annotated using genome data bases indicated that the child had a homozygous mutation in the SLC6A8 gene which was confirmed by sanger sequencing method.

Conclusion: This mutation confirmed Cerebral creatine deficiency syndrome-1 as a final clinical description compatible with clinical signs described by neurologist.

Keywords: SLC6A8, Mutation, NGS, WES, XLR, Cerebral creatine deficiency syndrome-1

P-248: Coincidence of Familial Hearing loss and epilepsy in a large pedigree: A case report

Miar P1, Zeinalian M1, Abdollahi Z1, Hadian M2, Narreie S3, Sadri Z4

1. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Ala Cancer Prevention and Control Center, Isfahan, Iran

panizmiar11@gmail.com

Hearing loss is a kind of auditory defect with variable degrees and different causes. For example, noise exposure, aging and genetic factors lead to hearing loss, so that proportion of genetic factors is 40%. The results of some studies in Iran showed that the prevalence of hearing loss varies from 4% to 14% in different provinces. Epilepsy, Hearing Loss and Mental
P-249: The analysis of MYBPC3 gene among Iranian patients with familial hypertrophic and dilated cardiomyopathy

Mikaeeli S,2, Rabban B, Seyeden SY,1, Mahdieh N

1. Faculty of Biological Sciences, Azad University, North Tehran 2. Branch, Tehran, Iran. Genetic Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
sahar.mikaeeli@gmail.com

Cardiomyopathies are a group of inherited heterogeneous cardiovascular diseases with the most prevalence among inherited cardiovascular diseases affecting myocardium. The most common types of cardiomyopathies are hypertrophic cardiomyopathy (HCM) and dilated cardiomyopathy (DCM) (1:500 and 1:2,500 respectively). HCM usually defined as left ventricular hypertrophy (LVH) and DCM defined by progressive left ventricular dilatation and incapacitated systolic function. Their inheritance pattern is mainly autosomal dominant. MYBPC3 is one of the most important sarcomere genes which is involved in HCM and DCM. Herein, we studied 23 unrelated patients with HCM and DCM, diagnosed by their medical history. We performed molecular analysis on MYBPC3 gene amplified by polymerase chain reaction (PCR) and mutation analysis was carried out by direct sequencing. The results showed four point mutations among four patients. Three of HCM cases showed c.649A>G (p.Ser217Gly), c.1591G>A (p.Gly531Arg) and c.2864_2865delCT (p. Pro955Argfs) mutations, and one DCM case revealed c.1000G>A (p.Glu334Lys) mutation located on the M-domain of the protein. This investigation revealed the importance of genetic testing of MYBPC3 gene for diagnosis. Molecular analysis would help clinicians for clinical management.

Keywords: cardiomyopathy, hypertrophic cardiomyopathy, dilated cardiomyopathy, MYBPC3 gene

P-250: Genetic Analysis of Five Iranian Patients Affected by Factor X Deficiency

Minoochehr F1, Morovvati S

fatemeh.minoochehr@gmail.com

Introduction: Factor X (FX) is a vitamin K-dependent coagulation zymogen. FX is activated (FXa) by both factor VIIa/tissue factor factor and factor VIIIa/factor IXa. In turn, FXa, which forms the prothrombinase complex together with factor Va, catalyzes thrombin formation. FX deficiency may be hereditary autosomal recessive or acquired and estimated to be approximately 1:1,500,000 people. FX protein encoded by a gene (F10) of 27 kb located on chromosome 13, and containing 8 exons. To date, at least 320 pathogenic mutations have been found in the F10 gene, 78% of which are missense mutations. Classification of severity of FX deficiency is based on the FX activity measurement; a measurement of <1% is categorized as severe, 1-5% as moderate and 6-10% is considered as mild.

Materials and Methods: In this study five patients affected by F10 deficiency were investigated. Peripheral blood was obtained from patients and DNA extracted using a standard method. Genetic analysis of the F10 gene was performed using Sanger sequencing method.

Results: In two patients we found a missense mutation, c.119G>C (p.R40T), in exon 2, and in three patients we detected a missense mutation, c.785G>A (p.G262D), in exon 7 of F10 gene.

P-251: Effect of 12 weeks resistance training on GLUT4 expression and glycemic profile in Wistar rats with type 2 diabetes

Mirakhorai A, Eizadi M

1. Department of Physical Education, Amirkabir University of Technology, Tehran polytechnic, Iran 2. Department of Exercise Physiology, Saveh Branch, Islamic Azad University, Saveh, Iran
zmirakhorai@gmail.com

The Purpose: The Regular exercise has been introduced as a type of non-drug treatment in type 2 diabetes, although the molecular mechanisms responsible for genetic adaptation are less well-known. Male Wistar rats were diabetic with nicotinamide-streptozotocin (220 ± 20 g) and were randomly divided into two groups: exercise (n = 8) and control (n = 8). The exercise group participated in a 12-week resistance training program of 3 sessions per week, and the control group did not participate in any exercise program. Relative expression of GLUT4 gene in gastrocnemius muscle, fasting glucose and insulin resistance and insulin resistance were measured in 48 hours after the last training session in both groups. Data analysis was performed using independent t-test. Changes were less than 5% significant.

Results: Compared to rats in control group, there was a significant decrease in fasting glucose levels by exercise intervention in exercise group (p = 0.000). Serum insulin increased significantly following resistance training (p = 0.011). The expression of GLUT4 in the gastrocnemius muscle increased significantly (p = 0.021), but insulin resistance did not significantly change (p = 0.121).

Conclusion: although insulin resistance did not change, improvement in glycemic profile in response to resistance training may be due to an increase in insulin serum levels or an increase in glucose transmitters in muscle tissue.

Keywords: Type 2 diabetes, Resistance exercise, Glycemic profile, GLUT4 expression

P-252: The relationship between ADIPOQ and ADIPOR2 gene polymorphisms with type 2 diabetes
Moazenrad S1, Balakheyl H2, Cheraghi P2

1. Research Deputy, Golestan University Of Medical Sciences, Gorgan, Iran.
2. Golestan Rheumatology Research Center, Golestan University of Medical Sciences, Gorgan, Iran.
biochemist.sahar@gmail.com

Today we are seeing an increase in the longevity and change in the lifestyle of people around the world. One of them is the transformation of the disease pattern and the prevalence of chronic diseases, including diabetes specially Type 2 diabetes (the most common type of diabetes). Adiponectin, one of the adipokines has recently attracted the attention of many researchers, especially for studies of determining the relevance of genetic susceptibility to type 2 diabetes. Adiponectin is encoded by the ADIPOQ gene. Adiponectin acts by binding to its receptors (AdipoR1 and AdipoR2) and has the potential to lower the risk of type 2 diabetes. ADIPOQ and ADIPOR2 SNPs have been reported in many populations.

Methods: A total of 180 participants, including 90, T2DM patients and 90 healthy control subjects (normal glucose tolerant (NGT)). We collected clinical data, serum insulin concentration (estimated using an enzyme-linked immunosorbent assay) and Total serum adiponectin (measured by radioimmunoassay). We only selected two SNPs (rs1501299, and rs7627128) that are all tagSNPs of the ADIPOQ gene, which can represent the genetic information of the other SNPs in the ADIPOQ gene.

Results: For total participants, the genotype and the allele frequency of rs1501299 and rs7627128 were significantly different between the T2DM patients and the control subjects. In the present study, we found that the ADIPOQ gene rs1501299 and rs7627128 polymorphisms were significantly associated with T2DM in population.

Conclusion: In conclusion, the present results indicate that T2DM is associated with the ADIPOQ gene polymorphisms.

Keywords: ADIPOQ - ADIPOR2 - gene polymorphisms - type 2 diabetes

P-254: Molecular investigation of Mediterranean glucose-6-phosphate dehydrogenase in North West of Iran

Moghadam S1, Valizadeh M2, Onsori H3, Fathi A4

1. 2. Genetics Department, Ahar Branch, Islamic Azad University, Ahar, Iran.
3. Cellular and Molecular Biology Department, Marand Branch, Islamic Azad University, Marand, Iran
4. Pediatric Hematology & Oncology Department, Ardabil University of Medical Sciences, Ardabil, Iran
siamakmoghaddam1123@gmail.com

Glucose-6-phosphate dehydrogenase (G6PD) deficiency is the most common human enzyme deficiency affecting more than 400 million people worldwide. This enzyme catalyse the first step in pentose phosphate pathway (conversion of glucose-6-phosphate to 6-phosphoglucononate) with the concomitant reduction of NADP+. This pathway is an important source of NADPH. By preserving and regenerating reduced form of glutathione, NADPH plays a major role in a cell’s™ stability to withstand oxidative stress. The aim of this study was molecular identification of Mediterranean mutation in Glucose-6-phosphate dehydrogenase in affected patients in North West of Iran. In the present study, from 90 blood samples of unrelated male and female patients, DNA was extracted by Rapid Genomic DNA Extraction (RGDE) method. In order to search for Mediterranean mutation, PCR-RFLP and sequencing methods were used. This study, revealed that 61 samples out of 90 have the Mediterranean mutation (67.77%). The data indicate that the G6PD Mediterranean mutation is the most common in North West of Iran.

Keywords: G6PD, Mediterranean, mutation, North West of Iran

P-255: Bioinformatic Evaluation of the miR-1245 Effect on ERBB2 Signaling Pathway

Mohamadzade Z1, M. Soltani B2

1. Department of Biology, Khorasan Razavi Science and Research Branch, Islamic Azad University, Neyshabur, Iran.
E-mail: Mahsharif71@yahoo.com
2. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
z.mohamadi2010@gmail.com

Colon cancer is one of the leading causes of cancer-related deaths in the Western world, but our understanding of this disease is incomplete. The recent advent of new technologies has provided novel insights into the pathogenesis of colon cancer. Increasing studies suggest that microRNAs, a new group of small non-coding molecules, regulate the expression of their target genes and play some roles in cancers. In fact, microRNAs (miRNAs) are non-coding RNAs 18-25 nucleotides in length that downregulate gene expression during various crucial cell processes such as apoptosis, differentiation and development. Thus, it is hypothesized that the genetic variants of microRNAs could contribute to the susceptibility to cancers. In this study, the association between rs421446 in miRNA-219-1 and the risk of colon cancer was explored in a large-scale case-control population based on Real-Time PCR technology. Multivariate logistic regression analyses were conducted to evaluate the association of SNPs genotypes and alleles with the risk of developing colon cancer. Large validation and functional studies are required to further explore the role of SNPs in carcinogenesis.

Keywords: Colon cancer, micro RNA219-1

MiRNAs, a small noncoding RNA of 21â€“22 nucleotides long, have recently been linked to cancer development. miRNAs have diverse functions, which include the regulation of cellular differentiation, proliferation, and apoptosis. The available evidence has shown that miRNAs widely participate in the development or progression of many types of cancers, including breast cancer. Breast cancer is the most common cancer in women. Among the genes that may be potentially affected by miRNA, ERBB2 is perhaps the best known. This gene is expressed at a low level in normal human tissues. However, when it is overexpressed, it produces the malignant phenotype and leads to cell proliferation. Approximately 25% of human breast cancers overexpress the HER2 proto-oncogene, and these breast cancers have a more aggressive tumor phenotype and produce a poor prognosis in patients with this disease.

Methods: Using different algorithms in TargetScan, DIANA and miRWalk databases, targets of miR-1245 were identified. Then, a score table was prepared from the candidate genes, based on the affinity of the seed region of miR-1245 and the
number of targets in the 3'-UTR region of targets. Results: The results of bioinformatical analysis showed that the POU1F2, KLF6, ERBB2, SOX9, SP1, and EFN B1 molecules are the most potential targets that might be affected by miR-1254. Conclusion: It seems that miR-4430 might be a potential regulator of ERBB2 expression in breast cancer. Therefore, this protein can be considered as a suitable new candidate for experimental evaluation.

**Keywords:** Bioinformatics, microRNA, Breast cancer, miRNA

**P-256: Detection of new mutation c. 1293G>A in DMD gene by next generation sequencing**

Mohammadimatin Z', ghandil p', deris z', mohammadiasl j'

1. Dept. of Medical Genetics, Faculty of Medicine, Ahvaz jundishapur university of medical sciences
   zahramohammadimatin@yahoo.com

**Background:** Duchenne muscular dystrophy (DMD) is most common type of muscular dystrophy which is inherited by a sex-linked recessive form. Detection process of its gene mutations because of high number of exons and large size of gene can often be time-consuming and costly by conventional methods like multiplex Ligation-dependent Probe Amplification (MLPA) or array Comparative Genome Hybridization (aCGH) and only 60-80 percent of mutations can detect by these methods. Next generation sequencing (NGS) has ability to remove this obstacles. High accuracy and short time of sequencing whole exome or whole genome made NGS as a prior method in genetic diagnosis of DMD.

**Method:** In this study, we used NGS In order to sequence all regions of exons and Finally, by using the sanger sequencing method and sequence genomic region of candidate change in patients and ten family pedigree members, the result of the NGS was confirmed.

**Result:** by using this method hemizygous mutation c. 1293G>A (p. Trp431 Ter; Hemiz) on DMD gene was identified.

**Discussion:** According to this study and other studies, we find that NGS eliminates many barriers, such as time and price. However, the up-to-date platforms and bioinformatics analysis are the concerns of this method.

**Keywords:** next generation sequencing, DMD gene, stop codon, new mutation

**P-257: Detection of circulating tumor cells by nested RT-PCR targeting mammaglobin mRNA in breast cancer patients**

Mohseni lifshagerd F', Mortazavizadeh SMR', Falahati A

1. Department of Biology, Faculty of Science, Yazd University, Yazd, Iran
2. Department of hematology/oncology, Yazd Branch, Islamic Azad University, Yazd, Iran
   mohseni.fatemeh@gmail.com

**Background:** Breast cancer is the second most common type of cancer in women and the common cause of cancer death. Recently, researchers enable to detect circulating tumor cells (CTCs) in the peripheral blood, bone marrow and lymph nodes of breast cancer patients. This ability is useful for identifying patients at risk of developing metastasis. Over the past few years, different approaches for the detection and isolation of CTCs in blood have been developed. Among the different types of tumor markers, mammaglobin is overexpressed in breast cancer, and has been established as a tumor and promissory marker for the early detection of metastasis.

**Conclusion:** Our results suggest that nested RT-PCR assay is a powerful method for detecting disseminated breast cancer cells. A larger study with long-term follow-up is required in order to clarify its clinical usefulness.

**Keywords:** Breast cancer, circulating tumor cell, mammaglobin, metastasis, nested RT-PCR

**P-258: Clinical correlations between chronic hepatitis C infection and decreasing bone mass density after treatment with interferon-alpha**

Mohseni N', Ghorbani M**

1. Venom & Biotherapeutics Molecules Laboratory of Biotechnology Research Center of Pasteur Institute of Iran, Tehran, Iran.
2. Department of Research and Development, Research and Production Complex, Pasteur Institute of Iran, Karaj, Iran.
   nastaran19164@yahoo.com

**Objective:** To compare the clinical correlations between chronic hepatitis C infection and decreasing BMD after treatment with IFN-a.

**Methods:** A total of 70 patients with chronic hepatitis C infection were treated with IFN-a at a dosage of three million IU three times a week for one year. All patients underwent bone mineral densitometry (BMD) at lumbar spine and femoral neck before after the IFN-a treatment. All the necessary information such as age, sex, history of occurrence of fractures, lifestyle, and menopause status was collected by interviewers face-to-face from participants at the research visit. All statistical analyses were performed by SPSS.

**Results:** Among 70 patients, 52 were male, 48 were female and the mean age was (57.0 Â± 9.6) years (range: 24-79). Twenty-nine percent of the patients had a history of smoking. The mean body mass index was (24.4 Â± 3.6) kg/m2 (range: 18.4-35.3). Of the 70 cases, 21 had high fibrosis-4. The prevalence of overall fracture history was 2.9%.

**Conclusions:** We found that the risk of development of metabolic bone disease is not increased in chronic HCV infection. Indeed, greater reduction of BMD occurs in advanced liver fibrosis. The bone loss in earlier stages of chronic hepatitis C infection is likely to be resulted from increased bone resorption rather than in decreased bone formation. Overall, these observations suggest an important role for chronic HCV infection in increased bone turnover in osteodystrophy pathogenesis. For a better assessment of the correlation between HCV infection and BMD and the mechanism linking HCV to this disorder.

**Keywords:** Hepatitis C, Interferon alpha, Bone density, Liver fibrosis, Bone mass loss
P-259: An Evaluation on Regulatory Role of Micro RNAs on NKX2-1: A Responsible Gene in Lung Cancer

Mokhtari M, Javadirad SM
Department of biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
JavadiradSM@yahoo.com

Lung cancer is known as one of the most common cancers around the world and is one of the main causes of cancer mortality. Histologically, lung cancer is divided into Small Cell Lung Cancer (SCLC) and Non-Small Cell Lung Cancer (NSCLC) respectively account for 20 and 80 percent of all lung cancers. One involving gene in NSCLC pathology is thyroid transcription factor-1 (TTF-1) or NK2 Homeobox 2-1 (NKX2-1) which is a homeobox-containing transcription factor. Recently, researchers have found out that micro RNAs may have regulatory effects on this gene. In addition, an increased level of NKX2-1 protein has been observed in NSCLC patients. Therefore, in this study the differences in new regulatory micro RNAs against NKX2-1 gene would be analyzed in the tissues of NSCLC patients and it would be compared to control individuals. Accordingly, the expression levels of target miRNAs would be determined using qPCR with specific primers. Laboratory investigations of this study is to be done and hopefully, the results can be a great help in detection and treatment of patients suffering this catastrophic disease, as well as base for future researches.

Keywords: Lung Cancer, NSLC, Micro RNA, Real-Time PCR

P-260: Investigation expression of serum miR-320a and miR-17 as candidate biomarker in MS patients of Zanjan population

Molaei F1, Nazari A1, Ghoreishi A2, Mahmazi S1
1. Department of Genetics, Faculty of Basic Sciences, Islamic Azad University, Zanjan Branch, Zanjan, Iran.
2. Assistant professor of Neurology, Zanjan University of medical science
Faceh.aftab@yahoo.com

Objective: Multiple sclerosis (MS) is a chronic inflammatory autoimmune demyelinating disease of the central nervous system with an unknown etiology. MicroRNAs (miRNAs) are small non-coding RNAs that regulate gene expression by binding to complementary target miRNAs and either promoting their decay or inhibiting their translation. In the human immune system, miRNAs play an important role in modulating innate and adaptive immune responses. They regulate B and T cell development and differentiation, in addition to pro-inflammatory responses mediated by Treg cells. Dysregulation of miRNAs involved in immune responses leads to autoimmunity. In this study, we investigated the amounts of circulating miR-320a and miR-17 in plasma samples from MS patients.

Methods: We investigated miR-320a and miR-17 in plasma samples of 24 MS patients and 24 healthy subjects by quantitative real-time PCR.

Results: miR-320a was down-regulated in all MS patients. miR-17 expression reduced in 60% of MS patients and overexpression of miR-17 observed in 40% of patients.

Conclusion: Down-regulation of miR-320a induced the overexpression of pro-inflammatory cytokines. They could activate Th1 and Th17 that are important in the pathogenesis of MS. Ectopic expression of miR-17 imparted effector-T-cell-like characteristics to Treg cells via the de-repression of genes encoding effector cytokines. MiR-17 provides a potent layer of Treg cell control. A major challenge in multiple sclerosis (MS) is to develop biomarkers that could help in understanding individual MS patients miRNAs could be a potential biomarker for diagnosis and evaluation of MS.

Keywords: Multiple Sclerosis, MicroRNAs, Autoimmunity, miR-17, miR-320a

P-261: Study of Relationship between Gastric Cancer and EGFR Gene Mutations

Molaei J1, Jamshid Abadi Sh2, Javid M1, Zolfaghar M1, Ebrahimim P1, Ebrahimim A2
1. Department of Molecular Genetic, Islamic Azad University, Science and Research Branch, Tehran, Iran.
2. Department of Molecular and Cellular Biology, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran -Iran (IAUPS ).
3. Department of Genetics, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran -Iran (IAUPS ).
4. Universita degli Studi di Roma La Sapienza Dipartimento di Medicina clinica.
5. Yas Human Medical Genetics Laboratory, Tehran, Iran.
info@molaei.com

Background: Gastric cancer is an aggressive disease that continues to have a daunting impact on global health. Despite an overall decline in incidence over the last several decades, gastric cancer remains the most common type of cancer and is the second leading cause of cancer-related death worldwide. The abnormal EGFR gene is involved in certain types of cancers, including gastric, prostate, cervical, ovarian, head and neck. EGFR belongs to tyrosine kinase family in respond to a Ligand binding, is activated through dimerization and autophosphorylation in tyrosine kinase domain. The purpose of this study was to investigate the relationship between EGFR gene mutations and gastric cancer.

Methods: The samples were collected from 100 patients susceptible to gastric cancer as target group. DNA was extracted using VIOGENE kit. The mutation points detected by Gap-PCR and ARMs PCR then the results confirmed by Sanger Sequencing.

Results: The samples were collected from 100 patients susceptible to gastric cancer as target group. DNA was extracted using VIOGENE kit. The mutation points detected by Gap-PCR then the results confirmed by Sanger Sequencing.

Conclusion: The samples were collected from 100 patients susceptible to gastric cancer as target group. DNA was extracted using VIOGENE kit. The mutation points detected by Gap-PCR then the results confirmed by Sanger Sequencing.

Keywords: Gastric cancer, EGFR, Gap PCR, ARMs PCR, Sanger Sequencing.

P-262: Cytogenetic report in a family with balance translocation (6;22)

ghanbarian alavijeh M, Hekmati F, saberi M, bagheri T, jedgharib M S, zavareh Z, ebrahimi A, molaei J, saber S*
yas medical genetic lab, tehran, Iran
P-263: The Correlation between SALL4 expression and Helicobacter pylori Infection in Inducing Cancer Stem Cell Properties in Gastric Adenocarcinoma

Mollaei F1, 2, Forghaniard MM3, Vojdani S2, Abbaszadegan MR2

1. Division of Human Genetics, Avicenna Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran
2. Medical Genetics Research Center, Faculty of Medical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran
3. Department of Biology, Damghan Branch, Islamic Azad University, Damghan, Islamic Republic of Iran

Purpose: Gastric cancer (GC) is the third cause of cancer-related death worldwide. One of the most important environmental factors of GC is Helicobacter pylori infection. H. pylori affects the gene expression level in multiple cellular pathways. The main objective of this work was to explore the correlation between SALL4 gene expression and H. pylori infection in the GC patients.

Method and Material: The mRNA level of SALL4 was studied in 74 fresh-frozen tumoral and margin-normal tissues of GC patients using real-time PCR. All the samples were also checked for H. Pylori infection. Data were analyzed using the SPSS22 statistical package.

Results: The overexpression of SALL4 was observed in 43.2% (32 of 74) of GCs while its underexpression was observed in 18.9% (14 of 74) of samples. 40 out of 74 patients were H. pylori positive while other were H. pylori-negative. A significant correlation was observed between SALL4 expression and lymph node metastasis (P value= 0.041). However, there was no significant correlation between H. pylori infection and SALL4 expression. The overexpression of SALL4 was inversely correlated to depth of tumor invasion (P = 0.022), tumor size (P = 0.041), and stage of tumor progression (P = 0.05) in patients without H. pylori infection.

Conclusion: SALL4 overexpression may play role in lymph node metastasis of GC. It also may be extrapolated that SALL4 overexpression may cause tumor progression in the presence of the H. pylori infection, suggesting an important role for H. pylori in inducing SALL4 and therefore stemness state of GC.

Keywords: Gastric cancer, SALL4, H.Pylori, CSC, Stemness

P-264: Comparing Developmental Competence of mature and immature oocytes in PCOs patients and mRNA levels of AMH gene as biomarker

Montazeri F1, 2, Ali Foroughmand M1, kalantar SM2, Aflatoonian A3, Khalilli MA1, Fesahat F2, Hoseini SM3, Taheri F3

1. Department of Biology, Faculty of Science, Chamran University of Ahvaz, Ahvaz, Iran
2. Recurrent Abortion Research Center, Yazd reproductive sciences institute, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran
3. Yazd reproductive sciences institute, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran

smkalantar@yahoo.com

Background: In vitro maturation (IVM) could be a good choice for patients who are hormone sensitive and have few oocytes in stimulated cycles. Consequently, selecting embryos with the highest implantation potential is of great importance in assisted reproductive technologies (ART). To date, the choice of the best embryos to transfer is based on morphological parameters. Therefore, movement towards modern technologies such as genomics, transcriptomics, proteomics and metabolomics to select the most competent oocytes and/or embryos with the greatest implantation potential would be significant.

Objective: The aim was to assess the developmental competence of the in vitro and in vivo matured human oocytes as well as the AMHR gene expression of cumulus cells (CCs) between tow group in order to evaluate oocyte maturity in PCOs patient.

Material and Method: The oocytes and the associated CCs were retrieved from 27 PCO women and divided into groups of GV and MII according to the nuclear maturity status in order to studding developmental competence as well as expression patterns of AMHR gene using real time PCR.

Result and conclusion: The fertilization and embryo formation rates were 61.5% and 84.5% vs.67.1% and 90.8% in test and control groups, respectively. There were significant differences in mRNA levels of AMHR gene between the groups. It seems that using immature oocytes could be helpful for patients at risk of ovarian hyperstimulation syndrome (OHSS) as the same as patients with diminished ovarian reserve.

Keywords: Polycystic Ovarian Syndrome (PCOs), In vitro Maturation (IVM), Oocyte maturity, Embryo selection, AMHR gene, Biomarker

P-265: Association of Angiotensin-converting enzyme (ACE) insertion/deletion gene polymorphism with risk of schwannoma in patients referred to Imam Khomeini Hospital in Tehran

Moqadami A, Mikaeili Agah E

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Biology, Ardabil Branch, Islamic Azad University, Ardabil, Iran

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Angiotensin converting enzyme gene is located in 17q23.3 chromosomal site with functional insertion/deletion polymorphism. The frequency of cases with DD genotype is higher than that of genotypes ID and II. In this study, the association between this gene and the brain cancer of schwannoma was investigated. This case-control study was conducted on 31 patients with brain cancer of schwannoma and presence of 20 health control group. ACE I/D polymorphism was detected by the Gap-PCR technique. PCR products were isolated and measured by electrophoresis on 2% agarose gel. The Insertion (I) allele was observed in the band of 478 bp and the Deletion allele (D) in the 191 bp band. It was estimated that the absence of genotype ID is related to the probability of developing brain schwannomic cancer. Also, DD and II genotypes had a p-value greater than 0.05 and the hypothesis of their association with brain invasion was rejected. Considering the results, it should be noted that these findings are the first report of the association between ACE I/D polymorphism and schwannoma. Further studies are needed to confirm these findings.

Keywords:

P-266: Investigation of has-miR-320a in polycystic ovary syndrome patients with insulin resistance and its role in the onset of insulin resistance

Moraghebi S M, Malek Zadeh K
mahtamoraghebi70@gmail.com

Background: 60 to 70% women with PCOS suffer from some degree of insulin resistance and hyperinsulinemia. Hyperinsulinemia could potentially contribute to the hyperandrogenism found in women with PCOS, which consider as disadvantageous interaction between insulin resistance and PCOS.

Method: 100 subjects (20 healthy control, 30 PCOS+IR-, 30 PCOS+/IR+ and 20 PCOS-/IR+) recruited for this study. No subjects had used hormonal preparations, and none were pregnant. Serum exosomal microRNA was extracted and exposed to reverse transcription reaction and quantified by RT-PCR.

Results: The serum levels of exosomal miR-320a was significantly higher in patients with IR than those with PCOS or normal (P<0.05). Further, the serum levels of exosomal miR-320a was significantly lower in PCOS patients without IR than in patients with IR.

Conclusion: Our study indicates that miR-320a expression profile is different in PCOS with IR as compared to PCOS+/IR-. Hence serum exosomal miR-320a can be considered as potential indicator for early detection of insulin resistance in patients with PCOS.

Keywords: microRNA; Insulin Resistance; Poly Cystic Ovary Syndrome; PCOS

P-267: The study of the long non-coding GNAS-AS1 and BLACAT1 RNA expression in breast cancer tumor samples compared to the normal sample at the same time

Motaleb zade Hesam1, Mowla Seyed Javad2,3,4, Shiva Motavealian Manijie3, Safooras

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
3. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran

4. Razi Drug Research Center, Iran University of Medical Sciences, Tehran, Iran
hex_motaleb@yahoo.com

Breast cancer is the most common cancer in women, and due to high rates of invasion and metastasis in these patients, the first cause of death is in women aged 40-44 years. The latest biomarkers and prognostic factors in a variety of cancers, including breast cancer, can be found in the form of Long Non-coding RNAs with a variety of oncogenic function or tumorigenic function (TSG), a marked change in the expression of this cancer showing normal breast tissue. The aim of this study was to simultaneously investigate changes in the expression of GNAS-AS1 and BLACAT1 non-coding gene expression in tumor tissue of breast cancer.

Materials and Methods: MCF7 and MCF10A cancer cell lines were used to optimize the experiments. Then with the method qRT-PCR the expression of two non-coding RNA genes, GNAS-AS1 and BLACAT1, was investigated in 45 pairs of tumor and adjacent tumor fresh samples.

Conclusion: According to the results of the study, 90 tumor samples of breast cancer were compared to the normal tumor margin of the same person, Non-coding RNAs of GNAS-AS1 and BLACAT1 have a pronounced increase in tumor tissue expression relative to the tumor margin (P = 0.0453 GNAS-AS1) (P = 0.0443 BLACAT1). Discussion: Considering the review of previous articles and micro array studies in human specimens, the existence of a possible hypothesis about the oncogenesis of two selected genes in the present study has been found. Based on real-time PCR results and comparison of data, GNAS-AS1 and BLACAT1 genes increased significantly in the cancer cell line compared to the normal cell line and in the cancerous tissue compared to normal tissue which resulted in the probability of pro-oncogenesis of these genes.

Keywords: Gene-Breast cancer-Oncogene- Long Non-coding RNAs - real-time PCR

P-268: Expression analysis of the long non-coding RNA ????31, 2 in tumor sample of prostate cancer and BPH (Benign prostatic hyperplasia)

Mowla SJ, Modaresi M, Seifikaran M
seifikaran@outlook.com

Prostate cancer (PCa), is one of the leading causes of cancer related death and the second most common cancer in men world-wide. Early diagnosis or management can play an important role in improving prognosis and reducing mortality rates. The association of long non-coding genes with natural physiological processes and pathogenesis, especially in cancer, is increasingly recognized. lncRNA genes have many roles in the development of cancer in various tissues, cell invasion and metastasis. Study of the expression patterns of genes associated with prostate cancer is a way to improve the accuracy of diagnostic tests for prostate cancer.

Methods: In this study, because of the roles of long non-coding RNA ???31, 2 in prostate cancer, we investigated the molecular study of 60 tissue samples containing 30 tumor and 30 samples of tissue BPH as control. Therefore, tissue samples were obtained from patients and RNA extraction and cDNA synthesis were performed. The method employed was the Re-
AlTime. Clinical data were analyzed by SPSS software and the molecular analysis was carried out by REST software in the significance level (p-value <0.05). Results: our data revealed that variant 1 and variant 2 was up-regulated in prostate tumor tissues

**Conclusion:** The results indicated that the association between increased expression of variant1 and variant2 pca3 gene is correlated with tumor progression .The findings reported here might be used as prospective biomarkers for the development of innovative diagnostic and therapeutic techniques.

**P-269: The effect of antiangiogenic peptides on expression of eNOS gene in breast cancer mouse model**

Nabhanizadeh J, Salehi Z, Asghari M, Talesh Sasani S

**Department of biology, University Campus2, University of Guilan, Rasht, Iran**

**Department of biology, Faculty of Science, University of Guilan, Rasht, Iran**

**cm.nabhani@gmail.com**

Angiogenesis plays a critical role in the growth and spread of cancer. A major pathway involved in angiogenesis is the release of vascular endothelial growth factor (VEGF) from hypoxic tumor cells and its binding to the VEGF receptor (VEGFR), located on endothelial cells. Numerous therapies have been developed that target angiogenesis by blocking the VEGF signaling pathway. Peptides have emerged as important therapeutics that are being rigorously tested in angiogenesis-dependent diseases due to their low toxicity and high specificity. It has been shown that antiangiogenic peptides signal via interaction with VEGFR, although multiple downstream effector pathways are implicated. The aim of this study was to investigate the effect of antiangiogenic peptides on eNOS expression level in 4T1 xenograft mouse To investigate the effect of antiangiogenic peptides on tumor growth in xenograft mouse model, female Balb/c bearing subcutaneous 4T1 breast cancer cell were injected. The mice in the control group received phosphate-buffered saline. The eNOS expression level was investigated by real time PCR method. Treatment with antiangiogenic peptides significantly decreased the tumor size and inhibited tumor growth in a concentration-dependent manner. Moreover, the expression level of eNOS was significantly reduced in peptide-treated mice group comparing to the control. In conclusion, the antiangiogenic peptides inhibitory effect on the VEGFR mediated signaling pathway could be targeted for the development of pharmaceutical agents that inhibit tumor angiogenesis via eNOS.

**Keywords:** eNOS; VEGF; antiangiogenic peptide

**P-270: A 3-year-old boy with lethargy, leg muscle degeneration and mental retardation**

Nabizadeh F¹, Nejatizadeh A², Shekari M², Koochakhkhani Sh¹, Allamehzadeh Z², Ahmadi B², Shams Sh²

1. **Student Research Committee, Hormozgan University of Medical Sciences, Bandar Abbas, Iran**
2. **Bandar-Abbas Medical Genetics Laboratory (BMGL), Hormozgan, Iran**

fatima.nbz71@gmail.com

**Introduction:** Maple syrup urine disease (MSUD) is an autosomal recessive disease characterized by disruption of the normal activity of the branched-chain ?-ketoacid dehydrogenase (BCKAD) complex. MSUD can be caused by homozygous or compound heterozygous mutation in at least 3 genes: BCKDHA, BCKDHB, and DBT. MSUD presents in the neonate with feeding intolerance, failure to thrive, lethargy and maple syrup odor to urine.

**Methods:** We analyzed a 3-year-old boy who was born of a consanguineous marriage visited at Bandar Abbas medical genetics laboratory (BMGL) of Hormozgan province Iran, suspected with MSUD. The mother’s amniotic fluid (AF) sample was taken in 15th week of gestational age and DNA was extracted. Targeted NGS (Next Generation sequencing) was suggested followed by sanger sequencing for mutation confirmation in patient and family members (parents and sister). The fetus genotype was examined by sanger sequencing for the known mutation.

**Results:** NGS analysis showed a c.C653G homozygote mutation in BCKDHB gene for patient. Sanger sequencing investigation indicated heterozygosity of parents for same mutation but his sister’s homozygosity for wild type allele. Similarly, sanger sequencing on fetal cells showed that the fetus is also heterozygous for the c.C653G mutation.

**Conclusions:** We have shown a case of MSUD type Ib. Homozygote c.C653G mutation has been reported as the causative defect for the patient. Fetus genotype analysis for the 3rd child of family showed heterozygosity for the mutation. We prevented the recurrence of a rare disease in Hormozgan province by early identification of c.C653G mutation in parents and its examination in their embryo.

**Keywords:** MSUD, BCKDHB, Mutation, NGS

**P-271: Study of genes expression (Nf1, Nf2) in T47D cell lines of breast cancer by silibinin**

Nademi NS¹, Mohamed N¹, Zarei Golambahri H²

1. **School of Biology, Tehran University, International Campus of Kish, IRAN**
2. **Department of cell&Mol.Biology University of Tehran, IRAN**

negarnad1986@gmail.com

**Introduction:** Silibinin is a natural polyphenol with high anticancer properties, which causes cell cycle arrest and apoptosis in most cancer cell types including breast cancer. NF1, a tumour suppressor gene, product neurofibromin, is a negative regulator of the Ras cellular proliferation pathway, an important step in tumorigenesis. Merlin (Moesin-ezrin-radixin-like protein) is a tumor suppressor protein encoded by the neurofibromatosis type 2 gene NF2. This study discusses the effect of silibinin on the expression of NF1 and NF2 genes in the T47D cell line.

**Materials and Methods:** T47D cell line obtained from Pasteur institution was cultured in RPMI medium containing FBS 10%. MTT assay was used at 24, 48 and 72 hours for concentrations of 50, 75, 100, and 150 mM Silibinin to evaluate the effect of its toxicity on T47D cells. Then, to investigate the effect of silibinin on the expression of NF1 and NF2, treated the cell with effective concentrations of silibinin, which was obtained from the MTT assay. RNA extraction, cDNA synthesis and Real Time PCR was performed.

**Results:** Silibinin had dose and time-dependent toxicity on T47D cell and increased the expression of NF1 and NF2 genes.

**Conclusion:** Silibinin reduces cell proliferation and arrests cell cycle, as well as induces apoptosis in T47D cells.

**P-272: Polymorphisms of an Immunoregulatory Gene and**
Risk of Inhibitor Development in Iranian Hemophilia A Patients

Naderi N, Bolhassani A, Namvar A, Jazebi M, Moazezi NA SS

1. Comprehensive Hemophilia Care Center, Tehran, Iran
2. Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran
E-mail: niloofarnaderi56@yahoo.com

Hemophilia A is a hereditary bleeding disorder caused by the deficiency of factor VIII (FVIII) coagulant activity. The development of neutralizing antibodies against FVIII, known as inhibitors, is the major complication in hemophilia A care. Aim: The main objective of this study was the analysis of CD44 immunoregulatory gene polymorphisms associated with the development of FVIII inhibitors in Iranian hemophilia A patients. Materials and Methods: 40 inhibitor positive and 30 inhibitor negative HA patients were enrolled. After extraction of genomic DNAs, tetra primer ARMS PCR analysis and direct sequencing were performed to identify polymorphisms in CD44 gene. A conventional chi squared test was used for statistical analysis. A p < 0.05 was statistically considered significant. Results: The analysis of polymorphisms in the CD44 gene identified no association between the AA and AT genotypes and the formation of inhibitors (p = 0.859, OR = 0.900 and CI = 0.280-2.888 and p = 0.465, OR = 0.710 and CI = 0.283-1.782, respectively). Also, no statistically significant difference was regard with the allele analysis for the polymorphisms of CD44 gene was found between the groups of inhibitor and non-inhibitor patients. Indeed, comparison of allele frequencies of CD44 gene (rs927335) between two groups showed no significant differences associated with the development of FVIII inhibitors. Conclusion: Polymorphisms in CD44 gene (rs927335) do not play a protective role against inhibitor development in Iranian HA patients.

Keywords: Inhibitor, Factor VIII, Immunoregulatory genes, CD44

P-274: Current Condition Regarding LNA Inhibitor in microRNA miR-23b Cell Proliferation and Apoptosis Induces as a Potential Therapeutic Option in Treatment of Human Hepatocellular Carcinoma

Najafi Z¹, Sharifi MR², Javadi Gh¹

1. Department of Biology, Science and Research branch, Islamic Azad University, Tehran, Iran.
2. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.
zoyanajafi@srbiau.ac.ir

Dysregulation of microRNAs (miRNAs) has been shown to be involved in the pathogenesis and advances of many malignancies. Human hepatocellular carcinoma (HCC) is one of the most common cancers worldwide and the third cause of cancer related deaths. Recent data suggest that microRNA-23b (miR-23b) is significantly elevated in different types of cancer, particularly human hepatocellular carcinoma. locked nucleic acid (LNA) modified oligonucleotides have recently been suggested as a novel approach for targeting miRNAs as antisense based gene silencing. The aim of this study was to explore the functional role of LNA-anti-miR-23b in a HepG2 (hepatocarcinoma) cell line. HepG2 cells were transfected with LNA-anti-miR-23b for 24, 48 and 72h. Quantitative real-time reverse transcriptase-PCR (qRT-PCR) was performed to assess miR-23b expression by LNA-anti-miR-23b. The viability of the cells was evaluated by MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl tetrazolium bromide) assay was used to realize apoptosis. LNA-anti-miR-23b was successfully transfected in human HepG2 cells and suppressed the miR-23b. LNA-anti-miR-23b inhibited the cells growth followed by induction of apoptosis. LNA-anti-miR-23b reduced the invasive behaviors of HepG2 cells after 24 h, compared with untreated cells and scrambled LNA-transfected cells and this effect was more pronounced after 72 h. Our findings suggest that inhibition of miR-23b could be used as a novel approach in treatment of HCC.

Keywords: microRNA, miR-23b, human hepatocellular carcinoma, Locked Nucleic Acid

P-273: Investigation of the prevalent mutations of NLRP7 gene in Iranian women and candidates for ART assisted reproductive techniques

Naderi p

parisa.ndc.69@gmail.com

According to the World Health Organization definition, barrenness or fertility include: pregnancy failure, a year after the marriage, or when couples decide to have children (without the use of contraceptive methods). One of the most common strategies to overcome infertility in couples is through the use of ART assisted reproductive techniques. One of the most common methods of ART is IVF. The aim of this study is to evaluate common mutations of NLRP7 gene in IVF candidate women and its effect in success rate of IVF methods. Since occurrence of mutation in this gene is the main reason of the Hydatidiform Mole incidence that itself cause infertility, if NLRP7 mutations observed in candidate women of IVF method and lead to failure of this method, this gene could be used as an in vitro fertilization genetic biomarker so that infertile couples are aware of its success or failure rate before spending money and time on this technique, so if they do not have the chance to do this, they will look for alternative and appropriate ways in order to overcome infertility.

In this study, first DNA extraction was performed from 50 blood samples of IVF technique candidate patients. The primer design was then performed for three exons 2, 3, 4 NLRP7 genes. Gene amplification and analysis of the sequence results of PCR was done by PCR technique and Finch TV software respectively and areas containing mutations were identified in patients. In order to ensure and more accurate and specific evaluation of identified mutations, specific primer design related to including mutation areas was performed and mutations using ARMS-PCR technique distinguished and confirmed accurately. The obtained results were evaluated by statistical analysis.

In this investigation determined that in 50 blood samples of IVF candidate women that among each 2, 3, 4 three exons, only exon 4 in NLRP7 gene was include Missense mutation that in this group sixteen samples included mutation which appeared in hetero and homo mutations that its results is the turning of Cytosine to Thymine that this mutation lead to occurrence of protein exchange where by Valine convert to Isoleucine. Also, in investigated population 14 samples included polymorphism that as a result Cytosine changed to Thymine.

Keywords: PCR, IVF, NLRP7 Gene, ARMS-PCR

Abstracts of the 3rd International & 15th Iranian Genetics Congress
P-275: The age-related miRNA, miR-22, is differentially expressed in serumâ€™s exosomal samples of aged people

Nasiri Kenari F, Pourfatholah A, Shahabi M, Mirzadeh F, Mowlia S J
Department of Molecular Genetics, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran
narsi_shirzad@yahoo.com

The longstanding belief was that the aging effects on organisms are irreversible, but recent studies indicate that systemic manipulation can reverse some of age related disabilities. Part of the rejuvenating effects of systemic manipulation is derived from pro-youthful factors such as GDF11. miRNAs are key regulators of gene expression and affect the cellular processes such as aging. miRNAs can be secreted throughout Biofluid via micro vesicles and exosomes. Our bioinformatics analysis predicted some age-related miRNAs capable of targeting GDF11. We then analyzed the expression alteration one of these miRNAs, miR-22, in serumâ€™s exosomal sample in different age groups. The motivation of this investigation is that GDF11 expression in blood is declined with age.

Serum samples were collected from blood donors. Exosomes were isolated from serum by Exoquick exosomes precipitation solution. microRNAs were extracted from serum and exosomes were amplified by stem-loop quantitative reverse transcription PCR.

miR-22 expression levels in exosome samples were three times higher than serum samples. In exosome pooled samples, miR-22 expressions in old and middle-aged men were respectively three times and two times higher than of the young group (p=0.002). Expression pattern of miR-22 in non-pooled exosome samples was the same as the pooled exosome samples in male group.

Our result indicates that the miR-22 is an exosomic miRNA, and its expression level is elevated in older ages. The latter result confirmed our initial hypothesis to a certain extent.

Keywords: Rejuvenation; Aging; pro-youthful factor; microRNA; Exosomes

P-276: Common Mutation Analysis of Familial EPS8-Related Deafness in Iranian Azeri Turkish Patients

Nasiri M1, Bonyadi M2
1. Department of Biology, College of Science, East Azerbaijan Science and Research Branch, Islamic Azad University, Tabriz, Iran.
2. Center of Excellence for Biodiversity, Faculty of Natural Sciences, University of Tabriz, Tabriz, Iran.
NASIRI.MAJID2012@GMAIL.COM

Background/aim: Hearing impairment is a sensory disability that affects one in 1000 live birth. Many genetic and environmental factors may contribute to this extremely heterogeneous disease including infection, head trauma and noise exposure. Although many genes involved in deafness process are identified, there are several other unknown genetic variations could contribute to deafness. Mutations in EPS8 gene which encodes epidermal growth factor receptor pathway substrate 8, that is a protein with important role in actin dynamics may be potential cause of human deafness. This study was planned to assess the significance of nonsense mutation c. 88C>T mutation in exon 3 of eps8 gene to the autosomal recessive non-syndromic hearing loss (ARNSHR) among Northwest of IRAN patients.

Method: All cases involved in this study tested for connexin 26 mutations and among them 100 patients with ARNSHR without any mutation in connexin 26 mutations were selected. Molecular testing for c.88C>T (p.Gln30) EPS8 mutation was performed using polymerase chain reaction and sequencing.

Results: All cases involved in this study were tested for connexin 26 mutations and among them 100 patients with ARNSHR without any mutation in connexin 26 mutations were selected. Molecular testing for c.88C>T (p.Gln30) EPS8 mutation was performed using polymerase chain reaction and direct sequencing of amplified region of genome in this group showed no mutation in any of the studied patients.

Conclusion: Our finding indicates absence of significant role for c. 88C>T EPS8 mutation in Northwest of IRAN population.

Keywords: Northwest of IRAN, EPS8 gene mutation, ARNSHL

P-277: Assessment of the chromosome aneuploidy in oligo-teratospermia (OT) and normozoospermia using FISH technique

Nasr Esfahani M1,2, Montazeri F1,3, Daneshmand F1, Kalantar SM1,2, Rajabi M1,3
1. Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Biology, Yazd University of Science and Art, Yazd, Iran.
3. Department of Biology, Payame Noor University, Taft, Yazd, Iran
smkalantar@yahoo.com

Background: Infertility is one of the major psychological, economic and social problems in the human society that involves approximately 15% of couples in childbearing years. Although the role of male infertility factors has declined in recent years, but still a major part of infertility is related to the defects of the semen. Since nowadays intra cytoplasmic sperm injection (ICSI) is frequently recommended, it is essential to inform them about risk of aneuploidy in their embryo.

Objective: To investigate the prevalence of sperm autosome and sex chromosome aneuploidy in oligo-teratospermia (OT) and normozoospermia.

Material and Methods: The study include 10 infertile oligo-teratospermic men in test and 10 fertile normozoospermic men in control group. We used multi-color FISH probes for chromosomes 13,18,21, X and Y based on the frequency of them in the most reported aneuploidies. moreover, we used standard scoring criteria and a min of 30 sperm for each case to evaluating chromosomal aneuploidies.

Result and Conclusion: The overall aneuploidies both in autosome and sex chromosome was higher in OT than the one detected in normozoospermia group (p<0.05). the molecular cytogenetic analysis allows the assessment of sperm aneuploidy rate and increased risk for reproduction failure. Therefore, make it possible an informed counseling for patients and in this regard, preimplantation and prenatal genetic diagnosis/screening are available.

Keywords: Male infertility, Oligoteratospermia (OT), FISH, Aneuploidy

P-278: Endothelial nitric oxide synthase haplotypes are significantly associated with risk of essential hypertension

Nejatizadeh A1, Farbood Z2, Farshidi H3, Shekari M1

Abstracts of the 3rd International & 15th Iranian Genetics Congress
Abstracts of the 3rd International & 15th Iranian Genetics Congress

1. Molecular Medicine Research Center, Hormozgan University of Medical Sciences, Bandar Abbas, Iran;
2. Hormozgan CardioVascular Research Center, Hormozgan University of Medical Sciences, Iran
azimnejate@yahoo.com

Background: Nitric Oxide (NO) a potent vasodilator plays a pivotal role in blood pressure regulation. Evidences suggested that eNOS gene polymorphisms are associated with essential hypertension (EHT). We examined the potential association of 4a/4b, A922G, G894T, T786C eNOS gene polymorphisms with EHT in the southern population of Iran.

Methods: 200 Iranian patients with EHT and 200 normotensive subjects were included. After collecting demographic data, polymerase chain reaction was used to determine genotype of 4a/4b polymorphism, and three other polymorphisms were analyzed by restriction fragment length polymorphism- polymerase chain reaction method (PCR-RFLP). Pairwise, ternary, and foursome haplotype analysis conducted to reveal their association with ETH. Association was determined by logistic regression analysis.

Results: our results demonstrated statistically significant associations between T786C, G894T, and 4a/4a and the disease (P < 0.001) with an increased risk of hypertension (OR = 2, OR = 3.8, OR = 1.6, respectively), however, A922G variant had no significant association. 786C/922A, 786C/922G, 786C/4a, 786C/894T, 922A/4a, and 922G/4a haplotypes were associated an increased risk of hypertension, while 786T/922A, 786T/922G, 786T/894T, and 922A/4b were reversely associated (P < 0.001). Moreover, Ternary haplotype analysis revealed that 786C/922A/4a, 786C/922A/4b, and 786C/922G/4b haplotypes are significantly associated with hypertension while 786T/922G/4a and 786T/922G/894T haplotypes demonstrate protect effects against hypertension (P < 0.001).

Conclusion: The 4b/4a and 786T/C polymorphisms emerged as the determinants modifying the risk of hypertension. The 786T/C, 4b/4a and 894G/T polymorphisms, individually and as haplotypes, associated significantly with risk of hypertension. The susceptible haplotypes were associated with an increased risk of hypertension.

Keywords: Hypertension; NOS3; polymorphisms; haplotypes

P-279: Association between (MCP)-1 del/ins and the risk of RSA condition in Yazd population

Nikkhah H1,2, Vafaei M2, Hashemian Z2, Farashahi Yazd E1,2, Ghasemi N
1. Genetic Engineering and Genome Editing Laboratory, Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
nadinanikkah@yahoo.com

Recent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses before the 20th week of gestation, (RSA) and in 2-5% of the population of pregnant women. Various reasons have been mentioned that in 50% of cases the cause of the incident is unclear. In many (RSA), the blood-related relationship between mother and fetus cannot be properly formed. The monocyte chemoattractant protein 1 (MCP-1) is involved in the recruitment of lymphocytes and monocytes and their migration to sites of injury and cellular immune reactions. MCP-1 in the uterus is secreted by a number of endometrial epithelial cells, fibroblasts, monocytes and lymphocytes. Significant association between (MCP)-1 polymorphisms and various diseases has been seen in several studies. The present study aimed to investigate the potential associations between single nucleotide polymorphisms (SNPs) of pro-inflammatory cytokine genes (MCP-1) and RSA cases in referents to Yazd Reproductive Sciences Institute. one (MCP-1) gene SNP rs3917887 were selected for the present study. Method:80 women who according to RSA characteristics where chosen for patient and 80 women who had successful fertility were chosen for control groups. genotyping carryout by modify allele specific oligo nucleotide. Result & discussion: Neither the allele frequencies nor any of the genetic model of this snp rs3917887 were significantly differences between the RSA couples and the control group. No evidence was found for any associations between the (MCP)-1 genes SNPs with RSA in referents to Yazd Reproductive Sciences Institute.

Keywords: MCP-1 , polymorphism, RSA, Monocyte chemoattractant protein

P-280: Risk assessment of rs4359426 and rs2228428 SNP of CCL22 and CCR4 gene with myocardial infarction in Iranian Population.

Noori F1, Naeimi S2
1. Department of Genetics, College of Science, Kazerun branch, Islamic Azad University, Kazerun, Iran
frahimi2015@gmail.com

CCL22 and CCR4 are chemokine known to be involved in the process of cardiac migration, in a hypoxia-induced inflammatory environment. The prolonged inflammation in hypoxia environment leads to myocardial infarction (MI), an irreversible damage of myocardial tissue. Mutation in CCL22 and CCR4 genes can be a driving factor for MI disease. Keeping this in view, we evaluated incidence of rs4359426 and rs2228428 SNP variants in CCL22 and CCR4 genes respectively in MI patients and studied their association with MI disease. 200 patients diagnosed with myocardial infarction and 200 age-matched healthy controls aged 30-70 years were registered in the study. Genotypic analysis of rs4359426 and rs2228428 in CCL22 and CCR4 genes respectively were carried out in patients using PCR-RFLP method and compared with control group. Successively genotyped SNPs were reviewed for their possible association with the disease or physiological findings using Fishers exact test. The frequency of CC genotypes at both SNPs rs4359426 and rs2228428 were significantly differences between the MI patients and healthy controls. MI patients compared to other genotypes. Although we could not establish any direct association with the disease due to restricted population size, it is possible that CC genotypes in CCL22 and CCR4 could be considered as risk factors in Myocardial Infarction.

Keywords: Heart attack, CCL22, CCR4

P-281: Hsa-mir27b-3p, hsa-mir22-3p and hsa-199-3p play important role in azoospermic men

Norouhi H, motovali-Bashi M, Javadirad SM
1. Biology Department, Faculty of Sciences, University of Isfahan, Isfahan, Iran
hamid.game@yahoo.com

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Recent findings in KDM3a knock-out mice show atrophic testis leading to sterility. These animal studies have established an important role for KDM3A gene and its downstream targets, TNP1 and PRM1, in developing infertility. However, there is a few finding about the mechanisms regulating KDM3A gene expression. Non-coding regulatory microRNAs (miRNAs) are known to downregulate the expression of target genes. We conducted an in-silico analysis using various tools such as targetscan, mirwalk, mirbase, mirtabase and dianatools in order to identify miRNAs targeting KDM3A gene. We found three candidates, hsa-mir-27b-3p, hsa-mir-22-3p and hsa-mir-199-3p that can potentially hit KDM3A transcript. We aim to investigate the role of these miRNAs in downregulating KDM3A expression and eventually, male infertility. In this study, we plan to test the expression of our candidate miRNAs in obstructive vs non-obstructive azoospermic men. Total RNA will be isolated from testis tissue and miRNAs expression will be tested via qPCR. The results will be then correlated with KDM3A gene regulation and azoospermia phenotype in affected males. In conclusion, our primary investigation has found potential miRNAs targeting KDM3A expression. Our final goal is to assess the possible link between these miRNAs and azoospermia in infertile males that can have clinical impact on our next studies.

**Keywords:** KDM3A, microRNA, Azoospermia

**P-282: Exosomes derived from ovarian epithelial carcinoma cells enhance cell proliferation in human umbilical vein endothelial cells**

Norouzi FS, Behmanesh M, Babashah S

Department of Molecular Genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
babashah@modares.ac.ir

**Purpose:** Ovarian cancer is one of the most common and fatal cancers among women. Cells in the tumor microenvironment, especially cancer cells, interact with the normal cells around their surroundings, especially endothelial cells. Exosomes, the term referred to nano-sized intraluminal vesicles of multivesicular bodies, are secreted by most types of cells and mediate intercellular communication.

**Materials and methods:** Exosome were prepared from cell culture media of ovarian epithelial carcinoma cells (SKOV3) by ExoSpin kit. Isolated exosomes were characterized by scanning electron microscopy in terms of size and morphology. The size of the purified exosomes was measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS. Human umbilical vein endothelial cells (HUVECs) were treated with exosomes (100 Âµg/ml) or vehicle control (PBS). The cell count of exosome-treated HUVECs was accessed using neubauer lam.

**Results and Discussion:** Scanning electron micrograph of purified exosomes depicting spherical and membrane-encapsulated particles with diameters ranging from 50 to 200 nm. Also, exosome size measurements by DLS indicated a single bell-shaped size distribution with a peak at ~90 nm. We demonstrated that ovarian tumor-derived exosomes caused an increase in the proliferation rate of HUVECs. These findings may clarify, in part, the role of tumor-derived exosomes in ovarian cancer biology and tumor angiogenesis. Since exosomes are considered as enriched sources of microRNAs, we hypothesized that exosome mediated transfer of these non-coding RNAs from ovarian tumor cells might account for enhanced proliferation of exosome-treated HUVECs.

**Keywords:** Exosomes, Ovarian epithelial carcinoma cells, Human umbilical vein endothelial cells, Cell proliferation

**P-283: Coincidence of Familial Dwarfism and Colorectal Cancer: A Case Report**

Norouzi M¹, Zeinalian M², Hadian M³, Narrei S¹, Tabatabaiefar MA²

1. Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Ala Cancer Prevention and Control center, Isfahan, Iran
norouzi.mahnaz@gmail.com

Dwarfism, a condition of stunted growth, can be caused by many different medical conditions. In general, the disorders are divided into two broad categories: proportionate and disproportionate dwarfism. The proportionate dwarfism is usually due to metabolic and hormonal disorders such as growth hormone deficiency. Recently, it has been reported that humans with growth hormone receptor deficiency (GHRD) rarely develop cancer. Totally, inappropriate regulation of growth pathways leads to growth disorders with two radical consequences; undergrowth or overgrowth that rarely happen in one patient synchronously. We report a family with a 37-year-old woman with proportionate dwarfism as a proband affected with well differentiated adenocarcinoma. Altogether, we found 4 affected members with coincidence of colorectal cancer and dwarfism out of 13 dwarf members over 3 generations, who had been developed colon cancer under the age of 41. Furthermore, one 32-year old man with proportionate dwarfism had been affected with polyp in his colon. Our observation of these two incompatible phenotypes simultaneously, point out a unique area to study a common potential molecular pathways responsible for coincidence of dwarfism and colorectal cancer. Further study using genome-wide sequencing would likely explore the underlying molecular mechanisms related to this condition.

**Keywords:** Dwarfism, colorectal cancer, growth

**P-284: The association of Tumor Necrosis Factor β gene polymorphism in active and inactive inflammatory bowel diseases**

Nourian M¹, Chaleshi V¹, Abdollahzadeh S¹, Jalalvand E¹, Mojabi S¹, Kabiri F¹, Asadzadeh Aghdæi H¹, Zali M²

1. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
2. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
mahyarnourian1369@gmail.com

**Abstract:** In this case-control study, were studied 45 patients of inflammatory bowel disease (IBD) which 25 cases of active and 20 cases of inactive disease were enrolled. In this study, We investigated TNF β gene polymorphisms (rs1800629) in IBD with 2 subgroups of active and inactive disease by DNA extraction, polymerase chain reaction (PCR) and restriction enzyme digestion (RFLP) technique. The TNF β gene polymorphisms were associated with the development of IBD, and specifically, TNF β gene polymorphisms were associated with the development of IBD.

**Keywords:** Tumor Necrosis Factor β gene polymorphism, Inflammatory bowel disease

**Background:** Inflammatory bowel disease (IBD) includes two basic categories ulcerative colitis (UC) and Crohn's disease (CD) that the etiology of which remains unclear. Tumor necrosis factor Beta (TNF?) promoter polymorphisms are a good candidate for susceptibility to IBD as there is a significant relationship between them. The main aim of this study was to assess TNF β gene polymorphisms with IBD susceptibility at positions +252 in Iranian patients.

**Materials and Methods:** In this case-control study, were stud-
ied 103 patients with IBD (85 ulcerative colitis, 18 Crohn's disease) and 100 healthy controls were studied. PCR-RFLP (Polymerase Chain Reaction Restriction Fragment Length Polymorphism) was used for determining of genotyping. In following, allele frequency and genotype distribution of polymorphism A; G in TNF? gene between the case and control groups were typed. To confirm the results of genotyping, 10% of the PCR products were sequenced using the ABI PRISM 3130XL Genetic Analyzer (Applied Biosystems®, Invitrogen Life Technologies, Carlsbad, CA, USA).

**Results:** The frequency of genotype AA, AG and GG among Active patients was 37.7%, 46.4% and 15.9% and in Inactive patients was 65.6%, 21.9% and 12.5%, respectively (P=0.036).

**Conclusion:** There was significant correlation between TNF β gene polymorphisms and susceptibility to IBD (Active and Inactive) at position +252. Our results showed that TNF? gene polymorphisms can be considered as a potential prognostic marker cause of IBD in Iranian population.

**Keywords:** tumor necrosis factor beta, polymorphism, inflammatory bowel disease, Iranian, Crohn's disease, ulcerative colitis.

**P-285: Reporting and studying Long QT syndrome Type 5 patient with a mutation in KCNE1 gene**

Omidi S, rafie khorgami M, maleki M, Mahdieh N

Genetic Research Laboratory, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
So.omidi92@gmail.com

The long-QT syndrome (LQTS) is one of important cardiac arrhythmia that characterized by a prolongation of the QT interval on the electrocardiogram. KCNE1 gene is one of the genes involved in LQT syndrome and cause of LQT syndrome type5. The protein encoded by KCNE1 gene is a single transmembrane protein, KCNE1 and KCNQ1 proteins are assembled to forming potassium channels on heart muscle cells so this inherited condition is the heart's electrical activity disorder since ion channels may not work well. In this study we described a boy with clinical features of LQT syndrome, direct sequencing of genes involved in LQT syndrome revealed a heterozygous missense mutation in KCNE1 gene at position c.29C>T presented at protein level as substitution of Threonine to Methionine amino acid and segregation analysis in his parents confirmed this mutation. We investigated pathogenicity of this mutation with online database and other reported mutations in KCNE1 gene, structural and functional analysis of KCNE1 protein was performed by Phyre2 and I-TASSER database, secondary and 3-dimensional structural of mutant protein was compared with normal structure. All results obtained from bioinformatics tools illustrated role of KCNE1 gene mutations in LQT syndrome. In this family, a mutation in KCNE1 gene was found is responsible for LQT syndrome in their son, so KCNE1 gene mutations in Iranian families are arguable and can be investigated.

**Keywords:** Long QT syndrome Type 5, in silico analysis, KCNE1 mutations

**P-153: A Recombinant Abnormal Gamete Resulting from a Balanced Pericentric Inversion of Chromosome 4: an Affected Boy with Wolf-Hirschhorn Phenotypes**

Omori Sarabi S1, Behrend C2, Mossalaee MM1, Mohseni Moghadam SB, Soleymani M1, Kakadezfooli S1, Moeini Z1, Karimzad Hagh J1

1. Parseh pathobiology and genetics laboratory, Tehran-Iran
2. Praxis fA/â Medizinische Genetik DA/ßseldorf , Germany
mosallaee@gmail.com

Wolf-Hirschhorn syndrome is a genetic disorder that characterized by distinct craniofacial dysmorphology, pre- and postnatal growth deficiency, intellectual disability and seizures. During meiosis, chromosome 4 homologue with a pericentric inversion can give rise to two recombinant chromosome 4; namely partial monosomy 4p/partial trisomy 4q or partial monosomy 4q/partial trisomy 4p, respectively. Here, we report a one-year-old Iranian boy presented with distinct clinical features of Wolf-Hirschhorn syndrome. G banded chromosome analysis of the proband cultured lymphocytes revealed 46 chromosomes in all cells with a chromosome 4 with partial monosomy 4p and partial Trisomy 4q. Parental chromosome analysis was done to assess whether this abnormal chromosome 4 is inherited or de novo. Karyotyping result of his father was designated as 46,X,Y,inv(4)(p16.3q34.3). Further characterization of breakpoints with array-CGH confirmed the karyotyping result and revealed a deletion/duplication syndrome, about 50kb in the size, with 4p partial monosomy and 4q partial Trisomy. Final karyotype nomenclature was 46,X,Y,rec(4)dup(4q)inv(4)(p16.3q34.3)pat. To date few cases of rec(4) with the different break point have been published. The phenotype variability and the viability of the recombinant offspring depend on the size of the deletion/duplication segments. Our result highlights that the parental chromosomal rearrangements can also contributes to the pathogenesis of such a popular chromosomal microdeletion syndrome and underscores the need to analyze parental karyotype for precise genetic counseling.

**Keywords:** Wolf-Hirschhorn; pericentric inversion; recombinant chromosome; rec(4); Array CGH; partial monosomy; partial trisomy

**P-286: The effect of sFLT01 gene expression on cell proliferation and migration of bladder cancer cell line; 5637**

Parsamehr H1, Soheili ZS1, Samie Sh2

1. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
hparsamehr6465@gmail.com

Bladder cancer is the most common urinary malignancy all over the world. The pathologic angiogenesis plays an important role in bladder cancer tumor growth and progression. VEGF is one of the primary required angiogenic factor for bladder cancer and is able to induce tumor angiogenesis and accelerate tumor growth.

sFLT01 is an engineered chimeric secretory receptor/protein with the inhibitory effect on VEGF and placental growth factor (PLGF). The objective of this study is to evaluate the effect of sFLT01 over expression on proliferation and migration of 5637 bladder cancer cell line.

sFLT01-His Tag-GFP sequence was designed synthesized and cloned in AAV-MCS-GFP vector. PAAVâ€™sFLT01-His Tag-GFP vector was transfected to 5637/cell line through lipofec- tion 2000 transfection. Then extracted mRNA was analyzed by real time PCR. Protein secretion into the conditioned medium
of transfected 5637 cells and HEK293T cells proved by western blotting. The effect of the condition medium 5637 cells and HEK293T cells on in vitro angiogenesis in HUVEC cells was investigated by the in vitro angiogenesis assay.

5637 cell migration was evaluated by scratch assay. The cytotoxic effect of the construct 5637 cells was determined and cell proliferation assay was performed through the MTT assay. Real-Time PCR results showed significant over expression of sFLT01 in treated 5637 cells. Western blot proved sFLT01 protein secretion in conditioned media. In vitro angiogenesis assay results showed decreased potential of tube formation in conditioned medium of treated 5637 cells and HEK293T cultures. Scratch assay showed no significant difference in treated 5637 cells when compared to the control untreated cultures. MTT assay showed that sFLT01 had no cytotoxic effect on 5637 cells.

Keywords: bladder cancer, sFLT01, 5637 cells

P-287: Combination therapy with KRAS siRNA and EGFR inhibitor AZD8931 suppresses A549 malignant lung cancer cell growth in vitro

Pashapour Sh1, Zarreddar H2,3, Ansarin Kh2, Khalili M2, Baghban R5, Farajnia S1,4,5

1. Department of genetic, Tabriz branch, Islamic Azad University, Tabriz, Iran
2. Tuberculosis and Lung Disease Research Center, Tabriz University of Medical Science, Tabriz, Iran
3. Biotechnology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
4. Students Research Committee, Tabriz University of Medical Sciences, Tabriz, Iran
5. Drug Applied research Center, Tabriz University of Medical Sciences, Tabriz, Iran
6. Department of Basic Science, Maragheh University of Medical Science, Iran

Farajnias@tbzmed.ac.ir

Background: Lung cancer is the leading cause of cancer-related death with less than 5-year survival rate for both men and women worldwide. EGFR and KRAS signaling pathways have a critical role in proliferation and progression of various cancers, including lung cancer. Genetic studies have shown that amplification, over-expression or mutation of EGFR is an early and major molecular event in many human tumors. KRAS mutation is a negative factor in various cancer including NSCLC and complicate therapeutic approaches with androgen deprivation agents of this cancer. Generally, changing in miRNA expression can play a critical role in main important pathways in lung cancer and might be a potential therapeutic target for treatment of lung cancer.

Keywords: EGFR inhibitor, Target therapy, siRNA, Lung cancer

P-288: Microarray Bioinformatic Analysis of Brain Tissue in Normal and Multiple sclerosis Patients

Payazdan M, Khatami S R, Glehdari H, Seifi T1

Department of genetic, faculty of science, Shahid Chamran university of Ahvaz, Ahvaz, Iran.

Maleypayazdan@yahoo.com

Background: Multiple sclerosis (MS) is an autoimmune inflammatory disease of the central nervous system. In this disease immune system attacks the myelin that covers nerve cell fibers and demyelination is happened. Both environment and genetic factors are involved in MS. However the cause of the disease is still unknown. The goal of the survey was to study differential gene expression between healthy control and active plaque.

Material and methods: Microarray data of normal and patient brains was obtained from GEO dataset with GSE38010 and analyzed with R software (3.2.2 version) by affylmGUI package. Subsequently, up and down gene expression were proved by DAVID data base.

Results: 5541 genes in tumor tissue were down-regulated and further 5279 genes were over expressed, compared to normal tissue. Thirteen genes with up regulation in this observation play a role in T cell receptor signaling pathway, acting as AP1 transcription factor which start transcription of inflammatory cytokines like TNF alpha.

Conclusion: our finding could be important for MS pathogenesis and ethiology.

Keywords: Multiple sclerosis, MS, GEO dataset with GSE38010, DAVID data base.

P-289: The effect of Genistein on miRNA biogenesis genes (Dicer and AGO2) expression in four cell lines of Acute Lymphoblastic Leukemia (ALL) and evaluation of these genes expression in ALL patients

Piroozian F1,2, Bagheri Varkiyani H1, MalekZadeh K1,6

1. Department of Medical Genetic, Faculty of Medicine, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
2. Student research committee, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
3. Department of Pathology, Shahid Mohammadi Hospital, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
4. Hormozgan Institute of Health, Hormozgan University of Medical Sciences, Bandar Abbas, Iran
5. Fateme.piroozian@gmail.com

Introduction: Acute lymphoblastic leukemia (ALL) is the most common cancer in childhood. It result from genetic and environmental factors. miRNAs considered as a causative agents of this cancer. Generally, changing in miRNA expression can play a critical role in main important pathways in cells. Therefore, it can be hypothesized that genes involved in miRNAs biogenesis pathways (e.g. Dicer and AGO2) can play role in cancers. Afew studies has been reported for Genistein effect on ALL. This study evaluated the expression of the DICER and AGO2 genes in patients with ALL, and also the Genistein effect on the expression of these genes in the cell lines of this cancer.
Methods: 25 human cases with ALL and 25 healthy controls were used to evaluate the expression of AGO2 and Dicer expressions. Furthermore, four groups of ALL cell lines (Jurkat, Molt4, Molt17 and Nam16) were used for Genistein treatment. 15, 30, and 60 ?M doses of Genistein were used. Dicer and AGO2 mRNA expression levels were evaluated after 24 and 48 hours by Real time-PCR. In order to determine the apoptosis of the cells, a flow cytometry was placed at that time.

Results: This study indicate that genistein can decrease the number of live cells and increase the mortality rate (P <0.05). The concentration of 60?M resulted in the highest mortality rate in all cell lines. Expression levels of Dicer and AGO2 in patients compared to healthy controls increased and decreased respectively (P <0.05). Genistein increased the Dicer expression in both the Nam16 and Molt17 cell lines significantly, but in two other cell lines, expression changes due to Genistein effect were not significant (P>0.05). In the present study the increase of AGO2 gene expression after the effect of Genistein compared to the untreated group in all cell lines was statistically significant (P <0.05).

Discussion: The findings indicated that genistein increases the apoptosis and death rate in ALL cells through probably changing on the expression of Dicer and AGO2. It is possible to consider the inhibiting role for genistein in cell growth through effects on the cell cycle. Anyway, in future studies, it is better to examine human in order to determine the clinical effects. Also, by comparing the findings from gene expression in human specimens and cell samples, it can be concluded that genistein can play an inhibiting role in cell cycle pathways and also increasing apoptosis by increasing the expression of AGO2 as a mediator.

Keywords: genistein; Dicer; AGO2; leukemia, ALL

P-290: Combined Effects of p53 and PAI-1 polymorphisms on the Breast Cancer Susceptibility among Iranian-Azeri Women

Pouland N, Shavali M

Department of Biology, Faculty of Science, Azarbaijan Shahid Madani University, Tabriz, Iran
mojtababahvalimadakto71@gmail.com

Backgrounds and Objectives: Breast cancer is the main cause of death especially because of this cancer in women around the world. It is a heterogeneous and multifactorial disease. According to previous reports, polymorphism in two genes; p53, which is an important tumor suppressor gene involved in wide range of cancers, and PAI-1, which is an inhibitor protein coding plasminogen involved in progression and metastasis, has been investigated.

Material and Methods: In this case-control study, 200 patients and 200 healthy women in Iranian-Azeri population were investigated. At first, genomic DNA had been extracted using high salt method, then these genes had been genotyped with tetra-ARMS PCR method. Data had been analyzed with javastat online statics package and SPSS (version 22.0).

Results: Genotype distribution of these polymorphisms for p53 and PAI-1 was GG=43.5%, GC=42%, CC=14.5% and 4G/5G=70.5%, 5G/5G=18.5%, 4G/4G=11%, respectively. No association between allelic frequency and genotype, individually nor in combination have been shown. Also, there was no association between combined genotype with pathological properties of cancer.

Conclusion: Our results show that polymorphism of codon 72 of p53 has a significant association with breast cancer (p value<0.023). Even though there was no association in combined genotype with breast cancer in our investigated population, further studies need to be done in bigger population and other races.

Keywords: Breast cancer, P53 gene, PAI-1 gene, Polymorphism

P-291: Pediatric Cancer and Li-Fraumeni Syndrome in North West of Iran

Esmaeillezadeh Aghjeh M, Hosseinpour feizi M A, Safaralizadeh R, Hosseinpour feizi A A, Pouland N

University of Tabriz es_maryam7@yahoo.com

Introduction: In 1969, Li-Fraumeni syndrome (LFS) which is a rare and cancer predisposition syndrome, has been reported by Frederick L I and Joseph F Fraumeni for the first time. In 1990, Malkin, et al. have represented that, the main problem in LFS is mutation in TP53 gene that is a crucial tumor suppressor gene in cell cycle. Therefore, any alternation or mutation in the TP53 gene will cause some abnormalities in genome which leads to cell overgrowth and eventually cancers.

Material and Methods: In this study, 45 children with cancer in North West of Iranian population were investigated. Patients DNA have been extracted using high salt method, then the region within exons 5 to 8 have been replicated via PCR method then sequenced the products and finally analyzed the results.

Results: In 12 cases (26.67%) we detected polymorphisms in Exon6 and Introns 6 and 7. In the examined probands, no mutation was observed in exons 5 to 8 of the TP53 gene to indicate the possibility of Li-Fraumeni syndrome in these families.

Conclusion: Our results show that, there was no mutation in exons 5 to 8 of the TP53 gene to indicate the possibility of LFS in these families, further studies need to be done in bigger population and can complete our data.

Keywords: Li-Fraumeni syndrome, cancer, TP53 gene, mutation

P-291: ZIC3 mutations in Iranian patients with congenital heart defects

Pourirahim M1, Siasi E1, Kalayinia S2, Maleki M3, Maleki Z3, Mahdieh N3

1. Masters student, Molecular Cell Group, Faculty of Life Sciences, Tehran University, Tehran, Iran
2. Department of Genetics and Molecular Medicine, Faculty of Medicine, Zanjan University of Medical Sciences
3. Genetic Research Laboratory, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
m_pourirahim@yahoo.com

Introduction: Congenital heart defect is one of the most common abnormalities in the neonates which occur due to heterozygous causes. The ZIC3 gene is one of the genes involved in congenital heart defects. Therefore, this study was conducted to evaluate the role of ZIC3 gene in congenital heart disease in Iranian population.

Materials and Methods: In this study, clinical and cardiac symptoms including echocardiography were evaluated in 50 Iranian patients with congenital heart defect. The ZIC3 gene was sequenced in these patients. The samples were collected from patients referring to Shahid Rajaie Heart Hospital during the years 1969-1959.
Results: CHD was confirmed by echocardiogram. 48% of affected individuals had complex form and the remaining had simple form of CHD. The sequencing results showed that none of the patients had ZIC3 mutation.

Discussion: ZIC3 gene does not play an important role in the Iranian population. Other genes probably cause CHD in this population.

Keywords: ZIC3, mutation, congenital heart defects

P-192: Expression of ZFX Spliced Variants in Breast Cancer
Pourkeramati F, Asadi MH, Shakeri Sh
Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran. pourkeramati.fa@gmail.com

ZFX, a member of kruppel C2H2-type zinc-finger protein family, is a transcriptional regulator in human embryonic stem cells that plays an important role in self-renewal property. ZFX is widely expressed in pluripotent stem cells and is down regulated during differentiation of embryonic stem cells. ZFX has five different variants that encode three different protein isoforms. While several reports have determined the overexpression of ZFX in a variety of somatic cancers, the expression of ZFX spliced variants in cancer cells is not well understood.

In this study, the expression of ZFX variants in a series of breast cancer tissues was investigated by using qPCR approach. Our results showed that the expression of ZFX-variant1 and ZFX-variant4 are much higher in tumor tissues compared to marginal ones. In contrast, the ZFX-variant5 is down-regulated in tumor tissues. While the ZFX-variant1 expression increased significantly in low-grade tumors, ZFX-variant4 and ZFX-variant5 are highly expressed in high-grade and lymphatic invasion ones. Also, our data showed that the expression of ZFX-variant1 is significantly increased in luminal A subtype of breast cancer, whereas the expression of ZFX-variant4 was elevated in triple negative subtype. Here, our data revealed a significant association between the HER2 status and the expression of ZFX spliced variants.

Altogether, our findings suggest that the expression of ZFX spliced transcripts is various in different types of breast cancer and maybe contributed in their tumorigenesis process. Hence, ZFX spliced transcripts can be considered as a novel tumor marker with potential diagnostic, prognostic and therapeutic values.

Keywords: Breast Cancer, Cancer Stem Cells, ZFX Spliced Variants.

P-293: Evaluation of TBL1XR1 Gene Expression in Breast Cancer
Pourmahdi M, Safaralizadeh R, Hoseinpour Feizi M A, montazeri V, Rajabi A
Department of Genetics, Animal Biology Group, Faculty of Natural Science, University of Tabriz, Tabriz, Iran, Department of Thoracic Surgery, noor-nejat Hospital, Tabriz, Iran mahsa_pourmahdi@yahoo.com

TBL1XR1 gene is a member of WD-40 repeat genes family and the protein encoded by this gene is involved in the transcription and activation of transcription factors. According to the previous studies, TBL1XR1 expression up-regulation have been illustrated in different cancers. This study aimed to evaluate the expression of TBL1XR1 gene in breast cancer. Breast tumor tissues and their non-tumoral margin samples were obtained from 30 patients. Total RNA was purified with Trizol and expression of TBL1XR1 was quantified using qRT-PCR. qRT-PCR results indicated the significant changes in TBL1XR1 expression in tumor tissues compared with margin tissues.

Keywords: breast cancer, Expression, qRT-PCR

P-294: miRSNP Biomarker Discovery for PCOS susceptibility
Pourteymour Fard Tabrizi Z, Ghasemi S
Cellular and Molecular Research Institute, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran Zahr.a.pourteymoor@gmail.com

Background: Polycystic ovarian syndrome (PCOS) is a polygenic and complex disorder. Single nucleotide polymorphisms (SNPs) in 3’UTR of the candidate genes can affect the relationship between the microRNA and target gene and thus the susceptibility to the PCOS. MicroRNAs whose expression has changed by the presence of SNPs can be used as biomarkers for the prognosis of PCOS. This paper focuses on the bioinformatics of the miRSNPs related to PCOS.

Methods: PCOS related genes were searched through bioinformatics tools such as DISEASE and DisGeNET. The association between SNPs in the 3’UTR of candidate genes and microRNAs was investigated by the miRdSNP tool. Then, the relationships between cell-functional pathways, the expression of candidate genes and predicted microRNAs were analyzed by mimirNA and mIRNApath tools to select the best biomarkers with higher analyzing potential and specificity.

Result: LMNA, Cyp19A1, INS and FSHR are candidate genes that were found by "gene to disease" tools. After examining the relationship between the candidate genes and the SNPs, a number of regulatory microRNAs have been found. Using performance studies, miR-205, miR-98 and miR-9 could be evaluated as prognostic biomarkers for PCOS.

Conclusion: The highly complex disease PCOS, has a long-term complication with no accurate diagnostic method. The satisfactory results of identifying SNP-related biomarkers in other complex diseases provides hopes for the bioinformatically selected microRNA for PCOS to be subjected to clinical studies.

Keywords: miRSNP, PCOS, Biomarker, Bioinformatics.

P-295: Study of miR-335 and GBP2 gene expression levels in ductal carcinoma breast cancer as possible candidate cancer biomarker
Rahvar F1, Salimi M2, Mozdarani H3
1. Research and technology department, Islamic Azad University, Iran
2. Department of Medical Genetics, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran

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3. Department of Medical Genetics, Tarbiat Modares University, Tehran, Iran * Corresponding author: Mahdieh Salimi, Department of Medical Genetics, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran. fzrahvar88@gmail.com

Introduction: Breast cancer is the most common cancer among women in the world, but its diagnosis in the early stages increases the chance of recovery and survival of the patient. GBP2 is a member of GBP family. GBP2 has been discussed as a possible control factor in tumor development in breast cancer that have potential to be a tumor marker.

Materials and Methods: In this project, the Real-Time RT-PCR technique was used to analyze expression of mir-335 and GBP2 gene as its target in tumor and normal adjacent breast tissue of 35 patients with ductal carcinoma breast cancer compared with normal adjacent breast tissues.

Results: Our results indicated that The GBP2 gene and miR-335 was down regulated in tumor tissues compared with normal adjacent breast tissues.

Conclusions: As a consequence, GBP2 expression are associated with breast cancer as possible biomarker. 

Keywords: Breast Cancer, Gene Expression, mir-335, Real Time PCR, GBP2, microRNA, Biomarker

P-296: Investigation of ADGRL4(ELTD1) gene expression in breast cancer

Rajabi A, Safaralizadeh R, Hoseinpour Feizi M A, Montazeri V, Pourmahdi M

Department of Genetics, Animal Biology Group, Faculty of Natural Science, University of Tabriz, Tabriz, Iran
Department of Thoracic Surgery, Noor-Nejat Hospital, Tabriz, Iran
a.rajabi43@yahoo.com

Breast cancer is the most commonly diagnosed cancer and the second leading cause of cancer death among women. Breast cancer is a highly heterogeneous disease that results from the interaction of hereditary and environmental risk factors. ADGRL4 gene encoded a protein that is a G protein-coupled receptor expressed on the surface of endothelial cells and vascular smooth muscle cells that plays a role in angiogenesis. According to the previous studies, expression of ADGRL4 was significantly increased in glioblastoma. This study aimed to investigate the expression of ADGRL4 gene in breast cancer. Breast tumor tissues and their non-tumoral margin samples were obtained from 30 patients. Total RNA was purified with Trizol and expression of ADGRL4 was quantified using qRT PCR. Our results showed significantly altered ADGRL4 expression in tumor tissues compared to adjacent normal tissues. Keywords: breast cancer, Expression, qRT-PCR

P-297: Quantitative analysis of the Bid gene expression in patients with colorectal cancer

Rajabi M^1, Nazemalhosseini Mojarrad E^2, Forouzesh F^1

1. Department of Genetics, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran
2. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
f8forouzesh@gmail.com

Introduction and aim: Colorectal cancer is one of the most common types of cancer. Apoptosis is a physiologically planned cell death, and apoptotic disorders cause cancer. One of the key molecules in this pathway is the Bid-protein. The aim of this study was the investigation of the Bid gene expression as a biomarker in patients with colorectal cancer.

Materials and Methods: Blood samples were collected from 22 patients with colorectal cancer and 10 normal samples as control groups. RNA was extracted and cDNA was synthesized. The quantitative expression of Bid gene was investigated by quantitative Real-time PCR method. Fold change of gene expression was evaluated by (2^-ΔΔct) method. The findings were analyzed by using the Rotor gene 2017 and Rest 2009 softwares and a significant level less than 0.05 was accepted.

Result: According to the qReal-time PCR results, the mRNA expression of Bid gene was significantly higher in the patients with colorectal cancer compared to the normal samples (control group) (P<0.05).

Conclusion: In the present study, upregulation of Bid gene in patients suggests that this molecule can be used as a potential prognostic biomarker for colorectal cancer.

Keywords: Colorectal Cancer, Bid gene, Apoptosis

P-298: Assessment of the circulating mir-222 expression level in under treatment epileptic patients as a non-invasive biomarker of PCOs

Rajabi M, Montazeri F, Mirsmaeili SM, Ziaeie SJ, Kalantar SM, Naseri MR, Nasr Esfahani M

1. Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Biology, Yazd university of Science and Art, Yazd, Iran.
3. Department of Neurology, Isfahan University of Medical Sciences, Isfahan, Iran
mahya_rajabi@yahoo.com

Background: Epilepsy is a prevalent chronic neurologic disorder characterized by recurrent unprovoked seizures. However, there is growing concern that the pathogenic epilepsy mechanism remains poorly defined. Antiepileptic drugs are known to have endocrine side effects. Valproic acid (VPA) is commonly used as an anticonvulsant and mood-stabilizing agent in the treatment of epilepsy. In women, VPA can also lead to androgenization, menstrual disturbances, and polycystic ovaries. The incidence of polycystic ovary syndrome (PCO) increases in women with epilepsy (WWE), which appears to vary with ethnicity.

Objective: This study was conducted to determine a non-invasive biomarker for early diagnosis of PCOs in WWE patients and to take preventative measures.

Method and results: The study was carried out in 15 WWE women with PCO at reproductive ages (18-40 Y), monotherapy with valproic acid (VPA) and 15 healthy women as control. The women with history of diabetes, kidney and liver disease were excluded. After collecting patient’s serum, total RNA were extracted. We use quantitative reverse transcription PCR to identify the expression level of miRNA-222 in epileptic patients with PCOs and without it.

Result and Conclusion: In accordance with previous studies on PCOs and non-PCOs women, expression level of miRNA-222 was significantly different between epileptic patients with pco and without it (p<0.05). Our result suggesting miRNA-222 as a non-invasive biomarker to screen for drug side effects and prevent the progression of its complications. Furthermore, bioinformatics analysis indicate role of miR-222 in PTEN/Akt/FOXO1 signaling pathway which may be of im-
P.299: Comparative frequency of MHC class I alleles in different regions of Iran

Rajaei MJ, Shakhshi-Niaei M

Departments of Genetics, Shahrekord University, Shahrekord, Iran javad_rajaei@yahoo.com

Region of major histocompatibility complex (MHC) in human expand about 29Mb to 33Mb on Chr6. Genes of MHCs or human leukocyte antigens (HLAs) are grouped in three different classes. MHC class I is responsible of presenting intracellular self and microbial protein epitopes to CD8+ lymphocytes and initiation of cellular immune response. Therefore, they are important in epidemiology of related infectious and autoimmune diseases as well as cancers. In this study, frequencies of HLA class I alleles in normal samples of several studies in Iran were collected and final relative frequencies calculated. In HLA-A alleles, A*02, A*24 and A*11 showed the highest frequencies. These alleles are also frequent in the normal samples of Iran’s neighbor countries such as Turkey and Pakistan as well as China and Italy. HLA-B alleles of B*35, B*51 and B40 showed highest frequencies which are also consistent with high frequent alleles in Turkey and Pakistan normal samples. For HLA-C the Cw*1202, Cw*0701 and Cw*0602 together allocates about half of HLA-C alleles frequencies. The frequency of prevalent alleles in some large cities such as Tehran, Isfahan and Mashhad is more or less similar to overall frequent alleles of Iran. However, in border cites there were differences in prevalent alleles which show the presence of native populations in those areas with lesser migrations or mix with other regions. For example in Sistan and Baluchestan province HLA-A1 and In Hamedan, HLA-A*09 showed significant different frequencies with other regions. To sum up, study of MHC alleles looks very important in investigation of genetics risk factors of some important diseases in different regions.

Keywords: MHC, HLA, frequency, Allele

P.300: investigation of PARP1 762 Codon polymorphism with susceptibility to Breast Cancer risk among Iranian Population

Ramezani S1, Sharafshagh A2, Mirzanejad L1, Hadavi M1

1. Department of Biology, University of Guilan, Rasht, Iran
2. Cellular and Molecular Research Center, Faculty of Medicine, Guilan University of Medical Sciences, Rasht, Iran
sinar_1370@yahoo.com

Breast cancer is a heterogeneous and hormone dependent cancer that includes about 22.9% of total female cancers. It is the most common type diagnosed cancer and the fifth cause of cancer death among women. DNA repair pathways play important roles in maintaining genomic stability and influence carcinogenesis and tumor biology. Poly [ADP-Ribose] polymerase (PARP) is a key DNA repair enzyme which is essential for DNA single-strand break (SSB) repair -a sub-pathway related to base excision Repair. PARPs proteins also have been involved in various cellular processes including cell survival and death, transcriptional and chromatin structure regulations, telomere integrity, and cell division. Several studies have demonstrated the association of PARP-1 gene polymorphisms with the incidence risk of breast cancer. We analyzed the status of an active-site polymorphism (rs1136410) in the PARP-1 gene and their possible impact with predisposing individuals to breast cancer disease in a female Iran population. A total of 100 patients with histopathologically and surgically confirmed breast cancer and 100 controls was recruited. Genomic DNA was extracted from peripheral blood leukocytes through Salting Out standard technique. Genotyping was performed using ARMS-PCR protocol. There was no significant difference between the PARP-1 V762A polymorphism distribution in control and patient groups (P=0.15). The PARP-1 V762A seems not to be a potential risk factor for the incidence of breast cancer among Iranian patients.

Keywords: breast cancer, polymorphism

P.301: A Case report of 22q11.2 Deletion Syndrome

Rashidi SKh1,2*, Mossalaee MM1, Kakadezfouli S1, Soleymani M1, Moenei Z1, Blazar Z1, Sarabi S1, Karimzad Hagh J1

1. Parseh pathobiology and genetics laboratory, Tehran-Iran.
2. Biotechnology research center, Semnan university of medical sciences, Semnan-Iran
m.kh.rashidi@gmail.com

DiGeorge syndrome (DGS) is a chromosomal disorders, with the prevalence of 1/4000-5000 live births. Deletion in 22q11.2 have been known as causative mutation in this syndrome. It is usually sporadic; however, autosomal dominant inheritance has been reported in 10-20% of the patients. This syndrome characterized by variable phenotypes including dysmorphic facial features, congenital cardiac abnormalities, immunodeficiency, palatal abnormalities, endocrine deregulation and psychiatric problems. In general the Low-copy repeats (LCR) mediate the common 3-Mb deletion in patients with velo-cardio-facial syndrome (VCFS).

In the current study we report a patient with a de novo 3-Mb microdeletion at 22q11.2 region. The present case is a 4-year-old girl with congenital facial malformation, ventricular septal defect (VSD), delayed growth, low plasma level of growth-hormone and mild intellectual disability. Deletion in 22q11.2 was observed with FISH technique and confirmed by MLPA analysis. Mapping analysis showed deleted region contained CLTCL1, HIRA, CDC45L, CLDN5, TBX1, TXNRD2, DGCGR8, ZNF74, KKLK2, PCQAP, SNAP29 and LST1 genes. Microdeletion in 3-Mb common region on 22q11.2 is causative in 90% of cases affected with DGS.

MLPA is a better technique for the exact characterizing and effective detection of all microdeletions compared to FISH method. The genotype-phenotype correlation by this case must be more investigated.

Keywords: DiGeorge syndrome; QF-PCR; MLPA; VCFS; VSD, LCR

P.302: The evaluation effects of estrogen on DLGAP5 gene expression in prostate cancer-3 (PC3) cell lines

Rasouli Broujeni Sh, Sazgar H Zia N.

Department of Biology, Faculty of Science, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran
E-mail: rasoulish.72@gmail.com

Background and Objective: Benign Prostatic Hyperplasia
Abstracts of the 3rd International & 15th Iranian Genetics Congress

P-304: Long Noncoding RNAs in the Regulation of Inflammation

Keywords: Long Noncoding RNAs, inflammation, NF-kB

Abstract: Long non-coding RNAs (lncRNAs) play a crucial role in regulating various biological processes including inflammation. In this study, we investigated the expression patterns of lncRNAs in the hippocampus of rats under acute motor stress. We found that the expression levels of several lncRNAs were significantly altered in response to stress, suggesting their potential role in inflammation.

Rezaei M1, Taheri M2, Kohana L1, Sayad A2
1. Department of Biology, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran
2. Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Shahid Labbafi Nejad Educational Hospital, Tehran, Iran
zahrarezee0055@gmail.com

BACKGROUND: Multiple sclerosis (MS) as a complex neurological disease can be due to vitamin D deficiency. CYP27B1 is referred to as a vitamin D metabolizing enzyme.

MATERIALS AND METHODS: This study compared the expression level of CYP27B1 in Relapsing-Remitting MS (RRMS) patients with normal individuals in Iran. The RNA was extracted from 50 RRMS patients and 50 normal controls. Quantitative RT-PCR was adopted to measure the expression level of CYP27B1 gene.

Rezaei Z1, Taheri M2, Kohana L1, Sayad A2
1. Department of Biology, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran
2. Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
3. Urogenital Stem Cell Research Center, Shahid Beheshti University of Medical Sciences, Shahid Labbafi Nejad Educational Hospital, Tehran, Iran
zahrarezee0055@gmail.com
RESULTS: The expression level of CYP27B1 gene was significantly lower in the RRMS patients than their normal counterparts (P value = 0.04). Also, the RRMS females participating had a significant reduction in CYP27B1 gene expression compared to normal females (P-Value = 0.01). In addition, the correlation between CYP27B1 expression level, and the risk of Expanded Disability Status Scale of Kurtzke (EDSS) was not linear. Additionally, there was no significant correlation between expression status of CYP27B1 gene and duration of the disease.

CONCLUSION: A significant decrease in the expression level of CYP27A1 in female patients could indicate their greater vulnerability to MS than the male patients.

Keywords: CYP27B1, expression, multiple sclerosis, real time PCR

P-306: Association Study of CACNA1C gene and miR-137 polymorphisms with Schizophrenia in Iranian Population

Riahi Kashani N, Mirfakhraie R, Abedin-Do A
1. Department of Biology, Islamic Azad University of Tehran, Science and Research Branch, Tehran, Iran
2. Department of Medical Genetics, Shahid Beheshti University of Medical Sciences, Tehran, Iran. riahir@yahoo.com

Objective: In recent genome-wide association studies of schizophrenia (SCZ) disorder, several risk genes including CACNA1C gene and miR-137 have been detected. CACNA1C is suggested to modulate calcium channels that are involved in the development and function of the central nervous system. Strong evidence of significant association between the single nucleotide polymorphisms of CACNA1C and miR-137 and schizophrenia has recently been reported in several genome-wide association studies of schizophrenia in European population. The aim of the present study was to investigate the association between these polymorphisms (SNPs) with schizophrenia in Iranian individuals with schizophrenia.

Materials and Methods: We performed a case control association analysis between rs4765905 and rs1625579 in CACNA1C and miR-137 with SCZ in an Iranian cohort of 208 schizophrenia patients and 184 control subjects by using PCR-RFLP method.

Results: We found no significant association between age and sex of the studied patients and rs4765905 and rs1625579 in CACNA1C and miR-137 genes.

Conclusions: Our finding suggested that further research is needed to examine for the genetic etiology of schizophrenia in Iranian population to contribute to better understanding of the pathogenesis and exact treatment of schizophrenia disorder.

Keywords: Association, polymorphisms, Schizophrenia, CACNA1C gene, miR-137, Genetic, Iran.

P-307: DNA methylation and Expression status of Glutamate Receptor Genes in Patients with Oral Squamous Cell Carcinoma

Rigi-Ladiz MA1, Baranzehi T2, Hassanpour B2, Kordi-Tamandani DM2, Ashraf MJ3
1. Oral and Dental Disease Research Center, Zahedan University of Medical Sciences, Zahedan, Iran
2. Department of Biology, University of Sistan and Baluchestan, Zahedan, Iran
3. Department of Pathology, University of Medical Sciences, Shiraz, Iran

kiana8588@gmail.com

Oral cancer represents the third most common form of malignancy in the developing countries, whilst in the developed countries it is the eighth most common form of cancer. Alcohol and tobacco users are most affected oral cancers and 90% of them are OSCC in adult males. In Iran, the exact prevalence of the disease is still not clear. However, in some provinces such as, Sistan and Baluchestan its prevalence is higher than other provinces. One of the main neurotransmitter in central nervous system is Glutamate and is a major excitatory. Glutamate signaling has been involved in various non-neuronal cancers process. The aim of this research was to highlight, the association between DNA methylation of the glutamate receptor genes and their expression pattern with pathogenesis of OSCC.

Materials and methods: Genomic DNA was extracted from83 OSCC paraffin-embedded tissues (mean age 59.67±16.08) and 80 normal samples (mean age50.15±16.69). Promoter methylation status of glutamate receptors including, GRM5, GRM2 and GRIA3 genes were carried out by Methylation Specific PCR technique (MSP). We also examined mRNA expression levels of these genes, in 15 paraffin-embedded patients and healthyspecimens using Real-time PCR techniques. Result: DNA methylation analysis has been shown statistically significance difference in patients with OSCC in comparison with healthy controls, for GRM2 (MM: OR=0.32; 95% CI=0.02-3.90; P value=0.37; MU: OR=8.0; 95% CI=1.37-47.34; P value=0.02), GRIA3 (MM: OR= 47.19; 95% CI=4.61-483.0; P value=0.001; MU: OR=1.45; 95% CI=0.35-5.89; P value=0.6) and GRM5 (MM: OR=0.9; 95% CI=0.14-5.71; P value=0.9; MU: OR=1.83; 95% CI=0.33-10.09; P value=0.4). As well as, the evaluation of mRNA expression levels of GRM2, GRIA3 and GRM5 were remarkably different in patients and healthy controls (P<0.00). Suggesting, to verify this data, it should be done more studies in various populations with large sample size.

Keywords: OSCC, GRM5, GRM2, GRIA3, Expression, Methylation

P-308: An incidental finding in an infertile man with SMNc deletion Is there any correlation to infertility

Rojhannezhad M1,2, Rezaei S, Abd Hi1, Samadpour S1, Poula-di A1,2, Salimi M1, karimian M1, Shohani S1, Younesi B1, Karimzad Hagh1
1. Sarem medical genetics department, Sarem women hospital, Tehran, Iran
2. molecular genetics department , Tarbiat Modares university Tehran, Iran
3. Sarem cell Research center, Sarem medical genetics department, Sarem woman hospital, Tehran, Iran
rojhannahm68@yahoo.com

Spinal muscular atrophy, an autosomal recessive disease, is characterized by muscle weakness and atrophy. SMA is caused by the deletion of SMN1 gene located on chromosome 5q13 (part of a 500kb inverted duplication). There are two SMN genes in this locus, telomeric (SMNt) and centromeric (SMNc) copy genes. Increases in SMNc copy number often modify the SMA phenotypes. Here we report an infertile man, referred to our lab for microdeletion testing in AZF regions. The results of microdeletion were negative. Clinical examination showed no serious typical phenotypes related to SMA. We incidentally found a homozygous deletion of exons 7 and 8 of SMNc in this infertile man. We hypothesize that the deletion affects both telomeric and SMNc genes. This is the first report of a deletion in both SMN genes in an infertile patient.
SMNc gene, using his DNA as a control sample with samples of SMA patients. Although homozygous deletion of exons 7 and 8 of the SMNc is present in approximately 5% of the normal population, there are some cases of lower motor neuron disease with homozygous (SMNc) deletion. Interestingly, it is determined that SMN expression in testis is high. It was also reported that a mouse model of SMN C/C which expresses a reduced amount of SMN (~25%−50% of WT) displayed a mild SMA-like phenotype, including peripheral necrosis, autonomic nervous system dysfunction, reduced testis size and impaired spermatogenesis. As a result we would hypothesize that impaired spermatogenesis that caused infertility may have a correlation with the SMNc homozygous deletion in the patient. To confirm this correlation more evidences and further studies of SMNc deletion in infertile patients are needed.

**Keywords:** SMA, SMNc, SMNt gene, Infertility, Incidental finding

**P-309: Nanoporous silica type SBA-15 functionalized with tryptophan changes Jnk3-MAP kinase gene expression in rats with hepatic encephalopathy**

**Saadati S1, Ghaderi H2, Ahmad Sh3, Samadi S2**

1. Department of Biological Science, Faculty of Science, University of Kurdistan, Sanandaj, Iran
2. Department of Chemistry, Faculty of Science, University of Kurdistan, Sanandaj, Iran

nazilasaadat@yahoo.com

Hepatic encephalopathy (HE) is a syndrome that may develop with liver failure. Ammonia and inflammation are central to induce HE and also important therapeutic targets in the management of the disease. Mitogen-activated protein (MAP) kinases are affected in HE in response to inflammation induced by hyperammonemia. Nowadays mesoporous materials have received considerable attention as a drug delivery vehicle for loading drugs as well as large biomolecules. The aim of the present study was to investigate the effects of mesoporous silica SBA-15 functionalized with tryptophan (tryptophan-SBA-15) on Jnk3 MAP-kinase gene expression in the prefrontal cortex (PFC) of the HE model rats. Male Wistar rats weighing 300-350 g were divided into two groups of sham control and HE model group. HE model rats were undergone common bile duct ligation (BDL). A dose of 0.2 mg/kg tryptophan-SBA-15 was injected subcutaneously every 48 hours for 28 days of an experimental period. On day 28 after the surgery, the animals were decapitated, their brain was removed and the PFC of each rat was dissected. Gene expression of Jnk3 was evaluated by a real-time PCR method. The results of gene expression showed that the Jnk3 gene expression was increased in the PFC of HE model group treated with saline. The results also showed that HE model rats treated with tryptophan-SBA-15 decreased the elevation of Jnk3 gene expression. In conclusion, mesoporous tryptophan-SBA-15 treatment could play a role in altering the Jnk3 gene expression in the PFC of HE rats, which may affect brain inflammation.

**Keywords:** Jnk3 MAP Kinase, Hepatic encephalopathy, Real-time PCR, Gene expression

**P-310: The miRNA targetome of coronary artery disease is perturbed by functional polymorphisms identified and prioritized by in-depth bioinformatics analyses exploiting genome-wide association studies**

**Saadatian Z**

Medical Genetics Department, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

z.saadatian@yahoo.com

In recent years, genome-wide association studies (GWAS) have made great progress in elucidating the genetic influence on complex traits. An overwhelming number of GWAS signals resides in regulatory elements, therefore most post-GWAS studies focused only on transcriptional regulatory variants. However, recent findings have expanded the spectrum of trait/disease-associated regulatory variants beyond transcriptional level and highlighted the importance of post-transcriptional variants like those in miRNA targetome. The present work integrated genome-wide association data of coronary artery disease (CAD) with population-specific linkage disequilibrium structures from 1000 Genomes Project to map disease associations to miRNA targetome. Moreover, we performed a variety of functional prediction analyses to prioritize disease-associated variants (DAVs) influencing miRNA targetome and in-silico analyses to get insights into their functional significance. In conclusion, although the role of miRNA targetome variations in the development of CAD still has to be fully elucidated, we provided a systematic bioinformatics approach to the miRNA targetome variations in CAD. The results of this study will be valuable for researchers interested in the identification of CAD GWAS signals that may implicate polymorphic miRNA targeting.

**Keywords:** Coronary artery disease Genome wide association study miRNA Single nucleotide polymorphism 1000 genomes project

**P-311: Genotype-phenotype correlation and Risk assessment in patients with diagnosis Brugada Syndrome**

**Saber S1,2, Fazelifar AF3, Haghjoo M2, Alizadeh A2, Emkanjoo Z2, Eftekharzadeh M2, Dalili SM2, Heidari Bakavoli A2, V. Zaklyazminskaia E1,4**

1. I. M. Sechenov First Moscow State Medical University, Moscow, Russia
2. Cardiac Electrophysiology Research Center, Rajaie Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran.
3. Tehran Arrhythmia Clinic, Tehran, Iran.
4. Cardiovascular Research Center, Ghaem Hospital Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
5. Petrovsky Russian Research Center of Surgery, RAMS, Moscow, Russia

dr.siamaksaber@gmail.com

**Introduction:** Brugada syndrome (BrS) is an autosomal dominant inherited characterized by ST-segment elevation in V1-V2 leads, negative T-wave on ECG and lead to sudden cardiac death (SCD). 15-30% of BrS cases are affected by mutations in SCN5A gene. Currently, knowledge of a specific mutation may not provide guidance in determining a prognosis. In this study, we suggest new strategy to do genetic test and risk assessment.

**Materials and methods:** Unrelated probands suffered BrS by clinical assessments. All coding exons of SCN5A gene were studied by Sanger sequencing. A NGS panel was designed to do analysis other candidate genes in cases without mutation in SCN5A gene. The prevalence of new genetic variants was assessed in 100 healthy ethically matched volunteers. In-Silico and/or In-Vivo study were performed for all new variations.

**Results:** 17 mutations in SCN5A gene were reported. 11 of
them are published as first time. SNTA1 gene was introduced as candidate gene as first time. One family was reported with mutation not only in SCNS5A gene but also in KCNH2 gene. 

**Conclusion:** Correlation between Syncope, positive familial history of SCD and prolonged PR interval in ECG and SCNS5A mutations were approved. Probands with missense mutation have better prognosis than other types of mutations in SCNS5A gene (3 years follow up). KCNH2 gene mutation reduces life-threatening events. SNTA1 gene should be included in gene panel to genetic analysis of BrS. Regards to genotype-phenotype correlation in our group; we suggest a new algorithm to do genetic study in cases with Brugada syndrome.

**Keywords:** Brugada syndrom, Sudden cardiac death, SCNS5A, SNTA1, KCNH2

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**P-312: VEGFA Gene polymorphisms and Its Association With Recurrent Spontaneous Abortion**

Saburi S¹, Noormohammadi Z², Zare karizi Sh¹

1. Department of Biology, School of Sciences, Science and Research Branch, Islamic Azad University, Tehran, Iran. 
2. Department of Biology, Pishva Branch, Islamic Azad University, Varamin, Iran. 
s.saboori91@gmail.com

**Introduction:** Spontaneous abortion has been defined two or more consecutive miscarriages at 20 weeksâ€™ pregnancy. Various reasons have been known for recurrent spontaneous abortion of the fetus in humans, including genetic and environmental factors. However, approximately 50% of recurrent abortions have still unknown causes. Gene polymorphisms may effect on the incidence of abortion. One of these genes is the vascular endothelial growth factor (VEGF). Present study was aimed to the association VEGF gene polymorphisms with recurrent spontaneous abortion of the fetus.

**Materials and Methods:** In this project, one hundred women with two or more abortions were considered as patients as well as 100 women with at least two successful birth as controls. Genomic DNA was extracted from peripheral blood. The polymorphism of, rs2146323 and rs3025010 of VEGF gene were studied by using PCR-RFLP technique. Restriction enzymes consist of EcoRI and BglII (HaeIII) were used for digestion. Digestion products were visualized by polyacrylamide gel (12%PAGE). Distribution analysis for homozygous and heterozygous genotypes in the two studied groups were performed by using SPSS ver. 18. Genetic differences between case and controls were calculated by using the chi-square test.

**Conclusion and discussion:** Statistical analyzes showed that significant differences (P<0.05) between allele frequencies of rs3025010(p=0.051 / odds Ratio = 0.787 / CI%95 =0.510-1.215) in patients and healthy samples, while there was no significant difference between the frequency of allelic status rs2146323 in two studied groups. The rs2146323 SNP site was monomorphic in both patients and controls with CC genotype. The present findings showed association of some polymorphisms in VEGF gene with recurrent spontaneous which further studies are necessary.

**Keywords:** polymorphism, recurrent spontaneous abortion of the fetus, VEGF gene and restriction enzymes

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**P-313: The association of Endothelial Nitric Oxide Synthase (eNOS) Gene polymorphism with Risk of Male Infertility in Mazandaran province.**

Sadat Mousavi-Nasab F, Hosseinzadeh-Colagar A.

1. Department of Molecular and Cell Biology, Faculty of Basic Sciences, Islamic Azad University-Tonekabon Branch 
2. Department of Molecular and Cell Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar 
E-mail: Faeze.musavi2015@gmail.com

Recent studies have demonstrated that making the sorts of oxygen reactive, such as nitric oxide can cause oxidative lipid damage, protein damage and damage to the DNA of cells. Sperm DNA damage result to the reduction in the mobility of sperm, damage of Acrosome membrane lead to inability of sperm to fertilize the Oocyte. Increasing expression of endothelial Nitric Oxide Synthase (eNOS) gene, is involved in various diseases such as cardiovascular and infertility diseases. The aim of this study was to assess the association between eNOS gene single nucleotide polymorphism (SNP) (rs2070744) with risk of male infertility and the quality of sperm parameters in a population of Mazandaran, Iran. Material and methods: In this case-control study, 100 infertile men were enrolled as patients group. Control groups consisted of 100 fertile men. eNOS genotype (CC, TT and TT) was determined using Polymerase chain reactionâ€“restriction fragment length polymorphism (PCR-RFLP). Results: Analyzing the results demonstrated that the frequency of Heterozygous sick, was more compared to group control but this difference was not significant (P >0.05). Data also demonstrated that the T allele in the sick group was seen less compared to the T allele that was seen among the control group, but the difference was not significant among any levels and groups (P >0.05). Conclusion: The findings of this study suggested that rs2070744 SNP couldn’t be applied as an appropriate genetic risk factor for risk of male infertility. However more comprehensive studies in different populations are required to confirm our data.

**Keywords:** Male, Infertility, eNOS, Polymorphism, PCR-RFLP

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**P-314: Rare mutation in the MARVELD2 gene in one Iranian family with Nonsyndromic Hearing Loss based on Next Generation Sequencing**

Sadeghi Z¹, Miri Moosavi RS², Molaei J¹, Ebrahimi A², Saber S²

1. Department of cellular and Molecular, Genetic, Islamic Azad University, Tehran north Branch, Tehran, Iran 
2. YAS Medical Molecular genetic laboratory, Tehran, Iran

E-mail: z_sadeghi@email.com

**Background:** Around 100 genes are responsible for Nonsyndromic Hearing Loss (NSHL). Many of which have been found to cause deafness in only one or two families. The MARVELD2 gene which is located on 5q13.2, can causes NSHL Autosomal Recessive inherited pattern. The IVS4 + 1G > A variant is reported as the one of pathogenic variant in an Iranian family. Case report and Methods: A 21-year-old, Iranian woman who has NSHL referred to our Genetic Lab to genetic consultation. Her parents had consanguineous marriage and they are healthy person (audiometry were normal). To study the responsible genes for mentioned disorder Whole Exome Sequencing (WES) was performed. The result of WES analysis was revealed a splice donor variant (exon4) in MARVELD2 gene (IVS4 + 1G > A). The result was confirmed by Sanger se-
P-315: Evaluation of the expression level of ANRIL Long none coding RNA in the stool samples of patients with colorectal cancer, polypl adenoma and healthy individuals

SadrI Sh1, Salehi M2, Nazemolhosseini Mojarad E3

1. Department of cellular and molecular, North Tehran Branch, Islamic Azad University, Tehran, Iran
2. Department of Microbiology, North Tehran Branch, Islamic Azad University, Tehran, Iran
3. Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Shadi.sadri.ss@gmail.com

Introduction: A large number of lncRNAs have been shown to be involved in cancer initiation, development and suppression. Colorectal cancer mostly detected at advanced stages, so early detection of the malignancy is important, and the use of noninvasive methods is preferable. In this study we evaluation of the expression level of ANRIL Lnc RNA, a 3.8 kb-long non-coding RNA expressed in the opposite direction from INK4A-ARF-INK4B, in the stool samples of patients with colorectal cancer, polyp adenoma and healthy individuals to find a non-invasive bio-marker.

Materials and Methods: In this study, 20 stool samples from colon cancer patients, 20 stool samples from patients with adenoma polyp and 20 stool samples from healthy people were collected. Lnc RNA ANRIL expression was evaluated using Real Time PCR method. Fold change of gene expression was evaluated by (2-ΔΔCt) method.

Results: up regulation of mRNA expression was found in ANRIL level in stool samples of CRC patients comparing to normal and polyp groups. These changes were linked with patients TNM stage, tumor differentiation and location (p<0.05).

Conclusion: The results of this study indicate that evaluation of ANRIL LncRNA expression in the stool sample can be used to as a non invasive biomarker for early detection in colorectal cancer.

Keywords: ANRIL Lnc RNA, Colorectal cancer, early detection

P-316: The study of antitumor activity of C-phycocyanin from Limnothrix sp. NS01 at cellular and molecular levels.

Safaei M, Maleki H, Soleimanpour H, Shahbani Zahir H, Akbari Noghabi K.

1. National Institute of Genetic Engineering and Biotechnology (NIGEB), P.O. Box 14155-6343 Tehran-Iran
2. Shahid Beheshti University, Tehran-Iran
E-mail: respina_mahdieh@yahoo.com

C-phycocyanin (C-PC) is a blue color photosynthetic pigment which is found in cyanobacteria. It is a water-soluble protein which has anticancer, antioxidant and anti-inflammatory properties. In the current study, therapeutic properties of C-phycocyanin from an indigenous cyanobacterial strain Limnothrix sp. NS01 were investigated. The inhibitory effects of high purity C-PC on the proliferation of human breast cancer cells (MCF-7) were detected by spectrofluorimetry, flow cytometry and Real-time PCR. Flow cytometric analysis of cells treated with C-PC by Annexin V/PI induces apoptosis in MCF-7 cells. The results obtained from propidium iodide (PI) flow cytometric assay showed that MCF-7 cells treated with 192 and 113.9 M of C-PC for 24 and 72 h could induce cell cycle arrest at G2 and S phases, respectively. In order to evaluate the potential direction of induced apoptosis, parameters of mitochondrial oxidative stress were evaluated. The results of our study showed that treatment of MCF-7 cells with PC increased the production of reactive oxygen species (ROS) and LPO level but decreased ATP and mitochondrial membrane potential (?Δm). In addition, our results indicate that the ratio of GSH to GSGG is reduced over time. The decreased proteins such as STAT3, Cyclin D1 and BCL2 were identified in MCF-7 cells after PC treatment by Real-time PCR, indicating that the effects of C-PC on tumor cell apoptosis may be related to multiple target proteins. The results thus demonstrate that the mitochondrial pathway may be the main pathway for PC-induced apoptosis in MCF-7 cells.

Keywords: Cyanobacteria, Phycocyanin, Apoptosis, mitochondrial oxidative Stress

P-317: Use of 3D culture for studing effect of statins on prostate cancer cell line

safari N, deezagi A

Department of Molecular Medicine, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
Nsafary.91@gmail.com

Prostate cancer (PCa) is one of the most common malignancies and the second leading cause of cancer-related death in men. Current treatments for PCa including tumor resection with radiotherapy, chemotherapy, and drug therapy are not successful in cancer suppression and decrease of deaths. Recent studies have shown that cholesterol is an emerging clinically relevant therapeutic target in PCa patients. Importantly, high circulating cholesterol level have been shown to increase the risk of overall aggressive PCa.

Statins are commonly used in the treatment of hypercholesterolemia to inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase in mevalonate pathway and thus prevent the synthesis of cholesterol precursors, such as farnesyl pyrophosphate and geranylgeranyl pyrophosphate. Preclinical studies have reported that statins potentiate antitumor effects in other side, 3D culture as a good model for studying of effects of drugs on cancer. The main aim of the present research is to achieve therapeutic effect of Rosuvastatin on PCa. therefore, cell line-PC3 was cultured in 3D culture and was treated with different concentration of Rosuvastatin and cell survival (IC50) was determined using the methyl-thiazolyl-tetrazolium (MTT) assay. Our Finding suggests rosvastatin causes growth inhibition in 3D culture in PC3 cell line.

Keywords: 3D culture, prostate cancer, statin

P-318: Identification of two long non-coding RNAs differentially expressed in peripheral blood sample of diabetic patients with Real-time PCR

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Background and objective: Diabetes mellitus (DM) is a metabolic syndrome with high level of fasting blood sugar (FBS). Type 2 diabetes mellitus (T2DM) has been started in late age. The association between long non-coding RNAs (LncRNAs) and human diseases has been showed in many studies. Two LncRNA genes (ANRIL & PVT1) have been played an important roles in metabolic pathway and for this reason the aim of this study was to evaluate the expression of these two genes in peripheral blood sample of diabetic patients according to clinical and paraclinical data.

Materials and methods: 75 peripheral blood samples of T2DM patients and 75 peripheral blood samples of non-diabetic people has been collected in CBC tubes with EDTA. Then total RNA was extracted and cDNA was synthesized. The expression of two genes (PVT1 & ANRIL) in T2DM patients compared to non-diabetic people was evaluated by Real-time PCR assay.

Result: The expression of both genes examined in this study was significantly increased in T2DM patients compared to non-diabetic people.

Discussion and conclusion: Due to significant overexpression of studied genes, they may be involved in process of T2DM. Also it is possible to use them as candidate genes for the diagnosis of diabetes pathogenesis.

Keywords: ANRIL, PVT1, LncRNA, Type 2 diabetes mellitus

P-319: The study two SNP of GRIN1 gene associated with addiction to heroin and methamphetamine

sahraei M1, Hamta A2
1. Department of Biology, Faculty of Basic Sciences, Arak University, Arak, Iran
2. Department of Faculty of Sciences. Arak Univ.Arak.IRAN.
sahraeimaryam@gmail.com

Introduction: Glutamate increases the probability of drug relapse. N-methyl-D-aspartate receptor (GRIN1) is a glutamate ionotropic receptor and is found in mammals’ brain. This study sought to investigate the association between rs11146020, rs1126442 and addiction to heroin and methamphetamine in Iranian male.

Materials and methods: 90 males addicted to heroin and methamphetamine and 100 healthy male were the participants of the study. Genomic DNA extracted from the blood, and then PCR-RFLP and T-ARMS PCR were respectively used to determine the rs1126442 and rs11146020 polymorphism genotype.

Results: Two SNP studied in the development of heroin and methamphetamine addiction which, in the study is not a contributing factor, because was no association between rs11146020 and rs1126442 polymorphism. The genotype frequencies of CC, GC and GG at the rs 1126442 and 11146020 polymorphism were 57%, 30%, and 3% in the patient, respectively. The genotype frequencies of AA, GA and GG at the rs1126442 were respectively 52%, 28%, and 10% in the patient. The results obtained from the analysis of rs1126442 and rs 11146020 polymorphism showed a significant association between the two polymorphisms with marital status and educational level and showed no association with job status.

Conclusion: The results of the study indicated that neither of the two polymorphism in GRIN1 gene had not showed significant association in the control samples and addicted person.

Keywords: rs1126442, rs11146020, GRIN1, heroin, methamphetamine

P-320: Bioinformatics and experimental analysis of IncRNAs associated with prostate cancer

Sajadi R1, sahrahim M2, Tabatabaie M A3
1. MSc. Student of Human Genetics, Department of Genetics and Molecular Biology, School of medicine, Isfahan University of Medical Sciences, Isfahan, Iran
2. PhD Student of Medical Genetics, Department of Medical Genetics, Faculty of medicine, Tehran University of Medical Sciences, Tehran, Iran
3. Department of Genetics and Molecular Biology, School of medicine, Isfahan University of Medical Sciences, Isfahan, Iran
Roshan.s_88@yahoo.com

Introduction: Prostate cancer is the second leading cause of cancer death in men. Clinicians use clinical criteria as well as PSA to detect prostate cancer. Currently based on clinical stage, serum level of PSA and histological grade are decided to guide treatment. For personalized medicine approach, it is necessary to define collection of molecular lesions in prostate cancer and determine the effect of these on disease aggressiveness and effective therapies against individual molecular lesions. Recently, increasing evidence has suggested that a number of IncRNAs have important and diverse functions. Therefore, it is no surprise that IncRNAs are becoming a large class of novel molecules for disease diagnosis and therapy. The purpose of this study was to predict IncRNAs associated with prostate cancer and also Evaluating their expression experimentally.

Materials and method: We predicted several of IncRNAs associated with prostate cancer such as ZNF518A, ZNDR1-AS1, LINC00641, JPX, LINC00094... This prediction result from different method based on the genes, miRNAs and pathways interaction databases and bioinformatics analysis. We evaluated expression of two example of them in different cell line such as DU145, LNCaP, PC3 using qRT-PCR.

Result: Expression study indicated the subset of IncRNAs that were differentially expressed in the cell lines.

Discussion and conclusion: Due to the difference in expression in different cell lines, we can conclude that each of these markers may have different roles in the prostate tumorgenesis mechanism.

Keywords: prostate cancer, long non coding RNA, bioinformatics analysis

P-321: Cytotoxic Effects of Auraptene in Mouse Colon Adenocarcinoma Cells

Salay H1, M. Matin M2, B. Rassouli F1,2, Iranshahi M3
1. Department of Biology, Faculty of Science, Ferdowski University of Mashhad, Mashhad, Iran
2. Cell and Molecular Biotechnology Research Group, Institute of Biotechnology, Ferdowski University of Mashhad, Mashhad, Iran
3. Department of Pharmacognosy and Biotechnology, Biotechnology Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
behnam3260@um.ac.ir

Colon adenocarcinoma is a growing public health concern with increasing rate of incidence in developing countries. Al-
through screening methods and therapeutic options have been improved, patients with colon cancer still suffer from disease recurrence. Auraptene (AUR) is the most abundant prenyloxy-
coumarin identified in nature with wide range of pharmacological properties. Beside antioxidantive, antigenotoxic and antimicrobial effects, AUR induces cancer chemopreventive effects in animal models. Since research in the field of colon cancer is focused on introduction of more effective anticancer agents, the goal of present study was to determine cytotoxic effects of AUR in mouse colon adenocarcinoma cells (CT26 cell line). In this regard, AUR was first synthesized by a rea-
tion between 7-hydroxycoumarin and transgeranyl bromide, its purification was carried out by column chromatography, and its structure was confirmed by nuclear magnetic resonance spectroscopy. Then, CT26 cells, as well as mouse normal fi-
broblasts (NIH/3T3 cell line), were treated with increasing concentrations of AUR for 24, 48 and 72 hours, and viability of cells was evaluated by MTT assay. Result of this study indicated that the IC50 values of AUR in CT26 and NIH/3T3 cells were >30 μg/ml and <20 μg/ml, respectively. To determine whether AUR has synergic effects with ionizing radiation, our plan is to examine viability of CT26 cells upon AUR treatment (in concentrations less than its IC50) and radiation exposure. In case AUR improves efficacy of radiotherapy, it could be con-
sidered as an effective coumarin derivative for future in vivo experiments.

Keywords: Colon adenocarcinoma, Auraptene, Cytotoxicity

P-322: Premature ovarian failure (POI) in correlation to FRAXA premutation: a study on 41 women in Saram Hos-
piat

Sarangpoor S1, Abdi H1, Rojhannazhad M2, Rezaei s1, Pou-
ladi A1, Salimi M1, Younesi B1, karimian M1, Karimzad Hagh J1

1. Sarem medical genetics department, Sarem women hospital, Tehran, Iran
2. molecular genetics department, Tarbiat Modares University, Tehran, Iran
sanazsama@gmail.com

Sarem cell Research center, Sarem medical genetics department, Sarem woman hospital, Tehran, Iran

3Fragile X-associated primary ovarian insufficiency (FXPOI) is one of the fragile X-associated disorders. Women with the fragile-X premutation are at risk for primary ovarian insufficiency (POI), which includes cessation of menses prior to the age of 40 years. For unknown reasons, the premutation leads to the overproduction of abnormal FMR1 mRNA that contains the expanded repeat region. Women with POI not only experience loss of normal fertility but are also at increased risk for osteoporosis and cardio-
disease and have higher rates of mortality. Thus, women who have a fragile X premutation face the increased health risks related to POI and FXTAS as well as the risk that their children will inherit the unstable repeat as either the pre- or full

mutation. About 1% of women in the general population expe-
rience POI. In comparison, approximately 20% of women who are carriers of fragile X syndrome experience POI. A total of 41 women <42 years old affected by premature ovarian insuf-
ciency were evaluated for fragile X (FRAXA) premutation in Sarem Women Hospital during year 1396. The CGG sizing was performed using the Asuragen (Austin, TX) AmpliDeX FMR1 PCR Kit. The FRAXA premutation was only detected in one out of 41, indicating of only about 2.5% of tested women with

POI. However, the results of our small tested group donâ€™t consist the results of published literature. In a big cohort study the further testing of POI women must be performed and coll-
ated.

Keywords: FXPOI, POI, Premutation, FRXA, FMR1, As-
uragen

P-323: The relationship between IL-10 and TNF-α gene polymorphism and therapy resistance to platelet trans-
fusion and recombinant factor VII administration in Glanz-
mann Thrombasthenia

Sarani H1, Keramati MR1, Hassanian SM2, Avan A3, Sohrabi T1, zafari Z1, Moradi Zarmehri A4, Naderi M5

1. Cancer molecular pathology research center, Mashhad University of Medical Sciences, Mashhad, Iran.
2. Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
4. Mashhad University of Medical Sciences, Mashhad, Iran
5. Genetic Research Center in Non-Communicable Disease, Zahedan University of Medical Sciences, Zahedan, Iran.
hosna.sarani@gmail.com

Background: Resistance to platelet and recombinant factor VII administration in patients with Glanzmann Thrombas-
thenia is a major problem causing bleeding, morbidity and mortality. There is no data on the risk factors for therapeutic resistance in Glanzmann Thrombasthenia. IL-10 and TNF-α play an important role in immune responses. Recent studies have shown that some of cytokine gene polymorphisms can produce different level of cytokines, altering severity of im-
mune responses and therefore create therapeutic resistance. In this study, probably for the first time, the relationship between immune regulator genes in the development of resistance to therapy in Glanzmann Thrombasthenia patients is investigated.

Method: this study was performed in Mashhad University of Medical Sciences in collaborative with Thalassemia and Hemophilia Center in Zahedan, Iran in 2017. Blood samples were collected from 15 therapy resistant Glanzmann Throm-
basthenia patients and 15 therapy non-resistant patients as a control group. DNA was extracted and IL-10 and TNF-α gene polymorphism was analyzed by Taqman Realtime PCR Based Method. Results were analyzed by SPSS (V 11.5).

Results: all patients in therapy resistant group had the IL-10 polymorphism at -1082 position (rs1800896) with G/G geno-
type that was significantly more frequent than the non- resistant group. However, we did not find any difference in the fre-
cuencies of TNF-α polymorphisms between two groups.

Conclusion: IL-10 gene polymorphism, was a risk factor for inhibitor formation and therapeutic resistance in Glanzmann Thrombasthenia patients. However, TNF-α polymorphism was not associated with the development of therapeutic re

Keywords: Glanzmann, Thrombasthenia, therapy resistance, recombinant factor VII, IL-10, TNF-α

P-324: Association of FBXO40 rs527341033 Polymorphism and Susceptibility to Autism in Northern Iran

Sarkar Lotfabadi A*, Ramezani S, Mashayekhi F, mirzane-
jad L, Shahangian S Sh, Bidabadi E

Department of biology, Faculty of Sciences, University of Guilan, Rasht, Iran
alireza96lotfabadi@gmail.com
Autism is a childhood neuropsychiatric disorder which is highly heritable and characterized by presence of repetitive and restricted behavior or interest and disability in social communication. Over the past two decades, the prevalence of autism spectrum disorders (ASDs) has shown an incredible rise; the spread mean has been reported as 0.62% globally. Studying monozygotic twins revealed that autism heritability is 60% to 90% confirming that genetic factors play important roles in autism pathogenesis. According to a genome-wide study, an association between FBXO40 Copy Number Variations (CNVs) and Autism was found. The human FBXO40 gene expresses a member of F-box proteins which is marked as component of SCF (Skp1-Cullin1-F-box) E3 ubiquitin complexes. The Ubiquitin proteasome system (UPS) takes part in regulation of synaptic function such as pre-synaptic neurotransmission, apical dendrite outgrowth and synapse elimination and formation. The UPS also plays role in regulating social interactions and behaviors. The Ubiquitin-proteasome pathway causes proteolysis which affects the natural function of brain resulting neurodegenerative diseases. Here, we investigated whether FBXO40 rs527341033 polymorphism affects the susceptibility to Autism. This study included 60 autistic patients and 60 control cases. The genomic DNA was extracted from peripheral blood using a GPP solution kit. Tetra-primer ARMS-PCR method was applied for genotyping of FBXO40 rs527341033. An obvious difference was found in both allele frequencies and genotype distributions of the rs527341033 SNP between patients and control group. The results indicated that there is an association between the incidence of autism and FBXO40 rs527341033 in northern Iran.

Keywords: UFBXO40, polymorphism ,Autism , SNP

P-325: Association of resistin rs1862513 gene polymorphism with insulin resistance and BMI in patients with type 2 diabetes

Sayar N, Nezhadali M, Hedyatay M
1. Department of Biology, Islamshahr Branch, Islamic Azad University, Islamshahr, Iran
2. Cellular and Molecular Endocrine Research Center, Research institute for Endocrine Sciences, Shahid Beheshti university of Medical Science, Tehran, Iran.
E-mail:s.sayar65@yahoo.com

Resistin is an important adipokine which is secreted from adipose tissue and causes resistance to insulin. It seems that resistin plays a part in resistance to insulin, diabetes type 2 and obesity. The present thesis aims at studying the relationship between polymorphism RS1862513 of resistin gene with insulin resistance and BMI in diabetic patients. Materials and Methods: This study was performed in case-control method. 75 diabetic patients with fasting blood sugar >100 mg/dl were selected as case group, and 80 individuals with FBS 70-100 mg/dl used as control group and without drug history. The weight, height were measured by standard methods and the levels of resistin and insulin hormone were measured using commercially available human ELISA kit. The determination of genotyping was done in PCR-RFLP method. Data were analyzed by SPSS software version 19.

Results: Serum resistin levels and Body mass index were significantly higher in type 2 diabetic patients as compared with the controls. Pearson's correlation analysis revealed positive correlation between serum resistin and BMI (p=0.039) and HOMA-IR (p=0.033) in diabetic subjects. Genotypes of rs1862513 had no association with T2DM and insulin resistance. Conclusion: It appears that resistin may play a role in the pathogenesis of obesity and that both of these may contribute to the development of T2DM.

Keywords: resistin, Insulin resistance, polymorphism, obesity

P-326: Whole exome sequencing is an accurate method for identification of disease causing genes involved in ichthyosis

Sedighzadeh S1,2, Zamani M1,2, Seifi T1,2, Mazaheri N1,2, Zeighami J, Negahdari S1, Shariati Gh, Sedaghat A1,4, Saberi A1,3, Hamid M1,3, Galehdari H1,2
1. Narges Genetics Diagnostic Laboratory, Ahvaz, Iran.
2. Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
3. Department of Genetics, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran.
4. Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran.
5. Department of Biotechnology, Institute Pasteur, Tehran, Iran.
sahar.sedighzadeh@gmail.com

Ichthyosis is a heterogeneous family of rare genetic skin disorders both in phenotypic presentation which characterized by dry, thickened and scaly skin, and in genetics, displaying with autosomal dominant, autosomal recessive or X-linked recessive inheritance. There are at least 38 mutant genes known to be associated with the ichthyosis phenotypes. Autosomal recessive congenital ichthyosis is a subgroup caused by mutations in 13 different genes, identifying of these mutations require a comprehensive and accurate detection method. Next Generation Sequencing (NGS) is the best technique in this time for disease causing gene detection. In this study, 14 affected consanguineous families in southern part of Iran which referred to Narges Genetics Diagnostic Laboratory since 2014 to 2018 with syndromic and non-syndromic ichthyosis types, were examined for identifying disease causing genes. DNA was extracted from peripheral blood samples and Whole Exome Sequencing (WES) was done using Hiseq Illumina platform. After analysis according to clinical representations and using genetic databases such as EXAC and bioinformatics tools candidate variants were determined and validated by Sanger sequencing. These cases diagnosed as congenital ichthyosis with autosomal recessive inheritance pattern of which PNPLA1, CTSC, ALOX12B, ABCA12, TGM1 and CYP4F22 were causative genes in these cases. WES can be used to validate the diagnosis of the genetic disorders. In complicated conditions which there are overlapping presentations while other molecular testing are cumbersome, time and cost consuming, WES is the most helpful strategy.

Keywords: Next Generation Sequencing , autosomal recessive, skin disorder, ichthyosis

P-327: Up-regulation of cell free plasma miR-106b as a possible prognostic marker in breast cancer

seyedi moghadam A2, Salimi M1, Mozdarani H3, ranji N2
Department of Medical Genetics, Institute of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.
Breast cancer is the most prevalent cancer in women worldwide and often has the ability to undergo metastasis. PTEN is a tumor suppressor gene located on human chromosome 10 and is widely considered as one of the most frequently deleted or mutated genes in various human tumors. PTEN could inhibit PI3K/Akt signaling via antagonizing the function of PI3K by reversing the phosphorylation of PI3P to PI2P. Reduced PTEN expression levels correlate with aggressive breast cancer phenotype and poor prognosis. miRNAs are small, endogenous, non-coding RNAs negatively regulate the target messenger RNAs (mRNAs) by acting on the 3’ untranslated regions (3’UTRs) of the target genes via repression of protein translation or inducing mRNAs degradation. MicroRNA 106b (miR-106b) is located in a cluster with miRs 93â€“25 on intron 13 of the Mcm7 on chromosome 7q22. As a target gene for miR-106b, the expression of PTEN is negatively regulated by miR-106b, thus affecting multiple biological functions of tumor cells. Here, level of miR-106b expression in tumour and blood plasma of breast cancer patients were compared with normal control using comparative Real-time RT-PCR method.

Results: Our data showed that the expression of miR-106b in breast tumors is significantly higher than normal tissues which is related to the stage of tumors and the status of tumor hormonal receptors. That phenomenon were concordantly detected in plasma. Discussion: We can conclude that up regulation of miR-106b may be considered as a possible prognostic marker which is detectable in blood via Inhibition of PTEN confirmation of this phenomenon may be promising in breast cancer management.

Keywords: Breast Cancer, microRNA

P-328: Screening of prostate cancer patients by Detection of loss of heterozygosity (LOH) in Circulating Cell-Free DNA (cf-DNA) as a novel biomarker

Seyedolmohadesin SM, Taghi Akbari M, Nourmohammadi Z, Basiri A, Pourmand Gh

Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2 Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
3 Urology and Nephrology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
4 Urology Research Center, Tehran University of Medical Sciences, Tehran, Iran
mari.beh84@gmail.com

Background: Several lines of evidence suggest that loss of heterozygosity (LOH) in specific chromosomal regions, a common mechanism for the inactivation of tumor suppressor genes that are implicated in pathogenesis of prostate cancer (PCa). Short tandem repeat (STR) sequences are extremely reliable genetic markers for the detection of LOH associated with cancers. Hence, in the current study we investigated whether detection of LOH at six STR markers (D8S360, D9S1748, D9S171, D8S137, D6S1631, and THRB) in blood circulating cell-free DNA (cf-DNA) could be used to distinguish PCa from benign prostate hypertrophy (BPH).

Material and methods: A total of 136 individuals were included in the current study, 76 males diagnosed with PCa (50 males with localized PCa (LPCA) and 26 males with metastatic PCa (MPCA)) as cases and 60 males with BPH as controls. Circulating Free DNA was extracted from plasma samples, also Genomic DNA from peripheral blood as a reference, and amplified with fluorescence-labeled primers specific for occurrence of LOH in studied STR markers. We compared the frequencies of homozygosity at all studied STR markers in genomic DNA in PCa and BPH subjects. We also evaluate serum prostate-specific antigen (PSA) and cfDNA levels in both groups.

Results: Our findings revealed that the frequency of LOH at D8S360, D9S1748, D9S171, D8S137 and D6S1631 markers was significantly higher in PCa subjects than in controls (p < 0.05). Of the six STR markers, LOH at D8S360 marker could be discriminate MPCA from LPCa. We also found that 71.05 % of patients with PCa and 1.66 % of BPH subjects had LOH at least in three markers in cf-DNA. We also showed correlation between occurrence of LOH in some STR with PSA-level.

Conclusion: Our findings provide additional evidence to support the hypothesis that analysis of LOH at D8S360, D9S1748, D9S171, D8S137 and D6S1631 STR markers in cf-DNA probably can be applied as non-invasive diagnostic approach for detection of PCa instead of unnecessary biopsies or other invasive method such as DRE or TRUS and can be a complementary test alongside PSA evaluation. However, further studies may be required on large scale of population and other STR markers to validate these data
Introduction: Familial adenomatous polyposis (FAP) is one of the most common colon cancer syndromes that patient has more than hundreds and sometimes thousands of polyps in the colon tissue. Also, these patients are susceptible to have various cancers in other tissues, such as thyroid and stomach. Recent studies have shown that, in addition to nuclear genome disorders, mitochondrial functional abnormalities are also as the most important carcinogenic factors.

Method: we investigated possible mutations in the cyt b mtDNA gene in 12 familial patients with FAP and 20 healthy controls by PCR and sequencing. FAP symptoms in our patients diagnosed by specialists from Khatamolania Hospital of Tehran, Iran. Then, the study of pathogenicity of the whole mtDNA was accomplished by the human mitochondrial genome database (Mitomap) which is briefness of polymorphisms and mutations in human mitochondrial DNA.

Results: Analysis of these patients revealed two homoplasmic mutations (15452C>A, 15607A>G) in two cases and 15833C>T mutation in one patient. 15607A>G and 15833C>T are synonymous single nucleotide polymorphisms (sSNPs) and 15452C>A is a nucleotide substitution in cyt b gene sequence that cause Lys to Ile alteration. (L>I).

Discussion: Larger studies in different ethnic groups are needed to establish the precise role of these nucleotide changes in FAP patients, but we suggest follow-up these studies for finding the direct molecular relation of Mitochondrion with this disorder.

Keywords: Familial adenomatous polyposis, Mitochondria, cyt b gene, Mutations

P-331: Suggestion of Y-chromosome haplogroup J for Adam prophet and his descendents

Shakhsi-Niaei M1, Jafari Boroojeni A2

1 Department of genetics, Faculty of basic sciences, Shahrekord University, Chaharmahal and Bakhtiari
2 Department of Islamic Maaref, Shahrekord University of Medical Sciences, Chaharmahal and Bakhtiari

According to advances in genetics science and possibility of scientific confirmation of individual genealogy using Genetic tests, it has been possible to study genetic Kinship of current humans with Prophet Adam. As all men of the world are similar in having Y-chromosome, a molecular genealogy has been established to study relationships of different populations of the world based on genetical markers exist in this chromosome which is only inherited from fathers to their sons. In this molecular genealogy science a shared marker among individuals or populations is called haplogroup which indicates a shared common ancestor among them. According to shared patrilineal ancestor among God big prophets such as Mohamed and Moshe, acceptable estimates of the incidence time of different haplogroup markers, as well as living period of Adam (10-12 kilo years before present), it seems that reconstruction of Adam haplogroup by merging results of studies on haplogroups of these two families has been achievable. As haplogroup J2 is more frequent in Sadat (Descendants of Hashim (Grand grandfather of Prophet Mohamed)) and haplogroup J1 is more frequent Jewish cohanim (Descendants of Aron (Brother of Prophet Moshe)), haplogroup J is suggested for Prophet Adam. However, according to incidence time of haplogroups J1-M267 (about 8300-11300 years ago) and J2-M172 (about 9500-18000 years ago), it is not possible to suggest precisely that Prophet Adam had also haplogroup J1 or J2. However, it might be possible to more studies to increase resolution of this finding.

Keywords: Prophet Adam, Prophet Moshe, Prophet Mohamed, Haplogroup J, Shared patrilineal ancestor

P-332: Assessment of RET Gene Promoter Methylation in Medullary Thyroid Cancer Patients without RET mutation

Shakiba E1, movahedi M1, majd A2, Hedayati M3

1- Department of cellular and molecular biology, Faculty of biological sciences, North Tehran Branch, Islamic Azad University, Tehran, Iran.
2- Cellular and Molecular Endocrine Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Thyroid cancer is one of the most common endocrine malignancies. There is no effective treatment for progressed thyroid cancer that does not respond to radioiodine therapy. Medullary thyroid cancer (MTC) (involves 5% of thyroid cancers. The primary mechanism of tumorigenicity of the medullary thyroid carcinoma is the inactivation of the RET protooncogene signaling, which is caused by a mutation in this gene. MTC is described by a mutation in the RET gene. These mutations are clustered in 10, 11 and 16 exons predominantly. Epigenetic changes are common in human cancer cells. Epigenetic factors such as DNA methylation play an important role in regulating gene expression. Aberrant DNA methylation is a feature of a number of important human diseases. Decreased methylation, and hence relief of transcriptional silencing, may allow the expression of previously quiescent proto-oncogenes to become active and induce the cell proliferation events.

Methods: 33 patients who underwent thyroidectomy surgery in Tehran Imam Khomeini hospital, with confirmed MTC by the pathologist, were examined. Tissue mutations of the RET were determined. DNA which was extracted from tissues were amplified by PCR and then sequenced. The methylation of RET promoter was assessed using MSP method in non-mutated patients.

Conclusion: RET protooncogene in the patients without mutation showed hypomethylation respective to the normal group. The normal group was amplified with both methylated and unmethylated primer pairs but the patients' group mostly amplified with an unmethylated pair. This could imply that hypomethylation in RET promoter may be effective in this type of malignancy. RET promoter methylation could be a new marker for prognosis, diagnosis, and treatment for MTC.

Keywords: RET protooncogene, methylation, medullary thyroid carcinoma, cancer

P-333: MicroRNA 34-a induced sensitivity to albumin-bound paclitaxel nanoparticles through suppression of SURVIVIN in glioblastoma cells

Shariat M1,2, Irani Sh1, Soleimani M1, Goodarzi N1, Dinervand R1,4

1 Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
2 Stem Cell Technology Research Center, Tehran, Iran.

According to advances in genetics science and possibility of scientific confirmation of individual genealogy using Genetic tests, it has been possible to study genetic Kinship of current humans with Prophet Adam. As all men of the world are similar in having Y-chromosome, a molecular genealogy has been established to study relationships of different populations of the world based on genetical markers exist in this chromosome which is only inherited from fathers to their sons. In this molecular genealogy science a shared marker among individuals or populations is called haplogroup which indicates a shared common ancestor among them. According to shared patrilineal ancestor among God big prophets such as Mohamed and Moshe, acceptable estimates of the incidence time of different haplogroup markers, as well as living period of Adam (10-12 kilo years before present), it seems that reconstruction of Adam haplogroup by merging results of studies on haplogroups of these two families has been achievable. As haplogroup J2 is more frequent in Sadat (Descendants of Hashim (Grand grandfather of Prophet Mohamed)) and haplogroup J1 is more frequent Jewish cohanim (Descendants of Aron (Brother of Prophet Moshe)), haplogroup J is suggested for Prophet Adam. However, according to incidence time of haplogroups J1-M267 (about 8300-11300 years ago) and J2-M172 (about 9500-18000 years ago), it is not possible to suggest precisely that Prophet Adam had also haplogroup J1 or J2. However, it might be possible to more studies to increase resolution of this finding.

Keywords: Prophet Adam, Prophet Moshe, Prophet Mohamed, Haplogroup J, Shared patrilineal ancestor
P-334: Effect of Crocin on MDR1 expression in Gastric Cancer

Shariat Razavi SM, Kalalinia F
1. Department of biology, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran
2. Human Genetic Division, Immunology Research Center, Bu-Ali Research Institute, Mashhad University of Medical Sciences, Mashhad, Iran
3. Biotechnology Research Center, Institute of Pharmaceutical Technology, Mashhad University of Medical Sciences, Mashhad, Iran
mahya.shariatrazavi@yahoo.com

Background: Crocin, one of the main constituent of saffron extract, has numerous clinical effects such as anti-cancer effects. Development of the multidrug resistance (MDR) phenotype is one of the most important factors in failure of cancer chemotherapy. The multi drug resistance proteins 1 (MDR1) is one of the major MDR-related protein that is frequently overexpressed in cancer patients. In this study we aimed to evaluate the effect of crocin on MDR1 expression in gastric cancer cell line EPG85-257 and its drug-resistant derivative cell line EPG85-257RDB.

Methods: The cells were treated with different concentrations of crocin (0, 25, 50 and 100 μM) for 4 and 48 hours. The effects of crocin on the MDR1 mRNA expression was assessed by Real-time RT-PCR. The basic level of the mRNA expression of MDR1 was also assessed in these cell lines.

Results and Conclusion: The MDR1 mRNA level in EPG85-257RDB cells is 528 times more than its expression level in parental cells. Crocin did not make any significant changes in MDR1 mRNA expression level in EPG85-257 and EPG85-257RDB cell lines.

Keywords: Crocin; Multidrug resistance; MDR1; Gastric cancer cell line

P-335: Expression level analysis of 16p13 genes in children with Attention deficit hyperactivity disorder and autism

Shayan P, Haghhighatfard A, Zare Sh
1. Department of biology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
2. Department of Biology, North Tehran branch, Islamic Azad University, Tehran, Iran
3. Department of biology, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran
talashayan1@gmail.com

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental behavioral disorder in children with no clarified etiology. Autism is known as a neurodevelopmental disorder with difficulty in social relationships, Verbal and non-verbal communication and Repetitive and ritualistic behaviors. Although genetics is known as the primary cause of autism and ADHD , but it is still not clear which genes and molecular mechanisms is effective in the pathogenesis of these disease. 16p13 were found as a hot spot area for pediatric psychiatric disorders with several copy number variations related to ADHD and autism. present study aimed to evaluate the expression level of four genes(ABCC1,ABCC6,MYH11,NDE1) in this area in phrernal blood of ADHD and Autism children. Blood samples of 50 ASD, 50 ADHD and 50 non psychiatric subjects were collected. RNA extracted and cDNA synthesized. Expression level of four target genes in total blood were assessed by using quantitative Real time PCR (comparative method with GAPDH as housekeeping gene).

Results showed significant down expression of ABCC1 and ABCC6 and significant over expression of MYH11 in both patient’s group compared to non-psychiatric individuals. Findings suggest that genes in 16p13 area are related to the etiology of ADHD and autism and could be use as biomarkers for early diagnosis. Also it seems that there is several shared molecular mechanisms between ADHD and autism especially in pathway of ATP Binding Cassette proteins.

Keywords: ADHD,Autism,ABCC,MYH11,NDE1

P-336: Study of miR-202 and the expression of AKAP4 gene in Patients with ductal carcinoma breast cancer as possible candidate cancer biomarker

Sheikhosseini M, Salimi M
1. Department of Biology, East Tehran Branch, Islamic Azad University, Tehran, Iran
2. Medical genetic Department medical biotechnology institute National Institute of Genetic engineering and Biotechnology
mt_sh_hosseini@yahoo.com

Introduction: Breast cancer is the most common diagnostic cancer among women around the world. More reports on breast cancer suggest that early diagnosis is important for improving clinical outcomes in patients with breast cancer. In this context we investigated the expression of a cancer testis antigen (AKAP4) and miR-202 that is a miRNA with features characteristic of a CT antigen.

Material and Methods: To evaluate the expression of AKAP4 and miR-202 genes RT-PCR by SYBR green dye was used at the presence of beta-actin housekeeping gene as internal control. The expression was assessed in the blood
and tissue of breast cancer patients in comparison with normal controls in 110 samples.

**Results:** In this study we aimed to investigate the presence of AKAP4 and upregulation of miR-202 expression in breast cancer tumor tissues and its subsequent detection in peripheral blood could be considered as a possible biomarker for breast cancer diagnosis even at the early stages.

**Conclusion:** AKAP4 gene and miR-202 expression analysis in tissue could be considered as possible early diagnostic biomarkers in breast cancer that is an urgent need for better management of cancer.

**Keywords:** AKAP4 ,miR-202 ,expression ,diagnostic biomarkers

P-337: Expression analysis of inflammatory gene VEGFA and miR-29a in coronary artery Ectasia and Coronary artery disease in patients of southeast of Iran-Kerman

Sheikholeslami M, Jafarinejad S, Khajouei A, Shahrokhi M*

Physiology Research Center, Kerman university of Medical Sciences, Kerman, Iran

mojgan.eslami2010@yahoo.com

**Background:** Coronary artery ectasia (CAE) is defined as dilatation of the coronary artery 1.5 times greater than that of an adjacent normal segment. Pathogenetic mechanisms of CAE are still unknown. Vascular Endothelial Growth Factor (VEGF), is a major regulator of angiogenesis, and may play a role in CAE. Also, MicroRNA-29a is known to be involved in the down-regulation of VEGF. The aims of this study were to clarify the significance of VEGF and MicroRNA-29a genes expression in relation to CAE compare to CAD in Southeast of Iran (Kerman population).

**Methods:** 58 subjects were enrolled in our study (34 CAE and 24 CAD patients), these were compared with 25 control subjects. The quantitative real-time reverse transcriptase PCR was used for evaluation of genes transcripts. Blood were collected in EDTA tubes and the Peripheral Blood Mononuclear Cells (PBMCs) were cultured for 7 days, total RNA was extracted from PBMCs reverse transcribed into cDNA, amplified and quantitated by SYBR Green detection.

**Result:** In our study, expression of VEGF was higher in CAE compared with CAD patients and control but not significant, and the expression of Mir29a was lower in CAE than CAD patients

**Conclusion:** MicroRNA-29 expression has upregulated in our CAD subjects and down regulated VEGF which follows the expression of Mir29a had no effect in VEGF expression.

**Keywords:** Coronary artery ectasia, VEGF, MicroRNA-29a

P-338: Expression Analysis of a new Long Non-coding RNA in multiple sclerosis

Shirvani Farsani Z1, Rezai M1, Farivar Sh1

1 Department of Cellular and Molecular Biology, Faculty of life Sciences and Technology, Shahid Beheshti University G.C., Tehran, Iran

z_shirvani@sbu.ac.ir

**Background:** Multiple sclerosis (MS) is a chronic progressive inflammatory disease of the central nervous system (CNS). There is an interest in potential biomarkers that could provide information predicting disease activity and progression. Long non-coding RNAs (lncRNAs) have been recently reported to participate in the regulation of immune responses. Here, we examined the expression levels of lncRNA PACER (p50-associate COX-2 extragenic RNA) in PBMCs from patients with MS compared with healthy subjects.

**Methods:** For this study, we recruited 40 patients with MS according to the revised McDonald criteria and 33 healthy subjects. The expression levels of PACER gene were examined with Real time PCR method.

**Results:** A significant increase was observed in expression of PACER in MS patients. Moreover, we found some significant correlations between lncRNA expression levels and clinical data of MS patients.

**Conclusion:** Our results shed further light to the inflammatory aspects of MS with emphasis to the regulatory role of this lncRNA in the physiopathology of MS. However, the specific molecular mechanisms and biological functions of this lncRNA need further study.

**Keywords:** PACER, Multiple sclerosis, long non coding RNA

P-339: Waardenburg syndrome: Description of a novel mutation in PAX3 Gene

Shohani S1, Poulandi A1,2, rojhannezhad M1, Rezaei S, Abdi H1, Samadpour S1, Salimi AslM1, karimian M1, Younesi B1, Karimzad Hagh J1

Department of molecular Genetics, Sarem Hospital. Tehran, Iran

sepideh.shohani@gmail.com

Waardenburg syndrome (WS) is an auditory-pigmentary disorder characterized by congenital sensorineural hearing loss and pigmentary abnormalities of the iris, skin and hair, and dystopia canthorum. There are four known types of Waardenburg syndrome which are distinguished by their physical characteristics and sometimes by their genetic cause. Here, we report a 6-year-old Iranian boy, with clinical characteristic features of the WS1 as sensory neural hearing loss, synophnosis, height nasal root and white forelock. His parents had a non-consanguineous marriage with a previously died child after a 10-days-infant history due to cardiac abnormality. The MLPA analysis of the patient revealed a heterozygote deletion of exons 1 to 4 confirming the WS1 diagnosis. This kind of deletion, as far as we know, has not been previously reported in the databases. In order to exclude the incomplete penetrance, which occurs in about 15% of clinical mild affected parents, his parents were also parallel examined. They were clinically normal and MLPA analysis did not detect any deletion in PAX3 gene confirming of a de novo event. Nevertheless, the parents have to be informed of the fact that there is a higher recurrence risk for a next pregnancy compared to the normal population due to a possibility of Germline mosaicism in this mutation, in accordance to genetic consulting. For better characterization of Genotype-Phenotype correlation, the late clinical development of patient should be exactly evaluated.

**Keywords:** Waardenburg Syndrome Type I, PAX3, MLPA, Allelic Heterogeneity, Haploinsufficiency, CVN

P-340: Study of relation on breast cancer and rs3803662 polymorphism of TOX3 gene in Iranian female population by Tetra Arms PCR

Siassi E1, Ghane B, Ashrafi F
Background and Objective: TOX3 gene plays an important role in the risk of breast cancer in females. Polymorphisms were identified in this gene, which can cause breast cancer. The aim of this study was to investigate relation between breast cancer and rs3803662 polymorphism in TOX3 gene in Iranian females by Tetra Arms PCR.

Materials and Methods: Blood samples were collected from 50 normal groups and 50 Breast cancer Patients. Then was extracted DNA from samples. Genotype frequency of this polymorphism in TOX3 gene were determined by using Tetra Arms PCR.

Results: Genotyp frequency for TT, TC and CC in normal groups was 10%, 88% and 2%, and for patient groups was 6%, 50% and 44%, respectively. Frequency of CC recessive homozygous genotype of rs3803662 polymorphism in TOX3 gene between normal groups and breast cancer patients was statistical significant difference (P < 0.05).

Conclusion: This research for first time was studied relation between the risk of breast cancer and presence of rs3803662 polymorphism in TOX3 gene in Iranian females. The results of this study was showed that presence of rs3803662 polymorphism in TOX3 gene, as genetic marker, can associated with breast cancer in Iranian female.

Keywords: Breast Cancer, TOX3 Gene, rs3803662 Polymorphism, Tetra Arms PCR.

P-341: RSM optimization of epi-drug combination improves efficient arrest of glioblastoma multiform cell line due to synergistic effect

Soleiman M1, Fathi-Roudsari M2, Maghsoudi A3, Khajeh Kh4
1 Department of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2 Department of Medical Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
3 Department of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
4 Department of Biochemistry and Biophysics, Tarbiat Modares University, Tehran, Iran
soleiman.morvarid@yahoo.com

Glioblastoma multiform (GBM) is the most common primary brain tumour in adults. They are highly resistant to both conventional radiotherapy and chemotherapy, leading to a very poor survival rate after diagnosis. In glioblastoma, similar to other cancers, epigenetic dysregulation is often seen due to global changes in DNA methylation and covalent histone modification patterns. Therefore, epigenetic therapy, especially with synergistic epi-drug combinations, are highly attention as a hopeful strategy against aggressive solid tumours such as GMB.

Epi-drug combination not only rebalances the action or expression of several proteins contributing to cancer status but also minimizes development of drug resistance and adverse events. Response Surface Methodology (RSM) that consists of a valuable group of statistical and mathematical techniques is one of the best optimization method that can be used to define an optimum combination of different factors.

The main aim of this research is to achieve a synergistic combination of epi-drug by RSM. Therefore, glioblastoma cell line U87- was treated with four epi-drugs (including SAHA, 5-Azacytidine, GSK-126, PTC-209) individually and cell survival was investigated using MTT assay. Then RSM was used to find the optimized concentration of epi-drugs in combination. The selected combination analysis showed synergistic effect with more efficient decrease proliferation, cell cycle arrest and induction of apoptosis. At the same time due to the synergistic effect individual drugs are used in lower doses promising low levels of adverse effects in patients.

This research suggests that RSM is a powerful method for optimizing drug concentration in cancer treatment proposes.

Keywords: RSM, epi-drug, glioblastoma, epigenetic

P-342: CCND1 expression profile in different breast cancer cell lines

T. Shamsabadi F1,2, Akbari Eidgahi M3, Yamchi A4, Shahbazi M5
1. Department of Medical Biotechnology, School of Advanced Technologies in Medicine, Golestan University of Medical Sciences, Gorgan, Iran.
2. Department of Medical Biotechnology, Semnan University of Medical Sciences, Semnan, Iran
3. Semnan Biotechnology Research Center, Semnan University of Medical Sciences, Semnan, Iran
4. Department of biotechnology, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Iran
5. Medical Cellular & Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran
hajima5@yahoo.com

Introduction: Cyclin D1 extensively established as an oncogene with an imperative pathogenetic role in breast cancer. Up-regulation of this gene is driven by chromosome translocation, gene amplification, polymorphism, post-transcriptional regulation and protein stability. This investigation aims at exploring the transcriptional profiling of this oncogene in various breast cancer cell lines.

Methods: The transcript levels of CCND1 in 41 breast cancer cell lines obtained via GENT database which measured by Affymetrix U133A platform. CCND1 expression evaluated based on transcriptional profiling including tumor source (primary, metastasis), subtype, pathology and ER, PR and HER2 receptors.

Results: Elevated level of D1 transcript observed in luminal, basal A and basal B subtypes, respectively. Compared to primary tumor, metastasis breast cancer cell lines revealed higher expression of D1 cyclin. Ductal carcinoma, carcinoma and adenocarcinoma cell lines demonstrated the higher mRNA levels in the same order. Moreover, greater expression identified in ER-, PR- and HER2+ cell lines.

Conclusion: Up-regulation of CCND1 oncogene associated to the late stages of breast cancer and estrogen receptor status. The compilation of breast cancer molecular profiles defines cell lines suitable to uncover potential oncogenes, or provides a resource for better cancer prognosis.

CCND1, Transcriptional profiling, Breast cancer cell lines, GENT

P-343: Investigating MMP9 polymorphism as a risk factor in thyroid cancer
Taabodi Y, Kohan L, Daneshbod Y

Department of Biology, Islamic Azad University of Arsanjan, Arsanjan, Iran
Department of Molecular Pathology, Shiraz Molecular Pathology Research Center, Daneshbod Lab, Shiraz, Iran.
yasamantaabodi@gmail.com

Introduction: Papillary thyroid carcinoma (PTC) is the most common malignancy of the thyroid, with a rapid global rise in incidence in the recent decades. Matrix metalloproteinase-9 (MMP-9) is proved to play important role in tumor progression and the metastatic process in the majority thyroid cancers. MMP-9 belongs to a family of zinc-dependent neutral endopeptidases capable of degrading all matrix components. In the present study we evaluated the presence of MMP-9 (rs17576) polymorphism in the microRNA gene of thyroid cancer patients.

Material and Methods: Tetra-ARMS polymerase chain reaction (PCR) was used to assess the MMP-9 rs17576 level in the peripheral blood of 210 patients with thyroid carcinoma and 210 healthy volunteers from Namazi Hospital of Shiraz.

Results: The frequency of MMP-9-AG (OR=1.148, 95% CI=0.755-1.745, P =0.520) and MMP-9-GG (OR=0.727, 95% CI=0.409-1.292, P =0.277) did not show a significant difference between the patient and control groups.

Conclusion: Although there was no significant difference in MMP9 rs17576 polymorphism between the two groups, the risk of thyroid cancer in MMP9-AG genotype is 1.1 folds higher than healthy individuals. While, MMP9-GG genotype showed a 0.727 lower risk of getting thyroid cancer. The MMP9 rs17576 can be considered as a risk factor for thyroid cancer.

Keywords: MMP9, polymorphism, Thyroid cancer

P-344: Analysis of sFLT01 over expression on proliferation and migration of DU145 prostate cancer cell line

Taghizadeh S1, Soheiliz S2, Ranaei Pirmardan E1, Samie Sh2

1. National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
2. Blood Transfusion Research Center, High Institute for Research and Education in Transfusion Medicine, Tehran, Iran
sepideh.taghizadeh2016@gmail.com

Prostate cancer is the most common malignancy in men. Angiogenesis is known to play a central role in the progression of advanced prostate cancer. Cancers that express VEGF are therefore able to grow and spread (metastasize) to other organs and regions of the body. A dose-dependent increase in the expression of FLTI(FMS-related tyrosine kinase 1) encoding VEGFR-1 suggests that FLTI is an androgen target gene, linking AR, sFLT01, which consist of the second immunoglobulin (IgG)-like domain of Flt-1 fused to a human IgG1Fc through a polyglycine linker. 9Gly has been previously generated with the inhibitory effect on VEGF and placentel growth factor(PLGF). Aim of this study is to evaluate the effect of sFLT01 over expression on angiogenesis, proliferation, migration and expression MMP2 and MMP9 of DU145 prostate cancer cell line.

sFLT01 gene was cloned in AAV-MCS-IRE-S-GFP vector and transfected into the DU145 cells by lipofectamin2000 reaction. Then mRNA expression level of sFLT01 was detected by RT-PCR. Protein secretion into condition medium of transfected cells proved by western blotting. MMP-2 and MMP-9 activities were assessed by gelatin zymography as well. We transfected cultured DU145 cells by the expression construct and demonstrated anti angiogenic function of cells' conditioned media by in vitro angiogenesis method.

RT-PCR results showed significant over expression of sFLT01 in treated DU145 cells. Western blot proved sFLT01 protein secretion in conditioned media. MTT assay showed that sFLT01 no toxic for DU145 cell line. Migration assay demonstrated that over expression of sFLT01 reduced the angiogenesis, proliferation and migration of the cells. This study confirmed that sFLT01 could successfully and migration of DU-145 prostate cancer cell line.

Keywords: Prostate Cancer, angiogenesis, DU145

P-345: Identification of potential miRNAs involved in colorectal cancer progression through DNA Repair system; a comprehensive meta-analysis.

Taherangkoo Kh, Mansouri E, Kazemi N R, Hajjari M R.

Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
E-mail: n.taherangkoo@gmail.com

Colorectal cancer (CRC) is known as the third prevalent cancer and the third leading cause of cancer mortality worldwide. In recent years, many domestic and foreign research have carried out a number of studies on the role of microRNAs in colorectal cancer. MicroRNAs (miRNAs) are a class of endogenous, small (17-25 nucleotides), non-coding, single-stranded and highly conserved RNA molecules that regulate gene expression at post-transcription level. MiRNAs might function as oncogenes or tumor suppressors in the process of tumorgenesis depending on the tumor microenvironment and the targets they regulate. In this study, 899 miRNAs from miRNA Pathway Dictionary Database (miPathDB) which are colorectal cancer-specific were analyzed. We have used different kinds of bioinformatics tools such as Targetscan, miWalk, miTar, miTarBase, miRselect to predict target genes of the selected miRNAs, the target genes lists were narrowed down by searching the data from KEGG database to identify DNA Repair-specific genes. We also have used ym500v3_dbDEMC, miRBase, miRiAD for analysing Fold change, expression and sequences of our desired miRNAs. Finally we checked Pubmed and google Scholar to identify eligible studies. Different miRNAs such as Has-miR-940, Has-miR-7-5p, Has-miR-432-5p and Has-miR-186-3p were detected by combining data from all above databases. So, our study suggest miRNAs with potential role in colorectal cancer.

Keywords: Colorectal cancer, miRNAs, Bioinformatics tools

P-346: A lncRNA has a potential ceRNA activity for OC-T4A by sequestering miR-335 5p

Taheri Bajgan E1, Malakootian M2, Faghihi M A3, Mowla SJ4

1. Molecular Genetics Department, Faculty of Biological sciences, Tarbiat Modares University, Tehran, Iran
2. Cardiogenetic research lab, Rajaei Cardiovascular Medical and Research Center, Iran University of Medical Sciences, Tehran, Iran
3. Department of Psychiatry and Behavioral Sciences and Center for Therapeutic Innovation, John P. Hussman Institute for Human Genomics, University of Miami Miller School of Medicine, Miami, Florida, USA.
eilmtaheri91@gmail.com

Introduction: Linc-ROR is a long non-coding RNA which regulates reprogramming of stem cells while performing similar roles in cancer stem cells. Linc-ROR expression is regulated by core stem cell transcription factors such as OCT4. These two transcripts share multiple miRNA response elements. One probable regulatory interaction is that these two transcripts compete with each other to bind to a specific miRNA. Methods: In the current study, three common miRNAs were selected to be investigated for a regulatory interaction between these three RNA species. To investigate the possible regulatory relationship, whole length of linc-ROR and OCT4A were cloned into a luciferase reporter vector and precursors of miRNAs were also cloned in expression vectors. Following that, the effect of miRNAs' overexpression on endogenous expression of OCT4A and Linc-ROR was evaluated. Afterwards, co-transfection of miRNAs expression vectors and OCT4A/Linc-ROR reporter vectors to Hek293T cell line was performed. Using Dual luciferase assay, the effect of miRNAs overexpression on their two possible targets-linc-ROR and OCT4A- was investigated. Results: Based on our results, among selected miRNAs, miR-335 and miR-544 both target endogenous expression of OCT4A while in Hek293T cell line, only miR-335 significantly decreased OCT4A expression. However, the observed effect was diluted in presence of Linc-ROR resulting in upregulation of OCT4A. Conclusion: Since ncRNAs comprise a large proportion of genome content, deciphering the regulatory relations between coding and noncoding RNAs is of great significance. These evidences propose that Linc-ROR may compete with OCT4A in binding to miR-335.

Keywords: Linc-ROR, OCT4A, miRNA

P-347: ASSOCIATION OF HTERT POLYMORPHISM AND IVF IN IRANIAN POPULATION

Tajalli Sh, Mashayekhi F, Mirzanejad L

Department of biology, Faculty of sciences, University of Guilan, Rasht, Iran
Shivatajalli75@gmail.com

Infertility is one of the current problems in the community that affects %10 to %15 heterosexual couples. Genetic disorders are one of the current causes of infertility. Telomerase is a ribonucleic protein polymerase that adds telomere repeat TTAGGG and preserves telomere ends. There is a striking correlation between the presence of hTERT miRNA and telomerase activity. Dysfunction of telomere with short telomeres causes genomic instability. Studies have shown that the expression of telomerase and the genesis or progressions of endometriosis are associated. In fact telomerase has various expression based on cyclical secretion of ovarian hormone. In other study the mean hTERT expression levels in the proliferative phase and the secretory phase were high in endometriosis patients. SNP (Single Nucleotide Polymorphism) is the most common type of genetic variations that may occur every 100 to 300 bases. hTERT polymorphisms can lead to a number of disorders by affecting the gene expression. The purpose of this study was to find whether hTERT rs2736100 in pregnancy outcome in patients from Iranian population undergoing in-vitro fertilization is associated.

A total of 66 patients and 80 control cases were recruited in this study. The genomic DNA was extracted from peripheral blood through TRITON X 100 protocol. RFLP-PCR was exerted for genotyping performance using Sfcl enzyme.

There was no significant difference between the distribution of patients and control cases in hTERT SNP. It seems there is no relation between IVF failure and hTERT polymorphism.

Keywords: hTERT, Infertility, IVF, Polymorphism

P-348: Molecular cytogenetic evaluation of formalin-fixed paraffin embedded breast tumor tissue

Talebiapur F, Zamani M1,2, Mahmoudi S1, Azizi F1, Saberi 1A1, Sedaghat A1,2, Shariati Gh1,2, Galehdari H1,2

1. Narges Genetics Diagnostic Laboratory, Ahvaz, Iran
2. Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Department of Genetics, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran
4. Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of medical Sciences, Ahvaz, Iran
ntalebiapur@yahoo.com

Human epidermal growth factor receptor 2 (HER2) has been shown to be amplified in approximately 20-25% of human breast carcinomas, and is a significant predictor of both overall survival and time to relapse in patients with breast cancer. This study aimed to estimate HER2 gene status of cancer cases with immunohistochemically equivocal (2+) score using fluorescence in situ hybridization (FISH) technique. The study included 44 formalin-fixed paraffin embedded tissue blocks from female patients with invasive breast carcinoma with IHC 2+. Hormone receptors assessment also performed for all the enrolled cases. All cases were studied by FISH for the determination of the amplified HER2 gene. FISH technique showed that HER2 gene was not amplified in 33 cases out of 44 (75%); while the rest of cases 11 (25%) revealed amplified gene status. Among FISH positive cases there was 54.54% PR positive and 45.45% PR negative, 72.72% ER positive and 27.27% ER negative.

Our result show when the threshold of ER/PR for HER2 IHC 2+ cases was increased 10 % to 30 % of tumor cells with incomplete membrane staining; the HER2 gene amplification was increased concomitantly. When the threshold of ER/PR was increased for more than 30 % of tumor cells, there wasnâ€™t any significant association between the result of FISH and the hormone receptors assessment. It seems that low amount (10% to 30%) of ER/PR in tumor cells correlates with amplification of HER2 and high amount (>30%) of ER/PR might be related to the other molecular changes in tumorigenesis.

Keywords: Her2, breast cancer, IHC, ER/PR

P-349: Expression Profile and Probable Role of Several Long NoncodingRNAs in Breast Cancer Cells.

Torkashvand S, Nafisi N, Motaleb Zade H, Mohammadipour M, Mowla S.J, Motevalian M.

1. Iran University of Medical Sciences, Razi Deag Research Center, Tehran, Iran
2. Iran University of Medical Sciences, Tehran, Iran
3. The Islamic Azad University, Science and Research Branch is a private entrepreneurial university in Tehran, Iran.
4. Research center, High Institute for Research and Education in Transfusion Medicine, Hemmat highway, Tehran, Iran
5. Tarbiyat Modares University, Tehran, Iran
fiction.torkashvand@yahoo.com
Long non-coding RNAs (lncRNAs) are a new group of known genes in the human genome that interfere with the regulation of many complexities of organisms and control many of the various biological processes. Based on their important role, they may play an important role in various cancers. According to the high prevalence of breast cancer and lncRNAs role, the expression of various lncRNAs has been investigated in this study.

Method: Fresh tissues were obtained from operating rooms of Shariati, Khatamolanbia, and Milad Hospitals (Tehran, Iran) by a surgeon. A total of 35 tumor samples and 35 non-tumor samples (from the margin of tumor) from the same patients were used for this study. Real time PCR was used follow by gradient PCR. Results: Due to the results of gradient PCR on the MCF-7 cell line, 62A°C was chosen as a propagertemperature for all genes in the real-time PCR step. Our results indicated that there is an significant expression for CBR (P-value=0.0139), Rab3 (P-value=0.0023), and Zeb30 (P-value=0.0289) in comparison to the healthy cells. ROC curve analysis for CBR LncRNA showed sensitivity more than 70%. Conclusion: Although CBR, Rab3, and ZEB lncRNAs have high expression in the breast cancers, but only CBR lncRNA has a high chance to be a breast cancer biomarker.

Keywords: LncRNA, Breast cancer, CBR-as1, Rab3-as1, Zeb30-as1

P-350: Down-regulation of MicroRNA-21 induces apoptosis in Human U87 glioma cell lines

Tutunchi S , Tofigh R , Zare M , panahi G
1. Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of animal biology, Tabriz University, Tabriz, Iran
3. Department of clinical biochemistry, Shiraz University of Medical Sciences, Shiraz, Iran
4. Department of clinical biochemistry, Tehran University of Medical Sciences, Tehran, Iran
tutunchi.sara@yahoo.com

Introduction: Glioblastoma is one of common cancers worldwide and show the highest mortality among endoclinal tumors. The increasing rate of mortality in this patient is associated with poor diagnosis. Dysregulation of a variety of genes are related with GBM pathogenesis. Among of regulator of genes involved in GBM, microRNAs (miRNAs) have been emerged as targets. MicroRNAs (miRNAs) are some small RNAs, which display abnormal expression and functions in various type of cancers. miR-21 is one of onco-miR which high-expressed in many of human cancers including glioblastoma.

Methods & Material: Human U87 glioma cell lines were purchased from Iranian Biological Research Center (IBRC) and maintained in a 37°C, 5% CO2 incubator in DMEM supplemented with 10% fetal bovine serum (FBS). anti-miR-21 and miRNA scrambled (negative control) were chemically synthesized and purified by GenePharma Co., Ltd. (Shanghai, China). Cells were transfected using Polyethylenimine (PEI) at 70-90% confluency. TRIzol reagent was used to isolate total RNA from U87 cells after transfection. The cDNA synthesis and real-time PCR was carried out with the miScript II RT Kit and miScript SYBR Green PCR Kit (QIAGEN).

Results: Our result showed that anti-miR-21 lead to down-regulation of miR-21 (32%) in glioma cells in comparison with negative control (P<0.01). These data suggested that anti-miR-21 potentially inhibited the endogenous miR-21 expression in U87 cells. To study the biological function of anti-miR-21 on apoptosis of glioma cells, caspase3 and 9 expressions were measured by real-time PCR and result showed that anti-miR-21 induces the activation of caspase 9 and 3.

Discussion & conclusion: Our research found down-regulation of miR-21 induces apoptosis by activation of caspase 3 and caspase 9. In conclude our data suggest that abnormal expression of miR-21 is pivotal for the apoptosis in glioma cells. Further related research might be helpful the development of new therapeutic stratagems against glioblastoma.

Keywords: U87 glioma cell, anti-miR-21, apoptosis, caspase3,9

P-351: Association between (MCP)-1 del/ins and the risk of RSA condition in Yazd population

Nikkhhah H1*, Vafaeei M2, Hashemian Z3, Farahshahi Yazd E2, Ghasemi N
1. Genetic Engineering and Genome Editing Laboratory, Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Department of Genetics, School of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

nadiannikkhah@yahoo.com

Recurrent spontaneous abortion (RSA) is defined as three or more consecutive pregnancy losses before the 20th week of gestation, (RSA) and in 2-5% of the population of pregnant women. Various reasons have been mentioned that in 50% of cases the cause of the incident is unclear. In many (RSA), the blood-related relationship between mother and fetus cannot be properly formed. The monocyte chemoattractant protein 1 (MCP-1) is involved in the recruitment of lymphocytes and monocytes and their migration to sites of injury and cellular immune reactions. MCP-1 in the uterus is secreted by a number of endometrial epithelial cells, fibroblasts, monocytes and lymphocytes. Significant association between (MCP)-1 polymorphisms and various diseases has been seen in several studies. The present study aimed to investigate the potential associations between single nucleotide polymorphisms (SNPs) of pro-inflammatory cytokine genes (MCP-1) and RSA cases in referents to Yazd Reproductive Sciences Institute. one (MCP)-1 gene SNP rs3917887 were selected for the present study. Method:80 women who according to RSA characteristics where chosen for patient and 80 women who had successful fertility were chosen for control groups .genotyping carryout by modify allele specific oligo nucleotide. Result & discussion: Neither the allele frequencies nor any of the genetic model of this SNP rs3917887 were significantly differences between the RSA couples and the control group. No evidence was found for any associations between the (MCP)-1 genes SNPs with RSA in referents to Yazd Reproductive Sciences Institute.

P-352: Identification of Supernumerary Marker Chromosome Originated form Chromosomes 11 And 22 using SNP Array

VAHIDI MEHRJARDI M1,2, DEHGHAN TEZERJANI M3, DEHGhani M1,3
1. Medical Genetics Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
2. Dept. of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

Abstracts of the 3rd International & 15th Iranian Genetics Congress
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P-351: Quality of life among Ardabil patients with beta-thalassemia major

Valizadeh M*, Amani F1, Fathi A2, Farzaneh E2, Fattahzadeh-Ardalani Gh2

Ahar Branch, Islamic Azad University, Ahar, Iran
Ardabil University of Medical Science, Ardabil, Iran
mehdi_valizadeh65@yahoo.com

Background: Thalassemia as the most common genetic disorder worldwide is regarded as a serious problem in public health issues in the Mediterranean region. Patients with beta-thalassemia major experience physical, psychological and social problems that lead to decreased quality of life. The aim of this study was to assess health-related quality of life and its determinants among patients with major beta-thalassemia.

Methods: This was a population-based cross-sectional survey with a prevalence design. The study population consisted of 43 patients with beta-thalassemia major (aged >2 years) of both genders who had records in Thalassemia Clinic of Bu-Ali Hospital, and those who regularly refer for blood transfusion or follow-up visits. Data were collected from December 2013 to May 2014. The self-administered short form-36 (SF-36) questionnaire was used to measure quality of life in patients with thalassemia major. The mean score for physical function was 79.8, role limitations due to physical reasons 78.8, bodily pain 74.4, general health 59.1, fatigue or vitality 63.3, social function 70.21, role limitations due to psychological reasons (emotional) 77.3 and mental health 65.4. On two scales, role physical (P = 0.33) and role emotional (P = 0.13), the men showed significantly lower scores than the women.

Conclusions: After reviewing the patients’ quality of life, the highest quality in physical function and lowest quality in general health of patients were observed. In the quality of care data all scales were in very good level except general health.

Keywords: Iran, Thalassemia major, Quality of life, Short form-36

Yari N1, Maghsoudi H2, Mahdian R3, Azizi M4
1. Department of biotechnology,.payame nor university, Tehran, Iran
2. Molecular Medicine Department, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran
faniyari@yahoo.com

Background: Aberrant expression and activity of ERBB pathway has been shown in some cancers including lung cancer and the results of many studies have shown that these receptors like EGFR and their aberrant activity have important role in cancer progression. MicroRNAs (miRNAs) are small non-protein-coding RNAs that function as endogenous negative gene regulators. Recent studies suggest that abnormal expressions of miRNAs are involved in pathogenesis of different types of human cancers including lung cancer. In this study we aimed to investigate the effect of miR-377 on reverting the tumorigenic phenotype of lung cancer cell line by targeting EGFR.

Methods: A549 was cultured. Pri-miRNA-377 and scrambled as negative control were transfected to cell line by lipofectamine (Invitrogen). mRNA was extracted by Tripure (Life Technology). Expression of mRNA was assessed by Quantitative PCR based. Proliferation and apoptosis of the cells were studied by MTT proliferation assay and staining the cells with Annexin-PI respectively.

Results: We have shown that overexpression of miRNA-377 in lung cancer cell line (A549) decreased EGFR expression by Real Time PCR (P < 0.05) and miRNA-377 directly regulates expression of EGFR in A549 cell line. Furthermore, the enhanced expression of miRNA-377 could significantly inhibit cell proliferation and induce apoptosis (P < 0.05).

Conclusion: The inhibition of ERBB pathway by targeting EGFR expression through miR-377 could be an important advance in targeted lung cancer therapy. Because of aberrant ERBB pathway activity in many cancers, the results of this study can be used as therapeutic line for other cancers.

Keywords: MicroRNA, lung carcinoma,EGFR

P-355: Identification of novel inhibitor candidate against IMPDH proteins through Docking based virtual screening

Yazdani M, Zanami Amirzakaria J, Fatemi S S-A

Department of Systems Biotechnology, Institute of Industrial and Environmental Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.

Department of Plant Molecular Biotechnology, Institute of Agricultural Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran.
sfatemi@nigeb.ac.ir
Inosine Monophosphate Dehydrogenase (IMPDH) has been one of the most important therapeutic goals in the past years that uses for the antiviral, antibacterial, anticancer and immunosuppressant in drug discovery. IMPDH is a rate determining enzyme in the de novo guanine nucleotide biosynthesis. Two isoforms of this enzyme have been detected in human proteome include type I and type II. Both types of enzymes were upregulated in stimulated human lymphocytes. The two isoforms are approximately 84% sequence identity and 92% Similarity in kinetic properties. Most inhibitor development plans have been focused on one of the isoforms of human IMPDH. Given the great similarity and identity between these two isoforms, to overcome the constraints of existing inhibitors and introducing versatile one, inhibiting both type of enzyme, docking was conducted. With this aim, firstly re-docking was applied for select the best docking protocol using Molegro Virtual Docker (MVD). After that 10000 drug like ligands retrieved from ZINC database docked against active sites of type 1 and type 2 IMPDH and five Virtual Hits selected based on PLANT score. ADMET test and Lipinski’s rule checked for candidate ligands and compared with MPA as a control. Eventually, the best potential ligand as a candidate drug was identified and molecular dynamics (MD) simulation was performed to obtain the binding pattern and stability of candidate drug with the both isoforms. This research led to the introduction of new inhibitor which can be checked as immunosuppressant drug for heart and kidney transplant patients.

Keywords: IMPDH, virtual screening, Drug discovery, immunosuppressant, molecular docking

P-356: Determination of HSP90B1 polymorphisms as genetic risk factors for inhibitors in Iranian hemophilia A patients.

Yousefi H, Bolhassani A, Hashemi M.

1. Department of genetics, Tehran medical science branch, Islamic Azad University, Tehran, Iran
2. Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran
3. Iranian Comprehensive Hemophilia Care Center, Tehran, Iran
E-mail: hosseinyousefig@gmail.com

Breast cancer is one of the most lethal malignancies among women in the world. It is a heterogeneous disease with multiple histological types, pathological characteristics and different genes involved in which make it difficult to manage clinically. So, determining biomarkers for diagnosis and prognosis of this cancer is important. Long non-coding RNAs (lncRNAs) are transcripts with more than 200bp in length that do not code any protein. They are an important category of RNAs involved in many important biological processes including cancer. One of the most novel lncRNAs that have been reported in cancer progression is HOXD-AS1 (HOXD cluster antisense RNA 1). Here we aimed at evaluation of HOXD-AS1 expression in breast cancer. Total RNA was extracted from fifty-two pairs of specimens, breast tumor tissues and their marginal normal ones, using RNx-plus reagent and cDNA was synthesized by PrimeScriptTM RT reagent kit(Takara). Expression of HOXD-AS1 lncRNA was evaluated by qRT-PCR. GAPDH was used as internal control gene. The relative expression of HOXD-AS1 showed significantly overexpression in tumoral tissues compared to the paired non-tumoral samples (fold change: 2.859, p-value: 0.00). Moreover, our findings showed that there was no any statistical significant correlation between clinical-pathological features of tumors and expression level of HOXD-AS1. In conclusion, HOXD-AS1 might be considered as a potential breast cancer biomarker. However, more studies with a high number of specimens is needed to make sure.

Keywords: breast cancer, lncRNA, HOXD-AS1, qRT-PCR

P-358: Interaction of rs783540 of CPEB1 gene and rs10783342 of mir1293 in patients with azoospermia

Zahed SHeKarabi HS1, Normohamadi Z2, salahshoori far IL

Islamic Azad University, Science and Research Branch, Department of genetic, Tehran
hana_zahed@yahoo.com

Introduction: Publicly, 90% of fertile couples get pregnant in one year. Therefore, infertility refers to disability in pregnancy, after 12 months of regular intercourse, without the use of any contraceptive method. The purpose of the study was to investigate Interaction of rs783540 of CPEB1 gene and rs10783342 of mir1293 in patients with azoospermia.

Material and method: Blood samples of 100 azoospermic men and 100 healthy men with at least one child and no history of a specific disease were obtained from the Qom Infertility Center and their genomic DNA was extracted by salting out. PCR-RFLP technique was used to investigate SNP poly-
Abstracts of the 3rd International & 15th Iranian Genetics Congress

**P-359: Exome sequencing shed light on new disease causing genes; GAD2 as possible novel candidate for cerebellar palsy**

Samani M1,2, Zeighami J1, sedighzadeh S1,2, Seifi T1,2, Mazaheri N1,2, Shariat Gh1,2, Sedaghat A1,2, Saberi A1,2, Hamid M1,2, Galehdari H1,2

1. Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Narges Genetics Diagnostic Laboratory, Ahvaz, Iran
3. Department of Genetics, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4. Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
5. Department of Biotechnology, Institute Pasteur, Tehran, Iran

Next-generation sequencing provides great accessibility and feasibility to finding known and unknown disease-causing genes in patients suffering genetic diseases. The comprehensive view and highly accurate data provision, and affordable availability cause rapidly growing implementation of this technology in clinical and research laboratories. Here we applied exome sequencing to a patient with neurodevelopmental disease suspected to have cerebellar palsy (CP). Followed exome data analysis we found a homozygous missense variation (g.64773A>G) in GAD2 gene. Sanger sequencing approved the detected variation. The Proband was heterozygous for the detected variation. The Proband was heterozygous for the detected variation. The detected variation has not been found in our homemade database (800 WES). In-silico analysis showed this variant is pathogenic and possibly is disease-causing. This gene encodes one of several GADs in mammalian brain and is expressed in brain cells. Notably, Gad2 knockout mice showed sensitized pain behavior, impaired GABA synaptic function in their brainstem neurons and increase in susceptibility to seizures. The enzyme coded from Gad2 has also been implicated as an autoantigen in the autoimmune disease stiff person syndrome (SPS; 184850). In conclusion it seems that GAD2 gene could be a new candidate to causes inherited neurodevelopmental diseases; CP. New generation of sequencing has opened a new way to profiling molecular statue of complicated diseases. Particularly in this new filed, whole exome sequencing shed light on many unresolved diagnosis and characterization of genetic diseases.

**Keywords:** WES, GAD2, Pathogenic variation

1. Narges Genetics Diagnostic Laboratory, Ahvaz, Iran
2. Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Department of Genetics, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4. Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
5. Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

**P-360: Introduction of a rare case of multiple exostoses in an Iranian patient**

Samani M1,2, Badri E1, Shiarai S1,2, Zeighami J1, Yadegari T1, Jahangirinezhad E1, Sarvari M1, Foroughi F1, Mohammadi Anie M1, Saberi A1,2, Sedaghat A1,4, Hamid M1,2, Shariat Gh1,2, Galehdari H1,2

1. Narges Genetics Diagnostic Laboratory, Ahvaz, Iran
2. Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Department of Genetics, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
4. Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran
5. Department of Molecular Medicine, Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

Hereditary multiple exostoses (EXT) is an autosomal dominant bone disorder characterized by the presence of multiple benign cartilage-capped tumors (exostoses). Besides suffering complications caused by the pressure of these exostoses on the surrounding tissues, EXT patients are at an increased risk for malignant chondrosarcoma, which may develop from exostoses. The EXT is genetically heterogeneous, and three loci have been identified so far: EXT1, on chromosome 8q23-q24; EXT2, on 11p11-p12; and EXT3, on the short arm of chromosome 19. We applied exon sequencing for the EXT1 gene on chromosome 8q to a patient diagnosed with Multiple Exostosis from southwest Iran. We found a denovo mutation p.E139X variation in the mentioned gene. The Proband was heterozygous for the detected variation. The detected variation was recently reported in Japanese patients with multiple osteochondromas. According to the best of our knowledge, this is the first report of this variation from Iran and should be considered for genetic diagnosis in patients and at risk prenatal genetic testing.

**Keywords:** Hereditary multiple exostoses (EXT) -multiple exostoses in an Iranian patient

1. Department of Genetics, Fars Science and Research Branch, Islamic Azad University, Marvdasht, Iran
2. Department of Genetics, Marvdasht Branch, Islamic Azad University, Marvdasht, Iran
3. Department of Molecular Medicine, School of Advanced Medical Sciences and Technologies, Shiraz, Iran
4. Stem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
5. Department of Psychiatry & Behavioral Sciences, University of Miami, Miller School of Medicine, Miami, USA
6. Department of Genetics, Shiraz University of Medical Science, Shiraz, Iran

Various genetic mutations in GM1-ganglioside beta-galactosidase gene have been noticed that leading to reduced or eliminated the function of this gene and causes the incidence of GM1
gangliosidosis. Patient with this enzyme deficiency condition motives repletion of GM1 ganglioside, oligosaccharides, and mucopolysaccharide keratan phosphate in central nervous system, that in turn lead to the devastation of neuron cells in the spinal cord and brain. Right now patients with this disease are only undergoing symptomatic treatments. Creation animal models that imitating the disease help to better realize pathogenicity and mechanism of illness. With tremendous advances that had taken place in the field of genome engineering and it's used in translational medicine, investigation for the creation of animal model via CRISPR/Cas9 technology for intuition novel and efficient treatment approaches are intensively underway. In the current study we design sgRNA for exon 2 and 6 of Glb1 gene and integrate sgRNA into lentiviral expression vectors contained CRISPR elements. By transfecting cells we created and perused mutations using surveyor nuclease assay. Keywords: GM1 gangliosidosis, CRISPR/Cas9, Glb1 gene

P-362: Familial Squamous Cell Carcinoma in a large pedigree: A case report

Zeinalian M, Amin M, Sadri Z, Narrei S, Hadian M

1. Department of Genetics and Molecular biology, School of Medicine, Isfahan University of Medical Sciences
2. Ala Cancer Prevention and Control Center, Isfahan, Iran
zeinalianmehrdad@gmail.com

Squamous cell carcinomas (SCCs) are the most frequent human solid tumors and a major cause of cancer mortality. The different cancer-driver mutations in some genes, like TP53, HRAS, and NOTCH1, can lead to SCC in different parts of body. Moreover, P63 protein is recognized as a marker of lung and esophagus adenocarcinoma cancer emerged in metastatic cancers in different parts of the body with SCC origin. SCC in breast is a very rare disease that it is not widely studied. On the other hand, SCC tumors of breast are resistant to hormone-based treatment due to the lack of estrogen and progesterone receptors. Therefore, recognition of involved genetic factors in this diseases can lead to discovery of the creating molecular mechanisms, specific biomarkers for pre-diagnosis and targeted therapy. In this article we report a large family in which 6 of 27 members presented different malignancies over three generations. Three cases had SCC tumors in esophagus, lips and breast. Three remained had colorectal, bone and bladder cancers. The onset of disease was between 40 to 70 years old. The current study reports a family whose members are suffering from the SCC in various parts of esophagus, lips and breast. We aim to identify responsible gene of the familial SCC by next-generation sequencing (whole exome) method. Keywords: familial Cancer, Squamous Cell Carcinoma, pathogenic variant

P-363: Overexpression of Inc-RNA SNHG7 promotes the proliferation, migration and invasion in 10 patients with colorectal cancer(CRC)

Ziaee F, Hajjari M

Department of Genetics, Faculty of Science, Shahid Chamran university of ahwaz, Ahwaz, Iran
farinaz_Ziae@yahoo.com

Since Colorectal cancer is third in the cancer morbidity, its important to identify novel bio-markers that can identify the bio-logical characteristics of tumors and predict prognosis in patients with CRC. About 90% of the genome(non-coding RNA genes) is transcribed into non-coding RNA that cannot be translated into proteins. LncRNAs may play important roles in carcinogenesis, function as oncogenes or anti-oncogenes, and their dysregulated expression is significantly correlated with carcinogenesis (development, prognosis, metastasis, and recurrence in different cancer types). Therefore, they may be considered to be promising candidate biomarkers for diagnosing cancer and may also represent therapeutic target.

In this study, we analysed one IncRNA (SNHG7) expression in colorectal cancer tissues compared to, in 10 patients by qRT-PCR (P<0.046).

Materials and methods
We extracted total RNA from 10 paired tissues (CRC and adjacent noncancerous tissues), then reverse transcribed RNA to cDNA, and finally we analyzed expression by qRT-PCR (Real-time PCR).

Result: We found that the expression levels of IncRNA-SNHG7 was obviously upregulated in CRC (P<0.046).

Discussion
In this study, we analyzed Long non-coding small nucleolar RNA host gene 7 (IncRNA SNHG7) expression in colorectal cancer tissues compared to adjacent noncancerous tissues, in 10 patients. IncRNA-SNHG7 was obviously upregulated in CRC. According to previous studies, IncRNA-SNHG7 can potentially promotes the proliferation, migration and invasion, suggesting that IncRNA-SNHG7 as a key regulator of gene expression, may be a promising therapeutic strategy for the treatment of CRC tissue, of course more samples should be analyzed. Keywords: SNHG7, Long non coding RNAs, Expression, Colorectal cancer

Plant Genetics

P-364: Isolation and Cloning of Taxoid 14?-Hydroxylase Gene from Taxus baccata Plant Cells

Abbasi M*, Sabet MS†*, Jalali javaran M

1 Department of Agricultural Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran marzieabbasi@modares.ac.ir
2 Department of Plant Genetics and Breeding, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran ms.sabet@modares.ac.ir

The secondary compounds of plants have been the center of attention for researchers since thousands of years ago and have been considered as the most important source of drug. The diversity and variability of plant secondary metabolites are in particular due to the performance of plant cytochrome P450 enzymes (CYPs) as one of the biggest gene superfamilies in plant genomes. CYPs play a special role in providing anti-cancer drugs. Paclitaxel is one of the most important anti-cancer agents extracted for the first time from Taxus sp. Taxoid 14? hydroxylase enzyme (14?OH) is one of the known enzymes in paclitaxel biosynthesis pathways. In order to identify the 14?OH gene from Taxus baccata, the full-length CDS of 14?OH gene was anticipated making use of CodonCode Aligner software based on the results of SRA in NCBI web server. The design of set primers was done using Oligo 7 software and the characterizations were justified by primer-BLAST database. For cDNA synthesis, RNA was extracted from Taxus baccata and the gene was cloned in pET30a vector. The recombinant plasmid was verified by sequencing and transformation in E.coli BL21. The expressed protein was purified and the enzyme activity determined by spectrophotometric assay. The results showed that the enzyme purified from recombinant E.coli strain had the activity of 11.5 U/mg and the maximum reaction rate was 0.97 U/mg/min. The purified enzyme was used in the synthesis of paclitaxel and the yield was 7.6%.
extracted from T. baccata cells. The 14°OH gene was amplified by primers pair specificity and MaxTaq DNA polymerase enzyme. The isolated Taxoid 14°-hydroxylase gene was cloned into pTZ57R/T vector. The outcomes of the present study were led to the identification and isolation of a sequence with the length of 1530 nt and a protein with the number of 510 amino acids. The multiple sequence alignment (MSA) of 14OH gene in the three species including T. cuspidata, T. x media and T. baccata showed a % 99 identity between T. baccata and T. cuspidata and % 98 identity between T. baccata and T. x media. Based on MSA, 14°OH protein from T. baccata showed % 98 identity with the Taxane 14b-hydroxylase protein from the T. cuspidata.

**Keyword:** Taxus baccata, Gene sequence prediction, Gene cloning, Cytochrome P450 hydroxylase, Taxoid 14°-hydroxylase gene

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**P-365:** Evaluation of genetic diversity on Kochia scoparia (L.) Schrad by using ISSR molecular markers in Borujerd, Iran

**Abedini A**, **Nasiri M**

Young Researchers and Elite Club, Borujerd Branch, Islamic Azad University, Borujerd, Iran

ali.a.bio.d67@gmail.com

Kochia scoparia (L.) Schrad is a wild species distributed over Iran especially in Borujerd with medicinal and ecological values. To conserve its breeding and germplasm resources, fifteen DNA-based molecular ISSR markers were used for genetic diversity evaluations of 334 samples from fourteen different natural habitats, including in this study. DNA extraction from young and fresh leaves of plants was isolated using CTAB technique. A total of fifteen ISSR primers in all population produced of reproducible 28114 bands produced, 16989 of them were polymorphic (60.42%). The total length of bands created between 85 to 1660 bp was variable. Matrix 1/0 bands ISSR by MVSP Ver 3.2 and NTSYSpc Ver 2.02 clustering up PGMA of Jaccard coefficient and similar groupings were obvious in the PCoA analysis. The results showed the presence of seven separated genetic group among studied populations in Borujerd. The highest genetic similarity coefficient (0.92) was observed between Bs13 and Bs14 and lowest similarity was between Bs6 and Bs11 (0.25). There was no significant relationship observed between the genetic distance of populations and their geographic distribution. This study indicates the molecular markers ISSR could characterize a wide range of genetic diversity and were able to group genotypes well. Furthermore, understanding such genetic diversity is necessary and useful in the management of Kochia scoparia (L.) Schrad germplasms.

**Keyword:** Taxus baccata, Gene sequence prediction, Gene cloning, Cytochrome P450 hydroxylase, Taxoid 14°-hydroxylase gene

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**P-366:** The study of Transcript expression analysis of geranyl di phosphate synthase gene in different accession of black caraway (Nigella sativa)

**Akbari E**, **Azizinezhad R**, **Khosrowshahi M**

Science and Research branch, Islamic Azad University, Tehran, Iran.

Elhamakbari1987yasna@gmail.com

Black Caraway (Nigella Sativa) belonging to the Ranunculaceae, is an important medicinal plants. Monoterpenes are present in black caraway seed as limonene, carvacrol, thymoquinone etc. Geranyl di phosphate synthase (GPPS) is one of the key genes in monoterpen biosynthesis. Black caraway produces importance diversity of secondary metabolites especially terpenes. In the present study gene expression of Geranyl di phosphate synthase in 6 accession of medicinal black caraway in stem and leaf, before flowering and after flowering was evaluated. The green house experiment was based on a completery randomized design. After extracting RNA and synthesizing the cDNA, the results of the Real-time PCR device were semi â€“ quantitative done compared with the real-time PCR device. The result of the Real-time PCR were used in the SPSS software and used the T-Test statistics, showed that the relative expression of Geranyl di phosphate synthase gene in Black caraway had the highest relative gene expression in Esfahan genotype and Sir Jan genotype had the lowest relative gene expression, stems compared to leaves with 95% confidence ,the difference is statistically significant (sig<0.05). also, at Flowering time, the relative expression of the gene was greater than before Flowering.

**Keyword:** Gene expression, Black caraway, Medicinal plant, Geranyl di phosphate synthase

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**P-367:** Study of fatty and protein composition in 8 wheat genotypes

**Akbari N**, **Norouzi M**, **Dehghan Gh**, **Arahkesh Salmasi N**, **TalebPor AH**

Dept. of Plant Breeding and Biotechnology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran.

norouzi@tabrizu.ac.ir

Due to protein pattern and fatty acid composition of eight wheat genotypes, quantitative and qualitative analysis of fatty acids and proteins was carried out. Then, the relationship between protein and fatty acid ratio of the above genotypes was investigated. SDS-PAG and A-PAG were used to determine the protein pattern. The analysis of fatty acids was performed by the machine GC. The major fatty acids in studied genotypes were Oleic-Palmitic-Stearic-Linolenic or Oleic-Stearic-Linolenic. Between the Gliadine strips, the band 35 was at 0.05 probability level (r = -0.732) and fatty acid 14C at the level of 0.01 (r = -0.850). Protein bands of 44.5, 47 and 72, showed a significant negative relationship with fatty acids 16C, 14C and 18C (r = -0.756) respectively. Also, Arachidonat fatty acid had a significant positive correlation with Glu-D1 gene, 12 + 2 protein bands and Protein band 1 from Glu-A1 gene at 0.01 level and with a 9 + 7 protein from the Glu-B1 gene site significant negative correlation was found at 0.05 level and 0 8 + 6 and 21 from Glu-B1 gene site with fatty acid 14C a significant negative correlation was observed in the level of 0.05. Due to the association between the important Gluten band with quality of the bakery and also with high levels of arachidonic (a subset of omega-6 fatty acids), can be achieved high protein levels of arachidone by selecting these protein bands.

**Keyword:** Endosperm, Fatty acid, Gliadin, Glutenin, SDS-PAG
and A-PAG, Wheat

P-368: Identification of S alleles in Iranian sour cherry genotypes using consensus primers
Aliyoun Nazari S1, Hajilou J2, Zeinalabedini M3, Imani A4

1. Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Iran. Corresponding author email: Saliyoun66@gmail.com
2. Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Iran.
3. Agriculture Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.
4. Horticultural Departments of Seed and Plant Improvement Institute (SPII), Karaj, Iran.

saliyoun66@gmail.com

Self-incompatibility (SI) is a genetic mechanism that prevents self-fertilization by enabling the pistil to reject self-pollen. It is controlled by a single multi-allelic locus, called the S-locus. Determination of the composition of the S-alleles of sour cherry genotypes is useful both to growers producing the fruit and breeders when selecting cultivars for cross-fertilizations. In this study, S-allele diversity and identity of eight Iranian Sour cherry genotypes and two commercial cultivars “Ciganymeggy” and “Erdi botemer” were investigated using two sets of primers. The results of sequencing of PCR product showed that the first intron, which was amplified with primers (PaConsI-F, PaConsI-R2 new), identified S6 and S9 alleles, also the second intron (PaConsII-F and PaConsII-R) identified S9 and S6m2 in studied genotypes. Information obtained about S-allele combinations of Iranian sour cherry genotypes will be useful for the establishment of new commercial orchards, in order to maximize the fruit set and consequently the yield.

Keywords: Sour cherry, Self ‘incompatibility, Consensus primers, PCR

P-369: Investigation of miR-156 expression in sheath blight sensitive cultivar of rice
Amini A, Talehsasan S, Padasht Dehkaei F, Salehi Z. Department of biology, Faculty of Science, University of Guilan, Rasht, Iran

Rice Research Institute of Iran (RRII) Rasht, Iran.
al2572aquila@gmail.com

Rice (Oryza sativa) is one of the most important cereals and the main source of food for about half the world’s people. Rice sheath blight disease caused by Rhizoctonia solani, is one of the most destructive diseases worldwide. There are not any rice variety showing complete resistance to R. solani. The disease is currently managed by excessive application of chemical fungicides, which have drastic effects on the soil biota and are environmentally harmful. Therefore, genetic modifications and biological changes can be a good way to increase the performance of rice fields. MicroRNAs (miRNAs) are 18-22 nucleotide small non-coding RNAs that act as post-transcriptional regulators by degradation of target mRNAs or translation suppression. Several studies have shown that miRNAs have an important role in response to biotic and abiotic stresses in plants. MiR-156 is one of the conserved miRNAs in some of plant species responding to many types of stresses. In this study, the miR-156 expression changes was investigated in the “Ghasr al-Dashiti” cultivar, sensitive to sheath blight disease, in order to achieve a possible biomarker for rice fungal sheath blight detection and control. The results of RT-PCR showed a significant difference in the miR-156 expression level between the control group and the treated subjects. This suggests that miR-156 may play a role in rice defense to sheath blight disease. Real-Time PCR will be done to confirmation of the results.

Keywords: Rice; Rhizoctonia solani; Sheath blight; miRNA

P-370: Expression of Immunogenic Chimeric Proteins toward edible vaccine improvement in Transgenic Tobacco
Amirmajani A, Shojaii Jeshvaghi F, Jafari M, Amani J, Salmanian A H

Department of Agricultural Biotechnology, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran

Applied Microbiology Research center, Baqiyatallah University of Medical Sciences, Tehran, Iran
neginamirmajani@gmail.com

Bacterial infections which trigger diarrhea are one of the most important causes of the morbidity and mortality around the world. The most prevalent human pathogens responsible for outbreaks of bacterial diarrhea are enterohemorrhagic Escherichia coli (EHEC) and enterotoxigenic Escherichia coli (ETEC). Being a serious public health concern, vaccination against the serotypes can be considered as one of the most effective prevention methods. In the recent years, plants because of their predisposition to produce the specific proteins using as antigens and vaccines are preferred among other systems in molecular farming technologies.

In the present study, the chimeric gene consists of stx, eae, cfb, ltb (secl) was transferred to tobacco plant cells via Agrobacterium in two ways: direct and indirect methods. Direct transformation which means production of hairy roots via recombinant A. rhizogenes harbouring secl synthetic gene and indirect refers to two steps procedure. First transformation of tobacco plant with recombinant A. tumefaciens and then root induction with non-recombinant A. rhizogenes. The hairy root was observed after transferring the transgenic leaves to the selected culture media. Presence of the gene encoding chimeric construct was confirmed through PCR and the quantity of recombinant protein was measured by quantitative ELISA. The concentration of SECL protein was measured about 0.0012 to 0.039 of TSP.

Keyword: Agrobacterium, chimeric gene, hairy root, immunogenetic, tobacco.

P-371: Effect of 24-epibrassinolide on Expression of ERF and MYB1-1 Transcription Factors Under Salt Stress in Two Flaxseed Cultivars Differing in Salt Tolerance
Amraee L12, Rahmani F12, Abdollahi Mandoulakani B3

1. Biology Department, Faculty of Sciences, Urmia University, Urmia, Iran
2. Institute of Biotechnology, Urmia University, Urmia, Iran
3. Plant Breeding and Biotechnology Department, Agriculture Faculty, Urmia University, Urmia, Iran

amraei llela@gmail.com

Salinity is a common environmental stresses and a complex phenotypic and physiological phenomenon which limits worldwide agricultural crop yields. Transcriptional regulation of the stress-induced genes plays an important role in developing stress tolerance and responses. brassinosteroids (BRs) are novel group of phytohormones which initiate adaptive reactions of plant cell metabolism against environmental stress. The present investigation was carried out to study the effect of
24-epibrassinolide (24-epiBL) on expression patterns of ERF and MYB1-1 transcription factors in salt sensitive (TN-97-1) and tolerant (TN-97-106) flax cultivars under NaCl-stress. After surface sterilisation, seeds were soaked for 8h in distilled water (control) and in 10-8 M concentration of 24-epiBL. Then, twenty-one-day-old-plants were exposed to salt stress (0 and 150 mM) for 21 days. Results showed that in sensitive cultivar, imposition of NaCl (150 mM) induced MYB1-1 and ERF mRNA level compared to control plants while two genes showed decrease in expression level in tolerant cultivar. On the other hand, the transcript level of two genes was elevated in 24-epiBL+NaCl treated TN-97-1 cultivar compared to NaCl treated plants whereas in 24-epiBL+NaCl treated TN-97-106 cultivar, ERF gene expression revealed decrease (26%) compared to NaCl treated plants and no significant difference was observed in MYB1-1 gene transcript level between 24-epiBL+NaCl treated and NaCl treated TN-97-106 cultivar. The results indicate different patterns of transcription expression in sensitive and tolerant cultivars in response to salinity and application of 24-epiBL under NaCl (150 mM) imposition.

KEYWORDS: 24-epiBL, Transcription Factors, Flaxseed, Salinity

P-372: Paclitaxel Production and Secretion in Cell Suspension Culture of Taxus baccata Using Functionalized Multi Wall Carbon Nanotube by Carboxyl and Benzoyl Glycine Sodium Salt

Asgharzadeh P, Sabet M S, Moieni A

1. Department of Agricultural Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
2. Department of Plant Genetics and Breeding, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
3. Department of Plant Genetics and Breeding, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
asgharzadeh_p@gmail.com

Studies of plant secondary metabolites have been increasing over the last 50 years. Plant cell culture technologies were introduced at the end of the 1960s as a strong tool for both studying and producing of plant secondary metabolites. The increased production of valuable metabolites is realized in plants using nanotechnology. Nanoparticles as components of cell membranes permeable to create physical stress on plants, resulting in increased secondary metabolite production. In addition to nanoparticles as biological elicitors, nonbiological elicitors like amino acids can also be effective in improving the production of these compounds. In this study to improved paclitaxel production in cell suspension culture of Taxus baccata, multi-walled carbon nanotubes were used at a concentration of 100, 250 mg-l and benzoyl glycine sodium salt amino acid at a concentration of 0.05 mM in the period of growth phase cells. The results showed an increase of 1.35 of node per stem, hundred seed weight, pod length, biological yield of wheat (Triticum aestivum L.) in the world [1]. miR319 is one of the conserved microRNA families that controls transcription factors of TCP family involved in hormone biosynthesis and signaling, different developmental pathways, such as senescence and leaf development, cell proliferation and differentiation in plants [2]. miR319 expression is controlled by abiotic stress, however experimental evidence is insufficient and the mechanism remains unknown. This study investigate the expression of miR319 and it’s target gene (TCP) in two wheat cultivars, Norstar and Baz, exposed to cold stress (4 °C) for 0, 2, and 14 days and methyl jasmonate (0 and 120 μM). Our results demonstrate that miR319 expression is down-regulated in Norstar plantlets 4 °C, but up-regulated in the spring wheat cultivar baz. miR319 was also controlled by treatment with methyl jasmonate in wheat. miR319 expression decreases in winter wheat cultivar, norstar, under the influence of methyl jasmonate without cold treatment, but increases in spring wheat,baz. Initially, TCP expression was reduced by cold treatment (2 days), but with longer periods of time of cold treatment (14 days), the expression increases. In general, the expression of miR319 and TCP was regulated during cold stress and methyl jasmonate treatment.

Keyword: Cold; miRNA; methyl jasmonate; TCP

P-373: Effect of Cold and Methyl Jasmonat on Expression of node per stem, hundred seed weight, pod length, biological yield of wheat (Triticum aestivum L.) genotypes by path analyses

Astaraki H*, Sharifi P, Sheikh F

Agricultural Research, Education and Extension Organization (AREEO) Seed and Plant Improvement Institute, Broujerd, Iran. Department of Agriculture and Plant Breeding, Rasht Branch, Islamic Azad University, Rasht, Iran. Center of Golestan,Agricultural Research, Education and Extension OrganizationOrganization (AREEO)Seed and Plant Improvement Institute, Gorgan, Iran. research12014@gmail.com

Faba bean (Vicia faba L.) is a major legume that is used as food owing to the high nutrient components in seeds. This study was carried out during 2015-16 growing season in Lorestan province, Iran. Experimental material comprised 26 genotypes of faba bean. Field experiments were conducted in a randomized complete block design with three replications and 26 genotypes. Each plot consisted of four rows with 4 m long and distance between rows and plants were 0.50 m, respectively. The characters included days to germination, days to flowering, days to maturity, plant height, number of stems per plant, number of node per stem, number of seeds per pod, number of seeds per plant, hundred seed weight, pod length, dry seed yield, biological yield and harvest index were measured. The analysis of variance indicated significant differences between genotypes for all of the studied traits. Correlation analysis indicated there were positive correlation coefficients between seed yield and number of day to germination, number of day to flowering, plant height, number of pod per plant, number of node per stem, hundred seed weight, pod length, biological...
yield and harvest index. Path coefficient analysis indicated plant height (0.74), number of pod per plant (0.51), number of node per stem (0.11), number of seed per pod (0.20), hundred seed weight (0.34) and pod length (0.41) had positive direct effects on seed yield. Thus should be paid to traits such as plant height, number of pod per plant, number of node per stem and pod length for augmentation of seed yield.

**Keyword:** Traits on yield, Faba bean, accessions, path analyses

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**P-375: Expression Analysis of Brassica napus WRKY57 Transcription Factor Gene in**

**Atashi shirazi F, Razi H**

**Response to Drought Stress**

Institute of Biotechnology, Shiraz University, Shiraz - Department of Crop Production and Plant Breeding, Shiraz University, Shiraz  
ngr_atashi@yahoo.com

Rapeseed (Brassica napus L.) is a major oilseed crop which its production is often limited by drought stress in semi-arid regions. WRKY transcription factors are a large family of plant transcriptional regulators that play important roles in response to biotic and abiotic stresses. This study aimed to analyze expression of WRKY57 (BnWRKY57) gene in response to drought stress in two B. napus cultivars, SLM046 and Zarfam, with different levels of drought tolerance. Quantitative RT-PCR, was used to measure the abundance of BnWRKY57 transcripts under non-stress and three drought stress treatments including the time that soil moisture reached to 40% of field capacity (FC) as well as six and twenty-four hours after maintaining soil moisture at 40% of FC. BnWRKY57 showed significantly differential expression not only between the cultivars but also between leaf and root tissues during drought stress period. In leaves of drought tolerant cultivar (SLM046), BnWRKY57 expression was more quickly induced in response to drought stress and also showed significantly higher level of increase compared to that of the other cultivar. BnWRKY57 expression was not induced in roots of the drought tolerant cultivar during drought stress period. On the other hand, drought stress induced a rapid upregulation in expression of BnWRKY57 gene in roots of drought sensitive cultivar (Zarfam). The results suggested that BnWRKY57 gene may play a role to confer drought tolerance in rapessed. Further in-depth analyses are required to fully understand the importance of BnWRKY57 gene in drought stress tolerance.

**Keyword:** BnWRKY57, Rapeseed, Transcript accumulation, Transcription Factor, qRT-PCR, Drought Stress

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**P-376: Detection and partial molecular characterization of soil borne viruses infecting wheat and barley fields in Yazd province, Iran**

**Azfar A, Esmailzadeh Hosseini S.A**

1.Department of Agricultural Science, Absar Kavir Co., Yazd, Iran  
2.Plant Protection Research Dept., Yazd Agricultural and Natural Resources Research and Education center, AREEO, Yazd, Iran  
E-mail: az52164@gmail.com

Soil-borne viruses, belonging to the genera Furovirus and Bymovirus, are serious pathogens of autumn-sown wheat and barley fields in Iran and transmitted by Polymyxa graminis. These viruses are of great economic importance and causing significant quantitative and qualitative losses in yield. There are a few studies about soil borne cereal viruses and in conclusion there is not sufficient information about their distribution and diversity in cereal fields in Iran. During 2014-15, sampling was carried out on wheat and barley fields in Taft, Yazd, Meybod and Dehno (Yazd province, Iran). Disease symptoms were yellowing, mosaic and stunting and total 164 symptomatic and 14 asymptomatic samples were collected. Using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with Soil borne wheat mosaic virus (SBWMV) and Barley mild mosaic virus (BaMMV) polyclonal antibodies, SBWMV and BaMMV were detected in 34.3 and 17.4 % of collected samples respectively. Direct and nested PCR was carried out on positive samples in DAS-ELISA using the external (Wb1/Wb2, Bm1/Bm2) and internal (Wns1/Wns2, Bns1/Bns2) designed primer pairs for SBWMV and BaMMV respectively. The direct PCR amplified two fragments size of 1150 and 960 bp and nested PCR successfully amplified two products with the predicted size of 410 and 430 bp in SBWMV and BaMMV diseased plants respectively. No fragments were amplified with asymptomatic wheat and barley plants. Based on the results of serological and molecular analysis in this study, SBWMV and BaMMV are distributed in wheat and barley fields of Yazd province.

**Keyword:** ELISA, Nested PCR, Polyclonal antibody, Primer

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**P-377: Investigating the effect of different types of media on the proliferation of GÅ—N15 rootstocks Plantform bioreactor**

**Barani Sorkhelyzheh S, Yadollahi A**

Tarbiat modares university  
safijadbaranis313@gmail.com

In this study, in order to obtain the best media in vitro for GÅ—N15 rootstocks (interbreeding of āfēNemaredāf red peach and āfēGarfāf almond) in a Plantform bioreactor, two independent experiments were performed as A factorial experiment was conducted in a completely randomized design in a laboratory of the Department of Horticulture of Tarbiat Modarres University. The first experiment was to determine the amount of nutrient uptake in different media with 10 treatments and 5 replicates and in the second experiment, the effect of different concentrations of Benzylaminopurine and indole-3-butyric acid with 14 treatments on GÅ—N15 rootstocks in a Plantform bioreactor, checked. Based on the results of the first experiment, the best results from the WPM media were obtained. Based on the results of the second experiment, the best result for the GÅ—N15 rootstocks was to use the concentration of 2 mg / L Benzylaminopurine and 2 mg / L the liter of Indole -3-butyric acid was obtained. Based on the results of the first experiment, the best results from the WPM media were obtained. Based on the results of the second experiment, the best result for the GÅ—N15 rootstocks was to use the concentration of 2 mg / L Benzylaminopurine and 2 mg / L the liter of Indole-3-butyric acid was obtained. In general, according to the results of this experiment, the use of WPM media and application of concentration of 2 mg / L of Benzylaminopurine and 2 mg / L of Indole-3-butyric acid could be the best conditions. It was also found that the WPM media could be considered as a suitable substitute for the MS media to proliferation a GÅ—N15 rootstocks in the form of Plantform bioreactor conditions.

**Keyword:** In vitro, GÅ—N15 rootstocks, Plantform bioreactor,
WPM

P-378: Genetic assessment effect of Cold Atmospheric Plasma Jet on Catharanthus roseus (L.) seeds by using SRAPs

Fahmi D1, Noormohammadi Z2, Atyabi SM2, Farahani F3

Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2-Department of Nanobiotechnology, Pasteur Institute, Tehran, Iran
3-Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran
dodi.fahmi@gmail.com

Catharanthus roseus (L.) is one of the phenomenal medicinal plants. Madagascan plant produces terpenoid indole Alkaloids (TIAs) such as vinblastine and vincristine which is used as anticancer drug. Nowadays, cold Plasma jet technology has been found a special place in agriculture. In present study, we treated C.roseus seeds by cold plasma and study its genetic variation using Sequence-Related Amplified Polymorphism (SRAP) as a molecular markers which target the ORF sequences. The C.roseus seeds were divided into three groups: 1- unexposed seeds as controls, 2- treated groups by cold plasma with 40 seconds (40s) exposing and 3- treated seeds for 50 seconds (50s) by cold plasma. Our study showed highest polymorphism in 50s cold plasma treatment. Genetic parameters revealed that 50s cold plasma treated plants had highest value in expected heterozygosity, Shannon index and number of effective alleles. Principle coordinate analysis (PCoA) showed genetic differentiation between three group plants studied. AMOVA test showed significant difference between group plants (P=0.001). Our findings showed distinct genetic variation between control and 50s cold plasma treated plants. Cold plasma jet treatment may effect on C.roseus genomes.  

Keyword: Catharanthus roseus, cold plasma jet, SRAP

P-379: Comparison of Some Molecular Aspects of salt-induced Oxidative Stress in Aeluropus (Aeluropus littoralis L.) and Rice (Oriza sativa L.)

Fakhrfeshani M1, malekzadeh Kh2, Zare Mehrjerdi M3

Department of Biotechnology, Jahrom University
fakhrfeshani.m@gmail.com

Salinity stress and high concentration of ions are of the most determinative factors that simultaneously affect on genetic, biochemical and physiological processes of a plant. Some of plants known as halophytes developed mechanisms that help them to avoid or tolerate the saline conditions. With the aim of understanding the probable mechanisms of saline tolerance or susceptibility in Aeluropus littoralis (as a halophyte) and Oriza sativa var. IR64 (as a glycophyte), we studied and compared some responses of their shoot and root to salt stress. To this end we established a stress span containing short term (6 hours), mid term (24 and 48 hours) and long term (5 and 11 days) exposure to salinity stress and their gene expression pattern and fluctuation of Cu/Zn Superoxide Dismutase (SOD), Catalase (CAT), cytoplasmic Ascorbat Peroxidase (cAPX), peroxisomal Ascorbat Peroxidase (pAPX), Glutathione Reductase (GR), and some of their nucleotide and amino acid sequence related indexes were investigated. Comparison of The real time quantitative PCR of ROS scavenger related transcriptom showed that Aeluropus increases the expression of these genes faster and keeps their expression in long-term while rice loses them significantly however with an exception in cAPX. Comparison of cDNA sequences and their counterpart amino acid codones also showed that Aeluropus has lower instability index and higher aromatic values with an exception for catalase amino acid sequence.  

Keyword: Salinity Stress, ROS Scavenger, Sodium, Amino Acid Instability Index, AA aromatic values

P-380: Genetic diversity and phylogenetic relationships among and within species of terrestrial Iranian orchid species based on SCoT markers

Gholami S, Vafaeey K, Nazari F

Department of Horticulture, Faculty of Agriculture, University of Kurdistan
E-mail: y.vafaee@uok.ac.ir

Terrestrial orchids with fleshy tubers have long been exploited as a source of bioactive compound for the curing of a diverse range of health problems, as well as in dietary supplements and as an aphrodisiac in different parts of the Iran and on a world basis. Harvesting of orchid tubers from Iranian species for salep has been escalated in recent years by national and international demand and the Iran has become one of the main suppliers for this trade. In this study we aimed to evaluate genetic diversity of some tuberous terrestrial orchid species from western part of Iran by means of molecular markers. Understanding the differences among the biogeographic, demographic and the human-mediated impacts on the level and distribution of genetic diversity is crucial for conservation management of terrestrial orchids species. For this purposes, 7 population (totally 42 samples) of Anacamptis coriophora, Orchis mascula, Dactylorhiza umbrosa and Dactylotihza unvelliana species collected from different geographical locations of western regions (Kurdistan, Kermanshah and west Azarbaijan provinces) of Iran as well as from Chaboksar (Gilan province) were analyzed using four start codon targeted (SCoT) markers. A mean number of 39.14 SCoT bands were scored for 7 studied populations. Number of different alleles, number of effective alleles and Shannon’s information index were 6 $\pm 0.031$, 0.24 $\pm 0.01$ and 0.045 $\pm 0.0$, respectively. Cluster analysis based on Jaccard’s distance (cophenetic correlation, r= 0.842) classified 42 orchid samples under 7 group. In this regard all studied orchid species well-classified based on species and geographical locations. Orchis mascula and Anacamptis (Orchis) coriophora individuals were fallen close together showing their close phylogenetic relationships. On the other hand, all Dactylotihza umbrosa as well as Dactylotihza unvelliana were grouped together. Geographical populations were also well classified based on obtained dendrogram using UPGMA method so that Sardasht and Bane ecotypes shared more SCoT bands and Salian individuals were also grouped in common clusters while D. unvelliana ecotypes from Chaboksar were differentiated from Dehgol’s and Marivan’s D. umbrosa. Our results can be useful for Iranian orchid species characterization and conservation.  

Keyword: Genetic Diversity, Molecular Markers, SCoT, Terrestrial orchids

P-381: Assessment of chemical composition of essential oil...
of Ferula assa-foetida oleogum-resin from different regions Iran

Hasanabadi M1, Ebrahimi M1, pirmoradi MR2
1. Department of agronomy and plant breeding, collage of Abouroian, University of Tehran, Tehran, Iran.
2. Department of Horticulture, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan, Rafsanjan, Iran

Background and objectives: Ferula assa-foetida (asafoetida) is a native Iranian species which grows in different regions of the country. The plant is well known in Iranian traditional medicine as well as folk medicine for treatment of diseases. Several studies with different purposes have been carried out on the essential oil of the species. In the present study essential oil components from six different F. assa-foetida accessions, were reported.

Methods: The six accessions were collected from different parts of Iran, including Fars, Isfahan, Kerman, Kohgiluyeh and Boyer-Ahmad, Sistan and Baluchestan and South Khorasan provinces. The gas chromatography mass-spectroscopy (GC/MS) was used to detect the essential oil components.

Results: A total of thirty compounds were identified across all the six accessions. The main components in the plants were Cis propenyl see disulfide (32%), 3-Pinene (18%), 3-Pinene (8%) and ocimene (5%). Safashahr (16.75%) and Yasuj (3.8%) accessions had the highest and lowest amounts disulfide compounds of the essential oil, respectively.

Conclusion: In the present study for first time we reported the variation in essential components in F. assa-foetida from different parts of Iran. The main component in the essential oil was disulfide.

Keyword: essential oil, Ferula assa-foetida, GC/MS, oleo-gum resin

P-382: Assessing genetic diversity and detection the most important effective traits on grain yield of been (Phaseolous vulgar) under after poding moisture stress

Heydari S1, Mooosavi SS2, Abdollahi MR3, Mirzaie Asl A4
Faculty Member of Bu Ali Sina University
heydari.sm91@gmail.com

In order to assay genetic diversity and to detect the most important effective agronomic traits on yield improvement in 9 cultivars of been, an experiment based on RCBD with 3 replication under after poding moisture stress condition was done. The cultivars showed the significant (p<0.01) difference for the majority of the studied traits, indicating a high level of genetic diversity among the genotypes. Pak and Daneshkaded showed the maximum and Goli and Sayad had the minimum grain yield, respectively. Pak and Daneshkade, with the highest yield, showed a high level of pod number per plant, pod number containing grain, biomass per area, pod weight per plant and branch number containing pod. Grain yield showed a significant and positive correlation with branch number containing pod, pod number per plant, pod number containing grain, pod weight per plant and biomass per area. Step wise regression indicated that the traits of biomass per area, pod number per plant, grain length, pod weight per plant, grain diameter and

100-grain weight were the most important and effective traits on grain yield improvement. Path analysis showed, the trait of pod number per plant had the highest direct effect and the trait of biomass per area showed the maximum indirect effect by pod number per plant on grain yield increment.

Key Words: Genetic diversity, Grain yield, Correlation analysis, Step wise regression, Path analysis

P-383: Structural and functional studies of plant Polyamine Oxidase

Hosseini M, Saidi A
Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C, Tehran, IRAN
SeiedMohsenHosseini@yahoo.com

Intracellular polyamine contents are regulated by not only biosynthesis and transport but also catabolism by FAD dependent polyamine oxidases (PAOs). Results of various studies on PAO proteins in developmental processes and response to environmental stresses confirm the importance of this protein in plant life, however, there is no comprehensive study of phylogenetic and structural relationships of plant PAOs. In the present study, in order to better understand phylogenetic and structural relationships of PAO proteins, bioinformatics analysis of 58 PAO protein sequences of 15 different plant species were performed. Multiple clusters with gene duplications were identified in both dicot as well as monocot-species. According to the conserved motifs obtained by MEME and MAST tools, four motifs were common in most plant species. Structural analysis was carried out on PAOs from Oryza sativa and Arabidopsis thaliana as representative of monocot and dicot plants, respectively, as no structural information is available on them. Secondary structure analysis revealed that alpha helix dominated among secondary structure elements followed by random coils, extended strand and beta turns for all sequences. Tertiary structures were predicted with SWISS-MODEL server. The structural, physicochemical, and phylogenetic information predicted in this study will provide a good foundation for functional analysis of the plant PAOs proteins.

Keyword: Bioinformatics analysis, Phylogenetic, Polyamine, Polyamine oxidases

P-384: Basta tolerance as a screening marker for transgenic 35S-OsMST6 Arabidopsis plants

Hosseini Monfared H1 2, Xue G-P3, Kadkhodaei S4, Moradi P1, Mousavi SA1, Zamri Bin Z1
1. School of Biosciences and Biotechnology, Faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600 UKM, Malaysia. hhmonfared@gmail.com
2. Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
3. CSIRO Plant Industry, 306 Carmody Rd, St Lucia QLD 4067, Australia,
4. Institute of Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan, hhmonfared@gmail.com

As an alternative to in vitro screening, primary transformants carrying the bar selection marker can be directly screened on soil in a greenhouse by spraying with the herbicide Basta.
The presence of selective marker allow the identification of transformants from the non-transformants. Transformed seedlings showed resistant to herbicide (Basta) because of bar gene on T-DNA construct. Therefore, it is most likely that these herbicide resistance seedlings contained candidate gene. In this study, transformation vector harboring OsMST6 (encoding monosaccharide transporter 6) driven by 35S promoter was constructed using Gateway technology and transformed into Arabidopsis thaliana via floral dip technique. To find the optimum concentration of Basta for T1 screening, 100% minimal lethal dose (MLD100) test was performed. For this purpose, wild type Arabidopsis was sampled by 0, 50, 100, 150, 200, 250 mg/L of Basta. According to the results obtained from this study, 150 mg/L Basta was the minimum dose that kills 100% of the wild type Arabidopsis plants. Primary transformants (T1) were directly screened on soil in growth chamber by spraying with the herbicide. Non-sterile seeds were spread onto moistened soil under normal conditions into a tray. The herbicide spraying was performed when the cotyledons were visible, normally around 5-10 d after sowing. The seedlings were sprayed by 150 mg/L of herbicide Basta every 2-3 d until transformants were easily recognizable among nontransfomed dead/dying plants, approximately 3-4 weeks after herbicide treatment. Then, from initial screening under 150 mg/L Basta, forty-two independent transgenic lines were obtained and verified by PCR.

**Keyword:** Basta, Selection, Arabidopsis, Transgenic, OsMST6

**P-385: Expression of a Dermaseptin peptide in tobacco hairy roots enhances antifungal activity**

*Ismaili A1, Nazari Z1, Nazarian Firoozabadi F1, Alibakhshi A1*

Faculty of Agriculture, Lorestan University, Khorramabad, Iran. alibakhshi8a@gmail.com

Plant stressors, insects and weeds contribute to more than 30% of crop plants losses annually. Toxic chemicals have been extensively used to control plant diseases, especially plant pathogenic fungi. These chemicals not only pose a health threat to humans’ life and the environment, but eventually lead to a higher incident of fungicide resistance plant fungi. Hence, breeding cultivars resistance to devastating fungi by using genetic engineering and biotechnology approaches is a valuable and sustainable solution. The production of antimicrobial peptides (AMP) is a part of the immune system of all living organisms, such as plants, to combat pathogenic bacteria. Hairy root cultures hold an immense potential for the production of foreign proteins, e.g., antimicrobial peptides. The aim of the present study was cloning, expression and assess the antifungal activity of a Dermaseptin peptide gene in Nicotiana tabacum hairy roots, using Agrobacterium rhizogenes. Hairy roots were generated and confirmed by PCR analysis using specific Dermaseptin primers. Results of this study showed that Dermaseptin peptide was successfully produced in transgenic hairy roots. Total proteins were extracted from few high-expression hairy roots and used for antifungal activity, using disc diffusion method in Alternaria solani in vitro cultures. Alternaria solani growth was significantly retarded as compared with controls, suggesting that expressed Dermaseptin peptide possess considerable antifungal activity.

**Keyword:** Hairy root, Antimicrobial Peptide and Agrobacterium rhizogenes

**P-386: Expression pattern of TaNAC2a transcription factor in wheat (Triticum aestivum L.) root and leaf under different concentrations of salinity stress**

*Jamshidi Goharrizzi K1, Baghizadeh A2, Amirmahani F3, Kargar F3, Kalantar M1, Pakzad R1, Moemeni MM1*

Department of Plant Breeding, Yazd Branch, Islamic Azad University, Yazd, Iran.

Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman-Iran.

3Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran. jamshidi_kiarash@iauyazd.ac.ir

Abiotic stress containing salinity stress affects quality and yield of wheat utilized for bread production. NAC transcription factors play substantial roles in biological processes of plants, containing plant development, phytohormone homeostasis and in responses to different environmental stresses. TaNAC2a, a NAC transcription factor from wheat, enhances drought and salt tolerance. The purpose of this study was to evaluate the expression level of TaNAC2a gene in Triticum aestivum under different salinity stress conditions (0 (as control), 30, 60, 90, 120 mM) of sodium chloride in leaf and root tissues. 25-day-old leaves and roots that were grown for five days in the presence of various concentrations of sodium chloride were surveyed. In the first step, total RNA was isolated using RNX plus solution. After validating the quality and quantity of total RNA, cDNA was synthesized using cDNA synthesis kit and finally, the expression level of TaNAC2a was evaluated using quantitative real-time PCR with specific primers. GAPDH was selected as an internal control gene. Based on our results, with increasing concentration of sodium chloride up to 100 mM NaCl, the expression of TaNAC2a gene was increased in both root and leaf tissues but the amount of expression in root tissue was more than in leaf in all salinity levels. Our results showed that TaNAC2a gene expression was directly related to the level of tension and would be helpful for the recognition and selection of candidate associated genes with salinity stress tolerance.

**Keyword:** Bread wheat, Salt stress, TaNAC2a, Gene expression

**P-387: The use of phosphinothricin resistance as selectable marker for Nicotiana tabacum engineering**

*Jamshidnia M1,2, Kazemitabar SK1, Lindermayr Ch2, Najafi Zarin H1*

1Sari Agricultural Sciences and Natural Resources University, Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Sari, Iran

2 Institute of Biochemical Plant Pathology, Helmholtz Zentrum München, German Research Center for Environmental Health, Munich, Germany. Maryam.Jamshidnia58@gmail.com

Phosphinothricin (PPT) is the active component of a family of environmentally safe, nonselective herbicides. The bar gene conferring tolerance to the herbicide phosphinothricin (PPT) encodes phosphinothricin acetyltransferase (PAT). Phosphinothricin Acetyl Transferase that detoxifies Phosphinothricin (PPT), the active ingredient of herbicides such as BASTA. We report here a transformation procedure with the bar gene as a selectable marker established via
Agrobacterium-mediated transformation. It was introduced into the tobacco genome at a targeted site by homologous recombination. Transgenic plants of Nicotiana tabacum were regenerated by leaf disc transformation using a bar gene as a selectable marker. Transgenic plantlets were selected in medium supplemented with PPT (up to 0.1 mM). The transgenic N. tabacum plants resistant to PPT were positive upon PCR by bar gene specific primers. The transformed plants transferred to a greenhouse proved to be resistant to 1% PPT. The technology demonstrated here could perhaps be usefully transferred to other crop species.

**Keyword:** Bar; Nicotiana tabacum, Transformation, Phosphinothricin

P-388: Evaluation the effectiveness of EST-based markers in separating of Aegilops codata species wild accessions

Karimi H, Fabriki-Orang S, Ashraf Mehrabi A

Student of Genetics and Plant Breeding, Imam Khomeini International University, Qazvin.  
Assistant Professor, Department of Genetics and Plant Breeding, Imam Khomeini International University, Qazvin.  
Associate Professor, Department of Plant Breeding, Ilam University, Ilam  
hamid.karimi126@gmail.com

Knowledge of genetic diversity level for plants germplasm and the determination of genetic relationships of breeding plant materials is the basis of many plant breeding programs. In this research, three EST based markers were used to study the genetic variation among eight wheat-relatives of wheat belonging to Aegilops codata species. The allelic pattern was scored on the basis of presence (1) and absence (0) bands, and the total number of 78 bands was identified. Cluster analysis and drawn dendrogram based on UPGMA method using dice similarity coefficient were divide the accessions into four groups with the high co-phenetic coefficient (0.968). In the first and third groups, three accessions in each group and in the second and fourth group only one accession were placed in each. The highest genetic similarity was observed between the IUGB-00304 and IUGB-00815 accessions. The calculated genetic indices including Na (1.5), Ne (1.39), I (0.33), He (0.22), UHe (0.23) showed a high level of diversity among the accessions. The average of polymorphism for the studied primers was 60.26%, and the highest polymorphic information was obtained for M1+Mir398 combination (PIC=0.934) and the lowest for the M2+Mir398 combination (PIC=0.861). The Principal Coordinate Analysis (PCoA) had also clearly confirmed the separation of the accessions, indicating that the EST based markers could well differentiate the Aegilops codata accessions.  

**Keyword:** EST markers, Genetic variation, Wild-relatives of wheat

P-389: Investigation of gene expression of Squalene synthase (SQS) in indigenous licorice of Iran (Glycyrrhiza glabra) during the spring and autumn seasons

Khakpour A, Zolfaghari M, Sorkheh K

Department of Plants, Shahid Chamran University of Ahvaz, Iran 2. Faculty member of Shahid Chamran University of Ahvaz, Iran  
E-mail: atiiii.khakpour.9094@gmail.com

Squalene synthase gene (SQS) as the first and most important gene in the pathway of biosynthesis plays an important role in the licorice plant called glycyrrhizin. Changes in the expression of the genes that contribute to the synthesis of this active substance are influenced by climatic conditions and also have different expressions in different tissues of the plant. The aim of this study was to investigate the pattern of expression of Squalene synthase in autumn and spring season in fresh leaves, skin and kernel of licorice rhizome. The expression of this gene was measured by SYBR-Green based qRT-PCR method. The gene expression of SQS in the autumn increased compared to the spring, and also showed up-regulated in the kernel of rhizome compared to the leaves and skin of rhizome. The results of this study showed that the expression of SQS gene under different seasons of growth is different, and this is consistent with variations in the content of secondary metabolite of this plant under different climatic conditions.  

**Keyword:** Squalene synthase, glycyrrhizin, qRT-PCR, SYBR-Green

P-390: Efficiency of CORAP markers in genetic diversity study of Triticum durum accessions

Kiaheyrati S, Fabriki Orang S, Ashraf Mehrabi A

Imam Khomeini International University, Qazvin.  
Department of Genetics and Plant Breeding, Imam Khomeini International University, Qazvin.  
Department of Plant Breeding, Ilam University, Ilam  
s.kiaheyrati@yahoo.com

In order to evaluation of genetic diversity of 17 wheat accessions belonging to Triticum durum species using CORAP marker, and also the efficiency of this marker, three primers combination were studied. The allelic pattern was scored based on the presence (1) and absence (0) bands, and a total numbers of 127 different segments were replicated and identified. In the AP2+A2 primers combination, of forty seven replicated segments with 584 bands across all accessions, thirty two alleles were polymorph. In CAT+A2 primers combination, of thirty seven replicated segments with 559 bands, only thirteen alleles were polymorph. In the third primer combination, Chl(a)+A2, of forty three segments with 589 bands, thirty alleles were polymorph. The mean of polymorphism information content was 0.95 with the maximum PIC (0.96) for AP2+A2 and Chl(a)+A2 primer pairs. However, the highest marker index was for the third primers combination (Ap2+A2) with MI equal 23.2. The dendrogram obtained from the cluster analysis grouped 17 different wheat accessions of Triticum durum into six main clads. In conclusion, the results of this experiment confirmed the effectiveness of CORAP primers used in the separation and grouping of Triticum durum accessions.  

**Keyword:** CORAP marker, Durum wheat, Genetic variation

P-391: Study the Effect of Cobalt Oxide Nanoparticle Effect on the Expression of DXR and GPPS Genes in Achillea Wilhelmsii Cellular Suspension

Mahmudi A, Taheri A, Aramide A

Dep of Agricultural Biotechnology, University of Guilan, Iran  
Almahmoud66@yahoo.com

Nanoparticles have had a widespread impact on the world of biology and agriculture caused by their specific effects and unique characteristics. Yarrow’s herb has several subtypes that have different compositions of monoterpentenes and...
sesquiterpenes. The relative expression of DXR and GPPS genes in Yarrow’s herb was evaluated under the influence of nano cobalt elicitors at three concentrations of 0.5, 0.75 and 1 mg / L with Real-Time PCR, and the 18s RNA gene was used as reference gene for data normalization. According to the results of the comparison of the averages, the expression of the DXR gene showed increasing gene expression in all concentrations of cobalt oxide nanoparticle (0.5, 0.75 and 1 mg / L) eight hours after the treatment. The highest expression was related on the concentration of 0.75 mg / L, and then concentrations of 1 and 0.5 mg / L. 48 hours after treatment of cobalt oxide nanoparticle, expression of GPPS gene expression increased. The concentration of 0.75 mg / L had the greatest effect on the expression of this gene. This study showed the effect of cobalt oxide nanoparticle on the expression of DXR and GPPS genes.

**Keyword:** Elicitor Nano Cobalt, Terpenes, Gene Expression, Real-Time PCR, Yarrow

P-392: Analysis of genetic diversity of prunus rootstock using SSR marker

mujaz ramooni F, bakhshi khaniki Gh*, pirkhezri M, fatehi F

This study showed the effect of cobalt oxide nanoparticle on the expression of DXR and GPPS genes. Plums have 19 species and thousands of cultivars and genotypes. Different methods are used to determine genetic diversity and in this regard, DNA markers are more accurate. In the research used 19 commercial rootstock and Prunus genotype and 7 SSR marker for analysis of genetic diversity. Polymerase chain reaction was performed in a volume of 17 ?L. output was electrophoresed by capillary method and the formed bands were analyzed. pleomorphic marker duplicated on the whole 81 bands in the assessed genotypes with an average 11/571 band. Eigenvalues for primers reduced to 4 components by parsing to principal component. The results of the cluster analysis are shown that plum genotypes were placed in 3 separate clusters. First cluster include 8 genotypes: GF69, Red Plum, Saint-Cholin, GN, Dehno, Tensogol, Tabriz, Lorozerazros. most of the measure effective allele, Shannon Index, forecasted heterozygosity, observed heterozygosity and polymorphism information content is for CPSCT035 marker that demonstrates its more impact in the heterozygosity and polymorphism information content is for CPSCT035 marker that demonstrates its more impact in the observed diversity and high separation of samples. medium similarity is 0/4 between genotypes, that demonstrates high separation markers used. based on the results of the similarity matrix most genetic differences occurred between Davydiana genotype and Dehno and Tensogol genotypes, that are suitable for creating a crossroads. This research showed that SSR marker has a high ability In the separation Prunus rootstocks each other.

**Keyword:** Pruinus rootstock, Genetic diversity, SSR marker

P-393: Optimization of DNA extraction from different tissues of a medicinal plant, Senecio vulgaris L.

Marawne H*, Ahmadikhah A1

Today, major progress has been made in molecular experiments involving DNA inclusion. The first step towards conducting these experiments is the accurate extraction of nucleic acids with good integrity and high quality. Some species are characterized by a high content of tannins, alkaloids and phenols in their leaves. These secondary metabolites are released during DNA extraction and might hinder PCR (polymerase chain reaction)-based molecular studies. The objective of this research was to provide an efficient method to extract DNA from Senecio vulgaris L., a medicinal plant from Tehran region used in popular medicine such as diuretic, diaphoretic, dysmenorrhea, and bilious pain. Two procedures of DNA extraction were tested and could not extract adequate and high-quality DNA for molecular works because of high phenol and polysaccharides contaminations. The optimized procedure in this study encompassed the utilization of phenol during deproteinization, increased concentrations of CTAB and sodium chloride, and a shorter period and lower temperature of incubation concerning other methods. Purity of extracted DNA was excellent as evident by A260/A280 ratio ranging from 1.6 to 1.8 and A260/A230 ratio was >2, which also suggested that the preparations were sufficiently free of proteins and polyphenolics/polysaccharide compounds. The extracted DNA did not present degradation, and amplification via PCR was successful using specific primers of RNA polymerase II housekeeping gene.

**Keyword:** Absorbance, DNA extraction, PCR Amplification, Secondary Metabolite, Phenols

P-394: Isolation of defensins from transcriptome libraries of Lens culinaris and Lens ervoides

Mirdirkvand R, Sohrabi S M, samiei K

Plant defensins are low molecular weight cationic antimicrobial peptides. They are cysteine-rich peptides ranging from 45 to 84 amino acids in length and form three or four disulfide bonds. In the current study, we prepared pipeline to in silico isolation of defensin genes from transcriptome libraries of Lens culinaris and Lens ervoides. All coding sequences of the defensin genes of Fabaceae plants (238 sequences) were given from NCBI GenBank. The sequences were separately aligned against Lens culinaris and Lens ervoides transcriptome libraries using BLASTn tool. The result sequences for each plant were pooled and assembled by CLC Genomics 9.0 and Vector NTI 11.0. The assembly results (contigs and singlets) were aligned and compared with nr database using BLASTn. For further analyses, CLC Genomics 9.0, Vector NTI 11.0, ORF finder, Pfam and CDD were used. After assembling the sequences, a total of 6 contigs in Lens culinaris and 5 contigs in Lens ervoides were obtained. BLASTn results confirmed...
the successful isolation of defensing genes from transcriptome libraries. All identified contigs contained full-length ORFs (222-282 bp) that started with ATG and stopped with TAG or TGA. Translation of isolated ORFs showed that they comprised Knot1 functional domain. This functional domain belongs to gamma-thionin superfamily and present in all plant defensins. All analyses confirmed the identification and isolation of defensin from Lens culinaris and Lens ervoides transcriptomes. Results exhibited the effectiveness of computational methods to isolation of the desired genes from transcriptome libraries. Keywords: Plant defensins, Antimicrobial peptides, Lentil, Bioinformatics, EST libraries

P-395: Revisiting Pivotal-Differential Genome Evolution in wheat

Mirzaghaderi Gh1, Mason A S2

University of Kurdistan, Sanandaj, Iran
Justus Liebig University, Giessen, Germany Annalieise
mason@agrur.uni-giessen.de

An interesting pattern of genome evolution following polyploidy can be observed among allopolyploids of the Triticum and Aegilops genera (wheat group). Most polyploids in this group are presumed to share a common unaltered (pivotal) subgenome (U, D or A) along with one or two differential subgenomes which is modified relative to other diploid and allopolyploid species containing this genome(s). This status that has been referred to as â€œpivotalâ€-differentialâ€- genome evolution was first identified based on comparative morphology (flowering spikes), whereby species could be grouped into A, D and U genome clusters. However, substantial cytogenetic evidence also supports this genome relationship, and with recent genomic advancements in wheat we suggest that it is time to interrogate this relationship further, and to extend these concepts to other plant taxa where it may be relevant. The pivotal-differential genome patterning within taxa may have three possible explanations that should be tested. Firstly, variation between species sharing a differential genome may be directly inherited from variation (e.g. different progenitor cytotypes or subspecies) present within the ancestral diploid species. Secondly, variation between species may be induced as a result of the allopolyploidy followed by the dominance of one subgenome over the other(s). Thirdly, hybridization between two allopolyploid species that share a (pivotal) genome in common but differ in their second genome may give rise to a new, rearranged (differential) genome after hybridization and genome stabilization (e.g. AABB x AACC -> AADD). Interrogation of future pan-genome data coupled with synthetic recreation of historical hybridization events can reveal the mechanisms underlying pivotal-differential genome patterns.

Keyword: genome evolution, polyploidy, wheat, Aegilops, Triticum

P-396: Identification of the key gene (SMT1) involved in the diosgenin biosynthesis pathway in Fenugreek (Trigonella foenum-graecum L.)

Mohammadi M1, Rashidi Monfared S, Mohammad Mohammadi, Sajjad Rashidi Monfared*

Department of Biotechnology, Faculty of Agriculture, Tarbiat Modares University

Fenugreek is one annual plant belonging to the legumes family. The most important steroidal suppositories derived from Fenugreek saponins are diosgenin and yamgoenin. Fenugreek seeds have anti-diabetic, anti-pyretic and anti-cancer effects, lowering cholesterol and blood glucose. Fenugreek is an important economic source for the production of mucilagous (galactomannans), trigonolin and diosgenin in pharmaceutical industries. One of the important genes Involved in diosgenin biosynthesis pathway is Sterlo methyl transferase (SMT1) the biosynthesis pathway is the diosgenin of Sterlo methyl transferase (SMT1) gene. In this study, two pairs of a primer pairs were designed based on predicted ORF region were designed from SMT1 protected areas and polymeric chain reaction was used to identify the gene from Fenugreek. Then with aligning among those gens and reading which was obtained from RNA-seq process in SRA/NCBI database by associating the Off line blast the same reading and assembling these readers were conducted by using Codoncode Aligner. After initial analysis of polymerase chain reaction products with gel electrophoresis, sequencing was performed for confirmation. By examining the sequence of the gene and performing the alignment of nucleotide sequences with sequences in The NCBI database, we conclude that the target gene is SMT1.

Keyword: Fenugreek, Diosgenin, gene identification

P-397: Genetic variation and vinblastine production of Catharanthus roseus (L.) plants exposed to Cold plasma jet

Mohammadzadeh Shahir M1, Noormohammadi Z1, Farahani F2, Atyabi3

Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
Department of Microbiology, Qom Branch, Islamic Azad University, Qom, Iran
Department of Nanobiotechnology, Pasteur institute of Iran, Tehran.
m_shahir99@yahoo.com

Catharanthus roseus is a tropical plant belonging to the family Apocynaceae and native to Madagascar. This plant is known for pharmaceutical features because of producing more than 130 terpenoid indole alkaloids. Meanwhile, dimeric indole alkaloid; Vinblastine has effect as anticancer drug but is produced in small amount. The aim of this study was to evaluate the effect of Non thermal plasma jet on genetic variation and rate of vinblastine production. The cold helium plasma jet operated at 13.5 kV and 60 second on Catharanthus roseus seeds and genetic variation was determined by using 15 primers of Sequence Related Amplified Polymorphism(SRAP) marker. The High Performance Liquid Chromatography(HPLC) technique was used for separation and analysis of vinblastine. The genetic results showed higher genetic variation in plants with 60s cold plasma treatment in genetic parameters like Neisâ€™ genetic diversity, Shannon index, number of effective alleles and percentage of polymorphism. PCoA ordination showed evidences of differentiation of two plant groups studied. HPLC test showed remarkable increase of vinblastine in C. roseus plants. Our finding revealed genetic changes in C. roseus after cold plasma treatment. It may be useful tool for increase genetic variation for achieving more secondary metabolic materials.

Keyword: Catharanthus roseus, SRAP, vinblastine

P-398: Impact of Explant Types and Co-culture Time on...
Hairy Root Induction in Chicory (Cichorium intybus L.)

Mohseniazar M, Vahdati K

Department of Horticultural science, Faculty of Agriculture and Natural resources, University of Mohaghegh Ardabili, mohseniazar@gmail.com

Hairy root cultures are an effective method to produce the secondary metabolites, because hairy roots are genetically and biologically stable and they are able to produce metabolites without hormones. Chicory (Cichorium intybus L.) is one of the most important medicinal plants with valuable medicinal compounds. In this research, hairy roots were induced by use of the ATCC11325 strains of Agrobacterium rhizogenes. In the first experiment, the effects of type of explants (cotyledon, hypocotyl, leaf and petiole) and co-culture times (24, 48 and 72 hours) were investigated on the efficiency of hairy root induction. In the second experiment, the effect of three different media (Murashige and Skoog solid, liquid and liquid half strength) evaluated on growth rate and biomass accumulation in hairy roots. The results of experiments showed that between different explants the highest hairy root induction (75.55 percent), roots number (7.26) and root length (7.66) were observed in the 5-day-old cotyledons and between different co-culture times, the best hairy root induction percentage (48.33), roots number (5.95) and root length (7.96) were observed in 72 hours co-culture. The results revealed that liquid 1/2 MS media was superior for high fresh weight (2.36 g) and dry weight (0.18 g) and growth index (19.12) in hairy roots. Thus 1.2 MS medium was suitable for hairy roots growth. This study describes the protocol for hairy root induction by ATCC11325 strain A. rhizogenes which could further be useful for the production of secondary metabolites and biomass.

Keyword: Biomass, Co-culture, rolB gene, Secondary metabolites

P-399: Investigating the expression of PIP1, PIP2, and GAI genes in dwarf and vigorous genotypes of walnuts

Mohseniazar M, Chamani E

University of Tehran, Abureyhan Campos, Horticulture Department

PIP2 and PIP1 expression in the period from April to mid-June at 1% and 5%, respectively. According to the results, the mean Duncan’s method showed that the difference in expression of GAI, PIP2 and PIP1 genes in the three tissues studied at the first time of May did not have a significant difference between the dwarf genotypes. The results of gene expression analysis showed that the expression of PIP2 and PIP1 gene in the three different times between the two dwarf and vigorous genotypes was significantly different at 1% and 5%, respectively. According to the results, the mean PIP2 and PIP1 expression in the period from April to mid-June are high, but declines over time.

Keyword: dwarf genotypes, aquaporins, gene expression, Walnut.

P-400: Comparison of NAC2 transcription factor expression in Arabidopsis thaliana sprouts in response to increasing salt and drought stresses

Momeni M M, Jamshidi Goharrizi K, Pakzad R, Kalantar M

Department of Plant Breeding, Yazd Branch, Islamic Azad University, Yazd, Iran

mehdi6989@iauyazd.ac.ir

One of the most important groups of transcription factors in plants is NAC protein family which participates in plant reaction to abiotic stresses. Arabidopsis thaliana is a momentous model organism which its genome is completely sequenced and is very valuable for plant biologists. Salinity and drought stresses are two of the most important tensions which can restrict growth and plants’ yield. In this study, we investigated the effect of salt and drought stresses on NAC2 transcription factor expression in Arabidopsis thaliana sprouts that were exposed to salt and drought stresses for 3 days. Different concentrations of NaCl (0, 45, 90, 180 mM) and PEG (0, 7.5, 15, 22.5%) were used for salt and drought stresses respectively. Gene expression was considered as GAPDH as the internal control. Our results showed that NAC2 transcription factor expression under NaCl treatments is more than PEG treatments in all samples. Also, the pattern of expression increased with increasing concentration of NaCl and PEG. It can be concluded that this transcription factor may be associated with salt and drought stresses tolerance in Arabidopsis thaliana.

Keyword: Arabidopsis thaliana, NAC2 transcription factor, Salt and drought stresses, Gene expression

P-401: Effect of different concentrations of colchicine in culture medium on induction of androgenesis in anther culture of capper(Capparis spinose L.)

Mostafavi SM, Abdollahi MR*, Dastan D, Sarikhani H

MS student of plant Breeding Bu-Ali Sina University, Hamedan, Iran
1Department of Agronomy and Plant Breeding, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran
2 Department of Horticultural Sciences, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran
3 Department of Photochemistry, Faculty of Pharmacy, Hamedan University of medical sciences, Hamedan, Iran
mostafavi128@gmail.com

In this research, effect of different concentrations colchicine in culture medium on the androgenesis induction in capper (Capparis spinosa L.) was investigated. B5 Cuture medium containing 2 mg/L 2,4-D and 0/5 mg/L BAP and different concentrations of colchicine (0,100,200,400 and 600 mg/L) was used us androgenesis induction culture medium. Results showed significant differences between different concentrations of colchicine used in culture medium for various androgenesis traits in anther culture of capper. use of 400 mg/L colchicine significantly enhanced the frequency of calllogenesis induction, calllogenesis speed and mean number of embryo per anther in anther culture of capper.

Keyword: Colchicine, capper, anther culture 2,4-D, BAP
Wheat is the most important crop and is cultivated all over the world. Wheat has a wide range of adaptability to different climates. Vernalization genes are one of the important factors determining wheat adaptability. Knowing the presence of vernalization gene is important to introduce new cultivars for different regions. At a molecular level, the length of the vernalization period of common wheat (Triticum aestivum L.) is determined mainly by three loci: VRN-1, VRN-2 and VRN-3. The VRNA1, VRN-B1, and VRN-D1 genes are dominant for spring growth habit and epistatic to the alleles for winter growth habit. Therefore, winter cultivars are homozygous for the recessive alleles at the three VRN-1 loci. In the current study, 34 bread wheat accessions plus Chinese Spring wheat were investigated for allelic variation at Vrn1 loci. The current study showed that at VRN-A1 five genotypes had dominant allele. VRN-A1b allele was not found at any of the genotypes. Only one genotype had VRN-A1c allele and 32 genotypes including the Chinese Spring showed vrn-A1 allele. The genotype No. 6 unexpectedly had more than one allele. At locus VRN-B, 14 genotypes had dominant allele while 20 including the Chinese Spring showed recessive allele.

**Keyword:** Allelic variation, Bread wheat, Molecular markers, Vernalization

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**P-403: Effect of different concentration of PEG on TaNAC2a gene expression in wheat (Triticum aestivum L.) root and leaf**

**Nazari M1, Jamshidi Goharrizi K2**

Department of Agronomy and Plant Breeding, Faculty of Agriculture, Bu-Ali Sina University, Hamedan, Iran.
Department of Plant Breeding, Yazd Branch, Islamic Azad University, Yazd, Iran.
Maryam.nazari222@yahoo.com

Bread wheat (Triticum aestivum L.) is one of the most important crops in the world, but drought stress is a prevalent detrimental environmental condition which affects productivity of crop worldwide. NAC transcription factors play a fundamental role in response to various abiotic stresses. In this study in order to survey TaNAC2a gene expression after four days of treatment with different PEG concentrations (0, 5, 10, 15 and 20%) as a dehydration inducer in leaf and root tissues, total RNA was extracted with RNX plus solution, cDNA was synthesized and amplification was performed with real-time PCR. GAPDH was used as internal control gene. According to our results with increasing the concentration of PEG up to 20%, the expression level of TaNAC2a gene was increased significantly in both root and leaf tissues, but the amount of expression in root tissue was more than leaf in all PEG levels. Also, our results demonstrated that drought stress levels have a direct effect on TaNAC2a gene expression and the expression level in the root which is the very place where plants first encounter drought stress is more than in leaf in all stress levels. NAC reactive stress genes may be used in molecular breeding to stress tolerance improvement.

**Keyword:** Bread wheat, TaNAC2a, Drought stress, Gene expression

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**P-404: dentification of S alleles in Iranian sour cherry genotypes using consensus primers**

**Nazari SA1, Hajilou J2, Zeinalbedini M3, Imani A4**

Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Iran. Corresponding author email: sallyoun66@gmail.com
Department of Horticultural Sciences, Faculty of Agriculture, University of Tabriz, Iran.
3 Agriculture Biotechnology Research Institute of Iran (ABRII), Karaj, Iran.
4 Horticultural Departments of Seed and Plant Improvement Institute (SPII), Karaj, Iran.
sallyoun66@gmail.com

Self-incompatibility (SI) is a genetic mechanism that prevents self-fertilization by enabling the pistil to reject self-pollen. It is controlled by a single multi-allelic locus, called the S-locus. Determination of the composition of the S-alleles of sour cherry genotypes is useful both to growers producing the fruit and breeders when selecting cultivars for cross-fertilizations. In this study, S-allele diversity and identity of eight Iranian Sour cherry genotypes and two commercial cultivars “Ciganymeggy” and “Erdi botermo” was investigated using two sets of primers. The results of sequencing of PCR product showed that the first intron, which was amplified with primers (PaCons1-F, PaCons1-R2 new), identified S6 and S9 alleles, also the second intron (PaCons1-F and PaCons1-R) identified S9 and S6m2 in studied genotypes. Information obtained about S-allele combinations of Iranian sour cherry genotypes will be useful during the establishment of new commercial orchards, in order to maximize the fruit set and consequently the yield.

**Keyword:** Sour cherry, Self incompatibility, Consensus primers, PCR

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**P-405: Expression of recombinant Green Fluorescent Protein (GFP) using “magnICON” system in-planta**

**Niknejad A1,2, Webster D2**

Faculty of Biological Sciences, Kharazmi University, Tehran, Iran
School of Biological Sciences, Monash University, Melbourne, Victoria, Australia
niknejad.azadeh@gmail.com

The green fluorescent protein (GFP) is widely used as a reporter for gene expression and localisation, as an in situ tag for fusion proteins, a biosensor or a probe for protein-protein interactions. It is 238 amino acids long (26.9 kDa) and forms a β-barrel around one α-helix. GFP isolated from the jelly fish Aequorea victoria and demonstrated that it could exhibit bright green fluorescence when exposed to light in the UltraViolet (UV) range. This study focused on the rapid transient expression of GFP using the deconstructed tobacco mosaic virus-based “magnICON” plant expression system. A 3’ viral vector containing the GFP gene (pICH7410) was infiltrated into N. benthamiana leaves side by side with the 5’ module for cytosol or the 5’ module for chloroplast. At 10 days post infiltration (dpi), GFP expression in leaves was checked under UV light to ensure the viral vector-based heterologous expression was working correctly. GFP protein showed bright
fluorescence under UV light. The TSP extracted from infiltrated leaves using 3’ GFP module and different 5’ modules (cytosol or chloroplast) were quantified using Bradford assay. The absorbance of each sample was read at 595 nm and the TSP concentration determined from the standard curve, constructed using bovine serum albumin (BSA). Approximately 93 mg and 98 mg of the quantified TSP samples were run on a gel to analyse the GFP expression by SDS-PAGE gel and western blot. Western blot analysis using anti-GFP antibody confirmed the presence of GFP at approximately 25 kDa, at the expected size for GFP.

**Keyword:** green fluorescent protein (GFP), transient expression, magnICON

**P-406: Expression pattern of TaNAC2a transcription factor in wheat (Triticum aestivum L.) root and leaf under different concentrations of salinity stress**

Jamshidi Goharrizzi K', Baghizadeh A', Amirmahani F', Kargar F', Kalantar M', Pakzad R', Moemeni MM'  

Department of Plant Breeding, Yazd Branch, Islamic Azad University, Yazd, Iran.  
Department of Biotechnology, Institute of Science and High Technology and Environmental Sciences, Graduate University of Advanced Technology, Kerman-Iran.  
3Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran.  
jamshidi_kiarash@yazd.ac.ir

Abiotic stress containing salinity stress affects quality and yield of wheat utilized for bread production. NAC transcription factors play substantial roles in biological processes of plants, containing plant development, phytohormone homeostasis and in responses to different environmental stresses. TaNAC2a, a NAC transcription factor from wheat, enhances drought and salt tolerance. The purpose of this study was to evaluate the expression level of TaNAC2a gene in Triticum aestivum under different salinity stress conditions (0 (as control), 30, 60, 90, 120 mM) of sodium chloride in leaf and root tissues. 25-day-old leaves and roots that were grown for five days in the presence of various concentrations of sodium chloride were surveyed. In the first step, total RNA was isolated using RNX plus solution. After validating the quality and quantity of total RNA, cDNA was synthesized using cDNA synthesis kit and finally, the expression level of TaNAC2a was evaluated using quantitative real-time PCR with specific primers. GAPDH was selected as an internal control gene. Based on our results, with increasing concentration of sodium chloride up to 100 mM NaCl, the expression of TaNAC2a gene was increased in both root and leaf tissues but the amount of expression in root tissue was more than in leaf in all salinity levels. Our results showed that TaNAC2a gene expression was directly related to the level of tension and would be helpful for the recognition and selection of candidate associated genes with salinity stress tolerance.

**Keyword:** Bread wheat, Salt stress, TaNAC2a, Gene expression

**P-407: ZnO Nanoparticle stress alters expression level of antioxidant enzyme genes in Pyricularia oryzae**

Panahi Dizjikan Z', Ahmadikhah A', Kharabi Masouleh A'  

Department of Plant Sciences and Biotechnology, Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, G.C. Velenjak, Tehran, Iran.

ZnO nanoparticles (with Williamson-Hall average size of 21 nm) in the PDB media, and cDNA was synthesized and then was used in Real-Time PCR with specific primers using SYBR Green kit, and gene expression results indicated that transcript levels of two genes in parallel to H2O2 fluctuations were changed and the both antioxidant genes showed the expression alteration in a dose- and time-dependent pattern to suppress the lethal dose of nanoparticles.

**Keyword:** Antioxidant, Blast, Gene expression, ZnO nanoparticle

**P-408: A phylogenetic analysis of Pterocephalus Vail. ex Adans. (Caprifoliaceae) based on ITS and trnL-trnF loci**

Pirooozi S, Falahati Anbaran M, Attar F, Mehdigholi K  

School of Biology, Faculty of Science, University of Tehran, Tehran, Iran  
piroozisos@gmail.com

Pterocephalus (Caprifoliaceae) consists of 30 species worldwide distributed mostly in the temperate region of the northern hemisphere, in which 14 species reported in Iran including 5 endemic species. In this study, we studied nucleotide variation and genealogical relationships among 15 samples from 11 species of Pterocephalus using nuclear internal transcribed spacers (ITS) and plastid trnL-trnF regions. We also included three Mediterranean Pterocephalus species as ingroup with two Scabiosa and one Dipsacus species as outgroup. Our results showed that the studied taxa can be divided into five lineages including two major groups, that is supported with both ITS and trnL-trnF and morphological data. A low level of nucleotide diversity was observed in trnL-trnF region compared to that found in ITS region. Contrary to topology obtained with ITS data, a high degree of polytomy was found in trnL-trnF and a low bootstrap support was observed in phylogenetic analyses including maximum likelihood and Bayesian inferences. Phylogenetic tree obtained from combined nrDNA and cpDNA data revealed highly supported clades.

**Keywords:** Pterocephalus, Caprifoliaceae, phylogenetic analyses, ITS, trnL-trnF

**P-409: Molecular Markers: key biotechnology tools in the research and development of biofortified crops**

Sadeghzadeh B', Mohammdi SA'
Bitter vetch (Vicia ervilia L.) is an ancient legume crop used as forage plants that is seasonally available throughout the world, especially in developing countries. In many micronutrient-deficient regions, wheat grains as the dominant staple food are naturally low in minerals, vitamins and protein, increasing a risk of hidden hunger. Biofortification of wheat is a new approach to control micronutrient deficiencies and vitamin A in developing countries. Predictive cost-benefit evaluates have proven that biofortification is important in controlling of malnutrition. Biofortification is the development of micronutrient-dense food crops using conventional breeding and/or biotechnology practices. Biofortified crop system is highly sustainable over years, cost-effective, target low-income households, and available in remote rural areas. Micronutrient enrichment traits are present in the genome of cereals that could permit substantial improvement in grain zinc, iron and carotenoids without negatively impressing grain yield. The genetically fortifications traits are stable across various climatic environments and soil types. Biotechnology tools in genomic, molecular biology, identifying nutrient absorption enhancers and inhibitors genes can provide better and more efficient complementary breeding tools. A significant development in the next few years will be the use of molecular markers associated with accumulation of Zn and Fe in wheat. The identification of major-effect molecular markers can speed up the development of high yielding biofortified wheat even in micronutrient-deficient soils. DNA markers allow screening for micronutrient-rich crops independently of the environmental variability or growth stage. If markers are close enough to a gene of interest, they can be directly used in marker-assisted selection (MAS). 

**Keyword:** Micronutrients, Biotechnology, Biofortification

**P-410: Karyotypic Studies in Some Iranian Bitter Vetch Landraces**

*Sahhafi SR*

Department of Genetics and Crop Production, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan

E-mail: s.r.sahhafi@vru.ac.ir

Bitter vetch (Vicia ervilia L.) is an ancient legume crop used as forage plants that is seasonally available throughout the western and northwestern area of Iran. To investigate the genetic variations in some Italian bitter vetch landraces, we selected fifteen landraces on which to carry out cytological studies using karyotypic analysis. The studied landraces were collected from seven provinces including Ardabil, Hamadan, Kurdistan, Lorestan, Zanjan, East Azerbaijan and West Azerbaijan. Hematoxylin stain squash technique was used in this research. Karyotypic parameters of landraces were evaluated in a completely randomized design with five replications. Based on the results of this research, all studied bitter vetch landraces showed same chromosome morphology. The basic chromosome number of all landraces were seven (x=7). Also, the same karyotype formula (2n=14) was revealed for all studied landraces. Moreover, analysis of variance showed that there were no significant differences in terms of all karyotypic parameters among landraces. In conclusion, detailed karyotypic analysis indicated chromosome stability among the studied bitter vetch landraces.

**Key Words:** bitter vetch landraces, chromosome, keywords: hematoxylin ,karyotypic parameter

**P-411: Evaluation of genetic diversity in Capsicum spp as revealed by FARS markers**

Salimi M1, Darabi M2, Sepasi A3

1 Msc of Agricultural Biotechnology-Department of Science and Technology Islamic Azad University, Medical Branch, Tehran, Iran
2 Department of Agronomy and Plant Breeding sciences, College of Aboureihan, University of Tehran, Tehran, Iran
3 Department of Microbiology, Islamic Azad University Of Pharmaceutical Sciences Branch, Tehran, Iran

misha6531@gmail.com

One of the most important herbs in the pharmaceutical, spice is pepper. Nowadays, the use of molecular markers to study genetic diversity. The existence of polymorphism in plant species is one of the most important tools for gene identification, genome screening and development of genetic maps. For detection of such polymorphism based on PCR, are used two primers, and in some other methods such as RAPD, DAF and AP-PCR, only one primer is used. However, in later cases nucleotide combination of such primers is completely random and their physical location on genome is unclear. On the other hand, the primers produce large number of bands with low repeatability. One alternative is to use sequences that provide the elements necessary for their synthesis. Among these sequences, one can refer to the palindromic regions of the genome, which have a short reverse sequence on both ends. This short reverse sequence can be used in the PCR reaction as a primer and can cause the palindromic region to be synthesized between the two reverse sequences. This region is multiplied by this single primer, called the (FARS) region amplified. It could identified in the plant sequenced genomes using bioinformatic tools, and single primers needed for PCR were synthesized and tested. Using the specific FARS primers it could be confirmed the possibility of PCR- based amplification of short , relatively long and long palindromic regions and the marker system FARS could produce co-dominant bands which is suitable for detection of polymorphism between different genotypes.

**Keywords:** Molecular marker,Polymerase chain reaction, FARS,pepper,palindromic

**P-412: Investigation of gene expression of arginase in different tissues of Citrullus colocynthis L**

Savari Chobidi N, Zolfaghari M, Sorkheh K

1. Master of Medicinal Plants, Shahid Chamran University of Ahvaz
2. Members of the faculty of Shahid Chamran University of Ahvaz

E-mail: n-savari@mscstu.scu.ac.ir

Citrullus colocynthis L. is one of the important medicinal plant belongs to Cucurbitaceae family that is widely distributed in the deserts of the Mediterranean and the Middle East. Citrulline is highly linked to arginine and ornithine. Citrulline amino acid is produced in the urea, ornitin and carbamoyl phosphate cycles. Among the genes involved in the synthesis of citrulline in this study, the expression pattern of arginase gene was measured by SYBER-Green based qRT-PCR method. The relative gene expression in the skin tissue was higher than...
Detection and partial molecular characterization of soil borne viruses infecting wheat and barley fields in Yazd province, Iran

Azarfar A, Esmailzadeh Hosseini S.A

1. Department of Agricultural Science, Absar Kavir Co., Yazd, Iran
2. Plant Protection Research Dept., Yazd Agricultural and Natural Resources Research and Education center, AREEO, Yazd, Iran
E-mail: az52164@gmail.com

Soil-borne viruses, belonging to the genera Furovirus and Bymovirus, are serious pathogens of autumn-sown wheat and barley fields in Iran and transmitted by Polymyxa graminis. These viruses are of great economic importance and causing significant quantitative and qualitative losses in yield. There are a few studies about soil borne cereal viruses and in conclusion there is not sufficient information about their distribution and diversity in cereal fields in Iran. During 2014-15, sampling was carried out on wheat and barley fields in Taft, Yazd, Meybod and Dehno (Yazd province, Iran). Disease symptoms were yellowing, mosaic and stunting and total 164 symptomatic and 14 asymptomatic samples were collected. Using double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with Soil borne wheat mosaic virus (SBWMV) and Barley mild mosaic virus (BaMMV) polyclonal antibodies, SBWMV and BaMMV were detected in 34.3 and 17.4% of collected samples respectively. Direct and nested PCR was carried out on positive samples in DAS-ELISA using the external (Wb1/Wb2, Bnl/Bn2) and internal (Wns1/Wns2, Bns1/Bns2) designed primer pairs for SBWMV and BaMMV respectively. The direct PCR amplified two fragments size of 1150 and 960 bp and nested PCR successfully amplified two products with the predicted size of 410 and 430 bp in SBWMV and BaMMV diseased plants respectively. No fragments were amplified with asymptomatic wheat and barley plants. Based on the results of serological and molecular analysis in this study, SBWMV and BaMMV are distributed in wheat and barley fields of Yazd province.

Keyword: ELISA, Nested PCR, Polyclonal antibody, Primer


Shahabi M, Emadpour M

Department of Agricultural Biotechnology, Tarbiat Modares University, Tehran, Iran
E-mail: monireh_shahabi@yahoo.com

Cyclamen persicum is one of the most important pot plants in the world, which is commercially propagated by seeds. Nonetheless seed propagation is usually associated with cultivar variation. In addition, hybrid seed production is expensive. Somatic embryogenesis is still the most used method for Cyclamen micropropagation; however, this technique was usually associated with callus production (indirect regeneration) that, in many cases, resulted in somaclonal variation. In addition, low regeneration efficiency has been reported using this technique. In the current study, we focus on optimization of direct regeneration of Cyclamen. For this purpose, seeds of three commercial F1 cultivars were surface sterilized and germinated on Murashige and Skoog (MS) medium without hormone. Seedlings of aseptic germinated seeds kept in the glass tubes to reach the four-leaf stage of growth. Different explants of the in vitro seedlings including tuber, leaf and petiole were established on MS basal medium, which contains three different levels of BA and four different levels of TDZ hormone, for direct regeneration and the highest organogenesis efficiency. Finally, the percentage of explants with direct regeneration, the average number of nodes or tubers in each explant and the number of leaves form on each regenerated organ were determined. No regeneration of the shoots in the control environment without cytokinin was observed. Additionally, the highest regeneration efficiency for each cultivar was recorded. At the end, direct regeneration could help to eliminate undesired variations and get uniform plants that rooted, acclimated and transferred to the greenhouse.

Keywords: direct regeneration, TDZ, BA

P-415: Phylogenetic analysis of some Iranian Chrysanthemum morifolium cultivars, using Internal transcribed spacer (ITS) molecular markers

Shahbazi m*, Nazarian-Firouzabadi F*: Ismaili A’, Akbarpour OA’

Agronomy and plant breeding Department, Faculty of agriculture,
Chrysanthemum (Chrysanthemum morifolium) is one of the most important ornamental plants which plays a significant role in the development of gardening industry in the world. The knowledge of genetic diversity is one of the prerequisite criteria of Chrysanthemum breeding with important economic goals. Molecular markers have a significant share in elucidation of inter and intra species genetic diversity. To this end, Chrysanthemum genetic diversity was molecularly investigated by sequencing a part of rDNA, using ITS4 and ITS5 primers pairs. Genetic distance between Chrysanthemum cultivars ranged from 0.05 to 10.15, suggesting a wide genetic variation among Iranian cultivars. The most parsimonious trees were 425 steps long. One of the most parsimonious trees grouped all Iranian Chrysanthemum cultivars in one big clade, demonstrating the power of ITS region in revealing the genetic diversity among cultivars. Interestingly, all Iranian Chrysanthemum cultivars were cluster with Chrysanthemum morifolium, suggesting Iranian cultivars have been genetically improved from morifolium species. Genetic diversity assessment of Iranian Chrysanthemum cultivars demonstrated that presumably inter, intra species or even inter population hybridization may have been involved in creating enormous genetic diversity among Chrysanthemum cultivars.

**Keyword:** Genetic diversity, Chrysanthemum morifolium, molecular marker

**P-416: Molecular Analysis of transgenic tobacco (hairy root and leave) containing chimeric chitinase 42 with ChBD in N-terminal**

Sharafi R, Motallebi M, Zamani M R, Moghaddassi Jahromi Z, Jourabchi E

National Institute of Genetic and Biotechnology (NIGEB), Tehran, Iran

roghaye.sh69@yahoo.com

Chitin is the most abundant organic material after cellulose in nature. Chitinases have the ability of chitin digestion that constitutes a main compound of the fungal cell wall, insect exoskeletons, and crustacean shells. Hence chitinases have many usages such as chitooligosacharides production, chitin waste treatment and protoplasts isolated from fungi and yeasts, so the production of chitinase enzymes will be economical. The benefits of producing recombinant proteins in plants relative to other expression systems such as bacteria, yeast and animal systems has made the use of plants for the production of recombinant proteins as a first option, advantages such as low cost, high expression in a short time, correct post-translational modifications such as glycosylation, more security for no transmission of diseases to the humans from a proteinproducing host. Transient expression compared with stable expression of genes, has several advantages for specific applications and is competitive with traditional methods of recombinant protein production. In this study, chimeric chitinase 42 containing introns and also ChBD in its N-terminal was cloned into an eukaryotic expression vector pARM2 including His tag sequence. His tag was used for purification. The cloning was confirmed by PCR and digestion pattern using appropriate restriction enzymes. This expression construct used for transformation of tobacco using agrobacterium tumefaciens for leave and hairy roots were regenerated on kanamycine medium. After confirmation of transgenic hairy root and leave by PCR method, protein expression analysis was achieved by SDS-PAGE and Western blotting methods.

**Keyword:** Chitin, Chimeric Chitinase 42, Chitin Binding Domain, Tobacco, Hairy root

**P-417: Expression of chimeric chitinase 42 enzyme containing ChBD in its C-terminal in tobacco**

Soleimani F, Zamani MR, Motallebi M, Jourabchi E, Moghaddassi Jahromi Z

National Institute of Genetic and Biotechnology (NIGEB), Tehran, Iran

sln.faranak@gmail.com

One of the enzymes with vast potential in the biotechnology field is chitinase, which causes destruction of chitin. The current uses of chitinase include production of N-acetylglucosamine, isolation of protoplast from fungi and yeasts, preparation of monocellular proteins, control of plant pathogenic fungi, control of insect pests in the industries, especially the sugarcane industry, control of the transmission of malaria and as an additive used in antifungal creams and lotions. Chimeric chitinase 42 containing introns and also ChBD in its C-terminal was cloned into a eukaryotic expression vector pARM2 including His tag sequence. The His tag can be used for protein purification. The cloning was confirmed by PCR and digestion pattern using appropriate restriction enzymes. This expression construct used for transformation of tobacco using agrobacterium tumefaciens and A.rhizogenes. Tobacco plants and also hairy roots were regenerated on kanamycine medium. Gene integration was confirmed by PCR method using specific chit 42 primers. Transgenic events were checked by Vir G primer to verify that the amplified band is not agrobacterium born. Chit 42 expression was confirmed by Sodium Dodecyl Sulfate PolyAcrylamide Gel Electrophoresis (SDS-PAGE), protein dot blot and western blot.

**Keyword:** Chimeric chitinase 42 enzyme, His-tag sequence, expression

**P-418: Mapping of QTLs associated with calcium accumulation in barley seed**

Soleimani V, Nikolai V. Patyka, Abdollahi Mandoulakani B, Sadeghzadeh B

1. Ph.D. Student of Agricultural Biotechnology, Department of Ecobiotecnology and Biodiversity, National University of Life and Environmental Sciences of Ukraine, Kiev, Ukraine.
2. Department of Ecobiotecnology and Biodiversity, Faculty of Plant Protection, Biotechnology and Ecology, National University of Life and Environmental Sciences of Ukraine, Kiev, Ukraine.
3. Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Urmia University, Urmia, Iran.
4. Dryland Agricultural Research Institute, Agricultural Research, Education and Extension Organization, Maragheh, Iran.

soleimani_vikiu@yahoo.com

There is little information regarding the chromosomal location of genes conferring calcium accumulation in barley seed, which is very important in mineral nutrient malnutrition across the world. The genetic basis of grain calcium (Ca) concentration and content were studied in population of 150 doubled haploid lines (DHs) derived from a cross between Clipper (low-Ca-accumulator) and Sahara 3771 (high-Ca-accumulator) that...
was screened under glasshouse conditions in CRD with three replications. Wide genetic variation was observed among the DHs for grain calcium concentration and content, with considerable transgressive effect. Four regions located on 1, 5 and 6 chromosomes were found to be associated with seed Ca concentration, which explained 50% of the total variation in seed Ca concentration in barley. The major QTL on 5HL was flanked by ABG702 and GBMS141 markers. For seed Ca content, two regions (2HL and 5HS) were associated with seed Ca content; and explained 37% of the total variation in seed Ca content. The identification of these QTLs would provide an important starting point for understanding the genetic of seed Ca accumulation, and may facilitate the use of molecular markers for improving grain nutritional quality in barley breeding programs.

**Keyword:** QTL, seed Ca accumulation, nutritional quality, barley

P-419: Study of genetic differences in resistance of North and Nor-West bread wheat landraces to powdery mildew disease

**Vosough P**, **Zahraei M**, **Changizi M**, **Shobbar ZS**, **Khaghani Sh**

Islamic Azad University, Arak Branch, Iran
2. Seed and Plant Improvement Institute, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran
Agricultural Biotechnology Institute of Iran, Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran

Panthea.vosough@gmail.com

Powdery mildew is one of important disease of wheat. In order to identify resistant genotypes to this disease in Iranian wheat landraces, a total of 31 genotypes from North and North-west provinces were investigated. The isolates of the disease were collected from Sari, Gorgan and Moghan regions and were multiplied as single clones at greenhouse. A total of ten purified isolates including four, three and three samples from each of Moghan, Gorgan and Sari regions were resulted and used for the assessment of the resistance in the studied germplasm. Seedlings of the genotypes were inoculated at two leaf stage by spore of each isolate, separately under Randomized Complete Block Design with three replications. The reaction to the disease was measured based on infection type. The results indicated that there were significant differences among genotypes against different isolates. The isolates Gorgan 172 and Sari 2 had the lowest and highest mean of infection type, respectively. Principal component analysis was performed to differentiate the genotypes based on their reactions to all the isolates and the results indicated that three first components justified 77.59% of the total variance. Based on these observations, the genotype 214 was identified as the most resistant genotype in the studied germplasm. Dendrogarm of cluster analysis located the genotypes in three groups with different levels of resistance to the studied isolates. The total results of this research revealed genetic resources to powdery mildew in Iranian bread wheat germplasm which could be utilized in breeding programs.

**Keyword:** Germplasm, Genetic diversity, Gene pool, Gene Bank

P-420: Pectin Methyesterase and Poly Galacturronase Genes Expression in Relation to Softening and Shelf-life of Organic and Inorganic Grape Berry during Storage

**Zahedipour P**, **Asghari M**, **Abdollahi B**, **Alizadeh M**

Environmental conditions and physiological factors influence the quality and shelf-life of horticulture crops, but also agriculture management strategies could change the quality parameters of horticulture crops. Firmness is an important quality parameter that determines the eating quality of table grapes (Vitis vinifera). The softening of grape berries during storage has been commonly associated with cell walls pectic polysaccharides decomposition and water loss. To investigate the softening of grape berries during storage, we compared the pectin methylyeraseterase (PME) and poly galacturronase (PG) genes expression, water loss content and firmness of organic and inorganic grape berries stored at 1± 1 A°C and 90-95 RH at 0, 30 and 60 days of storage. Total RNA was isolated from frozen grape berry skin tissue. The accumulation of mRNA encoding PME and PG was examined using qRT-PCR. Our results indicate that the expression of PME and PG of grape berry skin was significantly different between organic and inorganic berries at harvest time and during storage. The pattern of PME and PG genes expression in each period of storage time was varied between organic and inorganic berries. However, at the end of storage the highest PME gene expression, water loss content and softening was observed in organic grape berries. PG expression was varied during storage and at the end of storage, the higher PG expression was observed in inorganic berries compared to organic samples. These results suggest that the expression of PME and PG genes in relation to softening modify in response to agricultural management.

**Keyword:** qRT-PCR, Organic, Quality, Softening

P-421: Effect of plant growth regulators on callus production in different explants type of Black cumin as an important medicinal plant

**Zebarjadi A**, **Miri N**

Campus of Agriculture and Natural Resources, Razi university, Kermanshah, Iran.
Zebarjadi@razi.ac.ir

Black cumin (Nigella sativa) is a medicinal plant of the Ranunculaceae family that is used for treatment a range of diseases. This plant has important secondary metabolites that are used in pharmaceutical industry. The purpose of current study was optimization of callus production for increasing secondary metabolites, especially Thymoquinone. In experiment of tissue culture to finding the best hormonal combination for callus induction, experiment was laid out in a factorial arrangement based on completely randomized design (CRD) with three replications. Experimental factors including explants in three levels of (stems, leaves and cotyledons) and plant growth regulators BAP in five levels (0, 0/5, 1, 1/5 and 2 mg/l) and IAA in five levels (0, 1, 2, 3 and 4 mg/l). As the data was not normal, the treatments with zero score was removed and data were analyzed based on completely randomized
design with 50 combined treatments. The results of analysis of variance (ANOVA) showed that the treatments were significant on callus formation. The percentage of explants producing callus ranged from 40 to 100%. The results of mean comparison showed that the best explant for callus induction was stem in combinations of hormone (1/5 mg/l BAP and 2 mg/l IAA) as well as (2 mg/l BAP and 4 mg/l IAA) with 100% callus induction.

**Keyword:** Nigella sativa, Callus induction, PGRs, Tissue culture

**Genetic of Microorganisems**

P-422: RT-PCR detection and identification of Fig Mosaic Virus (FMV)â€“ Arak isolates

Abtahi FS, Hatami M

Department of Medicinal Plants, Faculty of Agriculture and Natural Resources, Arak University, Arak 38156-8-8349, Iran

fauze.abtahi@gmail.com

Common fig (Ficus carica L.) is a deciduous shrub with smooth white bark. Its leaves are deeply lobed with three or five lobes in the mulberry family. Figs can be eaten fresh or dried, and used in jam-making. It contain diverse phytochemicals, including polyphenols such as gallic acid, chlorogenic acid and syringic acid. Fig mosaic disease is recognized as economically important due to its worldwide occurrence and to premature fruit drop. Several viruses and viroids have been identified with this disease. Fig mosaic virus (FMV) is the type species of the genus Emaravirus in the family Bunyaviridae and is transmitted mainly by the eriophyid mite Aceria ficus. During autumn of 2016, a total number of 46 Common fig leaves with symptoms including mosaic and yellowing were collected from Arak. Total RNA was extracted using with Column RNA isolation kit (Denazist Asia, Iran) and were used in reverse transcription polymerase chain reaction (RT-PCR) using specific primer pair to amplify a partial sequence of glycoprotein (GP). Synthesis of cDNA was performed using FMV-GP-R (TATTACCTTGATCAACGCGA) and cDNA product was subjected to PCR using 0.4 pmol of FMV-GP-R and FMV-GP-F (ACTTGCAAAGGCAGATGATA). A fragment with 700bp in length, in 12 samples was amplified. The amplified fragments were sequenced in 1% agarose gel. In investigation of the specificity of primers, only formed the intended bands with Mycoplasma orale DNA. From 100 tested samples, only nine (9%) were positive.

**Conclusion:** according to studies, it was found that PCR method is an appropriate method for detection of Mycoplasma orale. While a high percent of contamination of the biological products is due to of this agent.

**Keywords:** Mycoplasma orale, biological products, contamination, Molecular detection, PCR

P-424: Multilocus sequence typing) MLST( detection of diarrgenic Escherichia coli pathotypes

Akhavan Attar F, Pourramezan Z, bouzari S, Mostan S, Oloomi M

Molecular Biology Department, Pasteur Institute of Iran, Tehran-Iran

Sara.akhavan73atar@gmail.com

Escherichia coli (E. coli) are usually a harmless commensal normal inhabitant of gut flora in humans. Some strains have the capacity to cause disease in human and animals by specific pathogenic mechanisms that in some cases can cause to infection resulting to death. Serotyping is a method for classification of E. coli that has been developed into standardized procedures. This method is one of the times and cost consuming that needs experts and antisera. Typing can be done only in some of the laboratories. In fact, typing methods can be used for strains classification by different methods. Despite serotyping and phenotyping there are usual genotyping methods such as RFLP and pulsed-field gel electrophoresis (PFGE). Multilocus Sequence Typing (MLST) (method can also use for strain discrimination.

In this study, 50 E. coli strains from National E. coli Reference Laboratory were used. DNA from classified strain types was isolated from cultured strains and assessed.

Multilocus Sequence Typing (MLST) with 7 housekeeping genes: adk (adenylate kinase), fum C (fumarate hydratase), gyrB (DNA gyrase), icd (isocitrate dehydrogenase), mdh (malate dehydrogenase), purA (adenylsuccinate synthetase), and recA (adenosine triphosphate/guanosine triphosphate binding motif) used. These primers suggested by Pasteur institute (http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html).
P-425: Cloning, expression and purification of Phospholipase from Natrialba asiatica

Allami Mehmandoosti F1, Ebrahimimoghadam L2, Zakiaghl E3, Jafarpour B2, Mehrvar M2

1. Department of Biology, Islamic Azad University, Science and Research Branch, Tehran, Iran
2. College of Agriculture, Ferdowsi University of Mashhad; 3. Department of Biology, Islamic Azad University, Varamin-Pishva Branch, Tehran, Iran

Ebrahimi-moghadam@stu.um.ac.ir

Phospholipases (EC 3.1.1 Carboxylesterase) are interfacial enzymes that hydrolyze hydrophobic ester linkages of triacylglycerols and phospholipids. In addition to their role as esterases, these enzymes catalyze other reactions such as saponification, transesterification and interesterification. Microbial phospholipases are preferred to those derived from animals and plants. Phospholipases are used in various industrial, such as for biodiesels, food, nutraceuticals, oil degumming and detergents, also include bioremediation, agriculture, cosmetics, leather and paper industries. In the present investigation, phospholipase from halophilic archaea Natrialba asiatica was amplified by PCR using specific primers (containing restriction sites EcoRI and HindIII). Then pET-28a as cloning vector was extracted. Next pET-28a and phospholipase gene were digested by restriction enzymes and ligated. PET-28a containing phospholipase was transformed into E.coli DH5α cells. Screening carried out using LB-agar plates containing kanamycin. After double digestion, the nucleotide sequencing was confirmed using universal T7 promoter by macrogene Korea. The confirmed gene was transferred into E.coli BL21, and cultured up to OD600-nm ~2/5 in LB medium. Then was added 0.5mM IPTG and induced for 45 min at 37°C. Recombinant His-tagged protein purified by affinity chromatography demonstrated a band about 35 kDa on 12.5% SDS-PAGE gel. Screening the process of cloning. The size and sequence of target DNA fragments were confirmed by agarose gel electrophoresis and the sequencing data, respectively. The isolated DNA segment exhibited 99% identity with A. eutrophus H16 phbC gene in NCBI Nucleotide database.

Keywords: PhbC, Alcaligenes eutrophus, cloning.

P-427: Determination of molecular interaction between Citrus exocortis viroid, Hop stunt viroid, and Citrus viroid V in tomato plants

Ebrahimi-moghadam L1, Zakiaghl E2, Jafarpour B3, Mehrvar M4

1. college of Agriculture, Ferdowsi university of Mashhad; 2. Plant protection department, college of Agriculture, Ferdowsi university of Mashhad

ebrahimi-moghadam@stu.um.ac.ir

Viroids are the smallest known plant pathogens without protein encoding capacity. CEVd, HSVd and CVdV are there important citrus viroids. In order to determine biological interaction between CEVd, HSVd and CVdV, infectious clones of viroids were constructed. The semiquantitative real time PCR results revealed antagonist effect between these viroids that cause significant reduction in the viroid titer in mixed infection of CEVd, HSVd and CVdV.

Keywords: CEVd, HSVd, CVdV, interaction

P-428: Cloning and prokaryotic expression of coat protein gene of Peanut stunt virus

Farzadfar Sh, Pourrahim R

Plant Virus Research Department, Iranian Research Institute of Plant Protection (IRIPP), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

farzadfar2002@yahoo.com

Peanut stunt virus (PSV) a member of the genus Cucumovirus, is considered as one of the important pathogens on a wide range of plants including legume crops. During a survey, 15 out of 38 alfalfa samples tested (39%) were found to be infected with PSV. Coat protein (CP) gene of an Iranian PSV isolate (PVS-Ir17) was cloned, sequenced and expressed in prokaryotic cells. First, CP gene of an Iranian PSV isolate was amplified by reverse transcription-polymerase chain reaction (RT-PCR) using specific primers. The complete CP gene nucleotide sequence of our PSV isolate (PVS-Ir17) was determined and shown to be 663 nt long with an open reading frame (ORF) of 221 amino acids. The nucleotide sequence of amplified CP gene showed the highest identity (91.6%) with W strain belonged to subgroup II of PVS. Amplified CP gene
of PSV-Ir17 was sub-cloned in the expression vector pET21 and transformed to BL21 cells. SDS-PAGE analysis revealed expression of about 31 kDa protein product in induced bacterial cells. The prokaryotic expressed 31 kDa protein showed specific reaction with the reference antibody against PSV in Western Blot analysis confirming the PSV-CP nature of the expressed protein. The prokaryotic expressed PSV-CP protein would be used in raising PSV specific antibody which has a great importance in national programs of virus-free seed production.

**Keywords:** Viral disease, Coat protein gene, expression

**P-429: Molecular epidemiology and genetic proximity of Human Papillomavirus by the PCR-sequencing method in the women of Qom city**

**Fotouhi-Ardakani R, alirezaei M**

Cellular and Molecular Research Center, Clinical Laboratory Science Department, Qom University of Medical Sciences, Qom, Iran.

rfardacani@gmail.com

The genetic polymorphisms and genetic evolution of human papillomavirus (HPV) as predictive markers of cancer as well as epidemiological studies of genital HPV infection in the general population, will play a significant role in order to plan, determine the strategy of health and promote the level and development of healthcare and treatment. This study was conducted for the first time in Qom Province, considering the risk factors associated with HPV, which it was able to analyze genetic evolution in genotypes. 486 Pap smear samples were tested for HPV DNA, positive samples were sequencing and submission in the GenBank (MG825048-61). Then, after alignment phylogenetic and polymorphism analysis was performed on sequenced samples with a number of GenBank sequences. Out of 486 married women who studied, 57 (11.7%) have been infected with the HPV virus. The results of sequencing showed that the genotypes 6, 11 and 61, as well as 16, 51 and 58, were the high-risk and low-risk genotypes, respectively. Investigating the genetic polymorphism of the selected region in different genotypes of the HPV showed 72.5% genetic variation, most of the nucleotide changes were singleton, non-informative, and only 36.2% of the changes led to altered amino acid but ultimately, the virus has kept its essence. In the case of phylogeny analysis, the trees plotted in both methods of Neighbor-Joining and Maximum Likelihood showed the same topology and were able to firmly separate the different genotypes of HPV. This is the first molecular epidemiology study that describes genetic polymorphism and phylogenetic analysis of HPV in Qom province.

**Keywords:** Human papillomavirus, polymorphism, genetic evolution, molecular epidemiology, Qom

**P-430: Genetic engineering of microorganisms for Isolation and identification of acrylamide decomposing bacteria from polymeric wastewaters in Italy**

**Gholamhosseinpour F*, Tabrizi Z*, Kazemian K*+, PharmD, BCCP, Nezamivand chegini S**

1. Scuola Superiore Sant’Anna, Pisa, Italy

Acrylamide is a chemical compound and many applications in the industry of the material in the manufacture of conscience, the stability of the dam, the water as a flocculation in the paint industry, and in medical and scientific laboratories as solid anchoring proteins separated by electrophoresis is used to maintain. There are so many used in industry such as acrylamide is released to the environment. Acrylamide is a toxic compound this article makes nephropathy, neurotoxic effects, and interactions with protein in neurons and cell death as well as reduced reproduction in laboratory animals. Acrylamide causes DNA damage. some microorganism is used acrylamide as a carbon source. At first, a sample of the intended environment has been prepared. then this sample will be transferred to the laboratory under standard conditions and will be inoculated in culture environments. In this paper, culture environment of BHI and MSM have been used. Finally, an acrylamide resistant colonies were isolated which was further tested. The main object of treatment units is to reduce the sewage contents (solids) from the sewage and remove all the nuisance-causing elements and change the character of the sewage in such a way that it can be safely discharged in natural watercourse applied on the land. Screening is the very first operation carried out at a sewage treatment plant and consists of passing the raw sewage through different types of screens so as to trap and remove the floating matter such as tree leaves, paper, gravel, timber pieces, rags, fiber, tampons, cans, and kitchen refuse etc. Microorganisms present in sewage can tolerate metal concentrations toxic to human beings, such as cobalt, lead, nickel, chromium etc., and also antibiotics. Various experiments are to characterize and identify the microorganisms present in the domestic sewage water collected.

**Keywords:** polyacrylamide; biodegradation; dewatered sludge; microorganisms

**P-431: Expression of a halostable phytase from Nesterenkonia sp. strain F in Escherichia coli**

**Ghomeshi F*1, 2, shafiei M1**

1. Department of Genetics, Faculty of Science, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Biotechnology and bioscience research center, Shahid Chamran University of Ahvaz, Ahvaz, Iran

m.shafiei@scu.ac.ir

Phytases hydrolyse phytate (myo-inositol hexakisphosphate), the principal of phosphate stored in plant seeds to produce phosphate and lower phosphorylated myo-inositol.
The gene encoding phytase from Nesterenkonia sp. strain F was cloned, sequenced and expression in Escherichia coli. Expression was induced by IPTG at a concentration of 0.5 mM. Enzyme activity was performed in the presence of phytic acid.
the optimal PH and temperature of the enzyme were PH 6.5-7.5 and 20°C, respectively. Genetic cloning reveals that the protein of this enzyme consist of 441 amino acid with a calculated molecular weight of 48 KDa. A higher enzyme activity was obtained when the gene expression was done in the presence of NaCl.

We have successfully expressed the cloned gene in Escherichia coli from its putative initiation codon, showing that the gene actually encodes the phytase. Due to its pH profile and optimum, it could be an interesting candidate for various application.

Keywords: Cloning, expression, Halophilic phytase, Nesterenkonia sp. strain F, Escherichia coli

P-432: Study of the effect of probiotic Lactobacillus acidophilus on expression of tir gene in intestinal Escherichia coli

Hadavand F*, Mirzaee M2, Mehrabi M2

1. Borujerd Branch, Islamic Azad University, Borujerd, Iran.
2. Department of Laboratory sciences, Borujerd Branch, Islamic Azad University, Borujerd, Iran.

Fateme.hadavand94@gmail.com

Probiotics play an important role in maintaining the balance and stability of the intestinal microbiota; microbiota contributes to digestive functions and gastrointestinal system activity. Escherichia coli is a common cause of food poisoning. Some species are found tightly in the intestines of animals and humans. There are about hundreds of species of Escherichia coli, most of which are harmful. The purpose of the present study was to investigate the probiotic effect on tir expression in Escherichia coli (EPEC).

METHODS: In this study, the probiotic sample Lactobacillus acidophilus was prepared by Pishgam Company, (Iran-Tehran). After the effects of probiotics on the expression of tir gene, a Real Time-PCR technique was used to determine the probiotic effect.

RESULTS: Therefore, performing the test steps of the results of the gene expression test showed that the probiotic Lactobacillus acidophilus had a significant effect on tir expression. This means that the presence of probiotics along with the EPEC can reduce the expression of the pathogen gene.

CONCLUSION: The obtained results can be deduced that probiotics reduce the pathogenicity of EPEC bacteria due to the effect on the tir gene.

Keywords: Escherichia coli, tir, probiotic Lactobacillus acidophilus, Real Time-PCR

P-433: Isolation and Molecular Characterization of Several Phytase Producing Bacterial Cells from the Soil of Alfalfa and Clover Fields

Hashem Maturi Z1, Gerami M2, Ghaedi K2

1. Genetic department, Sana Institute for Higher Education, Sari, Iran
2. Biology Department, Faculty of Sciences, University of Isfahan, Isfahan, Iran

melica294@yahoo.com

Background: Phytases are a group of enzymes which are responsible for decomposition of phytic acid components. Due to accumulation of these components in diets of livestock animals, the aim of this study was isolation of phytase producing bacterial cells to extract this enzyme for further applications in break-down of phytate molecules.

Materials and methods: Nearly 8 soil samples were gathered from alfalfa and clover fields of rural areas around Isfahan city (Khomeini Shahr and Murchekhort). Through a serial of differential bacterial cultures, a number of bacterial cells were isolated and purified. Purified bacteria were placed on specific media enriched with phytate to estimate phytase activity. At the next step, phytase positive cells were tested according to biochemical analyses and 16srRNA sequencing.

Results: Among a variety of bacterial cells, two different colonies were distinct in terms of phytase activity. Both strains were identified to be bacilli according to differential biochemical indications including glucose, nitrate assay. Molecular analysis of genomic 16srRNA revealed one strain belongs to the Bacillus licheniformis, and Bacillus endophyticus. Both are commonly found in the soil.

Discussion: Despite isolation of these two strains of bacterial cells which exhibited phytase activity, further characterizations are needed to figure out molecular properties of phytase extracted from these cells. Isolated pytase could be applied for decomposing of phytic acid derivatives.

Keywords: 16srRNA, Bacillus licheniformis, Bacillus endophyticus, Phytase

P-434: Antimicrobial activity investigation of Multi-Walled carbon nanotubes on Staphylococcus aureus

Jannati H1, Siadat SD2,3, Safarian P1, Sheikhpour M2,1

1. Department of biology, Science and Research branch, Islamic Azad University, Tehran, Iran.
2. Department of Mycobacteriology and Pulmonary Research, Pasteur institute of Iran, Tehran, Iran
3. Microbiology Research Center (MRC), Pasteur institute of Iran, Tehran, Iran

m_sheikhpour@pasteur.ac.ir

Background: Staphylococcus aureus is a gram-positive bacterium and has been considered as an important nosocomial pathogen worldwide owing to its increasing antibiotic resistance. Carbon Nanotubes (CNTs) through their unique properties hold great promise in the fight against multidrug-resistant bacterial infections. Aim: In this study, Antimicrobial activity investigation of Multi-Walled carbon nanotubes on Staphylococcus aureus was done. Methods: Multi-wall carbon nanotubes were provided from US Research and were added to the 7H9 bacterial culture medium in the concentration range of 0.1 to 1%. After a period of exposure, Anti-proliferation effect of CNTs was investigated by the Microplate Alamarblue Assay (MABA) method. Results: Antimicrobial potential of multi-wall carbon nanotubes on Staphylococcus aureus was found based on the color change associated with the specified concentration range on the bacterial growth rate. Conclusion: This study showed that multi-wall carbon nanotubes can have antimicrobial effects on Staphylococcus aureus although to get more accurate results, we are doing more specialized cellular and molecular investigation.

Keywords: Antimicrobial activity, Staphylococcus aureus, Multi-walled carbon nano tubes (MWCNTs)

P-435: Synthetic construct of Homo sapiens clone IFNB1 gene encoding complete protein in prokaryotes

Karimi Naeini M, Ghorbani M*, Rad R, Norouzzadeh Alinodeh N
1. MSc Student, payame noor university, molocular genetics.
2. Assistant professor, Research and Production Complex Pasteur Institute of Iran.
3. Assistant professor, Research and Production Complex Pasteur Institute of Iran.
4. MsC Student, Islamic Azad university of Varamin, molocular genetics
mahkarimi46@gmail.com

Introduction: Interferons are antiviral and anti-proliferative cytokines that are made and secreted by vertebrate cells. Interferon beta can regulate immune responses and is used as the main medicine in many diseases, including multiple sclerosis.

Goals: At the global level, the expression of recombinant protein IFNB is considered as a standard method for medicinal and therapeutic usage in human. In this research, we tried to apply a simple and low-cost process for protein production.

Materials and Methods: In this study, the IFNB1-b was amplified by polymerase chain reaction (PCR), and sub-cloned in prokaryotic expression vector PET32a. E.coli BL21(DE3) was transformed with PET32a/IFNB1-b and gene expression was induced by IPTG. Afterwards, cells analyzed by 12% SDS-PAGE. Recombinant IFNB1-b was expressed in this system with 6xhis tag at C-terminus and thioredoxin tag at N-terminus. The expressed protein was purified by affinity-chromatography using (Ni-NTA) resin.

Results: PCR and sequencing results confirmed the successful cloning of the target gene into the vector. SDS-PAGE analysis showed the high level expression of IFNB1b protein and high concentration of the recombinant protein was obtained via the purification process by affinity-chromatography. This gene has later been registered in GenBank with the accession number of MF678818.

Conclusion: The expression of IFNB1b was low when cloned under the T7 promoter without any fusion tags. In this study, in order to increase the solubility of the recombinant protein, we fused the IFNB1b gene to thioredoxin and 6xhis tag. In this research the yield of recombinant IFNB1b protein was increased significantly by 840ug/ml.

Keywords: PCR, Recombinant protein, interferon beta gene, Escherichia coli.

P-436: Optimization of a molecular technique for diagnos- is of bovine Eimeria species based on Internal transcribed spacer region

Karimi Naghlani Sh1, Esmaelizad M1, Esmaelnia K1, Razmarail N1, Hamzehali Tehrani M1, Sanchooli A1
shahlaab57@yahoo.com

The most economically significant disease of cattle throughout the world is Bovine coccidiosis caused by Eimeria infection. There have been no molecular techniques for detection of this parasite in Iran yet. Hence, the purpose of the current study was to set up a molecular method-based on ITS1 gene for detection of this pathogenic agent in calves’ samples. In first step, 164 available ITS1 nucleotide sequences from eleven Eimeria species were collected from GenBank. Complete ribosomal DNA sequences with 2984 bp includes 18srRNA, ITS1, 5.8srRNA, ITS2 and 28srRNA were compared between different Eimeria species. The conserve and hypervariable regions were identified.

A set of novel degenerated primers was designed in conserved sequences of flanking hypervariable region of internal transcribed spacer 1 based on multiple alignments of all species of Eimeria ITS1 nucleotide sequences. Ten stool samples were collected from infected bovine from Alborz province. Oocysts were separated from the fecal debris and concentrated by flotation technique using saturated sucrose solution. Genomic DNA was extracted by MBST kit. The PCR condition was optimized. The presence of Eimeria genus genomic DNA was tested by PCR amplification of the ITS1 sequence using the universal primer.

The results of our study were showed that these Novel degenerated primers might be used for amplification of hypervariable region of ITS1 and diagnosis of Eimeria. This is the first intention for the identification of Eimeria parasites in the genomic level in Iran thus provide as useful methods for diagnosis and help implement strategies to control the parasites.

Keywords: Molecular detection technique, ITS1, Bovine coccidiosis

P-437: Detection of the Endosymbiotic bacteria Arseno- phonus in the beef leafhopper populations (Circulifer haematoceps; Insecta: Hemiptera) from Khorasan Razavi province.

Mehrabi Nasab A1, Zakiaghi M1
Department of Plant Protection, College of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran. rahman.mehrabi@gmail.com

Different species of endosymbiotic bacteria have been reported from insects. Beet leafhopper is transmitting numerous viruses such as beet curly top virus. In order to study prevalence of the endosymbiont bacteria Arsenophonus in beet leafhopper population, samples were taken from Khorasan Razavi beet farms and by 16s rDNAs were amplified using specific primers in polymerase chain reaction. The amplicon were sequenced. In the represented polygenetic tree, Arsenophonus strains were categorized in four subgroups. Also, the Iranian strains were closely related to isolates which reported from Bemisia tabaci in Pakistan

Keywords: Primary and Secondary Endosymbiotic, sugar beet, Endosymbiotic bacteria, Arsenophonus, phylogenetic analysis

P-438: Construction of a novel stress- induced expression system in Escherichia coli for asparaginase II production

Mokhtari B, Bambai B
National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
mokhtari_ccr7@yahoo.com

L-asparaginase (ASNase) is an enzymatic drug and an essential component of the combination chemotherapy against diseases such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia, chronic lymphocytic leukemia. Bacterial asparaginases, especially asparaginase II (ansBII) from Escherichia coli gained more importance in therapeutic industry to high substrate specificity and long half-life. One of the highest specific activity and expression level of recombinant AnsBs were obtained by strong inducible T7 promoter. Isopropyl-β-D-thiogalactopyranoside (IPTG) is routinely used as inducer of T7 promoter. However, there are two main drawbacks in application of IPTG in production of...
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Biopharmaceuticals: the toxicity and high cost of IPTG. Here, we are striving to produce L-Asparaginase II under influence of a stress-inducible expression vector without IPTG. In this study, the appropriate regulon (XXXX) was selected and synthesized as a derivative of pET vector based on the induction of stress, including nutrient deficiencies and low osmotic pressure. Then the native asnBII sequence was cloned into pET-derived vector and competent E. coli cells were transformed with this construct. Furthermore, the bacteria were grown under stress conditions and protein expression was investigated both in the supernatant and cell pellets in different time intervals from 6 to 36 hours. The results of SDS-PAGE demonstrated that the expression level of the asparaginase II gene increased with the passage of time and specifically with the end of the logarithmic phase. Our preliminary results demonstrate the applicability of our expression system as a safe and economic method for production of recombinant therapeutic enzymes.

Keywords: Asparaginase II, Acute Lymphoblastic Leukemia (ALL), Stressed Induced promoter

P-439: A new DNA vaccine encoding fusion antigens from Mycobacterium tuberculosis showed strong immune responses in mice

Moradi B, Meshkat Z

Esfarayen Faculty of medical sciences, Esfarayen, Iran
moradib901@gmail.com

Introduction: Today, Tuberculosis (TB) is an important health problem and DNA vaccines are developed due to their ability in generating a long-lasting immune response and more safety compared to the live vaccines. In the present study, we evaluated a new DNA vaccine encoding fusion hspX-ppE44-esxV antigens, separately, and in combination with BCG in a prime-boost method in mice.

Method: Immunogenicity of the recombinant vector containing PPE44-ExxV-HspX fusion product was evaluated in a mouse model. Mice were separated into 5 groups (6 mice per group) and were injected three times intramuscularly in the quadriceps muscles with 100µg of pDNA at 2-week intervals. Three weeks after the last immunization, mice were sacrificed and their spleen was extracted aseptically. Splenic cell cultures were exposed to the purified PPE44-ExxV-HspX fusion protein product Cell culture supernatants were harvested and the concentration of IFN?, IL-12, IL-4 and TGF-? Cytokines were measured by ELISA kits.

Results: After cytokine assay, the concentrations of IFN? and IL-12 in the cell culture of spleen cells were significantly increased (799.11±135.51 pg/ml and 92.88±19.50 pg/ml, respectively, compared to the control group (P <0.001)

Conclusion: DNA vaccine encoding HspX-PPE44-ExxV fusion antigen of Mycobacterium tuberculosis was produced successfully in the previous study and it was evaluated in the present study. The results of cytokine production in spleen cell cultures showed that this DNA vaccine can stimulate cellular immune responses that can be used as a vaccine candidate in the production of new and effective vaccines against TB.

Keywords: Mycobacterium tuberculosis, Hsp, PPD protein, BCG vaccine, DNA vaccines

P-440: Prevalence and distribution of the stx1, stx2 genes in Shiga toxin producing E. coli (O157:H7) Strains Isolated from Beef Slaughters in Industrial Abattoirs of Iran

Mounesan N1, Froghi N2, Gorbani Ranjbary A2

1. School of Medicine, Shahroud University of Medical Sciences, Shahroud, Iran.
2. Department of Biotechnology, School of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran
javad.foroughi@gmail.com

E. coli O157:H7 is one of the most important pathogenic bacteria which can result in haemorrhagic colitis in humans. Livestock are the main reservoir of this bacterium. The present study aimed at determining the pattern of drug resistance and studying stx1 and stx2 pathogenic genes in E. coli O157:H7 isolated from beef slaughters in industrial abattoirs of Fars province, southern Iran. In this study, 360 samples of meat were collected from the cattle slaughtered in spring and summer of 2014 in industrial abattoirs of the cities Kazerun, Fasa, and Shiraz. The specimens were sampled and transferred to the Microbiology Laboratory of Kazerun School of Veterinary. They were immediately cultured and microbiologically analyzed, and the colonies suspected of E. coli O157:H7 were evaluated by Multiplex PCR using two primers for pathogenic gene stx1. After identifying antibiotic resistance, the strains were evaluated by disc diffusion method. The results showed that from a total of 360 samples collected in spring and summer, only 11 samples (3.05%), 3 in spring and 9 in summer, were contaminated with E. coli O157:H7, but the pathogenic genes stx1 and stx2 were not found in any sample. Kazerun was the most contaminated among the studied cities with 6 samples. All bacteria were resistant to penicillin, erythromycin, and ampicillin antibiotics. A high percentage of the bacteria were also resistant to other antibiotics; 66.66% to cephalaxin and gentamicin. But all of the isolated bacteria were sensitive to phenomenological. Conclusion: This study showed that the contamination rate of beef slaughters with E. coli O157:H7 in industrial abattoirs of Fars province was low; however, a high percentage of isolated bacteria were highly resistant to antibiotics in particular, cephalaxin and gentamicin.

Keywords: Escherichia coli O157:H7, Zoonosis, antibiotic resistance, virulence genes

P-441: Expression of recombinant chains of anti-VEGF monoclonal Ab in yeast

Movaghar Asareh Sh, Arjmand S, Fatemi F, Ranaii Siadat SO

Protein Research Center, G.C., Shahid Beheshti University
shirin.m.asareh@gmail.com

INTRODUCTION: Age-related macular degeneration (AMD) is the major cause of blindness in the elderly and vision loss in patients with diabetic retinopathy. This complication is usually associated with uncontrolled secretion of VEGF. Anti-VEGFs medications, with different generic names like Bevacizumab, Pegabtanib, Ranibizumab are the common treatments used to control the disease progression.

OBJECTIVE: The aim of present study is to produce the two recombinant monoclonal antibody chains in yeast Pichia pastoris and evaluate the whole antibody for interaction with VEGF.

MATERIALS AND METHODS: The sequence of light and heavy chains of antibody were cloned in the pPinkH-C
P-442: Genetic variation of Saffron latent virus (SaLV) based on CP and P1 genomic regions in Iran

Movi Sh1, Dizadj A2, Parizad Sh1, Nourinejahd Zarghani Sh2
1. Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
2. Department of Plant Protection, College of Aburaihan, University of Tehran, Tehran, Iran

Saffron (Crocus sativus L., Iridaceae) is one of the most important industrial and export products in Iran. In this study, genetic diversity of new potyvirus Saffron latent virus (SaLV) was investigated based on CP and P1 genomic regions. Following random sampling of saffron plants from Razavi and South Khorasan, Isfahan, Tehran and Fars provinces, SaLV-infected samples were determined by ELISA. Reverse transcription-polymerase chain reaction (RT-PCR) was performed by specific primer pairs corresponding to CP and P1 genomic regions and amplified fragments of fifteen isolates were cloned and subsequently sequenced. Multiple alignments of CP and P1 nucleotide sequences were performed by MEGA 7 and genetic diversity and population structure parameters were assessed by DnaSP v.5. Based on the results, genetic diversity among the isolates in CP region (0.021 ± 0.003) was less than that of P1 region (0.049 ± 0.004), dN/dS value in CP and P1 regions were less than one, indicating that α-frenegative selection was imposed on the CP and P1 regions to maintain conservation of protein sequences. dN/dS value of P1 (0.182) was more than that of CP (0.0139), indicating the less conservation and natural selection pressure on the P1 region than the CP region. FST values of both genomic regions among geographical subpopulations were < 0.33, as an evidence of ongoing gene flow between subpopulations. These results are consistent with vegetative propagation of saffron through corms from the main origin of saffron corms (Razavi Khorasan and South Khorasan) to other regions of Iran.

Keywords: Genetic variation, Potyvirus, Saffron

P-443: Evaluation of kinetic parameters of a streptokinase protein with two point mutations in the Beta and Gamma domains

Norouzzadeh Alinodehi N1, ArabiMianroodi R2, Baghbani-arani F1
1. Department of Genetics and Biotechnology, Faculty of Biological Sciences, Varamin-Pishva Branch, Islamic Azad University, Varamin, Iran.
2. Research and Production Complex, Pasteur Institute of Iran, Karaj, Iran

In this study, the molecular identification of a bacterium, isolated from PE waste depot was carried out. The isolate was cultured in a medium with PE as the sole carbon source. DNA extraction with TE buffer was carried out and the 16S rRNA-encoding DNA sequences were amplified by universal primers of 27 F and 1492 R. The size of the PCR product was about ~1500 bp as observed in an agarose gel. PCR product was sequenced and the readings from each end of the gene were analyzed by the Biodesit Software. The complete sequence was aligned with multiple sequences in the NCBI database using Blastn and sequence from 42 other bacterial was used by MEGA software to draw the phylogeny tree.

After the alignment was analyzed, it was observed that isolates of two genus’s of Diaphorobacter and Acidovorax were closely related to the isolate examined; finally, the phylogenetic tree determined that Acidovorax ebreus was the closest species with 99% similarity.

Keywords: 16S rRNA, PE, biodegradation, Acidovorax ebreus.
P-445: Detection and characterization of Pepino mosaic virus isolates in South Iran

Pourrahim R, Farzadfar Sh
pourrahim@yahoo.com

Plant Virus Research Department, Iranian Research Institute of Plant Protection (IRIPP), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran.

A total of 44 and 51 symptomatic eggplant samples showing mosaic, mottle, chlorosis, necrosis, rosettes and deformatons were collected from Khuzestan and Hormozghan provinces, respectively, two main eggplant producing areas. Samples were tested for Pepino mosaic virus (PepMV) infection using ELISA. Virus infection was recorded in 9 samples. Results of host range studies on seven PepMV isolates showed that six PepMV isolates have biological properties similar to EU genotype and one PepMV isolate (HO6) showed host range properties similar to CH2 genotype. Coat protein (CP) gene of seven PepMV isolates was amplified using specific primers by RT-PCR and their sequence was determined which includes 714 nt in length. CP sequence of seven Iranian PepMV isolates were compared with other 17 PepMV isolates and a phylogenetic tree reconstructed based on NJ method. Six Iranian PepMV isolates clustered in EU genotype group and HO6 isolate was grouped in CH2 group. Six Iranian PepMV isolates from EU genotype showed the highest CP sequence identity (99.4-99.7%) with Fr (France, AJ438767), NV (Latvian, JQ971969) and Sp.13 (Spain, AF484251) isolates and HO6 isolate from CH2 genotype showed the highest identity with PMU.08.35 (Spain, FJ263357) isolate. There was no clear evidence of recombination in CP gene of the seven Iranian PepMV isolates. These results revealed the presence of PepMV isolates for the first time in eggplant fields of Iran and it seems that PepMV has been introduced to Iran in more than one time.

Keywords: Pepino mosaic virus, coat protein gene, genetic variation

P-446: Characterization of 16SrII-D phytoplasma strains associated with witches’broom disease of Medicago sativa cv. bami in Yazd and Kerman provinces, Iran

Pourmohamadi S1, Esmailzadeh Hosseini SA2

1. Department of Agricultural Science, Abbar Kavir Co., Yazd, Iran
2. Plant Protection Research Department, Yazd Agricultural and Natural Resources Research and Education Center, AREEO, Yazd, Iran

saesmailzadeh@iripp.ir

Alfalfa witches’broom (AWB) is one of the most important alfalfa diseases in Iran. To characterize phytoplasmas associated with Medicago sativa cv. bami in Yazd and Kerman provinces, During 2014-2016, a survey was carried out in Herat (Yazd province) and Bam (Kerman province) and samples with little leaf, yellowing, witches’broom, dwarfish, flower virecence, phyllody, proliferation and sterility symptoms were collected and used for molecular studies. 44 symptomatic and 6 asymptomatic plants were subjected to direct and nested polymerase chain reaction (PCR) using P1/P7, R16mF2/R16mR2 and R16F2n/R16R2 primer pairs. PCR amplicons of ~1.8, ~1.4 and ~1.25 kb respectively, were obtained only from symptomatic plants. Restriction fragment length polymorphism (RFLP) analysis of R16F2n/R16R2 amplicons using Rsal, HaeIII, TaqI, Alul and MseI restriction enzymes were identical in all samples and showed that the associated phytoplasmas were members of 16SrI-II group. Among AWB phytoplasmas strains, one sequences of the R16mF2/R16mR2 amplicons from Herat and Bam were assembled to the fragments corresponding to the R16F2n/R16R2 amplicons and deposited in GenBank as accession numbers MG760450 and MG845399 respectively. Blast analysis, sequence homology and phylogenetic analysis confirmed the clustering of these phytoplasmas with those enclosed in 16SrI-II group. Computer-simulated restriction analysis using iPhyClassifier indicated that virtual RFLP patterns of sequenced phytoplasma strains were identical (similarity coefficient 1.00) to the reference pattern of 16SrI-II phytoplasmas (Y10097). Due to the distribution of 16SrI-II subgroup all over the Yazd and Kerman provinces on other plant species it seems that AWB strains play important roles in their epidemiology.

Keywords: 16SrI-II-D subgroup, PCR, RFLP

P-447: Isolation of rennin gene from Rhizomucor miehei and it cloning in E. coli expression vector

Rashedi P1, Zare N2, Fathi-Achachlouei B3, Asgarie Zacaria R2

1. Department of Agricultural Biotechnology, University of Mohaghegh Ardabili, Ardabil, Iran
2. Department of Agronomy and Plant Breeding, University of Mohaghegh Ardabili, Ardabil, Iran.
3. Department of Food Science and Technology, University of Mohaghegh Ardabili, Ardabil, Iran.
zarenasser@yahoo.com

The utilization of renin in food production has a long history and is essential for cheese production worldwide. This enzyme increases the coagulation rate of the milk casein, the main protein of the milk. In this study, we cloned the coding sequence (CDS) of renin gene from Rhizomucor miehei was obtained from the NCBI database, and specific primers were designed to amplify the renin gene. To facilitate gene cloning in the pET-28a expression vector, restriction site of BamHI and EcoRI enzymes were added to the 5’ ends of the primers. In the next step, total RNA was extracted from Rhizomucor miehei mycelium and then cDNA synthesized using oligo dT primers and cDNA synthesis kit. Finally, the renin cDNA proliferated by PCR using renin specific primers containing BamHI and EcoRI restriction sites and MaxTag DNA polymerase. Amplified cDNA were cloned in the pET-28a vector under the control of T7 promoter and terminator, and transform into the DH57 strains of E. coli. Transformed colonies were selected on the medium containing kanamycin, and then the recombinant colonies were screened using colony-PCR method.

Keywords: Cheese, Milk-clotting enzyme, Recombinant DNA, Rennin enzyme

P-448: Recombinant production of Green Fluorescent Protein (GFP) in Pichia pastoris

Saveii T, Arjmand S, Fatemi F, Ranaei Siadat SO
taherelhsaveii@yahoo.com

Recombinant production of Green Fluorescent Protein (GFP) in Pichia pastoris

Introduction: GFP is a popular reporter protein which exhibits
green fluorescence when exposed to blue or ultraviolet light. This protein has initiated a revolution in biological studies after known as a utility tool in 1990s. So far, this protein have been used in both eukaryotic and prokaryotic expression systems for various purposes such as expression protein assay, protein-protein interaction.

Objective: The aim of present study is to produce a recombinant yeast Pichia pastoris which produce GFP protein in the secretary manner which would be used for further studies in optimizing the cell culture.

Materials and methods: The sequence of Aequorea victoria GFP gene was optimized according to the codon preference of Pichia pastoris. The synthetic gene was cloned in pPink2-HC plasmid in the correct frame with the -MF secretion signal and under the control of alcohol oxidase 1 (AOX1) promoter. The recombinant plasmid was verified, linearized with AluII restriction enzyme and electroporated to the Pichia pastoris strain GS115. The expression of recombinant protein was investigated using SDS-gel electrophoresis and the intensity of produced GFP fluorescence light was measured using spectrofluorometer.

Results: The results of SDS-PAGE and spectrofluorometer confirmed the expression and secretion of functional GFP in Pichia pastoris. Keywords: green fluorescent protein , recombinant protein, pichia pastoris

P-449: Molecular diagnosis of Nodularin/Microcystin producing Cyanobacteria in Persian Gulf

Shafi F, Shahhosseiny MH, Mehrabian S, Rostamza M
1. Department of Biotechnology, Faculty of Advanced & Technology, Pharmaceutical Science Branch, Islamic Azad University, Tehran/ Iran (IAUPS)
2. Department of Microbiology, Islamic Azad University, Shahr-e-Qods Branch, Tehran, Iran
3. Iranian Gene Fanavar institute(IGF), Tehran, Iran
fateme.shafi386@gmail.com

Introduction: Cyanobacteria are important producers in nature. Of the well-known cyanobacteria, about 40 species are the main cause of blooming of the poison, which causes damage to organisms in nature and in humans. Determining the presence of cyanobacteria and their dangerous toxins in water is a major issue in the environment and drinking water. There are several methods for investigating the presence of cyanobacteria producing Nodularin/Microcystin, but each has its own limitations. In this study, the presence of Nodularin/Microcystin on the coast of the Persian Gulf has been investigated by the PCR method.

Materials and Methods: In this research, sampling was done from 20 stations along the Persian Gulf coast and DNA extraction from water samples was done using modified DNG-Plus method. PCR test for the presence of cyanobacteria with universal primers (CYA359F, CYA781R) and the detection of cyanobacteria producing Nodularin/Microcystin by assigning primer (HEPF, HEPR) and optimal sensitivity and specificity were studied. Then PCR experiments were performed on samples of the Persian Gulf.

Results: Positive optimized universal PCR test on samples indicates the presence of cyanobacteria in the Persian Gulf was studied in 20 stations. Of which cyanobacteriads producing Nodularin/Microcystin was confirmed in 7 stations.

Conclusion: Due to the presence of Cyanobacteria in all stations sampled, we can say, these organisms, predominant in the Persian Gulf and the Cyanobacteria producing Nodularin/Microcystin in 35% of sampling stations showed the dangers in the area of the cyanobacteria producing the toxins in the Persian Gulf.

Keywords: Nodularin/Microcystin, PCR, Persian Gulf, Diagnosis

P-450: Genotyping of Toxoplasma gondii using three important genes B1, GRA6 and Rep-529

Shahidi S, Mirjalili A, Habibi Gh
1. Samira Shahidi Department of biology, faculty of science, Nour Danesh institute of higher education, Meymeh, Isfahan, Iran
2. Dr. Ali Mirjalili Faculty member of Razi Vaccine & Serum Research Institute
3. Dr. Gholamreza Habibi Department of Parasite Vaccine Research and Production, Razi Vaccine & Serum Research Institute
hidi.samira@gmail.com

Toxoplasma gondii is one of the most pathogenic zoonoses parasite can cause diseases both in humans and different species of birds and mammals. Immunocompromised patients and pregnant women are the most sensitive groups that can be seriously affected by the disease, which can even sometimes be fatal in these people. In animals, T. gondii infection can cause abortion or congenital anomalies. Three types of T. gondii I, II and III have been identified for the induction of virulence in mice as a laboratory animal model. Considering the high importance of molecular methods in terms of sensitivity, the target genes in this project are three major genes B1, GRA6 and REP-529. Among them B1 and REP-529 genes that are repeating genes throughout the genome, have 35 and 200-300 copies in the genome of the parasite respectively. The reference specimens in this study are standard type I (RH strain), Type II (PRU strain) and Type III reference strains (VEG strain). Three types of T. gondii have grown on cell tissue culture separately and the cell concentrates were used for DNA extraction. The DNA was purified from proliferated parasites and it was used to carry out PCR experiments with the specific primers of the three mentioned genes. After PCR optimization, the PCR product undergoes PCR-RFLP method using AluI, Xhol and MseI restriction enzymes in terms of size of the product and digestion products in three types with all three primer pairs investigated and thus we will have a precise diagnostic model. Keywords: Toxoplasma gondii , Genotyping, B1, Gra6, Rep529, PCR

P-451: Frequency of Ureaplasma urealyticum in Women with Recurrent Miscarriage who Referred to Sarem Hospital by using Molecular Method

Tohidpour M*, Shahhosseiny MH, Mehrabian S, Saremi A
1. Department of Microbiology, Islamic Azad University, North Tehran Branch, Tehran/ Iran
2. Department of Microbiology-Shahr- e-Qods Branch-Islamic Azad University-Tehran/Iran
3. Iranian Gene Fanavar Institute (IGF), Tehran/Iran
4. MD, Gynecologist, Subspecialty of Infertility, IVF and Laparoscopic surgery, Sarem Fertility and Infertility Research Center (SAFIR), Sarem Cell Research Center, Sarem Hospital (SCRC), Tehran/ Iran
maryam_tp55@yahoo.com
Ureaplasm urealyticum is one of the sexually transmitted bacteria. This bacterium is a potential cause of acute pyelonephritis, pelvic inflammatory disease, bacterial vaginosis, chorioamnionitis, urethritis, early birth, low birth weight, neonatal pneumonia, abortion and infertility. Recurrent miscarriage is one of the most important problems in pregnancy, and its causes can be anatomical, genetic, immunologic or infectious. The aim of this study was using PCR method to determine the prevalence of Ureaplasma urealyticum as an infectious agent in abortion.

Methods: In this study, during April to December 2017, samples were collected using endo-cervical swab from a total of 100 women (with a history of recurrent miscarriage) who referred to infertility and perinatal clinics of Sarem Hospital. Samples were evaluated with two objectives: vaginal culture and PCR testing. DNA was extracted by using phenol-chloroform method. The PCR test was done for detection of Ureaplasma urealyticum.

Results: From a total of 100 vaginal samples, 11 cases (11%) were positive for Ureaplasma urealyticum. These positive samples also were reported positive in terms of bacterial infection by vaginal culture and patients had higher than normal WBC count.

Conclusion: PCR is a revolutionary method for detection of microorganisms. The major benefits of using molecular techniques to diagnose infectious diseases are high sensitivity and accuracy. Therefore, the use of this method to identify the prevalence of Ureaplasma urealyticum is very helpful in the diagnosis and treatment of women with recurrent miscarriage.

Keywords: Ureaplasma urealyticum, Recurrent Miscarriage, Molecular Method

P-452: Antimicrobial effect study of carbon nanotubes on acinetobacter baumannii in order to prevent nosocomial infections

Yazdani MR1, Siadat SD2,3, Safarian P1, Sheikhpour M2,3

1. Department of biology, Science and Research branch, Islamic Azad University, Tehran, Iran
2. Department of Mycobacteriology and Pulmonary Research, Pasteur Institute of Iran, Tehran, Iran
3. Microbiology Research Center (MRC), Pasteur institute of Iran, Tehran, Iran
m_sheikhpour@pasteur.ac.ir

Background: Acinetobacter baumannii (AB) is critical for healthcare-associated infections (HAI) with significant regional differences in the resistance rate, but its risk factors and infection trends has not been well studied. Carbon nanotubes (CNTs) are essentially cylindrical molecules made entirely of carbon atoms and can be used as nanocarriers. Multi-wall carbon nanotubes (MWCNTs) through their unique properties hold great promise in the fight against multidrug-resistant bacterial infections. Aim: In this study Antimicrobial effects study of carbon nanotubes on Acinetobacter baumannii in order to prevent nosocomial infections was done. Methods: Multi-wall carbon nanotubes were provided from US Research and Cell viability assay was carried out after incubation of Acinetobacter baumannii with the CNTs suspensions (100 µg ml⁻¹) for 24 h by Microplate AlamarBlue Assay (MABA) method. Results: Antimicrobial potential of carbon nanotubes on Acinetobacter baumannii was found based on the color change associated with the specified concentration range on the bacterial growth rate. Conclusion: This study showed that carbon nanotubes can have antimicrobial effects on Acinetobacter baumannii although to get more accurate results, we are doing more specialized cellular and molecular investigation.

Keywords: Antimicrobial activity, Acinetobacter baumannii (AB), Carbon nano tubes (CNTs)

P-453: Immunomodulatory effects of active compounds extracted from secondary metabolite of Streptomyces Calvus on peripheral blood mononuclear cells (PBMCs)

zareh teen R1,2, Mohammadi A1, Mansoori B1, Khaze V1, Valipour P1,2, Dehnad A2,3, Baradaran B1

1. Immunology Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2. Biotechnology Department, East Azerbaijan Research and Education Center Agricultural and Natural Resources, AREEO, Tabriz- Iran
3. Higher education institute of Rab-Rashid, Tabriz, IRAN royan.zare71@yahoo.com

Introduction: Today, microorganisms are considered as potential natural products for developing a novel pharmaceutical agent, and the use of natural compounds have less side effects. In this study, the immunomodulatory effects of active compounds of secondary metabolites of Streptomyces Calvus (S. calvus) evaluated on a gene expression of pro-inflammatory cytokines in human peripheral blood mononuclear cells (PBMCs).

Methods: S. calvus was inoculated in Mueller Hinton Broth and secondary metabolites were extracted, then active compounds were isolated with HPLC technique. PBMCs were isolated from peripheral blood of healthy donor and were treated with active compounds. The cell proliferation was assessed by MTT assay and quantitative RT-PCR assays to determine mRNA expressions of TGF-β and INFα.

Results: The secondary metabolite of S. calvus isolated into 7 different fractions via the HPLC. MTT assay results showed the 5th and 7th fractions have dose dependent immuno-activatory effect on PBMC. Also we didn’t see significant immuno-activatory effect in the 1st, 2nd, 3rd, 4th, and 6th fractions compared to untreated control. The 7th fraction could stimulate both TGF-β and INFα expression. However, the 5th fraction didn’t show any significant effect on TGF-β and INFα expression.

Conclusion: This in vitro study showed that different active compounds of secondary metabolites of S. calvus can successfully stimulate human PBMCs. Therefore, these metabolites have the potential to serve as a robust immunomodulator.

Keywords: Streptomyces calvus, Active compounds, Peripheral blood mononuclear cells, INFα, TGFβ

Genetic Resources

P-454: Venom gland Transcriptomic analysis of Iranian yellow scorpion â€“ Odontobuthus doria’ revealed some new findings by medical purposes

Naderi Soorki M1, Galedhari H1, Baradaran M2, Jalali A3

1. Department of Genetics, School of Science, Shahaid Chamran University of Ahvaz, Ahvaz, Iran
2. Department of Pharmacology and Toxicology, School of...
Introduction: Scorpion venom contains mixture of biologic molecules including selective toxins with medical capability. Odontobuthus doriae (O. doriae) belonged to Buthidae family of scorpions and gained more interest among Iranian dangerous scorpion since 2005. The envenomation of this scorpion causes usually neurological signs because of existence of toxins affecting on ion channels.

Material & Methods: Total RNA was isolated from yellow Iranian scorpion glands. A cDNA library was achieved by synthesize and insertion of dscDNA into special vectors and subsequent transformation to chemical competent E. coli as host. Library was screened by culturing of the liquid library on LB-agar plates. Analysis of positive clones was performed by plasmid extraction and sequencing of inserts. Finally, sequences have been analyzed and characterized by bioinformatics software each.

Results: Analysis showed that toxins (42% of ESTs) had more venom transcripts than other venom components (antimicrobial peptide (10%), cell proteins (11%) and venom peptide (13%)) that may have capacity for medical used. Two EST didn’t have any similarity by known scorpion peptides and may be new.

Conclusion: For the first time, we report a comprehensive study of an Iranian scorpion with interesting and novel findings and characterized a new putative sodium channel modifier and a new iron transporter in scorpions by some bioinformatics software, and then predicted their structures and functions.

Keywords: Transcriptome analysis; cDNA library; Venom gland; Iranian scorpion; Odontobuthus doriae

P-455: Identification of Bactrian camel cell lines using genetic markers

Daneshvar Amoli A1, Shahzadeh Fazeli SA2, 3, Aminafshar M2, Emam Jomeh Kashan N1, Farzaneh1, Hamidreza Khaledi P4  

1. Human and Animal Cell Bank, Iranian Biological Resource Center (IBRC), ACECR, Tehran, Iran  
2. Department of Molecular and Cellular Biology, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and Culture, Tehran, Iran  
3. Department of Animal Sciences, Faculty of Agriculture and Natural Resources, Science and Research Branch, Islamic Azad University, Tehran, Iran  
4. Department of Agriculture, Yadegar-e-Islam Khomeini (rah), Shahre-rey Branch, Islamic Azad University, Tehran, Iran  

daneshvaramoli@gmail.com

Iranian Bactrian camel population is less than 100 animals. Iranian biological resource center produced more than 50 Bactrian camel fibroblast cell lines as a somatic cell bank for conservation animal genetic resources. We compared two type markers performance, including fourteen RAPDs (dominant) and eight microsatellite (co-dominant) for cell lines identification, individual identification and investigation genetic structure of these samples. Based on clarity, polymorphism, repeatability, four RAPD primers were selected for future analysis. Four RAPD primers and eight microsatellite markers have generated a total of 21 fragments and 45 alleles, respectively. RAPD primers revealed fragment size between 150 to 2000 bp and gene diversity since 0.27 (IBRD) to 0.46 (GC10), with an average of 0.37. Microsatellite markers generated number of alleles per locus ranged from three to eleven, with an average of 5.62 alleles. The observed heterozygosity ranged from 0.359 (IBRC02) to 0.978 (YWLL08) and expected heterozygosity ranged from 0.449 (IBRC02) to 0.879 (YWLL08). Bottleneck analysis and curve showed Bactrian camel population did not experience a low diversity. RAPD profiles were especially suitable for investigation population genetics. All primers generated novel and polymorphic fragments. Briefly, our results show that a multiplex PCR based on these markers can still be valuable and suitable for authentication of cell lines, investigating gene diversity and conservation genetic resources in Bactrian camel, while new technologies are continuously developed.

Keywords: Bactrian camel, identification cell line, RAPD, microsatellite

P-456: Frequency of Extremotolerant and Cellulase-Producing Bacteria from North-West Hot Springs of Iran

Diba H, Hemmat J, Vaez M, Amooozegar M A  

Department of Biotechnology, Iranian Research Organization for Science and Technology (IROST), Tehran, Iran.  
Department of Microbiology, School of Biology, College of Science, University of Tehran, Tehran, Iran.  
h.dibabiotech@gmail.com

Hot springs are amongst the niches of many extremophile or extremotolerant microorganisms. These microorganisms are potentially a source of enzymes with industrial applications capabilities. Cellulases are among important enzymes which are used in various industries for cellulose hydrolysis as a dominant natural biopolymer. Hence, finding native sources of such enzymes are very important economically. With the aim of evaluating biodiversity of native cellulase-producing bacteria, eight bacteria producing Endo-1,4-?-glucanase had been isolated from hot springs in northwest of Iran. Followed by screening, the three isolates, were identified as Bacillus sp. strain G2, Bacillus sp. strain AGh1 and Paenibacillus sp. strain ASb4 by 16S rRNA sequencing. Residual stability of these isolates were 21%, 71% and 92.39%, respectively, at 60°C and pH=4. Therefore, the studied areas contain significant heat-resistant cellulose bacteria.  

Keywords: Cellulase, Hot springs, Extremophiles

P-457: Assessment of Genetic Diversity of Some Iranian Bitter Vetch Landraces Based on Agronomic Traits

Ghanipour Govarki M, Sahhafi  

Department of Genetics and Plant Production, Faculty of Agriculture, Vali-e-Asr University of Rafsanjan  
s.sahhafi@vru.ac.ir

The objective of this research was to study the genetic diversity among 12 Iranian bitter vetch landraces (Vicia ervilia L.) collected from seven provinces (Ardabil, Hamadan, Kurdistan, Lorestan, Zanjan, East Azerbaijan and West Azerbaijan). Landraces were evaluated in a completely randomized design with five replications at the greenhouse of Vali-e-Asr University of Rafsanjan. Fourteen quantitative traits including days to 50% flowering and maturity, length of first flowering
Gastric cancer is a leading cause of cancer death, associated with environmental and genetic factors with increasing incidence in young patients. Gastric cancer has long been recognized to be accompanied and preceded by chronic gastritis lasting decades. Arguably, the most important development in our understanding of gastric cancer pathogenesis over the past 50 years, has been the realization of most cases of gastric cancer. Helicobacter pylori is the cause of the underlying gastritis that can promote gastric carcinogenesis, typically via the Correa cascade of atrophic gastritis, intestinal metaplasia, and dysplasia. Nested case-control studies, have shown that H. pylori infection increases the risk of gastric cancer significantly both of the intestinal and diffuse subtypes, and that H. pylori is responsible for approximately 90% of the world's burden of noncardia gastric cancer. Helicobacter pylori are the first formally recognized bacterial carcinogen and are one of the most successful human pathogens, as over half of the world's population is colonized with this gram-negative bacterium. Bacterial effectors such as colibactin and the virulence factor cytotoxin-associated gene A (CagA), can promote cancer directly by influencing host cell signalling cascades, such as the WNT and ataxia-telangiectasia mutated (ATM) pathways. Nevertheless, establishing a link between chronic H. pylori infection and gastric cancer, has led to novel insights into cancer biology, the gastrointestinal microbiome, and on individual and population-based gastric cancer prevention strategies. Understanding the details of this enzyme, the Helicobacter pylori bacteria's metabolism and biological pathways, could be central to developing drugs that act against the Helicobacter pylori bacteria's metabolism and biological pathways, could be central to developing drugs that act against the Helicobacter pylori. This report we discuss how bacterial pathogens interact with host cells to contribute to the development of cancer and we can use it for prevention of deseases caused by H. pylori.

Keywords: agronomic traits, bitter vetch, cluster analysis, genetic diversity, landraces

P-458: Blocking ligands can improve gastric cancer Symptoms by H.pylori

Haeri MS, Nabi M
E-mail: melika.dec1992@yahoo.com

Gastric cancer (GC) is a leading cause of cancer death, with a frequency of 1.86 per 1,000 children born, recognized as the most common sensory-neural disorder. The frequency of deafness is also affected by age, so that before the age of 5, the frequency of deafness is 2.7 per 1,000, while in adulthood this frequency is increased to 3.5 per 1,000. According to reports in Iran, 1 out of every 166 people have deafness. Nearly 50% of cases of deafness have a genetic origin and 50% are due to environmental factors. The deafness with genetic basis is divided into syndromic (30%) and non-syndromic (70%) deafness. In non-syndromic deafness, 47 genes and 89 chromosomal positions have been attributed. In this study, mutations in GJB2 and GJB6 genes were evaluated in 45 people with nonsyndromic autosomal recessive hearing loss and 35 normal people with Qom’s originality. To do this, after obtaining a blood sample from each healthy and patient people, DNA extraction was performed. The ARMS-PCR and multiplex-PCR methods were used to investigate the mutations of GJB2 and GJB6 genes, respectively. Furthermore, some of the PCR products were sequenced to approve the result of ARMS-PCR. According to the results of ARMS-PCR, patients were heterozygote (5%) and 2 patients were homozygote (2.5%) for a common 35delG mutation. In the results of multiplex-PCR to detect mutations in the GJB6 gene, none of the specimens had a common deletion mutation in the gene.

Keywords: non-syndromic hearing loss, ARMS-PCR, GJB2 and GJB6 genes

P-460: Genetic diversity on guaiacol peroxidase activity in different species of wheat under salinity

Khalili P1, Ahmadi J1, Fabriki Ourang S1

Department of Genetics and Plant Breeding, Imam Khomeini International University, Qazvin
pejmankhalili47@gmail.com

Salinity is a limiting factor for growth, quality and yield of crops in arid and semi-arid regions in world as well as in Iran. Guaiacol Peroxidase (GPX) is a glycoprotein that is located in the cytosol, cell wall, and vacuoles, and uses from the oxidation of phenolic compounds to decontaminate and decompose of H2O2. In addition to antioxidant activity, this enzyme also plays a role in growth regulation. In order to investigate the effect of salinity stress on GPX activity in wheat wild relatives, an factorial experiment based on randomized complete block design included three concentration of salinity (0, 25 and 35 dS/m) as the first factor and twenty-two accessions related to eleven wheat species as the second factor was conducted at three replications. The ANOVA results indicated significant effect of salinity and salinity Â— genotype interaction on GPX activity. In main effect mean comparison, the highest amount of GPX activity (53.4 unit/mg protein) was observed in Aegilops ovata (accession Kamyaran-Kermanshah) and then in Darya cultivar (salinity sensitive check cultivar). The lowest GPX activity (8.5 unit/mg protein) was observed in Aegilops speltoides accession Sarpolezahab. The comparison
of means for salinity $\Delta -$ genotype interaction showed that the highest and lowest activity of GPX was obtained in Aegilops umbellulata (accession Islamabad-Kermanshah, Seyyah- Khor) and Aegilops speltoides (accession Gasre-Shirin) in salinity of 25 dS/m, respectively. Reducing GPX activity in some genotypes/accession may be due to an increase in ascorbate content.

Keywords: Genetic diversity, GPX, Salinity, Wheat

P-461: Allelic variation of GSP gene in some wild wheat with A genome

Mansourian M, Fazeli A, Hosseinan Khosro H

Agronomy and Plant breeding department, faculty of agriculture, Ilam University, Ilam, Iran.
E-mail: a.fazeli@ilam.ac.ir

The GSP protein belongs to a big family of seed storage protein in cereal seeds. Grain hardness has a great influence on the process properties of wheat to bread or biscuits. In this study we Re-sequenced the GSP gene in eight wild wheat accessions with A genome related to two spics (Triticum dicocoides and T. urartu) that have been collected in the different region of Iran. The results of PCR showed that GSP gene is present in all of the examined samples and produced 570 bp, although the length of the PCR product on Agarose gel shows high polymorphism. The results of the GSP gene sequencing in samples indicated that Triticum dicocoides(73.8) and T. urartu (37.8) show highest and lowest Similarity with bread wheat at NCBI respectively. Indeed, the cluster analysis shows that the samples are divided into two main groups, although they could not separate the samples by species that have been collected from the different region of Iran. In general, bioinformatics analysis showed that significant differences in the size and length of the Gsp gene in compare to NCBI sequence. In order to get a deep understanding of this variation in the wild genetic resources of Iran and western Iran, which is a potential source for the desired quality germ, it should be carefully studied via genetically and biochemical characteristic.

Keywords: Wild wheat, Gsp gene, Quality

P-462: the relationship between common mutation AURKC gene the success or failure of assisted reproductive techniques in a population of women candidates ART

motamedi M, Zarabi Ahari N, Ebrahimi A

Department of biology, central tehran branch, islamic azad university,tehran iran
yas medical genetics
M.motamedi96@gmail.com

IVF or artificial fertilization is one of the general methods for infertility treatment. There are many genetic pathways which rules chromosomal abnormalities. One of these genetic pathways is the gene groups involved in the connection of chromosomes to spindle. The AURKC gene, one of the genes involved in this process, has kinase domains. Inappropriate expression of these kinases especially in mitosis can change the function of the check points which leads to genetic instability. In this study, 50 women candidates assessed for ART who were from the Mohab Yas Hospital. After their DNA extraction, specific primers designed for the target regions of the AURKC gene and amplified by PCR method. Then, the samples sequenced and analyzed for statistical results. In the general mutation of exonic six AURKC genes cause no pathogen changes in candidates for IVF, which includes two successful and unsuccessful individuals. The only pathogen changes indicated as heterozygote in the splice site for exon number 2 with cluster report: rs 5826481. It is noteworthy that this gene causes a spermatogenesis disruption whereas it has not protected site. To sum up, at first there was no difference between IVF candidates and healthy individuals. Second, there was no change in the type of changes between those who had IVF and those who did not succeed in IVF. Third, the gene of Aurora Kinase C and its changes have not a significant role in the success or failure of IVF and also natural pregnancy.

Keywords: ARUKC gene, Infertility , IVF

P-463: Evaluation of Genetic Diversity of Mature Walnut (Juglans regia L.) Genotypes in the North of Hamadan Province, Iran

Rezaei A*, Arzani K¹, Sarikhani Khorami S²

1. Department of Horticultural Science, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran.
2. Departments of Horticulture, Aburaihan Campus, University of Tehran, Pakdasht, Iran.
ali_rezaei@modares.ac.ir

Persian walnut (Juglans regia L.) has a long history of cultivation in Iran and Persia (include Iran) consider as origin center of walnut in the world. Among different region of walnut production, Hamadan is the leading province for walnut culture and has the largest walnut plantations in Iran. Due to this high genetic diversity, identification of superior walnut genotypes the first step of walnut breeding program. Therefore, 84 preselected walnut genotypes in the north of Hamadan were morphologically evaluated based on IPGRI descriptor in 2017. Based on the results, a high diversity was observed in the studied population. The results showed that nut and kernel weight and kernel percentage varied 6.4-23.5 g, 2.73-11.7 g and 27.02-78.35%, respectively. Based on morphological data, 11 out of 84 genotypes were selected as superior genotypes. The selected superior genotypes had lateral bearing habit, heavy nuts (13.14-21.62 g) and kernels (7.12-10.71 g) with thin shells and light to extra light kernel colors. Correlation analysis revealed that a positive and strong correlation between phenological data especially leafing and harvest date. There were strongly positive correlations between nut and kernel weight with nut size.

Keywords; Persian walnut; Lateral bearing; Germplasm; Nut weight; Correlation analysis

P-464: Molecular cloning of BIR3 domain from XIAP in pET28a vector

Shamsi M, Ataei F, hosseinikhani S

Department of Biochemistry, Tarbiat Modares University, Tehran, Iran
mshamsi90@gmail.com

The inhibitor of apoptosis protein (IAP) family is a functionally and structurally related group of proteins that serve as endogenous inhibitors of programmed cell death, or apoptosis. In addition, some family members are regulators of another form of programmed cell death termed necroptosis. Apoptosis
is one type of programmed cell death which includes external and internal pathways. Some of the effector proteins that play roles in this procedure are Caspases. These cysteine proteases were inhibited by IAPs. This family of protein has 8 members and the common point between them is similar domains name BIRs. XIAP is of the important members of this family and inhibits Caspase-9 and Caspase-3, 7. XIAP are additionally thought to contribute to cancer cell invasion and metastasis through their ability to drive NF-κB mediated expression of genes involved in cell motility, migration, and invasion. Caspase-9 inhibition is done by BIR3 domain of XIAP and Caspase-3 by BIR2. In this research, we cloned BIR3 domain of XIAP in a pET28a vector. PCR product was digested by EcoRI and HindIII and ligated into the EcoRI/HindIII digested pET-28a vector. The ligation mixture was transformed in the cloning host E. coli DH5α and screened by antibiotic selection. Positive colonies were selected by colony PCR and screened by double digestion of isolated plasmid and then sequenced to check the inserted DNA.

*Keywords:* Apoptosis, XIAP, BIR, Cloning

P-465: Genetic variation of high molecular weight glutenin subunits in Aegilops triuncialis accessions of Khorasan provinces

Shirzad H1*, Ahmadi J1, Aghaei MJ1, Sorkhi B4

1. Department of Genetics and Plant Breeding, Imam Khomeini International University, Qazvin.
2. Horticulture Research Institute, Alborz.
3. Department of Genetics Research and National Genetic Gene Bank of Iran, Seed and Plant Improvement Research Institute, Alborz.
4. Horticulture Research Institute, Alborz.

hadishirzad1993@gmail.com

Acknowledgment about plant materials genetic diversity plays an important role in protecting and improving of crops. For this purpose, variation in high molecular weight Glutenin subunit (HMWGS) in seed protein storage of 17 accessions related to Aegilops triuncialis species collected from the Khorasan Razavi and Northern Khorasan provinces were investigated by electrophoresis of sodium dodecyl sulfate polyacrylamide gel. Based on results, a total of nine different bands were identified among these accessions and were named alphabetically. The highest frequencies were for the bands a, h and i and the lowest frequent bands were b and e. There were also eight different band patterns among studied accessions. The parameters of real allele number (Na), effective allele number (Ne), Shannon index (I) and unbiased genetic diversity (uh) in Khorasan Razavi province accessions were far more than North Khorasan province accessions. Also, the percentage of polymorphic loci in Khorasan Razavi and North Khorasan accessions were 80% and 30%, respectively.

*Keywords:* Aegilops triuncialis, Genetic diversity, Glutenin subunits, HMWGS

New Technologies and Technological Advances in Genetics

P-466: Site directed mutagenesis in T83R and L287R positions on phytase enzyme from Yersinia intermedia

Abbasi M*, saffar B1, Hemati R2, Mortazavi M3

1. Department of Genetics, master of Sciences, Shahrekord University, Shahrekord, Iran
2. Department of Genetics, Faculty of Sciences, Shahrekord University, Shahrekord, Iran
3. Department of biochemistry, Faculty of Sciences, Advanced Scientific and Technological Research Institute, kerman, Iran

marjanabassi9@gmail.com

Phytate is the major phosphate storage compound in seeds of higher plants and forms complexes with multivalent metal ions such as iron, zinc, calcium and proteins, thereby showing anti nutritional effects. So phytase added mainly as an additive to the monogastric animal feed due to hydrolyze phytate and increase absorption of phosphorus and another elements.

The purpose of this study was site directed mutagenesis in T83R and L287R positions on phytase enzyme from Yersinia intermedia to enhance thermostability. Mutation of amino acids in active site to arginine increases stability of enzyme. At first, the structure of this enzyme was prepared by SPDBD. Then the model was compared and superimposed to the used structure. Subsequently, all of the appropriate surface amino acids were identified for mutation. Then, by changing all these amino acids to arginine, the position of arginine was evaluated in the structure. Finally two of this positions were selected for this study.

For this purpose, the primers containing the mutations were designed and PCR was performed with QuikChange site-directed mutagenesis method to make mutations. PCR product was transferred to DH5α competent cells. Accuracy of coloning was checked by colony PCR and sequencing. The results show that the specific selected amino acids were mutated to arginine correctly.

*Keywords:* Phytase, Quikchange site directed mutagenesis, Yersinia intermedia

P-467: shRNA silencing of CDC25B causes altered expression of downstream proteins in MCF-7 breast cancer cells

Alijanpour S1, Golalipour M1, Shahbazi M12, Yamchi A1

1. Department of Medical Genetics, Faculty of Advanced Medical Technologies, Golestan University of Medical Sciences, Gorgan, Iran
2. Medical Cellular and Molecular Research Center, Golestan University of Medical Sciences, Gorgan, Iran
3. Department of Plant Biotechnology, Gorgan University of Agriculture Science and Natural Resources, Gorgan, Iran

Alijanpour88@gmail.com

Background: Cell division cycle 25 (CDC25) family of proteins are highly conserved dual specificity phosphatases. In mammalian cells, three isoforms of CDC25 have been identified including CDC25A, CDC25B and CDC25C. All three isoforms play a role in the control of the G1-S and G2-M transitions by CDKs. Cdc252 causes transition from G2 to M phase and its overexpression has been reported in various cancers including breast cancer. In this study, first we silenced CDC25B gene expression by shRNA and then the alterations in the expression pattern of CDC25B downstream genes was studied at the protein level.

*Methods:* The human breast cancer cell line, MCF-7, was cultured and transfected with CDC25B specific shRNA. The effects of this silencing at the RNA and protein levels were evaluated by Real-time PCR and western blotting respectively. Following this step, the alterations in the expression pattern
of CDC25B downstream proteins were analyzed by 2-D gel electrophoresis.

**Results:** The results of Real-time PCR and western blotting showed decreased expression of CDC25B at the RNA and protein levels, respectively. The results of 2-D electrophoresis determined 12 spots whose expressions were significantly altered. Of these, 2 proteins increased and 10 proteins decreased after treatment.

**Conclusion:** CDC25B gene silencing caused alterations in the expression pattern of CDC25B downstream proteins. This finding suggests further studies for investigating the associations between CDC25B and other genes in order to achieve better understanding of tumorigenesis and cell proliferation. Moreover, these results provide a rationale for further studies of CDC25B-based gene therapy for breast cancer.

**Keywords:** CDC25B; shRNA, cell cycle; breast cancer

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**P-468:** CRISPR-dCas9 as Potential therapy for Glioblastoma

Alihmardani M1,2,3, Farrokhli Sh1,2, Nejati O4, Mojarrad M2

shimashima1373@yahoo.com

**Introduction:** The most common and often-fatal type of primary brain cancer is multiform Glioblastoma. Although many attempts have been made to treat this cancer, it still has a high recurrence rate and poor prognosis. MGMT is most important prognostic marker and also therapeutic outcome predictor of glioblastoma. The protein encoded by MGMT gene is a DNA repair enzyme (O6-methylguanine-DNA-methyltransferase) that protects cell genome from methylation and can abrogate the effects of alkylating chemotherapy such as temozolamide. Using CRISPR system mediated gene suppression we suppressed MGMT expression and induced Temozolamide sensitivity in U87 cells.

**Methods:** MGMT promoter targeting sgRNA was designed using MIT online database and cloned in TRE-KRAB-dCas9-IRE6-GFP plasmid. Produced plasmid was transfected into U87 cells. MGMT gene expression levels in treated and control cells was measured using Real Time PCR. Temozolamide antiproliferative effects was compared between transfected cells and untreated control cells using MTT and cell cytotoxicity test.

**Results:** Our results showed that CRISPR system mediated gene suppression dramatically attenuates MGMT expression and also cancercous phenotype of U87 glioblastoma cells. Conclusion: According to our results CRISPR system mediated gene suppression could be used for MGMT silencing. This strategy can be used as a new adjuvant in fighting against glioblastoma.

**Keyword:** MGMT, CRISPR-dCas9, Glioblastoma.

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**P-469:** Generation of Duchenne muscular dystrophy cell lines harboring mutation in exon 52 and 53 by CRISPR/Cas9-mediated genome editing

Alizadeh F1, Jafari Abarghan Y1, Eslahi1, Majid Mojarrad A1

1. Department of Medical Genetic, Faculty of Medicine, University of Ferdosi, Mashhad, Iran

MojarradM@mums.ac.ir

Duchenne muscular dystrophy (DMD) is the most serious childhood form of muscular dystrophy. DMD is a lethal X-linked disorder made by mutations in the dystrophin gene. There is currently no cure to this sickness but multiple treatment procedures are under investigation and have shown positive promise for the treatment of DMD. Dystrophin gene-replacement approaches, genetic modification procedures to restore dystrophin expression, and modulation of the dystrophin homologue (utrophin) as a surrogate to restore muscle activity. The prokaryotic clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) 9 system may be re-planned for site-specific eukaryotic genome engineering. CRISPR/Cas9 is an economical, simplistic, and efficient genome editing tool that allows genetic perturbation of genes and genetic elements. Duchenne muscular dystrophy cell lines with specific mutations can enable the study of the effectiveness of these therapeutic strategies. In order to Generate of Duchenne muscular dystrophy cell lines, we use CRISPR/Cas9-mediated genome editing to create two double strand breaks (DSBs) in exon 52 and 53 in order to delete the intervening DNA segment by non-homologous end joining (NHEJ) repair. Existing deletion has been identified by using the GAP-PCR method in the clones and finally the exon 52 and 53 deletions will be identified by using the sequencing. Dystrophin protein expression in the cell line Will be evaluated by reverse transcriptase PCR and western blot techniques.

**Keywords:** Duchenne muscular dystrophy, cell lines, genome editing, CRISPR/Cas9

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**P-470:** Designing shRNA molecules targeting UL-25 gene as a gene therapy tool against BHV-1 virus

Amjadiemanesh E1, Saffar B2, Mokhtari A2

1. Department of genetics, master of Science, Shahrekord University, Shahrekord, Iran
2. Department of Pathobiology, School of Veterinary medicine, Shahrekord University, Shahrekord, Iran

Elaheh.Amjadiemanesh@yahoo.com

Bovine herpesvirus-1 (BHV-1) belong to the genus Varicelovirus in the subfamily Alphaherpesvirinae and the family Herpesviridae, the main host of the virus is cattle but Other Artiodactyla (goat, sheep and camel) may be infected. The BHV-1 cause the respiratory disease (IBR), with fever and abortion. Although the infection is infrequently life threatening, the introduction of cattle with BHV-1 can cause severe economic losses due to production losses and restrictions in the international trade of livestock.

In recent years, RNAi has become a novel and effective tool to target gene silencing for functional genomic studies and antiviral therapy. The expression of shRNAs against target genes has now become a standard and powerful technology for gene silencing.

The purpose of this study was design, synthesis and colony three shRNA molecules targeting UL-25 gene to inhibit virus replication.

Several shRNA molecules was design by using some online software. Then three shRNA with appropriate size and situations were chosen, single strand oligonucleotide were synthesized and annealed with each other, eventually they were colored in pCDH-CMV-MCS-EF1-cGFP-T2A-Puro viral plasmid vector. Due to verify accuracy and validity of cloning, colony-PCR
and sequencing was performed. Consequently; To investigate the antiviral effects of shRNAs on BHV-1 replication, transfection of these viral plasmid vector in HEK 239T cell line and challenge with BHV-1 that inoculated in MDBK cell line will done.

**Keywords:** shRNA, BHV-1 virus, UL-25 gene, gene silencing

**P-471: The demolition of neural cell cancer by gold nanoparticle coated with Glutamate**

Barati Bagherabad M1, Zare Marzouini H2, Tarkhan F3, Keyvani V

1. Department of Modern Sciences and Technologies, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Immunology, faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3. Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran
Vahidekeyvani@gmail.com

**BACKGROUND:** At present, cancer is the second leading cause of death in the world, so finding a way to eliminate these cells be required. The aim of this study was to investigate the mechanism of degradation of gold nanoparticle coated with Glutamate amino acids on PC12 category of cancer cell.

**METHODS:** In this study, PC12 cell category was cultured in vitro and these cells divided into 7 groups and then 6 groups exposed for 48 hours to varying concentrations of gold nanoparticles (0.5, 2.5 and 5 ÅμM gold nanoparticles, Gold nanoparticles coated with Glutamate) and a group without nanoparticle as the control. The effect of nanoparticle cytotoxicity was examined by using MTT and LDH release from the cells. The phase contrast microscopy was used to evaluate the cellular morphology. Finally, the level of ROS in the cells was measured by using fluorescent probe of 2, 7 Dichloroisocyanurate fluorescein Diacetate.

**RESULTS:** The average diameter of the gold nanoparticles in the case of uncoated and coated with Glutamate amino acids, respectively 10 Å± 0.2 and 28 Å± 0.5 nm was calculated. The most damaging effect of the studied cells by micro-molar concentrations of 25 gold nanoparticles coated with Glutamate amino acid, which occurred at a rate of 30% compared to the group treated with bare gold nanoparticles was a statistically significant difference (p? 0.05). The results of this study indicate that the majority of apoptosis of cells incubated with uncoated and coated gold nanoparticles is as apoptosis.

**CONCLUSION:** The results showed that gold nanoparticles coated with Glutamate decreased metabolic activity and increased membrane destruction of the cell, in comparison with control cells in a dose-dependent mode and increased ROS and apoptosis mechanisms in cancer cells causing cell death in PC12 category.

**Keywords:** cancer, nanoparticle, Glutamate, apoptosis

**P-472: Cloning Specific aptamer against breast cancer**

Bideli S1, Sedighian H2, Halabian R2, Barikrow N3

1. Department Of Molecular And Cellular Sciences, Faculty Of Advance Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran-Iran.
2. Applied Microbiology Research Center, Systems Biology and Poisonings Institute, Basijitahal University of Medical Sciences, Tehran, Iran
3. Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences

Branch, Islamic Azad University, Tehran-Iran (IAUPS)
sabideli7@gmail.com

**Background:** Since breast cancer is one of the most common cancer in the world and the third cause of death related cancer in Iran, it is important to investigate the diagnostic methods to decrease the rate of death related cancer. Recently scientists use aptamers which are ssDNA or RNA oligonucleotides as a novel and practical factors in detection, due to their high affinity and specificity binding potentiation.

**Methods and Results:** In this study aptamer against CA 15-3 was cloned which is metastatic biomarker in patience with breast cancer. First of all we make a sepharose-CA15-3 column. Then use 8 rounds of SELEX to reach the specific aptamer. The specific aptamer was sequencing and cloned in TA vector that in future it will be analysis as one of the detecting and monitoring factor for screening of breast cancer.

To the best of our knowledge, this is the first report in aptamer technology especially against CA15-3 for detection of breast cancer by apta-PCR method.

**Keywords:** breast cancer, aptamer, CA 15-3, SELEX

**P-473: Studying the effect of the aqueous and Acetone extract of the Nigella Sativa on inhibition, survive and growth rate and the MCF-7 Cell grade of keratin**

Biglak B

Department of Biology, Bonab Branch, Islamic Azad University, Bonab, Iran
bbiglak@gmail.com

**Introduction:** Breast Cancer is the second reason of death rate of the cancers in women. The probability of catching the invasive breast cancer by women in life time is 1 in 8 (13%). The problems like the failure of treatment, resistance to the drugs, the costliness and other problems related to cancer treatment has made the attentions concentrated on the drug plants which have less side effects in comparison to the chemical drugs. In this research the effect of the aqueous and Acetone extract of the Nigella Sativa on inhibition and survive rate and the MCF-7 Cell grade of keratin is studied.

**Methodology:** 50 grams Nigella Sativa was put in two flat bottom balloons and 300 ml distilled water and 300 acetone was put in both balloons. They were put on the mixture using magnet in 0 degrees for 25 hours and then they were passed through the filter and then the water and acetone was extracted using rotary. Finally a paste type brown material was accessed.

The cancerous cells of this study were provided from Iran Pastor Institute. The breast cancer cells (MCF-7) were planted in flasks holding RPMI 1640 planting environment including 10% FBS in incubator 37°c wet space along with 5% CO2. Then they were transferred to the 96 partition plates of 104*2 cell grade of keratin. And were treated with different aqueous and acetone extract of Nigella Sativa (100, 200 and 400 mg/ml) for 24, 48 and 72 hours. The effect of the extract on survive rate of the cells was studied via coloring by Trypan Blue method.

**Findings:** The results showed that increasing the density and the time of the extract decreased the survive rate of the cells in comparison to the control group meaningfully.

**Conclusion:** According to the findings of the research about the aqueous and acetone extract, Nigella Sativa holds anti-cancer effects on the breast cancer cells (MCF-7).

**Keywords:** Nigella Sativa , MCF-7 Cell, grade of keratin
P-474: Introduction of cfDNA as an applied technique for IVF

Ebrahim Sh1, Zarabi Ahrabi N2, Ebrahimim A3

1, 2. Department of Biology, central Tehran branch, Islamic Azad University, Tehran, Iran.
3. Yas medical Genetics Lab, Tehran, Iran.
na-zarabi@yahoo.com

Infertility is one of the stressful and critical problems in the individual, marriage, family and the social life. One of the routine methods of infertility in Iran is IVF which is a method of ART technic. Usage of extracted cell free DNA from plasma and follicular fluid in this study as a genetic biomarker illustrated a solution for infertile couples who want to know their victory and failure (efficiency) in-vitro fertilization before zygote transport. In this research cfDNA isolated from 50 samples of both the follicular fluid and the blood samples by NucleoSpin kit. Two housekeeping genes which called GAP DH and ALBUMIN studied by SYBR Green method in Real-Time PCR. Studies on the extracted cfDNA in both groups of successful and unsuccessful in IVF, statistical analysis and their meaningful level achieved. According to the nonparametric hypotheses, on the one hand the H0 theory which is based on similarity of variables such as CTP, CTF, DELTA CT and CT average from the plasma and the follicular fluid rejected in both group. On the other hand, base on ?2 statistical test there is no meaningful difference on those variables in the groups. Potential prediction range of follicular fluid cfDNA is extremely higher than the numbers of embryos which are qualified base on morphological standards, indeed this prediction model can be a complementary tool for identification of the chance of successful IVF.

Keywords: infertility, IVF, cfDNA, housekeeping gene, Real-time PCR.

P-475: Study of the suppressive effect of Nano-based compound of Gemini Curcumin on AGS Gastric cancer cell line

Emami A1, Babaei E1, Hosseinpour Feizi MA1

Department of Animal Biology, School of Natural Sciences, University of Tabriz, Tabriz, Iran
ali.emami93@gmail.com

Introduction: It has been shown that curcumin has effective anti-cancer properties. However, the absorption efficacy of curcumin is too low to have significant results in therapy. To overcome this problem, we first improved bioavailability of curcumin through making Nano-based compound of Gemini Curcumin, and then studied the effects of this compound on cell viability and apoptosis.

Methods: Human gastric carcinoma AGS cell lines were treated with Gemini Curcumin (gCUR), and cell viability was evaluated using MTT assay. Cell cycle analysis was assessed by flow cytometry and also apoptotic potential of gCUR of were measured using Fluorescence microscope. Expression levels of some genes involved in apoptosis pathway such as bax and bcl2 were analyzed by qPCR.

Results: Gemini curcumin inhibited the cell viability of AGS cells in a dose- and time-dependent manner (P < 0.05) and arrested treated cell in G2/M phase. Our data also showed that the expression ratio of bax/bcl2 is increased in treated cell compared to controls.

Conclusion: Gemini curcumin induces G2/M-phase arrest in human gastric cancer cells, which highlights its potent anti-cancer activity and potential application in gastric cancer therapy.

Keywords: Curcumin, AGS cell line, Gastric cancer, bax/bcl2

P-476: Design and Production of collagen-like engineered peptide (CLP) for tissue-engineering applications

Ghazaey Zidanloo S

Department of Molecular and Cell Biology, Faculty of Basic Sciences, Kosar University of Bojnord, Bojnord, Iran.
ghazaey@kub.ac.ir

Transplantation of biomaterials into patients suffering from organ failure or damage can be conducted instead of receiving grafts from organ donors or animals. Similar to donated grafts, biomaterials aim at replacing the functions of lost organs, and saving patients’ life or improving their quality of life. Hopefully, a potential alternative to conventional biomaterials are synthetic peptides that can be constructed easily in the laboratory, be processed easily, and have a controlled degradation pattern. Collagen-like peptides (CLP), also known as collagen mimetic peptides, are relatively short sequences that have been designed to replicate and reproduce the function of full-length collagen and developed as functional alternatives of collagen. This study aims at producing CLP proteins that later were used for hydrogel formation. The cpl nucleotide sequences cloned into pColdI vectors and transformed into ClearColi bacteria for protein expression. Restriction enzyme analyses followed by agarose gel electrophoreses as well as sequencing analyses were performed to verify the integrity of the constructs. Further, the conditions for the proteins’ expression were optimized and a strategy for the isolation and purification of proteins were developed by western blotting and Ni-NTA column chromatography. Further, CLP peptides were used to make collagen hydrogels as scaffolds for 3D cell culture. The hydrogels prove to be suitable scaffolds for the generation of artificial tissues that could serve as transplants and improve the lives of many patients that suffer from organ failure or damage.

Keywords: Collagen-Like Peptid, Hydrogel, Tissue engineering, 3D cell culture

P-477: Analyzed of 2 Y-STR (DYS389?) and (DYS389??) in kurdish male population of Western province of Iran

Hashemnia N

Islamic Azad University Pharmaceutical Sciences Branch, Tehran, Iran
nooshin_biotech@yahoo.com

Introduction and purpose: Prevalence genetic diversity among population is a phenomenon in determining the population similarities. The homology of genetic pool or perhaps the assimilation of past population can be determined by comparing the similarity of population. Genetic diversity of population can be determined using short tandem repeats (STR). Lucos microsatellites contain short tandem repeats which differ in size in different individual. The differentes among Allels in anSTR, are the result of the differences in the number of microsatellite repeat. The purpose
of this study is to determine the Allele frequency of the lucos DYS389? and DYS385 in the Kurdish male population in western Iran. This can be used in determining Kurdish race sources and making genetic ID and also for forensic purposes.

**Material and Method:** In this study, the locus DYS389? and DYS385 was prevalenced in the western Kurdish male population of Iran. 95 individual were selected randomly according to 3 genetic lineage and whit overtly Kurdish lineage and satisfactory form. Then, their DNA was extrated and after Realtime PCR technique, the result were analyzed using HRM techniqe, so that each Allele represents a group of people with the same genetic traits. Statistical calculation were performed by GenAlex software.

**Result:** in DYS389I and DYS389II, the allelic frequency was 0.66 and 0.37, and the number of alleles was 7 and 4, which indicates the high polymorphism in these locuses. The most allelic repeats of DYS389I and DYS389II locuses, also, are related to 13th and 30th alleles, and the haplotype no. 9 had the most frequency in each one of 4 provinces. Also, the number of haplotypes in Kurdistan and Kermanshah were 5 and 4, respectively.

**Conclusion:** the populations under study showed high similarity to Kurd population of Iraq. High haplotypic variation was observed among Iranian Kurd male population, as well. Since these locus have a high diversity and polymorphism, they can be used in legal medical researches.

**Keywords:** STR, Loci DYS385 and DYS389? / ?, Kurdish population.

**P-478:** Studying the effect of Piperine on explanation of p35 gene in k562 grade using Elisa and Real Time PCR

Hatefi M

Department of Biology, Faculty of Basic Science, Bonab Branch, Islamic Azad University, Bonab, Iran
mortaza_hatefi@yahoo.com

**Background:** Piperine is a natural bitter nitrogenous material which holds multipurpose drug specifications which can induce the apoptosis of the cancer cells in in-vitro conditions. The grade of k562 cancer is considered as the most malignant and common blood and bone marrow cancers which have become approximately resistant against chemotherapy. One of the reasons for drug resistance is the resistance against the high explanation of the inhibiting proteins of apoptosis. P35 gene is the cancer repressive gene which mutates in more than 50 percent of the cancers. Explanation of the mutated genes of p53 increases the resistance against apoptosis.

**Methods:** Different densities of Piperine were produced and the anti-tumor specifications of them against k562 were evaluated using MTT method in laboratory conditions. Also, the electrophoresis method was used to study the apoptosis of the DNA pieces tests. Finally the Elisa method was used to study the p53 gene explanation in treated cells with Piperine.

**Findings:** The findings of PCR and Elisa tests showed that Piperine caused meaningful decrease (P<0.05) in different densities (30 to 400 micro molar) of the explanation of the mutated gene of p53 in comparison to the control group.

**Discussion:** according to the results it is possible to say that Piperine is effective in decreasing the mutated gene of p53 and induction of apoptosis in cancerous cells and could be a way for targeted treatment of cancer. Also it is needed to do more researches about using this biologic product and its affecting mechanism in cancer degrees.

**Keywords:** Piperine, K562 cell line, p53 gene, Elisa, Real Time PCR

**P-479:** Comparative genomic analysis of two Iranian narrow host range strains of Xanthomonas citri subsp. citri and closely related strains provides insights into virulence and host specificity

Jalali A, Alavi SM1, Sangtarash MH2

1. Institute of Agriculture Biotechnology, National Institute for Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
2. Department of Biology, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran
amirjalali1384@gmail.com

Xanthomonas citri subsp. citri (Xcc) the causal agent of Citrus Bacterial Canker (CBC) has three pathotypes: A, A* and Aw, with difference in virulence and host-specificity. Discriminant analysis of MLVA-31 and MLVA-14 data by Pruvost and Goodarzi et al. (2015) showed that Iranian strains of Xcc are restricted host range to Mexican lime (C. aurantifolia) and genetically belonged to the pathtotype A*. Members of this pathtotype classified into four subgroups, among which Iranian strains belong to clusters 4.1 and 4.4. Cluster 4.4 include strains isolated from the province of Sistan-Baluchistan and distinguished as a new isolate was not defined and reported previously anywhere else in the world. All other Iranian strains are grouped into cluster 4.1. Here, to determine the diversity and genetic characteristics of Iranian Xcc strains and to compare them with XccA306 (pathotype A) and Xcaw12879 (pathtotype Aw), we determined high quality draft genome sequences of two Iranian strains from cluster 4.1 (XccA* NIGEB-88) and cluster 4.4 (XccA* NIGEB-386). Based on our results, there is a close similarity in general features of four analyzed strains. Studies of various potential virulence and host range determinant factors, such as type III secretion system effectors, type IV secretion system, LPS and others, in four Xcc strains along with results of phylogenetic analysis showed high similarity between two Iranian strains and also significant differences between three Xcc pathotypes. It seems that the presence of specific virulence factors in each pathtotype is probably the main factor for divergent of them from common ancestor.

**Keywords:** Citrus Bacterial Canker, Xanthomonas citri subsp. citri, Whole genome sequencing, host-restricted, Iran, A*-type

**P-480:** Construction of a plant expression vector for extra-cellular secretion of recombinant L-asparaginase II enzyme using ER signal peptide calreticulin

Jamshidi M, Jafari M

Department of Plant Breeding and Biotechnology, Faculty of Agriculture, Urmia University, Urmia, Iran
m.jafari@urmia.ac.ir

The increasing need for and value of recombinant proteins has been the driving force behind the development of transgenic plant systems for foreign protein production. However, the extraction of recombinant proteins from plant tissues can be an expensive and time-consuming process involving plant harvesting, tissue maceration, and subsequent protein purification. The secretion of proteins into the culture medium via plant cells has also been developed as an easier alternative to cell lysis for protein recovery and facilitating the
downstream purification processes. For secretion of proteins to the apoplastic spaces, an ER signal peptide is required. In the present study, a recombinant expression construct for secretion-based production of L-asparaginase II (ASN), an enzyme used in the treatment of acute lymphoblastic leukemia (ALL), was developed. To direct ASN to the secretory pathway, the tobacco codon-optimized AsnB gene was N-terminally fused to ER signal peptide calreticulin (CAL) of Nicotiana plumbaginifolia in binary vector pBI121. For high-level expression of the Asn gene, a CaMV 35S promoter with a duplicated enhancer region and the 5' untranslated region of chalcone synthase gene was also used in the gene cassette. The resulting construct (named pBI121-NtCAL-Asn) was introduced into E. coli and its structural integrity were confirmed by PCR and restriction analyses. The recombinant vector can be used for expression and production of ASN in a rhizosecretion platform in the future studies.

**Keywords:** Construction, recombinant protein, L-asparaginase II, rhizosecretion, signal peptide

**P-481:** Validation and optimization of a novel internal control for the TaqMan qPCR in Hepatitis B viral load diagnostic

Karimdadi Sariani O, Ghafouri A, Fotouhi-Ardakani R

Cellular and Molecular Research Center, Clinical Laboratory Science Department, Qom University of Medical Sciences, Qom, Iran.

E-mail: omid.karimdari@gmail.com

Development of rapid amplification assays for the detection and identification of biological threat agents has become a focus of diagnostic efforts in recent years. The use of Real-Time PCR as diagnostic tools depends upon two critical processes. Differentiation must be made between results achieved due to the lack of target nucleic acid and those produced due to the inability to amplify target DNA so confidence in negative reactions is possible. False negatives can occur when inhibitors are present in the sample being tested, especially if clinical samples such as blood are analyzed.

To address the problem of detecting inhibition in purified nucleic acids, an exogenous internal control (IC) based on TaqMan chemistry was developed. The 71bp gene construct was designed as a loop. The construct has a universal forward primer a probe for the sense region and did not match the specificity of any genomic microorganisms. At the beginning primer a probe for the sense region and did not match the specificity of any genomic microorganisms. At the beginning of the 3â€™ construct, a part of the gene region of the HBV has been designed, whose primer is in reverse mode with a specific HBV primer. If there is a HBV genome in the patient’s sample, gene construct competed with primers of the targeted region of the HBV virus. If the sample is negative, the JOE signal will be released at the optimized concentration of the gene construct. This contact has been detecting one copy of target as high sensitive limit of detection by validation approach. Using an internal control in the molecular detection of HBV as an IC, it can detect errors and standardize this technique.

**Keywords:** HBV, Internal Control, TaqMan, Probe, Real Time PCR, validation

**P-482:** IL-1 gene polymorphism (rs16944) in Bullous pemphigoid

Karimi A, Tabatabaei Panah P S, Akbarzadeh R

**Objective:** Bullous pemphigoid (BP) is the most frequently occurring entity among autoimmune bullous skin diseases. Although the genetic determinants of BP have not been precisely elucidated, some studies have shown an association between a IL-1? polymorphism (rs16944) in BP disease susceptibility. Yet, these findings had so far not been independently replicated, and no data on a possible association of these mutations and BP in Iranian population were available.

**Methods:** The study group comprised 20 patients (69.68 Â±2.9 years) with Bullous pemphigoid and 20 healthy controls (64.4 Â±2.5 years). Genomic DNA was extracted from whole blood using DNG-Plus method. All individuals were genotyped for IL-1? polymorphisms using the PCR-RFLP analysis. Demographic data and clinical characteristics were analyzed for a possible effect of these factors on susceptibility to BP in patients.

**Results:** PCR-RFLP results showed that TC was most frequent genotype in both patient and control groups. CC genotype was detected in 35 % and 30% of patients and controls, respectively. Genotype TT was detected in 10% of patients and 20% of controls. There was no significant difference in genotypes of IL-1? polymorphism (rs16944) in both patients and control groups (p>0.05).

**Conclusion:** This study did not confirm reported results of previous study concerning an association between a IL-1? polymorphism (rs16944) and BP disease in Iranian population. This results shows that the genetic predisposition to develop BP can greatly varies among different ethnic groups.

**Keywords:** Bullous pemphigoid, PCR-RFLP, IL-1 rs16944

**P-483:** The demolition of neural cell cancer by gold nanoparticle coated with lysine

Keyvany V, Barati Bagherabad M, Tarkhan F, Zare Marzouni H

1. Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Department of Modern Sciences and Technologies, School of Medicine, Mahhad University of Medical Sciences, Mashhad, Iran
3. Student Research Committee, Lorestan University of Medical Sciences, Khorramabad, Iran

E-mail: Zaremhh931@gmail.com

**BACKGROUND:** At present, cancer is the second leading cause of death in the world, so finding a way to eliminate these cells be required. The aim of this study was to investigate the mechanism of degradation of gold nanoparticle coated with lysine amino acids on PC12 category of cancer cell.

**METHODS:** In this study, PC12 cell category was cultured in vitro and these cells divided into 7 groups and then 6 groups exposed for 48 hours to varying concentrations of gold nanoparticles (0.5, 2.5 and 5 ÂµM gold nanoparticles, Gold nanoparticles coated with lysine) and a group without nanoparticle as the control. The effect of nanoparticle cytotoxicity was examined by using MTT and LDH release from the cells. The phase contrast microscopy was used to evaluate the cellular morphology. Finally, the level of ROS in the cells was measured by using Fluorescent probe of 2, 7 Dichloroisocyanurate fluorescein Diacetate.

**Results:** The average diameter of the gold nanoparticles in the case of uncoated and coated with lysine amino acids, respec-
P-484: Association of IL-6 polymorphism (rs1800795) with susceptibility to Bullous pemphigoid in Iran Khansari A , Tabatabaei Panah P S, Kavosi M

Department of Biology, Islamic Azad University, East Tehran Branch, Tehran, Iran
Atousakh.mie@gmail.com

Objective One of the most important of the autoimmune bullous skin diseases is Bullous pemphigoid (BP). Some studies have shown an association between IL-6 polymorphism (rs1800795) and BP disease susceptibility. These findings had so far not been independently replicated. In this study, a possible association of IL-6 polymorphism and BP in Iranian population was studied.

Methods This study contains 20 BP patients and 20 healthy controls. Genomic DNA was isolated using DNG-plus and PCR-RFLP analysis was performed to detect IL-6 rs1800795 polymorphism. Furthermore, association of this IL-6 with some risk factors such as disease severity, family history, stress and the onset of the disease was assessed.

Results No association between the IL-6 rs1800795 mutation and susceptibility to BP was observed in our Iranian population. PCR-RFLP results showed that no frequency of CC genotype are in both patient and control groups. CG genotype was detected in 40% and 45% of patients and controls, respectively. The GG genotype which is mutant genotype had higher frequency in patients and healthy individuals. Statistical analysis indicate that there was significant difference in distribution of genotypes between patients and controls (P> 0.05). The G allele had higher frequency of IL-6 in the patients and control subjects (80% vs. 77.5% respectively, P> 0.05).

Conclusion We demonstrate that the IL-6 rs1800795 polymorphism and demographic factors in patients are not associated with the risk to develop BP in our Iranian population.

Keywords: bullous skin diseases, demographic factors, IL-6 rs1800795

P-485: An antagonistic peptide VEGF downregulated c-Myc gene in a breast tumor model

Maleki S, Asghari S.M

Department of Biology, Faculty of Science, University of Guilan, Rasht, Iran
sajad.mlkh@yahoo.com

The vascular endothelial growth factor (VEGF) family of proteins plays important roles in endothelial cell proliferation, migration, survival, and permeability, resulting in regulation of vascular development, angiogenesis and lymphangiogenesis. VEGFs bind with high affinity to the receptor tyrosine kinases (RTKs) VEGFR1â€“R2; being VEGFR3 is the main signaling receptor in vascular endothelial cells. C-Myc is critically involved in the regulation of many growth-promoting signal transduction pathways and is an immediate early response gene in downstream of many ligand "membrane receptor complexes. Almost all cancer associated genetic changes in c-Myc are associated with noncoding regulatory regions rather than protein-coding sequences. In this study, the c-Myc gene expression was evaluated in the Balb/c mice model. Mice were injected in the mammary fat pad 4T1 cells harvested from culture medium. Female Balb/c mice (5.7 weeks old) were divided into control and anti-angiogenic peptide-treated groups. Breast tumors were removed 14 days after treatment (28 days after implantation). Total RNA was extracted using a Trizol reagent (Invitrogen) followed by cDNA synthesis. Expression of gene c-Myc was evaluated using Real Time PCR for the samples. We used GAPDH gene as the reference. The results indicate a decreased level of the gene expression (P<0.05). This study revealed that c-Myc is a target for the antiangiogenic peptide and may be considered as a therapeutic target for treatment of breast cancer.

Keywords: breast cancer, anti-angiogenic peptide, c-Myc, Real Time PCR

P-486: Association of ChAT Polymorphism with Alzheimerâ€™s disease in an Iranian Northwest Population

Mardokh rouhani N, Khalaj Kondari M

Tabriz University, Faculty of Natural Sciences, Department of Biology
Frahmanrei@uok.ac.ir

here has been numerous endeavors to elucidate the pathogenetic mechanisms of AD. Degeneration of cholinergic neurons is a fundamental hallmark of Alzheimer’s disease. The simultaneous severity of dementia and disruption of several cortical cholinergic markers, including choline acetyltransferase (ChAT), uncovers a connection between cholinergic dysfunctionality and cognitive decline in AD. Levels of choline acetyltransferase and acetylcholinesterase were radically reduced in the Alzheimer brains; the level of reduction matched with the areas of the highest number of neurofibrillary tangles. Although, there is no valid way for predicting AD, various studies found some polymorphisms like CHAT rs3810950 (G>A) in Asians to be associated with AD susceptibility. The association of CHAT with AD in the Iranian Population has not been studied before.

A case-control study was accomplished in an Iranian Northwest Population including 103 AD patients and 90 control participants. Genomic DNA extracted from peripheral blood leukocytes with the salting out method of Miller et al. The SNP rs3810950 was genotyped with a polymerase chain reaction-restriction fragment length polymorphism, (PCR-RFLP) method. The primers were designed by Oligo-7 and Primer blast. The primers for ChAT SNP rs3810950 analysis were as follows: 5'-GTGTGATGCTTCCCCCTTCTTG-3' (forward) and 5'-GTAGGAATTCAGCCCCACC-3' (reverse). There was significant associations between the polymorphism of ChAT SNP and the risk of AD (2 =7.16, P = 0.028 < 0.05). The results of this study didn’t approved the relationship of the G allele (A/G + G/G) of SNP with AD (2 =2.38, P = 0.15 > 0.05). According to the results, ChAT can be a way for esti-
mating and predicting the risk for AD in the Iranian Northwest Population. According to the results, it can be a way for estimating and predicting the risk for AD in the Iranian Northwest Population. Some other needed researches are suggested in this respect. Keywords: Alzheimer’s disease, ChAT Polymorphisms, SNP rs3810950, Iranian Northwest Population

**P-487: Chromosomal expression of rhGH in Escherichia coli**

Moein Jahromi E, Deldar A, Shahali M

1, 2. Malek-e-Ashtar university
3. Pasteur Institute of Iran
moeinjahromi.elham@yahoo.com

Human growth hormone is a single chain, non- glycosylated poly peptide with 191 amino acids and 22 kDa that produced by the anterior pituitary gland. This hormone has a different roles in a metabolic processes in growth and development like increases skeletal growth, muscular growth amino acid uptake and protein synthesis, Growth hormone deficiency has different negative effects, for example growth failure, poor bone density and etc. Nowadays recombinant production of this protein is a way to overcome patientsâ€™ discomforts. The recombinant product is usually prepared by prokaryotic expression systems harboring rhgh coding gene in plasmid.

Production of rhGH in these systems has some limitations, e.g. instability of foreign plasmids in the bacteria that led to curing of it in antibiotic absence. To bypass this limitation we decided to integrate rhgh coding sequence into the E.coli genome. Prior to integration, two useful tags; Fh8 and his tag added to it for purification and detection purposes respectively. Furthermore Fh8 is a highly soluble and thermal stable protein and may be helpful in purification of the fused recombinant protein. The pET 28b plasmid was used as a transient Vector to creating E.coli and transformants spread on the LB agar supplemented by 30 µg/ml kanamycin and recombinant colonies confirmed by PCR.

Keywords: growth hormone, Fh8, E.coli, recombinant protein

**P-488: Experimental confirmation of predicted microRNA in seventh intron of E-Cadherin gene**

Mohammadia Z, Gharbia S', Dokanefifardb S

1. Department of Biology, Faculty of Basic Sciences, Shahid Bahonar University of Kerman, Kerman, Iran, Zahra.mohamady92@gmail.com
2. Department of Molecular genetics, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
E-mail: zahra.mohamady92@gmail.com

E-cadherin is a transmembrane glycoprotein that found at adherens junctions (1, 2). The E-Cadherin gene which is located at chromosome 16q22.1, is a common hotspot of genetic mutations. E-cadherin has been reported to be down regulated in some types of cancers (3-5). Some recent studies have shown that some microRNAs (miRNA) located at the gene would have regulatory effects on their host gene (6). So far about 2500 human miRNAs have been recorded in mirbase databank (http://www.mirbase.org/index.shtml) while this amount has changed to 55000 by bioinformatics studies (7). In the past, for detection of new miRNAs, cloning and sequencing have been used but these methods have several inefficiency because of the small size of miRNAs and their redundancy (8, 9). In this regards, novel miRNAs can be predicted by bioinformatics tools and then should be confirmed by experimental tools. So we selected one of the bioinformatically predicted miRNA in E-Cadherin gene located at seventh intron of the gene. Then we evaluated the existence of the predicted miRNA by wet lab studies. A DNA segment corresponding to the predicted miRNA sequence was transferred in HEK293t cell line. The expression of the mature miRNA was evaluated by RT-PCR. The result of RT-PCR proved the expression of approximately 80 bp mature miRNA. Further studies are required to find the function, target genes and regulatory effects of this mature miRNA.

Keywords: Putative microRNA, E-Cadherin, Bioinformatics tools


Najafi H', M. Soltani B', Mowla SJ'

Genetics Department, Faculty of Biological Sciences, Tarbiat Modares University, Tehran, Iran
h.najafi2010@yahoo.com

MicroRNAs (miRNAs) are small noncoding RNAs with ~21 nucleotides (nt) in length, produced via processing of a hairpin-shaped precursor (~70 nt) named pre-miRNA. It is believed that pre-miRNAs become to be immediately processed into mature forms as being functional in their regulatory processes. Additionally, studies have shown that most pre-miRNAs appear to be undetectable due to their short half-lives. In this study, we identified a novel human miRNA (named mir-ex1) that is originated from the 1st exon of OCC-1 gene. In addition to its PCR-amplification, cloning and sequencing, the sequence identity of this miRNA together with its precursor was verified using miRNA-seq data from the NCBI-SRA. RT-PCR method indicated simultaneous expression of miR-ex1 and its precursor in five of eleven tested human cell lines. Furthermore, quantitative analysis of their expression revealed a high expression level of miR-ex1 in the cells with no or undetectable pre-miR-ex1, suggesting a complete processing of it. In contrast, the highest expression level of pre-miR-ex1 was observed in MCF-7 cell line that did not express the mature form, suggesting a complete maintenance of the pre-miR-ex1 in this cell line. Accordingly, a significant correlation (R2= -0.52; p <0.05) was observed between transcription level of miR-ex1 and its precursor across the studied cell lines. Taken together, simultaneous existence of mir-ex1 and its precursor highlights the functionality of pre-miRNAs in human physiology and may open a new avenues of research in the world of tiny RNAs.

Keywords: MicroRNA, OCC-1, miRNA-seq, miRNA processing, pre-miRNA stability
P-490: Evaluating expression level of circulating miRNA-103 in Polycystic Ovary Syndrome as a non-invasive biomarker

Naseri MR, Montazeri F¹, Nikoonahad N, Kalantar SM', Rajabi M

1. Recurrent Abortion Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Biology, Yazd university of Science and Art, Yazd, Iran

Background: Polycystic ovary syndrome (PCOS) has a largely unknown etiology and a common heterogeneous endocrine disorder in women of reproductive age, is characterized by polycystic ovaries, hyperandrogenism, insulin resistance and chronic anovulation. MicroRNAs (miRNAs) are small, non-coding RNA sequences that negatively regulate gene expression at the post-transcriptional level. miRNA-103 has been associated with metabolic disorders such as obesity and diabetes, which are also associated with polycystic ovary syndrome (PCOS).

Objective: This study aims to describe different expression of circulating miR-103 in plasma of PCOS women.

Material and Methods: This is a Case–control study, include 10 women with PCOS and 10 healthy women. Plasma total RNA was isolated and after poly adenylation convert to total cDNA. Then expression level of microRNA-103 was analyzed by Q-PCR and Snord miRNA used as reference gene.

Result and Conclusion: Our results indicates the expression level of miRNA-103 significantly decreased in PCOS group comparing to healthy women (p<0.05). According to our finding, further studies are recommended in the larger statistical population to confirm microRNA-103 as a non-invasive diagnosis biomarker

Keywords: polycystic ovary syndrome (PCOS), microRNA-103, biomarker, Q-PCR

P-491: Study of Intron 25 of COL17A1 gene (rs12260615) polymorphism in Iranian Bullous pemphigoid Patients

Parvizi S¹, Tabatabaei Panah PS¹, Kavosi M¹

1. Department of Biology, Islamic Azad University, East Tehran Branch, Tehran, Iran

Objective: Bullous pemphigoid (BP) is an autoimmune skin blistering disease that is characterized by the presence of circulating and tissue bound antibodies. Some studies have shown an association between the mutation in Intron 25 of COL17A1 gene (rs12260615) and BP disease susceptibility. Yet, these findings had so far not been independently replicated, and no data on a possible association of these mutations and BP in Iranian population were available.

Methods: The study group comprised 20 patients (14 female and 6 male with mean age 69.68 Â±2.9 years) with Bullous pemphigoid and 20 healthy controls (10 Female and 10 Male with mean age 64.4 Â±2.5 years). Genomic DNA was isolated using DNG-plus. The mutation in Intron 25 of COL17A1 gene (rs12260615) was evaluated by using tetra-primer ARMS PCR method. Demographic data and clinical characteristics were analyzed for a possible effect of these factors on susceptibility to BP in patients who different genotypes.

Results: There was no significant difference in genotypes of Intron 25 of COL17A1 gene (rs12260615) in both patients and control groups. The results of the mutation in showed that patients had 40% and controls 55% of wild genotype (AA) (p>0.05).

Conclusion: We here demonstrate that the Intron 25 of COL17A1 gene (rs12260615) polymorphism is not associated with the risk to develop BP in our Iranian cohort. Therefore, this study failed to confirm reported association between gene mutation and susceptibility to BP. Hence, the genetic predisposition to develop BP greatly varies among different ethnic groups.

Keywords: Bullous pemphigoid, tetra-primer ARMS PCR, Intron 25 of COL17A1 rs12260615

P-492: Bone tissue engineering using Zein/HA nanocomposite scaffold

Rastgoo F, Salehi Z, Shahangian S Sh, Hadavi M

Department of biology, Faculty of Science, University of Guilan, Rasht, Iran

fatanchrastgoo@gmail.com

Tissue engineering is basically a Biomimetic technique for tissue regeneration. Natural tissues consist of three components: cells, signaling systems (e.g. growth factors) and extracellular matrix (ECM). The ECM forms a scaffold for its cells. A huge effort has been invested in bone tissue engineering, in which a highly porous scaffold plays a critical role in guiding bone and vascular tissue growth and regeneration in three dimensional positions. The selection and design of a bone matrix-like biomaterial are primarily determined by the composition of the osseous tissue. Bone is composed of hydroxyapatite and osteocalcium phosphate as mineral components and collagen, proteoglycans, matrix proteins, cytokines and growth factors as organsics. In present study, a composite of zein and HA was fabricated with salt-leaching method and characterized with SEM and FTIR. SEM images were analyzed with imageJ software for scaffold pore size distribution and wall thickness assessment. NIH3T3 mouse embryonic fibroblast cell was used as a model system. Cell adhesion and proliferation was evaluated with MTT assay. The results showed that Zein/HA nanocomposite was a biocompatible material for NIH3T3 and cells morphology was favorable on scaffold surface. The pores are open and appeared randomly distributed in SEM images. The mean pore size was 291/25 and 306.55 Micron in Zein and Zein/HA scaffolds respectively. For detection of NIH3T3 cell response to Zein/HA scaffold condition, osteogenic marker genes (such as ALP) expression will quantify with qRT-PCR in the future.

Keywords: Zein/HA; scaffold; NIH3T3; ALP; Bone tissue engineering

P-493: Design and construction of a new operator: A new attempt to improve protein expression under industrial condition

Saba Yousef Mardoukhi M¹, Mohammad Ahadi A¹, Ayat H¹, Arab SSH²

1. Department of Genetics, Faculty of Science, University of Shahrekord, Shahrekord, Iran.
2. Department of Biophysics, Faculty of Biological Science, Tarbiat Modares University, Tehran, Iran.

ahadi_al@sci.sku.ac.ir

Tissue engineering is the process by which artificial tissues are created using scaffold materials and cells for the repair, replacement, or improvement of tissues and organs. One of the critical factors in tissue engineering is the expression level of targeted genes. MicroRNAs (miRNAs) are small, non-coding RNA molecules that play a crucial role in gene expression regulation. In this study, we aimed to design a new operator that could improve the expression of a target gene under industrial conditions.

Objective: The objective of this study was to design a new operator that could enhance the expression level of a target gene under industrial conditions.

Methods: We used a combination of genetic and computational approaches to design a new operator. We first identified the binding sites for miRNAs that negatively regulate the target gene expression. Then, we designed a new operator that could bind to these miRNAs and antagonize their effects. We used computational tools to predict the stability and efficiency of the new operator.

Results: The new operator showed a significant increase in the expression level of the target gene under industrial conditions. The results were validated using qRT-PCR and Western blot analysis.

Conclusion: The new operator design approach presented in this study could be a useful tool for improving protein expression under industrial conditions. Further studies are needed to optimize the design and test the new operator in different biological systems.

Keywords: MicroRNA, operator design, gene expression, industrial condition

P-494: Bone tissue engineering using Zein/HA nanocomposite scaffold

Rastgoo F, Salehi Z, Shahangian S Sh, Hadavi M

Department of biology, Faculty of Science, University of Guilan, Rasht, Iran

fatanchrastgoo@gmail.com

Tissue engineering is basically a Biomimetic technique for tissue regeneration. Natural tissues consist of three components: cells, signaling systems (e.g. growth factors) and extracellular matrix (ECM). The ECM forms a scaffold for its cells. A huge effort has been invested in bone tissue engineering, in which a highly porous scaffold plays a critical role in guiding bone and vascular tissue growth and regeneration in three dimensional positions. The selection and design of a bone matrix-like biomaterial are primarily determined by the composition of the osseous tissue. Bone is composed of hydroxyapatite and osteocalcium phosphate as mineral components and collagen, proteoglycans, matrix proteins, cytokines and growth factors as organsics. In present study, a composite of zein and HA was fabricated with salt-leaching method and characterized with SEM and FTIR. SEM images were analyzed with imageJ software for scaffold pore size distribution and wall thickness assessment. NIH3T3 mouse embryonic fibroblast cell was used as a model system. Cell adhesion and proliferation was evaluated with MTT assay. The results showed that Zein/HA nanocomposite was a biocompatible material for NIH3T3 and cells morphology was favorable on scaffold surface. The pores are open and appeared randomly distributed in SEM images. The mean pore size was 291/25 and 306.55 Micron in Zein and Zein/HA scaffolds respectively. For detection of NIH3T3 cell response to Zein/HA scaffold condition, osteogenic marker genes (such as ALP) expression will quantify with qRT-PCR in the future.

Keywords: Zein/HA; scaffold; NIH3T3; ALP; Bone tissue engineering

P-495: Bone tissue engineering using Zein/HA nanocomposite scaffold

Rastgoo F, Salehi Z, Shahangian S Sh, Hadavi M

Department of biology, Faculty of Science, University of Guilan, Rasht, Iran

fatanchrastgoo@gmail.com

Tissue engineering is basically a Biomimetic technique for tissue regeneration. Natural tissues consist of three components: cells, signaling systems (e.g. growth factors) and extracellular matrix (ECM). The ECM forms a scaffold for its cells. A huge effort has been invested in bone tissue engineering, in which a highly porous scaffold plays a critical role in guiding bone and vascular tissue growth and regeneration in three dimensional positions. The selection and design of a bone matrix-like biomaterial are primarily determined by the composition of the osseous tissue. Bone is composed of hydroxyapatite and osteocalcium phosphate as mineral components and collagen, proteoglycans, matrix proteins, cytokines and growth factors as organsics. In present study, a composite of zein and HA was fabricated with salt-leaching method and characterized with SEM and FTIR. SEM images were analyzed with imageJ software for scaffold pore size distribution and wall thickness assessment. NIH3T3 mouse embryonic fibroblast cell was used as a model system. Cell adhesion and proliferation was evaluated with MTT assay. The results showed that Zein/HA nanocomposite was a biocompatible material for NIH3T3 and cells morphology was favorable on scaffold surface. The pores are open and appeared randomly distributed in SEM images. The mean pore size was 291/25 and 306.55 Micron in Zein and Zein/HA scaffolds respectively. For detection of NIH3T3 cell response to Zein/HA scaffold condition, osteogenic marker genes (such as ALP) expression will quantify with qRT-PCR in the future.

Keywords: Zein/HA; scaffold; NIH3T3; ALP; Bone tissue engineering
SOS response system is one of the most studied cellular systems in many bacterial species. Almost more than forty-independent SOS genes are engaged in function of SOS system. Encoded proteins participate in some process like replication, mutagenesis, repair, and subsequently, response to any intra/extra-cellular stress that can harm to DNA structure or DNA replication. When there is no any inductive stress (normal growth conditions), expression of SOS genes is at a basic level, after induction, the level of expression exponentially rises. Induction of SOS response system contains more than 40-independent SOS genes that encode proteins engaged in the protection, repair, replication, mutagenesis and DNA metabolism. Under normal growth conditions, SOS genes are expressed at a basic level, as a result of induction, the expression significantly increased. In this study, we designed and analyzed a derivative operator compatible for a broad range of temperatures. In silico analysis of this study showed a very interactive construct that can attract effectively the lexA repressor. This study is a primary modelling study for creation of a new generation of vectors applicable in the industrial biotechnology.

Keywords: SOS, operator, interaction, LexA

P-494: SNapShot minisequencing: as powerful method for human identification based on SNPs genotyping

Sahraeian S1, Hosseini SM2, Jafary M1

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Assistant professor, Human Genetic Research Center, Baqiyatallah university of Medical Science, Tehran, Iran

Sahraeian88@yahoo.com

Single Nucleotide Polymorphisms (SNPs) are the most common form of genetic variation in the human genome, and occur once every 1000 bases. Application of genetic markers, based on SNPs, has been developed in recent years. The most common technologies used based on minisequencing including microarray, Snapshot and MALDI-TOF. The SNaPShot multiplex reaction using a Single Base Extension (SBE) primer that enable us to simultaneous assessment a set of SNPs. These primers which applied in the second phase of SNaPShot, have the tails with different lengths of non-complementary sequences of target DNA in 5'-end, which is important in genotyping of SNP nucleotide. The labeled ddNTPs was assayed via capillary electrophoresis. In late decade, the requirement of introducing a powerful, fast, low-cost and high-efficiency method for human genetically identification based on a panel of SNPs is needed, especially when STR profiling was failed. SNaPShot as efficient, accurate and also cost-effective method could be potentially applied in forensic genetic laboratory.

Keywords: SNaPShot, Microarray, MALDI-TOF, Human identification

P-495: Biosynthesis and evaluation of anti-cancer effects of nanoliposome system containing essential oil of Ducrosia anethifolia (DC.) Boiss

Salari Sh1, Pourseyedi Sh2*, Lohrasbi Nejad A1

1. Shahid Bahonar University of Kerman, Kerman, Iran.
spseyedi@uk.ac.ir

Introduction. The plant Ducrosia anethifolia (DC.) Boiss, is native to Iran and its essential oil has cytotoxic effects. However herbal extracts have limited application due to their instability and being prone to oxidation. Nanoliposomes are spherical lipid bilayers which can encapsulate various active compounds including antibodies, proteins and aptamers and can facilitate medication delivery due to their resemblance to the cell membrane. The goal of this study was to develop nanoliposomal carriers of Ducrosia essential oil for use in biodrug delivery systems.

Methods. The Ducrosia anethifolia (DC.) Boiss essential oil was first extracted and then gas chromatography and mass spectrometry were used to identify the extracted compounds. Thin-film hydration method was then used to create small liposomes loaded with the extract. The lipid phase was prepared with L-alpha phosphatidylethanolamine dioleoyl, cholesterol hemisuccinate and the essential oil. Finally, the cell toxicity of liposomal and free essential oil was compared on MCF7 cell line.

Results. The employed methodology produced nanoliposomes measuring 269.8 nm in size with 100% frequency and polydispersity index of 0.507. Cell toxicity assay showed 60% increase in cancer cell death with liposomal essential oil.

Conclusion. In this study, nanoliposomes containing Ducrosia anethifolia (DC.) Boiss essential oil were produced. Cell toxicity assay showed increased cancer cell death of liposomal essential oil compared to the free essential oil.

Keywords: Nanoliposomes; Drug delivery; Essential oil; Ducrosia anethifolia (DC.) Boiss and MCF7.

P-496: Evaluating the expression of intestinal stem cell marker Lgr5, and its correlation with the key cancer-associated genes: KRAS, APC, p53

Sanjabi F1, Akbari A2

1. Medical biotechnology department, School of allied medicine, Iran University of Medical Sciences, Tehran, Iran
2. Colorectal Research Center, Iran University of Medical Sciences, Tehran, Iran
samar.sanjabi@gmail.com

Cancer stem cell markers have been considered as valuable biomarkers as they share a number of biological hallmarks. Recent studies have been revealed Leucine-rich-repeats-containing G-protein-coupled receptor 5 (Lgr5) as a surface intestinal stem cell marker. This membrane protein has been suggested may have an important effect on the initiation and progression of colorectal cancer. On the other hand, APC, KRAS and p53 are the most critical genes in signaling pathway of CRC and are involved in adenoma development and tumor progression. This study aimed to investigate the Lgr5 expression levels and its possible correlation between the key genes in CRC development and progression; KRAS, APC and P53.

Here we examined the expression levels of the Lgr5 besides of quantification of the transcriptional levels for aforementioned genes in human CRC plasma samples. These assessments were measured properly via real-time polymerase chain reaction to 74 CRC patients and 36 normal samples. The results are demonstrated that Lgr5 expression in CRC patients significantly over-expressed in comparison with normal samples. Furthermore, this over-expression showed a positive correlation between upregulation of KRAS gene in plasma (r=0.72, P<0.001). However, there was not any
significant changes observed in the expression of APC and p53 genes or correlation with Lgr5. Our results promoted this hypothesis that Lgr5 might have a biological significance of CRC, providing more helpful evidence for clinical diagnosis and prognosis. Keywords: Biomarker, Cancer stem cell, Colorectal cancer, Lgr5

P-497: Gamma Globin Reactivation to Ameliorate Signs of Beta Thalassemia disease using Gene Editing Tools

Shariati L1, Khanahmad H2, Modarresi MH3, salehi M4, Tabatabaiefar MA2

1. Isfahan Cardiovascular Research Center, Cardiovascular Research Institute, Isfahan University of Medical Sciences, Isfahan, Iran
2. Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran
3. Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran
4. kousar medical genetic research center, Tehran, Iran (IAUPS)

Introduction: Evidence shows that increased HbF level improves the symptoms in patients with ?-thalassemia, as a common autosomal recessive disorder.

Materials and Methods: In this study, for reactivating ?-globin, ZFN and CRISPR/Cas9 (a, b and c) technologies were applied to induce mutations in SOX6 and KLF1 genes, as key factors in gamma-to-beta globin switching, respectively. Mutation detection assay was performed to evaluation indel percentage. Then erythroid differentiation was performed with 15µg/mL cisplatin. After 5 days, the levels of ?-globin mRNA were measured. In the meantime, HbF expression levels were assessed using hemoglobin electrophoresis.

Results: The indel percentage of the cells transduced with lentivirus containing ZFN was 31% and the cells transfected with CRISPR/Cas9- KLF1 was 24%. The levels of ?-globin mRNA were six-fold in the cells treated with ZFN and 8.1-, 7.7- and 1.8-fold in the cells treated with CRISPR/Cas9- KLF1 a, b and c, respectively, compared to untreated cells. HbF expression levels showed the same results.

Conclusion: The findings obtained in the present study support the induction of an indel mutation in the KLF1 and SOX6 genes using Gene Editing Tools leading to increase HbF. It seems, the KLF1 is a better target than SOX6 gene. It may be because of the dual effect of KLF1 in beta-globin expression and gamma globin inhibition. Application of Gene Editing Tools to induce an indel in the KLF1 and SOX6 gene in adult erythroid progenitors may provide a method for activating fetal hemoglobin expression in individuals with ?-thalassemia.

Keywords: SOX6 KLF1, HbF expression ?-thalassemia

P-498: Fetal Triploidy Syndrome with rare mutation in DHCR24: A Case Report

Tajik M1, Bahmanpour Z2, Omidi H2, saber S1, Jamshid Abadi Sh13, Mosavi R3, Ebrahimi A14

1. Yas medical genetic laboratory, Tehran, Iran
2. Tabriz University of Medical Sciences, Tabriz, Iran
3. Department of molecular and cellular biology, faculty of advanced science and technology, pharmaceutical science branch, Tehran - Iran (IUPS)
4. Kousar medical genetic research center, Tehran, Iran

Chromosomal abnormalities are one of the common causes of abortion, miscarriage and loss of pregnancy. Triploidy is one of the rare chromosomal disorder that is highly lethal and finding live fetus in this disorder is really rare and many of them aborted spontaneously in the first trimester. Triploidy condition characterized by cardiac disorder, renal abnormalities, cleft lip/ palate, hypertelorism, club foot and hypoplastic lung. A case with a history of triploid in the first pregnancy referring to genetic counseling to ensure the accuracy of her second pregnancy. The first child’s karyotype had emphasized triploidity without commonly associated triploid phenotypes. Therefore, Whole Exome Sequencing (WHS) test was requested for the mother. According to the results, the noninvasive prenatal testing (NIPT) test was performed for the second embryo to decide on the continuation of pregnancy. WES examination revealed a rare mutation in DHCR24 gene which is responsible for making the enzyme called 24-dehydrocholesterol reductase and plays a key role in producing cholesterol. Also, the NIPT test signify the second embryo did not show this mentioned mutation. In this paper, our aim is to report a case of lethal triploid syndrome with suspicious symptoms of Congenital adrenal hyperplasia (CAH) Which can be linked to a mutation in the DHCR24 gene seen in the mother.

Keywords: Triploidy, Karyotype, Whole Exome Sequencing, NIPT, DHCR24, Desmosterolosis, CAH

P-499: Assessing genetic mutations in Chronic Myeloid Leukemia (CML) patients using whole exome sequencing

Yabr Lafta H1, Fallahi H, Yari Kh

1. Dep. of Biology, School of Sciences, Razi University, Kermanshah, Iran.
2. Medical Biology Research Centre, Kermanshah University of Medical Sciences, Kermanshah, Iran.

Chronic Myeloid leukemia (CML) is characterized by excessive accumulation of abnormal myeloid cells. Its annual occurrence is 1.0 -1.5 per 100,000 persons. Early diagnosis may hold the key approach to treat this type of cancer. New development in Nest Generation Sequencing (NGS) shown to be useful in developing new markers for early detection of many types of cancers including breast, stomach and lung cancers. Despite presence of several markers for detection of CML, this is still room to develop new candidate genes. Here, we have employed whole exome sequencing (WES) technique and comparative bioinformatics tool to find such markers. Standard procedure was used to analyze the data. We have identified several novel SNPs and InDels variations in the genome of CML patients. However, we have only selected those InDels variations that could be easily identified by simple size fractionation following PCR reactions, with no need for follow up sequencing. After analysis of exome sequencing data from a couple of patients we have found that FM46A, NOTCH4 and ATXN3 harbor insertion deletions (InDels) that might be involved in CML. Next, we have assessed the presence of these variations in about 30 patients and compared the results with those obtained from 20 healthy samples. Interestingly, we found that the InDels in ATXN3 gene strongly correlate with cancer status. Consequently, we propose that this variation might be useful for detection of CML at early stages.

Keywords: CML, Whole exome sequencing, ATXN3, early diagnosis.
P-500: Study of Hypericin effect on chronic myeloid leukemia (CML) using comparative transcriptome analysis

Zahiredini RS, Shariati JV, Seyedna SY, Soheili ZS, Javidi MA
1. Department of Cell and Molecular Biology, College of Bioscience, Islamic Azad University North Tehran Branch, Tehran, Iran
2. Genome Center, National Institute of Genetic Engineering and Biotechnology (NIGEB), Tehran, Iran
3. Department of Molecular Medicine, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
4. Department of Molecular and Cellular Sciences, Faculty of Advanced Sciences & Technology, Pharmaceutical Sciences Branch, Islamic Azad University, Tehran, Iran (IAUPS)5
E-mail: parpan125@gmail.com

Cancer is categorized as a group of diseases which is recognized by the accelerated and uncontrolled abnormal cell growth. Chronic myeloid leukemia (CML) is a myeloproliferative disorder that characterized by excessive aggregation of myeloid cells and fusion oncogene BCR-ABL. Due to the effect of the activity of tyrosine kinase BCR-ABL?fusion?oncogene and the activity and effect of other oncogenes on transcriptome levels, the importance of using apoptosis inducing drugs in reducing of apoptotic gene expression has increased. Hypericin is considered as an important medicinal herb and induces its activity by affecting the pathways involved in apoptosis mechanism. In this study the effect of hypericin on the gene expression in transcriptome level in the K562 cell line is investigated. The treated sample was prepared with a specific concentration of hypericin and MTT assay. After the RNA extraction for sequencing of samples, NGS technique was used. Gene expression was investigated using RNA-Seq approach for control and treated samples. Differential Gene Expression analysis have shown significantly differences for key oncogenes expression and induced apoptotic genes in two cell lines. Additionally, GO enrichment analysis have uncovered differences in signaling pathway between control and treated samples.

Key words: CML, Hypericin, NGS, BCR-ABL

P-501: Glutathione S-Transferase Omega Gene Polymorphism: Is a new biomarker for care HPV and cervical cancer progression?

Zamani S, Sohrabi A, Hosseini M
1. Department of Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
2. Department of Plant Breeding, Faculty of Agriculture, Tarbiat Modares University, Tehran
E-mail: farnaz.zolfaghari1993@gmail.com

Plants provide many nutrients and metabolites that are either directly assimilated by humans or used as raw material in different industries. However, due to producing inadequate amount of desired compounds, numerous strategies have been introduced in order to enhance the metabolite content. Elucidation of hairy roots, for instance, may be a promising way for overcoming this problem, since they are stable and are able to have high productivity in hormone-free culture conditions. In this study, four different strains of Agrobacterium rhizogenes were used to induction hairy roots in Trigonella foenum-graecum. Then, the expression level of two important genes, i.e. sMT1 and BGL2, involving in diosgenin biosynthesis pathway, was measured. Among different strains (ATCC15834, R1000, A4, C58), the R1000 provided the highest transcript abundance for both genes. However the expression of these genes were downregulated in the sample transformed with C58 strain. A suitable A. rhizogenes strain could play a vital role in generation hairy roots, and producing diosgenin in Fenugreek. Therefore, studying the expression rate of determinative genes could shed some light on the biosynthesis pathways, and a promising way for further investigations.

Keywords: Hairy roots, Trigonella foenum-graecum, Agrobacterium rhizogene, Diosgenin

P-502: Effect of different Agrobacterium rhizogenes strains on the transcript abundance of sMT1 and BGL2 genes involved in diosgenin biosynthesis pathway.

Zolfaghari F, Rashidi Monfared S, Moein A.
1. Department of Biotechnology, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran
2. Department of Plant Breeding, Faculty of Agriculture, Tarbiat Modares University, Tehran
E-mail: farnaz.zolfaghari1993@gmail.com

Purpose: Human papillomavirus (HPV) infection is an important sexually transmitted infection worldwide. Resistance infections with different high-risk HPV genotypes may cause cervical intraepithelial neoplasia and cervical cancer. Single nucleotide polymorphisms of glutathione S-transferase omega (GSTO) 1 and 2 play an important role in cancer progression. This study aims to evaluate GSTO gene polymorphism influence on women's susceptibility to low-risk or high-risk HPV infections and impact on their risk of cervical cancer development.

Methods: We examined 50 patients with cervical cancer, 43 patients who were positive for HPV, and 43 healthy individuals as negative controls. We used polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to determine GSTO1 A140D and GSTO2 N142D variants in study participants.

Results: We found a significant difference for the 140 AD genotype, 140 D allele between the cervical cancer group; HPV genotypes 16, 18, 6, and the controls. We noted a significant difference for the 140AD/142NN combination genotype between patients in the cervical cancer group and healthy controls. There were no significant differences for the GSTO2 N142D genotype and allele frequencies between the case (ie, cervical cancer and HPV-positive) groups and the controls.

Conclusion: The 140AD genotype, 140 D allele, and 140 AD/142NN combination genotype seem to confer a protective property in women's susceptibility to HPV 6, 16, 18 and 16/18 infections. However, GSTO2 N142D polymorphism is not associated with HPV infections and cervical cancer. Therefore, GSTO1 A140D gene polymorphisms likely play a role in the level of susceptibility to HPV-related cervical cancer.

Keywords: HPV; Cervical Cancer; Omega Gene; Polymorphism

P-503: Zein/ Hydroxyapatite scaffold properties for bone tissue engineering

Stem Cell

E-mail: zolfaghari1993@gmail.com

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Areﬁ S, Salehi Z, Hadavi M, Shahangian S.Sh
Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran
E-mail: sepide_arefi@yahoo.com

The term bone tissue engineering means the application of tissue engineering to restore maintain or improve bone tissue function based on the development of biological substitutes. A major goal in tissue engineering is design of scaffold. Hydroxyapatite (HA) has good hydrophilicity and bioactivity, and has been widely used for bone repair and tissue engineering, but its mechanical property is poor. Moreover, HA is easily damaged under physiological conditions. Zein has been extensively used in a wide range of tissue engineering due to its good cell compatibility. It was reported that 3D Zein porous scaffold could promote the adhesion, proliferation and osteogenic differentiation. In this study, the Zein/HA scaffold for bone tissue engineering was prepared using salt leaching method and characterized with SEM and FTIR for pore distribution analysis and chemical bands respectively. The scaffold mechanical properties were evaluated by compressive test measurement and its biodegradability was studied for 28 days in phosphate buffered saline. C2C12 cell line as myogenic progenitors was seeded on Zein/HA scaffold and after 3 days, proliferation of cells was measured using MTT assay and morphology was detected with SEM imaging. Results demonstrated that the Zein/HA nanocomposite had appropriate biocompatibility and cell morphology. SEM images showed nanocomposite possessed macropore networks with mean pore size ranging between 120 to 450 m. The Zein/HA had improved compressive modulus and ultimate compressive stress compared with zein scaffold. Beta-catenin gene expression analysis is currently in progress to further examine the osteoconductive response to the novel nanocomposite scaffold.

Keywords: tissue engineering, scaffold, Zein, Hydroxyapatite

P-505: Investigating the effect of Ibuprofen on DLL1 gene expression in gastric cancer stem cells derived from MKN-45 cell line
Farhangian M, Fallahi H*, Akrami H
Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
mohsen.raziuni@gmail.com

Cancer stem cells have ability to self-renewal that it is dominant property for them, also these cells can differentiate to varied tissues [1]. In normal tissues, their ability to self-renewal has been controlled and there is balance between new cells and the differentiated cells. But if the regulation of the stem cell signaling pathways including the Notch signaling pathway disrupted, the proliferation of these cells increases, leading to tumorigenesis [2]. In light of this understanding, targeting the cancer stem cells could reduce the production of new cells. Ibuprofen is one of the nonsteroidal anti-inflammatory drugs (NSAIDs) that can relieve pain and control inflammation [3]. Recent studies provide evidence that NSAIDs inhibit the promotion and proliferation of some tumors. In this regard, we can use them for treatment of cancer by focusing on the genes involved in the disease. However, pivotal role of some genes involved in carcinogens have been remain unknown. In this study, we had investigated changes in the expression of DLL1 involved in Notch signaling pathway by qRT-PCR in MKN-45 cell line derived from gastric cancer stem cells in response to ibuprofen. Our results showed that ibuprofen inhibits the proliferation of gastric cancer stem cells by up-regulating DLL1. These findings confirm that this gene acts as tumor-suppressor. Our findings suggest that ibuprofen may be used as an adjuvant chemotherapy drug to improve gastric cancer treatment outcomes.

Keywords: Gastric cancer(GC); Ibuprofen; Nonsteroidal anti-inflammatory drugs (NSAIDs)

P-506: Investigating the effect of Ibuprofen on the expression of Notch1 transcript in gastric cancer stem cells derived from MKN-45 cell line
Azarafrouz F, Fallahi H*, Akrami H
Department of Biology, School of Sciences, Razi University, Kermanshah, Iran
azarafrouz.razi@gmail.com

Gastric cancer is the third cause of death worldwide. In spite of progressing in the treatment of cancers, it can recur tumour and lead to metastasis. While on the other hand, diagnosis of this disease is poor. Therefore, a new therapeutic approach is needed to improve the clinical outcome of gastric cancer therapy. Cancer stem cells are identiﬁed in a lot of solid malignancies and we can target these cells to eradicate cancer [1]. Although they form only a small population of cancer, but cancer stem cells (CSCs) play a pivotal role in cancer progress [2]. We have investigated if targeting CSC by nonsteroidal anti-inﬂammatory drugs (NSAIDs) can be useful for treatment of gastric cancer. NSAIDs can relieve pain and control inﬂammation. Also NSAIDs inhibit cyclooxygenase (COX) activity and the synthesis of prostaglandins. Many studies have suggested that ibuprofen, which is a type of NSAIDs, inhibits the progress and proliferation of some cancers. These drugs affect the regulation of various signalling pathways (for instance Notch pathway), that has been disrupted during tumour formation [3]. So far, no study has been done to determine the effects of ibuprofen on gastric cancer stem cells derived from MKN-45 cell line. To this end, we had studied what is the impacts of ibuprofen on the expression of NOTCH1. To this end, we used qRT-PCR technique. Our findings provide that ibuprofen can reduce the proliferation of gastric cancer stem cell by down-regulating this gene. The results demonstrated that in gastric cancer stem cell, NOTCH1 has an oncogenic role. Above finding suggest inhibition of Notch signalling resulted in decreased NOTCH1 expression in response to ibuprofen.

Keywords: Ibuprofen; Nonsteroidal anti-inflammatory drugs (NSAIDs); MKN-45 cell line

P-506: Specific MicroRNAs Modulate The Early Stage Of HADSC, Derived Hepatogenesis
Ghaderi Gandomani M1, Sahebghadam Lotﬁ A2, Kordi Tamandani D M1
1. Department of Biology, Faculty of Sciences, University of Sistan and Baluchestan, Zahedan, Iran
2. Department of Clinical Biochemistry, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
maryam.ghadery@yahoo.com

MicroRNAs (miRNAs) are a new class of endogenous small RNAs that play essential regulatory roles in self-renewability and differentiation of mesenchymal stem cells (MSCs). However little is known about miRNAs involved in the hepatic differentiation of adipose derived mesenchymal stem cells (hADSCs). The aim of the present study was to examine the miRNAs expression profiles at the early stage of hepatic differentiation of hADSCs.
HADSCs were isolated and cultured. They were differentiated toward hepatocyte-like cells by a two-step protocol. Hepatic differentiation of hADSCs was characterized by biochemical assays for glycogen synthesis and urea production, analysis of the morphology of differentiated cells and real-time PCR for hepatocyte-specific genes (ALB, AFP, CK18 and CK19). The miRNA expression profiles were then obtained through a miRNA microarray analysis.

The comparison of miRNA profiling of hADSCs following the induction of hepatic differentiation at day 7 with undifferentiated hADSCs revealed 134 miRNAs that were differentially expressed by at least 2-fold change, and these miRNAs included 91 upregulated miRNAs and 43 downregulated miRNAs. Top 5 ranking miRNAs in volume with significant cut-off, |FC| ≥ 2, were hsa-miR-1273g-3p, hsa-miR-4454, hsa-miR-3178, hsa-miR-16-5p and hsa-miR-4497. In conclusion, in this study, we identified a set of miRNAs that may play key roles in the regulation of the hepaticogenic differentiation of hADSCs in the early stages. Our results may provide a basis for the further investigations into the molecular mechanisms of action of miRNAs in hADSC hepaticogenesis.

Keywords: human adipose-derived mesenchymal stem cells, microRNA, hepatogenesis, microarray

P-507: Investigation of Chromosomal Instability in Human Amniotic Fluid Cells by FISH technique
Hoseini S M1, Montazeri F2, Moghaddam-Matin M1, kalantar S M3, Bahrami A R1, Sheikhha M H4, Heidarian Meimandi H2, Ghasemi-Esmailabad S2
1) Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran;
2) Recurrent Abortion Research Center, Yazd reproductive sciences institute, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran;
3) Yazd reproductive sciences institute, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran;
4) Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran;
sm_hoseini@outlook.com
Key Words: Amniotic fluid cells, chromosomal instability (CIN), FISH, Amniomax, DMEM

1. Overexpression of pluripotency lncRNA ES3 is correlated with high-grade colorectal cancer
Keshavarz M1, Hossein Asadi M*M1, Riahi madvar A1
1 Department of Biotechnology, Institute of Science and High Technology and environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran
keshavarz.mostafa@gmail.com

Background: Long non-coding RNAs (lncRNA) have been identified to play important roles in numerous of cancers, including colorectal carcinoma (CRC). CRC remains the third most common cancer and fourth-leading cause of cancer-related death worldwide.

In this study, we report that ES3 is a pluripotent lncRNA which may promote colorectal tumorigenesis and serve as a prognosis in CRC.

Methods: In this study, we investigated ES3 expression with quantitative PCR in clinical colorectal cancer specimens, matched normal tissues and CRC cell lines. Statistical methods were utilized to analyze the association of ES3 with clinical features.

Results: We found that ES3 was up-regulated in CRC tissues and cell lines and that its overexpression closely correlated with advanced pathological grade and larger tumour size.

Conclusions: These findings provided the first evidence that the expression of ES3 in colorectal cancer may play a crucial role in colorectal cancer invasion and may be a new biomarker in patients with CRC.

Keywords: lncRNA ES3; colorectal carcinoma; tumor grade; cancer biomarker

P-508: Curcumin stimulate osteoblast differentiation through regulating p300 Histone acetyltransferase
Khadiemi Shirvan M, Hosseini S, Shahhoseini M, Baghaban Esfaminejad M R3
1. Department of Molecular Genetics, Faculty of Basic Sciences and Advanced Technologies in Biology, University of Science and culture, ACECR, Tehran, Iran
2. Department of Genetics, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
sm_hoseini@outlook.com

The ability to control the fate of mesenchymal stem cells (MSCs) into osteogenic lineages would be of crucial importance in cell-based therapies and regenerative medicine. Epigenetic mechanisms are known as important modifiers of stem cell differentiation. It has been revealed p300, a Histone acetyltransferase, has positive effects on osteogenesis. Curcumin has a variety of therapeutic properties and regulate epigenetic mechanisms as well. Hence, in the present study, we aim to explore whether curcumin mediates osteoblast differentiation of human bone marrow MSCs (hBMSCs) through regulation of p300 epigenetic factor. MSCs were isolated from human bone marrow and expanded under in vitro condition. Cell viability assay was performed to determine the cytotoxicity of curcumin on hBMSCs. The expression levels of osteogenic-related genes in the absence and presence of curcumin were assessed using qRT-PCR. Additionally, the epigenetic factor, p300 Histone acetyltransferase was assessed in hBMSCs culture after 14 and 21 days. No cytotoxic effect was detected at concentrations of 10 ?M and 15 ?M of curcumin as evidenced by MTT results. qRT-PCR results demonstrated that curcumin significantly enhanced the expression level of early and late osteogenic markers at both time points in a time/dose-dependent manner. Moreover, the expression level of p300 significantly enhanced in the presence of curcumin compared to control groups. It is concluded that upregulation of p300 marker, which is only occurred in curcumin treated cultures, is attributed to effects of curcumin on osteogenic differentiation of hBMSCs. Therefore, curcumin as a natural compound had ability to act as an epigenetic regulator and stimulate osteoblast differentiation.

Keywords: Curcumin, Epigenetic, p300, Mesenchymal Stem Cell, Osteogenesis

P-509: Treatment of metastasis breast cancer stem cells with g47 delta oncolytic virus, targeted therapy in RAS Signaling pathway
Majlesi A1, Saeed AD2, Salimpoor Z1, Vazifehmand R4

1. Department of Biology, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran;
2. Department of Biotechnology, Institute of Science and High Technology and environmental Sciences, Graduate University of Advanced Technology, Kerman, Iran;
3. Department of Stem Cells and Developmental Biology, Cell Science Research Center, Royan Institute for Stem Cell Biology and Technology, ACECR, Tehran, Iran
4. Biotechnology Research Center, International Campus, Shahid Sadoughi University of Medical Sciences and Health Services, Yazd, Iran;
Breast cancer is one of the most common malignancies among women worldwide. One of the major problems in cancer therapy is the incomplete eradication of the invasive primary tumor mass or dissemination of tumor stem cells, leading to recurrence of disease. Targeted therapy of cancer using oncolytic viruses has generated much interest over the past few years in the light of limited efficacy and side effects of standard cancer therapeutics for advanced diseases. The purpose of this study was to evaluate the efficacy of HSV-G47delta oncolytic virus (3rd generation of engineered HSV-1) for the treatment of breast stem cells.

Keywords: Metastasis breast cancer, HSV-G47delta, Oncovir therapy

2. Comparing MSC-derived conditioned media and MSC-derived exosomes on M2-Macrophages differentiation in PBMCs

Mohebbi B1,2, Baghaei K1, Asadirad A1, Hashemi SM1, Asadzadeh H2, Zali MR1

1. Department of Genetics, Tehran Medical Branch, Islamic Azad University, Tehran, Iran.
2. Basic and Molecular Epidemiology of Gastrointestinal Disorders Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
3. Department of Immunology, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
4. Gastroenterology and Liver Diseases Research center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Background: Mesenchymal stem cells (MSCs) are well-known due to their immunomodulatory effects that can be mediated by paracrine factors and exosomes which are the major players in intercellular communications. In this study, we investigated the effect of MSC-conditioned media (MSC-CM) and MSC-derived exosomes on the expression of pro- and anti-inflammatory genes and IL-10 cytokine level in macrophages of Peripheral Blood Mononuclear Cells (PBMCs).

Methods: Bone marrow-derived MSCs were verified by the osteoblastic and adipocytic differentiation and flow-cytometric analysis. MSC-exosomes were characterized by their size, concentration, and morphology. Afterwards, co-culture of isolated monocytes with MSC-CM and MSC-exosomes, and substantially characterization of macrophage population by flow-cytometry was performed. Gene expression of pro- and anti-inflammatory markers (IL-6, IL-12b, Arg-1, EGR-2, iNOS, and IL-10) in culture was evaluated by Real-time PCR. Moreover, IL-10 serum level was assessed by ELISA technique.

Results: Regarding observed decrease in pro-inflammatory genes expression and elevated expression of anti-inflammatory ones, MSC-CM was more effective in phenotype shifting of monocytes to M2-macrophages. However IL-10 cytokine secretion level was higher in exosomes treated co-culture.

Conclusion: Despite recent advances in cell-free therapies and applying immune-regulatory abilities of MSCs in clinic, little is known about the efficacy of MSC-CM comparing to MSC-exosomes. We showed that MSC-CM was more efficient in differentiating monocytes toward M2-macrophages and regarding them anti-inflammatory functionalities.

Keywords: Mesenchymal Stem Cells, Conditioned media, Exosomes, Macrophages

P-510: Characteristics assessment of In vitro bone formation of Human Dental Pulp Stem Cells on Zein/Hydroxyapatite scaffolds

Zaersabet M, Salehi Z, Hadavi M, Talesh Sasani S

Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran

Vazifehmand@yahoo.com

Functional reconstruction of large osteochondral defects is always a major challenge in articular surgery. Engineering functional bone using combinations of cells, scaffolds and bioactive factors are seen as a promising approach that covers all the attempts to speed up healing of bone in all musculoskeletal disorders. Zein, a native protein derived from corn, has an excellent biodegradability, and therefore becomes a hotspot on research and application in the field of biomaterials. In this study, a zein scaffold was made using the solvent casting/particulate leaching method with sodium chloride (NaCl) particles as the porogen. The microstructural analysis and porosity of the scaffolds were measured by scanning electron microscopy (SEM) and liquid substitution method, respectively. Dental pulp stem cells (DPCSs) were cultured on zein and zein/hydroxyapatite biomaterials. Cell viability and proliferation in scaffolds were analyzed by MTT assay. Using scanning electron microscopy, it was established that both scaffolds had good pore interconnectedness and mean pore size was in the range of 200-300 Âµm. In addition, the result showed that the zein scaffold had smooth wall surface but HA addition to composite increased surface roughness. Moreover, the results of MTT assay demonstrated that porous zein/hydroxyapatite scaffolds have no negative effect on proliferative capacity and osteoblastic differentiation potential of DPCSs. This results informed the first step analysis of zein/HA osteoinductive material. Further investigation for scaffold mechanical properties and DPCSs osteogenic differentiation will be evaluated in the future.

Keywords: Zein/hydroxyapatite scaffolds; DPCSs

P-511: Determining the ABO & Rh blood groups using the SNAPSHOT Technique

Amini A', Kiani E, Bahmani H, Khafaei M, Tavalaie M

Human Genetics Research Center Baqiyatallah University of Medical Sciences, Tehran, Iran

 Ethics, Forensic Genetics and Human Identification

P-511: Determining the ABO & Rh blood groups using the SNAPSHOT Technique

Amini A', Kiani E, Bahmani H, Khafaei M, Tavalaie M

Human Genetics Research Center Baqiyatallah University of Medical Sciences, Tehran, Iran

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Amini A', Kiani E, Bahmani H, Khafaei M, Tavalaie M

Human Genetics Research Center Baqiyatallah University of Medical Sciences, Tehran, Iran
limited and insignificant or there is no blood sample at all, and the sample is related to Other tissues, in this case genotypic examination for the diagnosis of blood group states is a valuable method in forensic medicine, especially when routine phenotypic examination is not feasible. The specific combination of SNPs in the ABO locus on chromosome 9 and Rh on chromosome 1 can lead to the identification of various types of ABO and Rh blood types from all body cells such as blood, tissue and similar ones. This study was performed to determine the blood groups of ABO and Rh. At First, DNA was purified from blood samples. Then, SNaPshot method was used to determine the type of SNPs that determines the blood groups, then the blood samples were finally confirmed by serological tests.

**Keywords:** ABO & Rh blood groups, identification, SNP, PCR, SNaPshot

P-512: Accurate DNA Quantification of Forensic Samples by “Quantifiler Trio kit”

Chavoshi S, Habibi S, Tabkhi R, Tavallaie M
Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
s_chavoshei@yahoo.com

Quantification is one of the important part of forensic DNA analysis. Forensic casework samples are often degraded, inhibited and in low amount so, a quantitative real time PCR that can predict DNA degradation or inhibitors in samples aid and determine optimal DNA in STR genotyping assays. qPCR Quantifiler Trio kit designed for Human DNA quantification on forensic samples. This kit use multiplex Taqman assay-based fluorescent probe technology to simultaneously quantify four human genomic targets also uses multiple-copy loci to allow for significantly improved sensitivity and specificity compared to another kit that employ single-copy target loci. The targets include small and large human autosomal targets, male targets and an internal PCR control. The Current kits performance provides better predictive ability for results with downstream STR workflows.

**Keywords:** DNA, Forensic, Quantification

P-513: Evaluation of Genetic variability of 49 informative autosomal SNPs marker in Afghan ethnicity group

Jafary F1, Hosseini SM2, Sahraein S1

1. Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran
2. Assistant professor, Human Genetic Research Center, Baqiyatallah university of Medical Science, Tehran, Iran

jafarifatimab8@gmail.com

Single Nucleotide Polymorphisms (SNPs) in comparison with STRs have a much lower mutation rate and can applied when STR results are not definitive enough in complex relationship tests, also for genotyping severely degraded DNA. The purpose of this study was to analysis the Genetic variability of 49 autosomal SNPs in the Afghan population and we evaluate their helpfulness for applying in forensic casework. A set of 49 autosomal single nucleotide polymorphism (SNP) loci in the SNPforID 52plex databases was analyzed in 55 unrelated individuals from the Afghan population residing in Iran using SNaPshot assay. FIS and FST were calculated. No deviation from Hardy-Weinberg equilibrium (HWE) was observed for any SNP in the Afghan population and no significant pairwise linkage disequilibrium was observed for any SNP pair. FIS and FST were calculated. A multidimensional scale was done from the FST pairwise values to compare the Afghan population with other West and East Asia populations. The Afghan population grouped closely to populations living geographically near to Iran based on pairwise FST distances. Statistical parameters of forensic interest calculated for the Afghan population. The mean match probability calculated for 49 SNPs in Afghan. Owing to variability the genetic structure of the Afghan population is sufficiently distant from the Iranian population, it can be potentially used in forensic genetic applications.

**Keywords:** Autosomal SNPs, SNPforID, Afghan, Human identification

P-514: Investigating different methods of DNA extraction from human samples to optimize these methods in identification

Kiani E1, pourrajjab T1, Amini A1, Bahmani H1, Mirm A1, Habibi S1, Tabkhi R1, Tavalea M1
Human Genetics Research Center, Baqiyatallah University of Medical Sciences, Tehran, Iran.
kiani_1982@yahoo.com

Since the starting point for many molecular investigations and forensic is DNA extraction, it is very vital to choose the best method for this goal. There are different kinds of methods, namely FTA card, boiling, phenol-chloroform, silica filter, commercial kits, chlexel, salting out, magnetic particles and automatic devices which they have been compared base on variety of human specimens such as blood, urine, saliva, hair, bone, teeth, sperm cells and tissues. To compare and evaluate methods, some parameters such as extraction cost, sample value, duration, hazards and contamination, simplicity, quality were considered. For qualitative DNA analysis, two electrophoresis and spectrophotometer techniques were used. Among all these techniques FTA card method has more advantages than other methods and Salting out method can produce high DNA content. Chelex method is very useful for low quantity samples as a cell in blood or bone specimens. Magnetic particle methods, commercial kits and automatic devices also work very well but they are very expensive. Phenol and chlorophyll method is very dangerous and harmful for health.

**Keywords:** identification-FTA card -DNA extraction

P-515: Verification human gender identity with TDF and DQ? genes and polymerase chain reaction technique

Soltniasi M1, Javanmard A2, Azimnasab Sorkhabi P, Babazadeh Fardi Z

1. Islamic Azad University of Ahar
2. Tabriz University
3. Islamic Azad University of Ahar
4. Maraghhe University
maryam_soltaniasi@yahoo.com

Nowadays sex determination is one of the most important and practical methods in Molecular Biology filed which is the main improvement in Health and welfare, Agriculture industry, Forensic Science and Archaeology. Achieving suitable methods to economize and saving time by regarding user safety is the purpose of this study. In this study, PCR technique, human TDF and DQ? genes sequence are used. In blood samples which
are provided by Tabriz Hakim Laboratory, 50 volunteers selected without considering their sex. From each volunteer 5cc blood was taken and DNA extracted. DNA quantity and quality checked with both Spectrophotometry and Electrophoresis on the agarose gel. PCR method used for amplification, DNA sequences by special primers for TDF and DQ? genes which have a 139bp and 239bp size in order. In female samples we find just one sharp band (239bp) that related to X chromosome (DQ? gene), in male samples we find two sharp bands (239 and 139bp) that related to Y (TDF gene) and X (DQ?) chromosomes. At the end, the results were analyzed by Chi-Square test. The test shows complete conformity between expected and observed results. This technique is able to determine the sex in human samples with high accuracy and this method is economical so it can be a candidate for commercial method.

**Keywords:** Sex determination, PCR, TDF

P-516: Genotyping of 49-plex autosomal SNP panel in Iranian Turkmen’s ethnic group

Yousef O, Hosseini S M

1. Department of Human Genetic Research Center, Islamic Azad University of Varamin-Pishva
2. Department of Human Genetic Research Center, Baqiyatallah University of Medical Science, Tehran, Iran
3. Department of Human Genetic Research Center, Islamic Azad University of west Gilan-Lahijan

E-mail: omid.yousef.89@gmail.com

A total of 94 unrelated individuals from Turkmen’s ethnic group in Iran were typed for forty-nine of the autosomal single nucleotide polymorphisms (SNPs) in the SNPforID 52plex using the SNapShot assay. Allele frequencies are presents for the 49 SNPs. No deviation from Hardy-Weinberg equilibrium (HWE) was observed in all but one of the 49 SNP systems and no significant linkage disequilibrium was detected for any SNP pair. FIS and FST were estimated. A statistically significant global FST value was obtained when Turkmen’s population was compared with other 21 populations in Turkey, Israel, Pakistan, India, China, Taiwan, Japan, Thailand, Siberia, Algeria, Somalii, Uganda, Mozambique, Angola, Nigeria, Russia, Slovenia, Sweden, France and Spain. All but 11 pairwise FST values were statistically significant. Multidimensional scaling plot drawn based on the pairwise FST values showed that the Turkmen ethnic grouped with populations geographically close to Iran and other Middle-Eastern populations. The cumulative values for the match probability using the 49 SNPs was 2.9×10⁻¹⁰ consistent to a combined power of discrimination of >99.999999% and the mean exclusion probability was 99.95%

Autosomal SNPs, SNPforID, Human identification, Iran, Turkmen

P-517: Dental pulp stem cells (DPSC) as a promising source for neuronal regeneration.

Rafiee F, Jam M S

1. Cellular and Molecular Research center, Basic Health Sciences Institute, Shahrekord University of medical Science, shahrekord, Iran
2. Cellular and Molecular Research center, Basic Health Sciences Institute, Shahrekord University of medical Science, shahrekord, Iran

The incidence of age-related neurodegenerative disorders such as Alzheimer’s diseases and Parkinson’s diseases is increasing in parallel to the significant enhancement of the average life expectancy of many populations around the world. Neurodegenerative disorders are characterized by neuronal loss where the death of neurons can be triggered by a variety of stimuli. Two CNS mitogens, the epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF) have been implicated in the proliferation, survival, self-renewal, cellular migration and differentiation of neural progenitor species. Stem cells are self-renewing multipotent progenitors with the broadest developmental potential in a given tissue at a given time. However the developmental origin of the stem cells plays a pivotal role in this regard. A great deal of attention has focused recently on stem cells due to their therapeutic potential. The DPSCs seem to be better candidate for both the differentiation of neuroprogenitors and the expression of neuronal markers which may be due to the ectodermal origin of these very promising multi-potent stem cells.

**Keywords:** DPSC, Neurodegenerative disorders, stem cell
Breast cancer (B.C) is the most common female malignancy and is the major cause of death in middle-aged women. Therefore, early detection can play an important role in disease prevention. About 5 to 10% of the cases are due to an inherited mutation in two major genes, BRCA1 and BRCA2 which transmits as an autosomal dominant form. Genetic testing enables us identifying patients at increased risk of developing B.C

The aim of the study was to identify the causative mutation of early B.C in a family with 9 affected members.

Methods: Linkage analysis was performed with the help of STR markers linked the BRCA1 and BRCA2 genes to indirectly track the mutation. Then the candidate gene was subsequently sequenced to find the mutation.

Results: Linkage analysis showed that BRCA1 gene is segregating with the disease. Sequencing results showed a novel heterozygote (c.3607 C>T, P.R1203 X) variant in BRCA1 gene. The variant was heterozygote in all affected members and was not present in healthy members of the family.

Conclusions: The newly identified variant caused a truncated protein which is not active and cause disease. Genetic testing is useful for the preventive interventions for families with high risk of the disease. Identification of these novel mutations helps in developing a mutation to program for early breast cancer screening. Early-onset B.C (less than 45 years) and a limited family history are sufficient to justify mutation screening with a detection rate of over 25% in this group.

Keywords: breast cancer, BRCA1 gene, sequencing, Iran

Gastric cancer is the fourth most common malignancy and the third leading cause of cancer death worldwide. Micro RNAs are single stranded molecules which can inhibit the expression of mRNA by binding to the 3' UTR region. It has been demonstrated that miRNAs control several cancer related mechanisms. Several studies revealed that miRNAs play key roles in many cancers such as gastric cancer. A single nucleotide polymorphism (SNP) is the most common type of genetic variation which may occur every 100-300 bases. It has been recognized that SNPs might play role in cancer. Since miRNAs are small functional units, single base changes (i.e. SNP) in both the precursor elements as well as the mature miRNA sequence may result in the evolution of new microRNAs through altering their biological function. The relation between structural changes and ectopic expressions of miRNA, diagnosis and incidence of gastric cancer has been previously proven. Here, we investigated whether the miR-559 rs58450758 polymorphism affects the susceptibility to gastric cancer.

Keywords: Gastric cancer, Polymorphism, miRNA

Establishing a powerful expression system for production of recombinant proteins is a demanding concern in chloroplast biotechnology. Microalga as a unicellular photosynthetic organism, is a proper platform for large-scale production of recombinant proteins known as molecular farming. Chlamydomonas reinhardtii as a model microalgae has a single chloroplast with 80 copy of the genome, which occupies %70 of the cell. This bio-system has several advantages for production of recombinant proteins, such as a very fast doubling cycle within a few hours, lower production costs in comparison to similar culture systems, the ability to grow in controlled conditions, which facilitates the production of biopharmaceuticals. In some cases, the constitutive expression of foreign proteins in chloroplasts lead to harmful phenotypic effects. Therefore existence of an inducible genetic system to control the expression of such genes is a valuable tool. Theophylline-responsive riboswitch (RS) is a synthetic RNA sensor that switches on the translation in presence of theophylline. In the current study we designed a construct based on the RS, for inducible expression of the GFP as a reporter gene, based on the previously published pME16 vector. The expression vector will insert the transcriptional cassette in the microalga chloroplast genome at the upstream of the psbH gene. For the final confirmation of the correct cloned construct in the E.coli, digestion with restriction enzymes, colony PCR and eventually sequencing will be used. The algae strain CC-125 cultivation was optimized on the TAP medium. In the follow up research, the construct will be transferred into the chloroplast genome of Chlamydomonas.

Keywords: recombinant proteins / Microalga / molecular farming / riboswitch

Background: MicroRNAs (miRNAs) are an extensive family of small (18–24 nucleotide), endogenous, single-stranded non-coding RNAs, which target the 3'-untranslated region (3'-UTR) of specific mRNAs to promote their degradation or repression of translation. miRNAs regulate important cellular processes such as proliferation, apoptosis, mobility, cell cycle progression, and differentiation, and their altered expression is associated with various cancers. Among those miRNAs, miR-143 shows tumor-suppressive activity in some human cancers.
Lung cancer is the leading cause of cancer deaths worldwide and metastasis is the major cause of death in lung cancer patient.

**Methods:** Human lung cancer cells (A549) were cultured at 37°C in 5% CO2 with RPMI 1640 media supplemented with 10% fetal bovine serum (FBS). Transient transfection of miRNA precursors or inhibitors was carried out using Lipofectamine 2000 according to the manufacturer’s protocol. Migration of human lung cancer cells in culture was determined by the “scratch” assay. For this, cells were seeded into a six well tissue culture dish. Cells in monolayers were scratched in a single straight line using a pipette tip. The migratory distance was measured under a microscope equipped with a camera.

**Results:** Intrinsic miR-143 expression was significantly decreased in lung cancer cells compared to non cancerous epithelial cells. Restoration of miR-143 led to inhibit migration and invasion of human lung cancer cells. These data suggest that miR-143 suppress pathways relevant to tumorigenicity and cancer progression.

**Conclusion:** The existing experimental evidence suggests that it is worth testing Mira-143 as a cancer therapeutic agent since the results of this study demonstrate that Mira-143 has the ability to inhibit migration and invasion of human lung cancer cells.

**Keywords:** Lung cancer, MicroRNAs ,Mira-143,Scratch assay

**P-523:** Evaluating the effect of supernatant bacteria, Lactobacillus gasseri on bcl-2 and bax gene expression in thyroid cancer cells

soleimani Rahim Abadi A, Mazlomi M A, Farahmand M

Department of biology, East Tehran Branch, Islamic Azad University, Tehran, Iran.
Anoosheshsoleimani66@gmail.com

Thyroid cancer is one of the most common endocrine malignancies. Among the most important genes involved Bax and Bcl-2 are two most common genes which are studied. Bcl-2 family are divided into two subfamily, one inhibits apoptosis and strengthens the other, therefore mutation in such the gene can cause cancer. Today, bacteria including lactobacilli, are known to be the major contributor in happening of so many diseases, including cancer.

**Materials and Methods:** The supernatant of Lactobacillus gasseri virus was prepared in several volumes of cell suspension (7.81, 15.6, 31.25, 62.5, 125, 250, 500 ?l) which is obtained from 1200 ?l from the cell culture medium. The effect of Lactobacillus gasseri supernatant was examined on SW1736 cell line of thyroid cancer cells by MTT method. The expression of Bax and Bcl-2 in the thyroid cancer cell line SW1736 contains cell culture supernatant, effects of Lactobacillus gasseri virus. This was performed using Real Time PCR.

**Results:** treatment of thyroid cells with different volumes of cell suspension revealed, the higher volumes of cell culture supernatant, the more cytotoxicity observed. Relative suppression of Bcl-2 in contrast to induction of gene expression of Bax was observed in higher concentration of supernatant. Comparing to standard group, the results were statistically significant.

**Conclusion Overall:** Lactobacillus gasseri supernatant can be used as a drug candidate in the treatment of thyroid cancer.

**Keywords:** Thyroid cancer, Lactobacillus gasseri, Real Time PCR, Bax, Bcl2

**P-524:** Association of Matrix Gla protein gene (MGP) polymorphisms with incidence of idiopathic kidney stone

Tabeshmehr P, Abbasi J, Jafari N

Department of Biology, Basic Science Faculty, Arsanjan Branch, Islamic Azad University, Arsanjan, Iran

Introduction: Kidney stone disease (KSD) with a high incidence rate among populations results from urinary tract stone formation. Inheritance and environmental reasons are the underlying causes of kidney stone development. Matrix ?-carboxyglutamic acid Gla protein (MGP) is a local natural calcification inhibitor secreted primarily by chondrocytes and vascular smooth muscle cells in the arterial tunica media. Presented here are the results of an association study between MPG rs1800801 and rs4236 polymorphisms and idiopathic kidney stone incidence.

**Material and Methods:** A total of 400 study subjects (200 KSD and 200 age and sex matched controls) were included in this study. All DNA contents were extracted. The rs1800801 polymorphisms were determined using Tetra-ARMS-PCR assay and the rs4236 polymorphisms were assessed by RFLP-PCR and Hae? restriction enzyme.

**Results:** Subjects with the rs1800801 AA genotype indicated a significantly higher risk for KSD (OR=1.9; 95% CI=1.04-3.4; P=0.036). The frequency of rs4236 G allele was statistically different between KSD patients and controls (OR=1.6; 95% CI=1.22-2.2; P=0.001).

**Conclusion:** According to the results of the present study, the rs1800801 AA genotype might be a risk factor for idiopathic kidney stone and the rs4236 G allele might be associated with the incidence of mentioned disease.

**Keywords:** Kidney stone disease, Matrix Gla protein, Polymorphism, rs1800801, rs4236

**P-525:** Association of HLA-DQA1*0102, -DQB1*0602 haplotypes in different genders of Khuzestan Province

delfan N1, Galehdari H1, shafiei M1, Khatami SR1, Tahereh Seifi1, Mohaghegh M1, Zabihi R1, Ghanbari F1, latifi T1, Majdinasab N2, Shariati Gh3

1. Department of Genetics, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
2. Department of Neurology, Faculty of Medicine, Ahwaz Jundishapur University of Medical Sciences, Ahwaz, Iran.
3. Department of Medical Genetics, Faculty of Medicine, Ahvaz Jundishapur, University of Medical Sciences, Ahvaz Iran

Introduction: The human leukocyte antigen (HLA) system or complex is a gene complex encoding the major histocompatibility complex (MHC) proteins in humans. HLA are proteins or markers found on most cells in human body. Many studies show that MHC gene variants are associated with some diseases. This survey on Khuzestan population included the frequency of HLA haplotypes. HLA typing is used to match patients and donors for bone marrow or cord blood transplants; thus, reporting the association of HLA-DQA1*0102 and -DQB1*0602 haplotypes in normal population according to gender, were the goal of the study.
Methods: This study included 242 healthy individuals without any disease. HLA-DQA1*0102, and DQB1*0602 alleles were genotyped, using the polymerase chain reaction amplification with sequence specific primers (PCR-SSP) method. The relationship of HLA alleles with gender examined. SPSS software was used for the statistical analyses.

Result: DQB1*0602+DQA1*0102+ as a two allelic haplotype showed no meaningful association with females compare with males (47.82% vs. 38.88%, p = 0.338, OR = 1.440 [95% CI = 0.681-3.046]).

Conclusion: The study on resident in southwest of Iran gives some information about the HLA haplotype that has high frequency in both genders of this population. As DQB1*0602+-DQA1*0102+ haplotype has association with susceptibility to autoimmune diseases like MS, in some population; so, having high frequency of this haplotype may put the population in danger.

Keywords: HLA-DQA1*0102, HLA-DQB1*0602, Haplotype; PCR-SSP, Iran.

P-526: Optimization of ziziphora extract on reduction of Claudin_1 gene expression in the AGS cell line

Nayyeri S, Deilami Khiaban Z

Faculty of Basic Sciences, Zanjan Branch, Islamic Azad University, Zanjan, Iran

The study of the gene of the Claudin family is of particular importance in cancers, especially gastric cancer, because research has shown increased expression in cancer cells. The aim of this study was to evaluate the claudin-1 gene expression in gastric cancer cells after treatment with Ziziphora extract. The advantage of this extract is its anti-cancer properties and the absence of side effects.

The aim of this study was to optimize the effect of ziziphora extract on expression of the Claudin_1 gene in the gastric cancer

The AGS cells were incubated 37°C containing 5% CO2 with 85% humidity DMEM with 10% FBS. The cells were treated with concentrations of 800, 1200, 2000 µg/ml of ziziphora for 72 hour. Extraction of RNA, synthesis of cDNA has been done using kit. The study of claudin-1 gene expression was performed by Real time PCR and also GAPDH gene was used as the internal control

The results of Real time PCR data shows in 72 hours treatment reduction of 5.8 and 10 fold have shown with concentrations of 800 and 1200 µg/ml of ziziphora respectively, but no change in the concentration of 2000µg/ ml was observed.

It seems that herbal medicines are effective in preventing tumor growth with respect to the effect and less side effects, are a good alternative to chemical drugs. Ziziphora extract in 1200 µg/ml concentration, reduced the expression of claudin-1 remarkably. The results of this study can be used to design an anticancer drug, especially gastric cancer.

Keywords: Claudin_1, Gastric Cancer, ziziphora, AGS

P-527: Lack of association between gastric cancer and hopQ alleles in Helicobacter pylori

Kazemi E, Kahrizi D

Fertility and Infertility Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

Helicobacter pylori use a number of mechanisms to survive in the stomach lumen. The presence of these bacteria in the stomach can lead to gastritis and reduction in stomach acid production. Acute inflammation can directly damage to the peripheral cells that are responsible for the secretion of acid. The risk of developing gastric carcinoma is associated with the heterogeneity of Helicobacter pylori virulence factors (such as cagA). The HopQ is one of the outer membrane proteins involved in bacterial adherence to the gastric mucosa and has been suggested to also play a role in the virulence of H. pylori. This gene has been shown in two types. The purpose of the current study was to investigate the association between different H. pylori virulence hopQ alleles (types I and II) and patients with gastroduodenal disorders. For this purpose, 58 stomach biopsies, of the patients with gastric cancer and 100 saliva samples from healthy individuals were collected. Then genomic DNA was purified and PCR for was done for desired genes via specific primers. The H. pylori infections were diagnosed by PCR for GlmM gene. Then frequencies of hopQI+, hopQII+ and hopQI+ hopQII+ genotypes were determined in H. pylori-infected cases. Statistical analysis showed that there were no significant differences between healthy and diseased ones for genotypes hopQI+, hopQII+ and hopQI+ hopQII+.

Keywords: Gastric cancer, HopQ genotyping, Helicobacter pylori

P-528: Evaluation of silver nanoparticles effects on Bap gene expression for biofilm formation in isolates of antibiotic-resistant Acinetobacter Bumanni by real time PCR method

Piri Gharagheh T, Sadat Shandiz SA*

1. Department of Biology, East Tehran Branch, Islamic Azad University, Tehran, Iran
2. Department of Biology, Central Tehran Branch, Islamic Azad University, Tehran, Iran

Background and aim: Acinetobacter bomanni is one of the most common opportunistic pathogen in hospital that is resistant to many antibiotics due to the production of biofilm. This study aimed to evaluate an anti-biofilm activity of silver nanoparticles (AgNPs) on clinical antibiotic resistant Acinetobacter bumanni. Materials and methods: In this experimental study, Acinetobacter bumanni were isolates from 100 clinical samples. After identification of Acinetobacter bumanni strains and determination of antibiotic resistant profiles, biofilm producer isolates were determined using PCR method. The Minimum inhibitory concentration (MIC) of strains against AgNPs was determined. After 24 hours exposure of strains with subMIC concentration of AgNPs, RNA extraction and cDNA synthesis was performed. Finally, evaluation of Bap gene expression was measured using real time PCR method.

Results: out of 100 clinical isolates, 12 isolates were belonged to Acinetobacter bumanni and all of strains were resistant to antibiotics except colistin. PCR results show that 12 isolates have Bap gene and they were biofilm positive. Real Time PCR results show that after treatment of isolates with subMIC concentration of AgNPs, all of strains had a significant reduction in Bap gene expression (P<0.05).

Conclusion: According to anti-biofilm effects of AgNPs, it seems that AgNPs can be used as drug candidate in pharmaceutical industries.
Keywords: Acientobacter bummani, Silver nanoparticle, Biofilm.

P-529: Repetitive elements sequence (REP/ERIC)-PCR based genotyping of Iranian isolates of Cylindrocladium buxicola Henricot

Zamani SM1, Mojerlou Sh2, Ghamari Zare A3*

Boxwood blight disease, caused by Calonectria pseudonaviculata (syn. Cylindrocladium pseudonaviculata, C. buxicola) is responsible for considerable damages on Buxus spp. in nurseries, gardens and wild boxwood populations in Iran and the world. Knowledge of the population structure and genetic diversity of the fungal pathogen can help to implement effective disease management strategies. In this study rep-(REP and ERIC) PCR genotyping were used to assess genetic heterogeneity in a collection of 21 representative isolates of a total 52 Iranian isolates of C. pseudonaviculata, which showed the highest pathogenic and morphological diversity, originating from 12 forest areas in three Northern provinces of Iran. Cluster analysis using UPGMA method and Jaccard’s coefficient showed that, although results were comparable, REP fingerprints discriminated the isolates better. The rep-PCR genotyping showed that isolates from different geographical regions produced identical rep-profiles indicating limited genetic diversity. The concatenated dendrogram of REP- and ERIC-PCR fingerprints clustered the isolates into four major groups. In each group, majority of the isolates from Mazandaran, Gilan and Golestan province exhibited similarities ranging from 95% to 99%. Similarity of fingerprints among isolates from different geographical regions revealed existence of a limited number of clonal groups of C. pseudonaviculata in Iran, and suggesting a potential epidemiological link. Overall, these data indicated a low level of genetic diversity in the Iranian C. pseudonaviculata isolates population similar to that reported in other countries, so it seems that in the disease control, the use of resistant cultivars is one.

Keyword: Calonectria pseudonaviculata, Iranian isolates, rep-PCR, Genotyping.

P-530: Investigation of the interaction of natural compounds with senp1 protein

Taghvaei S, Sabouni F, Minuchehr Z

National Institute of Genetic and Biotechnology (NIGEB), Tehran, Iran

*s_taghvaei@nigeb.ac.ir

Investigation of the interaction of natural compounds with senp1 protein

*Somayeh Taghvaei, Farzaneh Sabouni, Zarrin Minuchehr
National Institute of Genetics Engineering and Biotechnology

Introduction: SUMO proteins regulate many cellular processes by attaching covalane to the substrate and sumoylation (1)(2). Sumoylation is a reversible process that is performed by SENP proteases (3).

Disturbances or changes in SUMO is correlated with a variety of diseases including cancers (prostate and thyroid), neurodegenerative syndromes, diabetes, viral infections and related growths (1) (5) (4) (6)

HIF-1α desumoylation by SENP1 under hypoxia conditions increases the HIF-1α stability and increases the expression of target genes of this factor (7).

SENP1 enables another oncoenic signaling pathway, such as androgenetic receptor transcription. This information and the rest of the information provided the potential importance of SENPs as diagnostic markers and also makes this category of enzymes an interesting therapeutic goals in which is identified in human tumors

SENP1 causes the desumoylation of HDAC1, P300 and AR. Natural products are capable of removing ROS and protecting cellular components like DNA, proteins and lipids from oxidative stress.

Material and Methods: We examined the interaction of some natural compounds with SENP1 protein through molecular docking. These compounds showed good binding and compared with normal SENP1 inhibitor as shown in the table below.

<table>
<thead>
<tr>
<th>Binding energy name</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betanidin</td>
<td>-8.41kcal/mol</td>
</tr>
<tr>
<td>Shikonin</td>
<td>-7.25</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>-6.78</td>
</tr>
<tr>
<td>Momordin</td>
<td>-6.3</td>
</tr>
</tbody>
</table>

Discussion and Conclusion: smaller Compounds such as betanidin, or with the many electrostatic and van der waals interactions, like caffeic acid, they showed lower binding energy and stronger interactions.

Keywords: SENP1, Molecular Docking, Cancer, Natural Compounds
Objective: The autoimmune disease is a disease that is spread by the immune system. Bullous pemphigoid (BP) is (the most frequently occurring entity among autoimmune bullous skin diseases and is clinically known and superficial wounds in the skin before the plaques and local ulcers. Although the genetic determinants of BP have not been precisely elucidated, some studies have shown an association between a IL17-RA polymorphism rs2291951. (Yet, these findings had so far not been independently replicated and no data on a possible association of these mutations and BP in Iranian population were available.

Methods: This study contains 20 BP patients and 20 healthy controls. Genomic DNA was isolated using DNG-plus and PCR-RFLP analysis was performed to detect IL17-RA polymorphism rs2291951 polymorphism. Several relevant information such as demographic data, age, gender, (…) or clinical characteristics were analyzed for a possible effect of these factors on susceptibility to BP in patients.

Results: There was no significant difference in genotypes of IL17-RA polymorphism rs2291951 (in both patients and control groups) p(0.05).

Conclusion: This study did not showed an association between a IL17-A polymorphism rs2291951 (and BP disease in Iranian population. This results shows that the genetic predisposition to develop BP can greatly varies among different ethnic groups. IL17-RA rs2291951,Bullous pemphigoid ,Autoimmune disease,polymorphism
بحث

1. دانشجویی دکتری زیستی اصلاح اصلاح نزدیک گروه گسترش تقلید عمومی دانشگاه تهران
2. دانشجو دکتری زیستی اصلاح نزدیک گروه گسترش عمومی دانشگاه تهران
3. دانشجویی دکتری زیستی اصلاح اصلاح نزدیک گروه گسترش عمومی دانشگاه تهران

2. مقدمه و هدف: گاما اینترفرون یک سایتوکاین پیچ و چرخش دارد که این یکی از مراکز عضوی در جوهرکاری رضیون می‌شود. گاما اینترفرون در رابطه با سرطان ریه، سرطان پوستی و سرطان ادراری مورد بررسی قرار گرفته است. هدف از این مطالعه بررسی حضور گاما اینترفرون در خون مبتلا به سرطان ریه، سرطان پوستی و سرطان ادراری بود.

3. کاهش و افزایش: در این مطالعه، در بیش از 100 نمونه خون از بیماران سرطانی، حضور گاما اینترفرون در خون مبتلا به سرطان ریه، سرطان پوستی و سرطان ادراری بررسی شد.

4. نتایج: نتایج نشان داد که حضور گاما اینترفرون در بیش از 100 نمونه خون از بیماران سرطانی، به طور معنی‌داری تفاوت بین بیماران سرطانی و کنترلی وجود دارد.

5. نتیجه‌گیری: در این مطالعه، حضور گاما اینترفرون در خون مبتلا به سرطان ریه، سرطان پوستی و سرطان ادراری، به طور معنی‌داری تفاوت بین بیماران سرطانی و کنترلی وجود دارد.

کلمات کلیدی: گاما اینترفرون، سرطان، ریه، پوستی، ادراری.
یک هفته بعد از تنش سرمای، به‌طور قابل‌توجهی مواردی از میزان تحمل به سرمای مورد بررسی و مطالعه قرار گرفتند. بالاترین تحمل به سرمای مورد بررسی در قالب رقیم به عنوان ارقام مقاوم و متحمل به سرمای معرفی شد. هر یک از این ارقام به‌طور فردی شناسایی گردید. مؤثرترین راه برای شناسایی این ارقام مربوط به برد باری پتیکه است. به‌طور کلی، نیمه استوایی که احتمال وقوع سرمای زیر صفر وجود دارد، بهترین حالت از نظر سبزینگی، وجود یا عدم وجود ساختار درگیر در مقاومت به سرمای مربوط به سرما است. در جدول‌های مربوط به پتیکه، برد باری پتیکه به عنوان نمونه‌بندی‌های با رتبه بالا انتخاب شد. به‌طور کلی، نیمه استوایی که احتمال وقوع سرمای زیر صفر وجود دارد بهترین حالت از نظر سبزینگی، وجود یا عدم وجود ساختار درگیر در مقاومت به سرمای مربوط به سرما است. در جدول‌های مربوط به پتیکه، برد باری پتیکه به عنوان نمونه‌بندی‌های با رتبه بالا انتخاب شد.
Multiple sclerosis (MS) is an autoimmune disease, of the central nervous system (CNS) that affects young adults. The cause of MS is unknown; however, susceptibility to MS is thought to result from complex interactions between genes and environmental factors. Allelic variation in HLA particularly class II is one of the biomarkers related to increase susceptibility to the disease. The age at onset of disease from first to sixth decade of life is variable. Epidemiological studies have suggested a minor genetic component to the age at onset of MS. The aim of our study was to determine frequency of HLA-DRB1*15 allele and influence on age at onset in a population of patients with multiple sclerosis in Iran. In this study, the presence of DRB1*15 allele was investigated in 70 Iranian multiple sclerosis patients and compared with 70 healthy individuals. HLA typing for this allele was performed by manual polymerase chain reaction (PCR) amplification with allele-specific primers (PCR-ASP) method. The results show that, Compared with healthy controls, the frequency of HLA-DRB1*15 was significantly higher in MS patients with different geographic area in Iran (PV=0.007) . In addition, no significant correlation was observed among HLA-DRB1*15 allele with age at onset of MS patients. In conclusion, this study showed that the Iranian people with HLA-DRB1*15 allele are far more likely susceptibility to MS and this allele could be as a biomarker for earlier prognosis of MS disease. However, this allele was not interfered in the age at onset of this disease.

Keywords: Multiple Sclerosis, HLA-DRB1*15, Allele-Specific PCR, age at onset
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