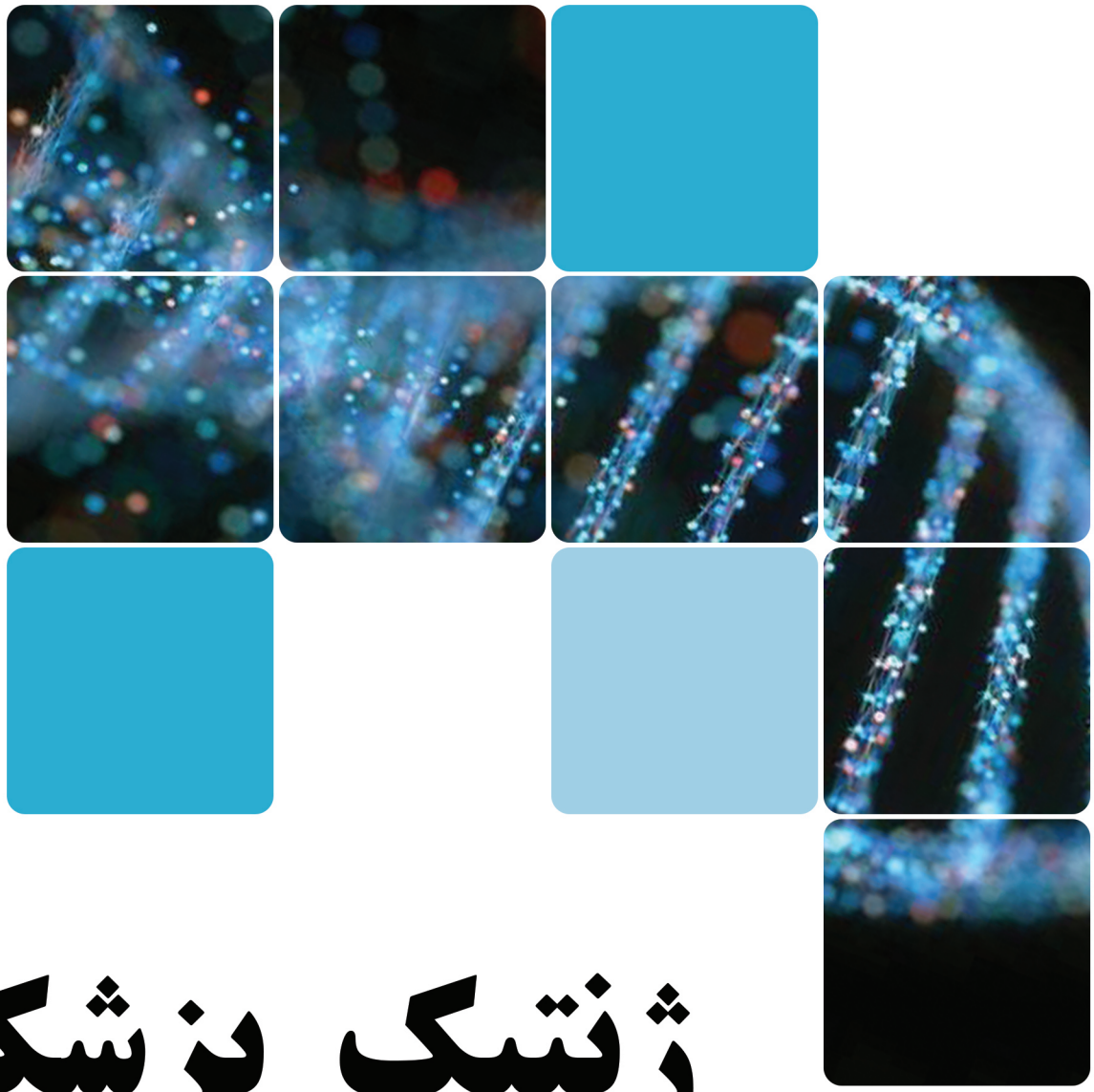
۸مین

سمینار و وبینار سراسری و بین المللی

ایران– مشهد– ۱۱ الی ۱۲ اسفند ماه ۱۴۰۰



ژنتیک پزشکی

(تشخیصی –تحقیقی)

وبینار سراسری / بین المللی

۸مین سمینار

ژنتیک پزشکی

تشخیصی - تحقیقی

Medical Genetics

1-Day-Seminar for Research and Diagnostic

زمان برگزاری: ۱۱ و ۱۲ اسفند ماه ۱۴۰۰

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۱- سیتوژنومیک

۲- ژنتیک مولکولی (کلاسیک و NGS)

۳- ژنتیک بالینی

۴- ویرایش ژنی و مداخلات ژنتیکی (CRISPR)

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۲- اعضای هیئت علمی و دانشجویان ژنتیک پزشکی

۳- پزشکان متخصص ژنتیک، کودکان، نورولوژی، زنان و زایمان، پریناتولوژی، داخلی و غیره.

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۵- دانشجویان / فارغ التحصیلان و اعضای هیئت علمی پزشکی مولکولی

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اعظم مجرد

تینا زراعتی

حسین آیتی

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	۹:۱۵-۹:۳۰	Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran	دکتر علیرضا پاسدار	Prevalence and clinical presentation of the genetic ocular disorders referred to a genetic counselling clinic in Mashhad
	۹:۳۰-۹:۴۵	Faculty Member at The Department of Dermatology,Thomas Jefferson University.	دکتر حسن وحید نژاد	Genetic approach to skin disorders
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Founder mutations of east Iran population	دکتر مجید مجرد	**Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran**	۱۰:۱۵– ۱۰:۳۰	
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CEP104 gene may involve in pathogenesis of a new developmental disorder other than Joubert syndrome	دکتر علی رشیدی نژاد	**Maternal, Fetal and Neonatal Research Center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran**	۱۰:۴۵– ۱۱:۰۰	
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Genetic study of more than 2000 patients affected with various genetic diseases, By Whole Exome Sequencing (WES) technique in Isfahan, Iran	دکتر منصور صالحی	Cellular, Molecular and Genetics Research Center, Isfahan University of Medical Sciences, 8175954319, Isfahan, Iran.	۱۲:۱۵- ۱۲:۳۰	
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Functional Evidence for ITSN1 Involvement in Development of Intellectual Disabilities	دکتر محمد حدادی	Department of Biology, Faculty of Basic Sciences, University of Zabol, Zabol, Iran	۱۲:۴۵- ۱۳:۰۰	
The clinical and exome sequencing data of patients with familial Parkinson Disease in Iran	آقای محمد سودیاب	Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran	۱۳:۰۰- ۱۳:۱۵	
A novel CFTR gene frame shift variant causing cystic fibrosis in a large Iranian family	دکتر محمدرضا دهقانی	Medical Genetics Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.	۱۳:۱۵- ۱۳:۳۰	
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A 26.92 Mb interstitial deletion at 7q32.3q36 in an Iranian patient with multiple anomalies	دکتر محمدرضا عباس زادگان	Medical Genetics Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran	۱۳:۳۰- ۱۳:۴۵	
Report of Chromosome Abnormalities in individuals with Consanguineous marriage referred	دکتر فرخنده بهجتی	Sarem Fertility & Infertility Research Center (SAFIR), Sarem Women's Hospital, Iran	۱۳:۴۵- ۱۴:۰۰	

to Sarem Women's ' Hospital , Tehran, Iran		University of Medical Sciences (IUMS), Tehran, Iran.		
Case report of translocation (21; 14) in relation to infertility and birth of a girl with karyotype 46, xx, der (21) add (14) (q13) del (21) (q11.2) In a family	خانم محدثه خوش اندام	Department of Reproductive Biology, Academic Center for Education, Culture, and Research (ACECR), Qom branch, Iran	۱۴:۰۰– ۱۴:۱۵	
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	زمان/ Time	موضوع/ Title	سخنران/ Lecturer	انتساب/Affiliation
بخش اول: ژنتیک پزشکی				
اعضای پانل: آقای دکتر مجرد، آقای دکتر عباس زادگان، آقای دکتر پاسدار، آقای دکتر غیور کریمیان، آقای دکتر کراچیان				
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	۸:۳۰–۸:۴۵	Department of Medical Informatics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.	دکتر تکتم دهقانی	SMARTDX: A NGS Data analysis platform for clinical laboratory
	۸:۴۵–۹:۰۰	Kidney Transplantation Complications Research Center, Mashhad University of Medical Sciences, Mashhad, Iran	دکتر عباسعلی زراعتی	Autosomal recessive polycystic kidney disease: late-onset renal enlargement and proteinuria with rare PKHD1 mutation.
	۹:۰۰–۹:۱۵	Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran	خانم عطیه اصلاحی	Early-infantile onset epilepsy and developmental delay caused by bi-allelic GAD1 variants
	۹:۱۵–۹:۳۰	Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran	دکتر فریدون عبدالملکی	Recurrent familial hydatidiform mole as a rare clinical problem
	۹:۳۰–۹:۴۵	Department of Genetics and Molecular Biology, School of Medicine, Isfahan University	خانم سحر بیات	A novel ARV1 mutation in an Iranian family with developmental and

epileptic encephalopathy-38		of Medical Sciences, Isfahan, Iran.		
Introduction of a Five-lncRNA Signature as a diagnostic biomarker in Gastric Cancer based on TCGA Data	اقای محمود قانعی	Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran	۹:۴۵– ۱۰:۰۰	
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SPG4 and SPG11 are the most common types of hereditary spastic paraplegia (HSP) in Iran	دکتر آفاق علوی	Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran	۱۰:۳۰– ۱۰:۴۵	
A prenatal diagnosis of frame shift mutation in CEP135 gene associated with primary microcephaly in an Iranian family	خانم زهرا نصرپور	Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran	۱۰:۴۵– ۱۱	
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The application of mesenchymal stem-cells regarding the disease treatment	دکتر احسان صبوری	Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran	۱۱:۳۰– ۱۱:۴۵	
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Progressive protein aggregation in retinitis pigmentosa type 11 patient iPSC-derived retinal pigment epithelium and its reversal through activation of autophagy	دکتر سینا مظفری جوین	Medical Genetics Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran	۱۲:۳۰– ۱۲:۴۵	
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GJB2 mutations in Iranian Azeri population with autosomal recessive nonsyndromic hearing loss (ARNSHL): First report of c.238 C>A mutation in Iran	دکتر بهزاد داورنیا	**Medical Genetics and Pathology, Ardabil University of Medical Sciences, Ardabil, Iran**	۱۳:۴۵– ۱۴:۰۰	
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چکیده

سخنرانی ها

Oral Presentations

Abstracts

Prevalence and clinical presentations of genetic ocular disorders referred to a genetic counseling clinic in Mashhad

Alireza Pasdar, MD, PhD, Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Email: pasdara@mums.ac.ir

Globally, the incidence of genetic ocular disorders is estimated at one in 1000. Genetic ocular disorders include a broad spectrum of diseases that affect individuals of all ages. They can present in the form of isolated disease, with systemic features, or as part of a syndrome or in non-syndromic forms with all inheritance patterns. Genetic counseling services have been strongly recommended by the World Health Organization (WHO) as a potential for screening and identification of involved genes. The objective of this study was to estimate the prevalence of genetic ocular disorders in patients who were referred to the eye genetic counseling clinic.

This was a single-center, retrospective observational study at Khatam-al-Anbia Eye Hospital (Mashhad, Iran) from 2018 to November 2021. A total of 118 index cases over a four-year period were analysed.

Inherited retinal disorders (IRDs), as a broad group of retinal degeneration, was accounted for the highest percentage of patients (51.32%). This consisted of non-syndromic retinitis pigmentosa (RP) (24.77%), syndromic RP (7.96%), macular dystrophy (7.96%), Leber congenital amaurosis (LCA) (4.42%), cone and rod dystrophies (2.65%), retinal dystrophy (1.76%), leber hereditary optic neuropathy (LHON) (0.88%), and foveal retinoschisis (0.88%).

Other common disorders included Usher syndrome, congenital glaucoma, decreased visual acuity, Stargardt, Bardet Biedl syndrome with a frequency of 9.73%, 4.42%, 5.3%, and 5.3% and2.65% respectively. In the current study, congenital cataract manifested as syndromic (3.53%) and non-syndromic (2.65%) forms.

Other cases with ocular manifestations included two cases (1.76%) each of von Hippel–Lindau disease (VHL), optic dystrophy, albinism, and inborn errors of metabolism (IEM) and one case each (0.88%) of Marcus Gunn phenomenon, leukocoria, cystoid macular edema (CME), Familial Adenomatous Polyposis (FAP), magalocornea, nystagmus, dominant optic atrophy, and microphthalmia were detected.

This study highlights that IRDs are the most common presenting complaint to the genetic counseling clinic in the setting of Mashhad, Iran.

Keywords: Genetic counseling; Genetic eye disease; Prevalence; Frequency

Application of Local Geographic Genetic Disorders Map; a Case of PND for Not Previously Diagnosed Familial Deafness (NSHL)

Arash Pooladi[1,2], Farzad Soleimani[1], Esmail Rahimi[2], Golaleh Karbasi[1], Masoud Alasvand[1], Fardin Fathi[1], Fereidoon Abdolmaleki[1,2]

1- Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran

2-MAAD Genetics Polyclinic & Lab., Sanandaj, Kurdistan, Iran

Correspondent: arash.pooladi@gmail.com

farzad.soleimani1@gmail.com, rahimi.esmaeil88@gmail.com, Gelare_633@yahoo.com, alasvand1100@gmail.com, farfath@gmail.com, f.abdolmaleki@gmail.com

Background and Objectives:

Geographical distribution of genetic diseases and their prevalent mutations in each region can be used for health problem definition, guideline development and more rapid and low cost diagnosis. Local geographical disorders map/database, has also been used extensively in epidemiology for disease surveillance and intervention monitoring. One of the impotent genetic disorders is Deafness. Deafness is clinically and genetically heterogeneous. This vast heterogeneity has made it difficult for genetic researchers to find a specific mutated gene in affected individuals from different ethnic groups. In this paper we are presenting a Prenatal Diagnosis (PND) case for Non-Syndromic Hearing Loss (NSHL) by our local map data (based on ethnicity and ancestral region) for a main genetic variant previously found and confirmed in a large pedigree of a Kurd village in Kurdistan province of Iran known as “silent Village”.

Material and methods:

Using the local data set that link to the EHR (Electronic Health Record) software, we can detect the genetic cause of the deafness in the family of a pregnant woman with 16w gestational age from the Karaftoo village and candidate her fetus for PND by amniosynthesis in a short time by two direct and indirect methods.

Results:

In our previously WES data of this region, a c.1180 C>T mutation in MITF was established as a cause of non-syndromic hearing loss in this pedigree. This mutation converts arginine residue at position 394 of the MITF protein to stop codon. The penetrance of this gene is estimated as 80% in two main family cluster of this large pedigree. In the first step, the parents were tested and the affected father is heterozygote for the above variant. Fortunately, the result of the genetic testing of the tested fetus is normal homozygote (wild type) and after several months was delivered with a normal newborn audiogram.

Discussion & Conclusion: Retrievable recording of the genetic data of the various regions of the country in the diagnostic genetic centers can play an important role for reducing the burden of genetic disorders and contribute to the birth of healthy newborns.

Keywords:

Local Regional database, regional genetic disease map, Deafness, Prenatal Diagnosis, PND, Non-Syndromic Hearing Loss, NSHL.

Identify the founding mutations of the eastern Iran population

Atieh Eslahi [1], Shima Farrokhi [1], Farzaneh Alizadeh [1], , Yasamin Yousefi [2], Majid Mojarrad [1*]
1- Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2- Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
Email Addresses:
*Corresponding author
Alizadehf951@mums.ac.ir
Yasaminsmartgirl@gmail.com
Eslahia951@mums.ac.ir
Farrokhish961@mums.ac.ir
majidmojarrad@gmail.com

Identification of founder mutations plays an important role in the process of genetic counseling and diagnosis of rare inherited diseases.

Due to having numerous ethnic and demographic minorities, the existence of different genetic barriers and the high frequency of consanguineous marriages, Iran has several founding mutations in demographic minorities. In this presentation, the founding mutations identified in the last decade in the population of the east of the country are introduced. During the last 10 years, at least four different founding mutations have been identified in isolated demographic minorities of Khorasan and Sistan and Baluchistan, which can be referred to as mutations causing hypermagnesemia and muscular dystrophy. Considering these mutations in the counseling and diagnosis process can help Reduce the financial and time costs of diagnosing diseases.

The first report of germline mosaicism of EHMT1 gene mutation in Iranian Population

Mohammad Miryounesi 1
PhD, Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
Email Addresses:
miryounesi@gmail.com

Background: Kleefstra syndrome 1 (KLEFS1; OMIM#610253), a rare autosomal dominant disorder, is caused by submicroscopic deletions in the chromosomal region 9q34.3 or mutations in the *euchromatic histone methyltransferase 1* gene (*EHMT1*). Missense, nonsense, splice site variants and small deletions and duplications have all been described in *EHMT1*. KLEFS1 is clinically characterized by core phenotypes comprising developmental delay/intellectual disability (DD/ID), (childhood) hypotonia, and characteristic facial features. Moreover, additional clinical features are observed in some patients.

Case presentation: We describe two Iranian siblings with Kleefstra syndrome. The parents are healthy, consanguineous and have five children; two of them are affected. There is no family history of similar problem. Both patients presented with motor delay, speech delay, mild learning disability, hypotonia at birth, synophrys, obesity and also behavioral and psychiatric problems. Peripheral blood samples were collected from affected siblings and their family members. Whole-exome sequencing identified a heterozygous nonsense variant in *EHMT1* (NM_024757.5: c.3046C>T, p.R1016X). Sanger sequencing confirmed this novel variant in both sisters. The parents and the healthy brother were also analyzed and all were normal homozygous. Online prediction tools including CADD and Mutation Taster suggest this variant as a damaging mutation. Combined Annotation Dependent Depletion (CADD) tool showed that this variant would be deleterious with a score of 40. The prediction of Mutation Taster showed it was disease causing.

Conclusions: Our two cases are the first report of germline mosaicism of the *EHMT1* gene defects in Iranian population. This report provides an emphasis on the importance of considering prenatal diagnosis (PND) in autosomal dominant disorders.

CEP104 gene may involve in pathogenesis of a new developmental disorder other than Joubert syndrome

Reza Shervin Badv1, Mojdeh Mahdiannasser2, Maryam Rasoulinezhad1, Laleh Habibi3, Ali Rashidi-Nezhad4,5*
1- Children's Medical Center, Pediatrics Center of Excellence. Tehran University of Medical Sciences, Tehran, Iran
2- Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran
3- Ronash Medical Genetics Center, Tehran, Iran
4- PhD, Maternal, Fetal and Neonatal Research Center, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
5- Genetic ward, Yas Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran
***** Corresponding author: arashidinezhad@tums.ac.irAbstract

Aim

The *CEP104* gene (OMIM: 616690) encodes the centrosome protein 104 (CEP104) that is involved in cilia function. Mutations in this gene have been described in four patients diagnosed with Joubert syndrome (JBTS) 25. Here, we challenged the concept that mutations in *CEP104* gene are only involved in the development of JBTS 25.

Method

In a clinical setting, whole-exome sequencing (WES) was applied to investigate pathogenic variants in patients with unexplained DD/ID.

Results

WES revealed a novel homozygous non-sense mutation in the *CEP104* gene (NM_014704.3:c.643C>T; NP_055519.1:p.(R215*)) in a girl with mild intellectual disability, hypotonia, imbalanced gait, and minor facial dysmorphisms. Her brain MRI data did not show MTS or any other brain anomalies.

Interpretation

Our study introduced a novel mutation in the *CEP104* gene that result in an ID phenotype other than JBTS25. Comparison of her phenotype with those of 8 previously published DD/ID patients

harboring mutations in *CEP104* genes revealed that more than half of them did not show JBTS related symptoms. Therefore, we suggest that the *CEP104* gene might also be involved in a disorder other than JBTS 25, a point that deserves to be emerged in the OMIM database.

Keywords: CEP104; Joubert syndrome; molar tooth sign; whole exome sequencing; Intellectual Disability

Novel Genomic Approaches to Neurogenetics Rare Disorders

Ehsan Ghayoor Karimiani
MD. Ph.D. in Medical Genetics, Next Generation Genetic Polyclinic, Mashhad, Iran
Molecular and Clinical Sciences Institute, St. George's, University of London, Cranmer Terrace, London, UK
Correspondent: eghayoor@gmail.com

Abstract

Introduction: The practice of genomic medicine stands to develop a research approach to clinical management. To realize this goal will require discovering the relationship between rare variation at each of the protein-coding genes and their consequent impact on individual health and expression of hereditary disorders. Here, we present novel genes and related syndromes based on the rare disorders cohort over 2000 families that were assessed at Next Generation genetic Polyclinic.

Materials & Methods: Families with Mendelian patterns compatible with genetic disorders such as neurodevelopmental, metabolic, and muscular disorders have been investigated. Expert specialists and clinical geneticists have done a complete clinical and paraclinical examination. Genomic DNA was extracted and evaluated through next-generation sequencing and followed by bioinformatic analysis. Parents and healthy offspring were assessed for the candidate gene variants.

Results: We delineated loss of *UGP2* in the brain leads to severe epileptic encephalopathy. Biallelic variants in *PCDHGC4* cause a novel neurodevelopmental syndrome with progressive microcephaly, seizures, and common anomalies. Pathogenic bi-allelic variants in *IPO8* in connective tissue disorder associated with cardiovascular defects, skeletal abnormalities, and immune dysregulation. Moreover, we are reporting families clinically diagnosed with Stickler syndrome carrying a different novel biallelic loss of function variants in *COL9A3*. Additionally, we showed that loss of *TNR* causes a nonprogressive neurodevelopmental disorder with spasticity and transient opisthotonus. We have also reported novel genetic variations in other genes such as (*GAD1*, *MADD*, *MFSD2A*) based on computational prediction and functional studies.

Conclusion & discussion: The progression in diagnosing rare disorders has undergone considerable in the past decade. The utility of genetic testing will also rely upon the further elucidation of the complications of genetic and allelic heterogeneity and the impact of rare and common variation at a locus, and advances in functional annotation of identified variants. To further, workup with next-generation technologies, using several cellular tools is essential for precise phenotype definition and to understand the underlying disease mechanisms

Keywords: Genetic, Novel genes, Rare disorders

Molecular profiling for precision cancer therapies

Marjan Yaghmaie
Associate Professor of Medical Genetics
Oncology, Hematology and Cell Therapy Research Institute, Shariati Hospital, Tehran University of Medical Sciences
m-yaghmaie@sina.tums.ac.ir
marjix58@gmail.com

Background: Use of next-generation sequencing (NGS) to identify clinically actionable genomic targets has been incorporated into routine clinical practice in the management of advanced solid tumors. Molecular profiles obtained on tumor DNA and RNA can guide the clinical management of cancer patients.

Method: For tumor profiling the scientists evaluate the clinical application of capture-based NGS techniques. This test identifies alterations in 300-500 genes, tumour mutational burden and genomic signatures as microsatellite instability. The decision to obtain the NGS assay for a particular patient was done according to investigator's choice

Result: The actual number of patients starting a targeted agent based on NGS results is low but it provides substantial information in terms of providing additional treatment options, identifying resistance conferring mutations and facilitating clinical trial enrollment. Optimal time of testing, early or late in disease course, financial implications of testing and using targeted therapy and survival benefit of targeted therapy need further studies.

Conclusion: Moleculr tumor profiling by NGS can provide diagnostic or prognostic information, identify a potential treatment regimen or targeted therapy, and determine eligibility for the following: i) a Food and Drug Administration (FDA) approved medication for that tumor type, ii) a medication available as off-label treatment for the specific molecular alteration in a nonapproved tumor type, or iii) a targeted therapy available in clinical trials with investigational agents based on an identified molecular alteration. Considering the lack of large prospective clinical trials that certified its clinical utility, the risks of overdiagnosis and increase costs without survival benefits are real.

Keyword: Precision medicine, Tumor profiling, solid tumors

A deletion in the LCA5 gene in an Iranian family with Leber congenital amaurosis

Mohammad Yahya Vahidi Mehrjardi 1, 2, Zahra Farshchian Yazdi 2, 3 ,Kimia Monshizadeh 2,3, Fatemeh Razavi 2,3.
1- Medical Genetics Research Center, Shahid sadoughi University of Medical Sciences, Yazd, Iran.
2-Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
3-Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
Email Addresses:
mmvahidi@gmail.com, farshchiyanzahra@yahoo.com, kimia.monshizadeh@yahoo.com,rzvfatemeh742@gmail.com

Background and Aim: One of the most severe kind of retinal dystrophy is Leber congenital amaurosis (LCA) that its indications start in the beginning of the life and 5% of inherited retinal dystrophies is caused by it .The clinical features of this disorder include nystagmus, profound visual loss, and poor pupillary reflexes. Although its inheritance pattern is autosomal recessive, dominant form of LCA has been reported. This is a heterogenous disease and mutation in over 25 genes are detected as its cause of it. One of these genes is LCA5 that encodes the ciliary protein lebercilin and its mutations account for about 2% of LCA. Here we aim to find genetic variants influencing LCA

Method: First, for two siblings of a consanguineous Iranian family that were reported with similar clinical characterization to LCA ,the SNP arrays were performed. After detecting the mutation, in order to confirm the new finding, PCR and Sanger sequencing were performed for first and second degree members of the family.

Results: SNP arrays identified a homozygous deletion in the first non-coding exon of LCA5. Both siblings were homozygous for this mutation. The results of PCR and sequencing of family members confirm this new mutation and both parents were heterozygous for this deletion of LCA5 gene.

Conclusion: Due to the importance of LCA among retinal dystrophy disorders, finding more mutations and variants in the causative genes can be of considerable help to specialists and patients. In this study, we found new deletion among Iranian population which hopes to provide more diagnosis and prevention of this disease. However, the findings of the current research are required to be replicated in other patients with LCA.

Keywords: Leber congenital amaurosis, LCA5 gene, SNP arrays

Diagnosis of Different Types of Early Infantile Epileptic Encephalopathies with Whole Exome Sequencing: A Three Year Cohort Study

Paria Najarzadeh Torbati1, Mehran Beiraghi Toosi2, Mohammad Doosti1, Najmeh Ahangari1, Shima Imannezhad3, Farah ashrafzadeh3, Narges Hashemi3, Mojtaba Safi1, Ehsan Ghayoor Karimiani*1,4,5

Department of Medical Genetics, Next Generation Genetic Polyclinic, Mashhad, Iran.

Pediatric Neurology Department, Ghaem hospital, Mashhad University of Medical Sciences, Mashhad, Iran

Department of Pediatrics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Innovative medical research center, Mashhad branch, Islamic Azad University, Mashhad, Iran.

Molecular and Clinical Sciences Institute, St. George's, University of London, Cranmer Terrace, London SW17 0RE, UK

paria22njz@gmail.com, Beiraghitm1@gmail.com, doosti.m@gmail.com, najmeh.ahangari@gmail.com, Shiimaa83@gmail.com, fashrafzadeh@yahoo.com, hashemin1984@gmail.com, mojtaba.safi@gmail.com, eghayoor@gmail.com

Corresponding Author: Ehsan Ghayoor Karimiani*1,4,5, eghayoor@gmail.com, Tell: +989153102327

Background: Early Infantile Epileptic Encephalopathies (EIEEs) are the most severe end of the spectrum of early onset epilepsies which usually lead to progressive psychomotor impairment. They start with different types of severe seizures in the early infantile period which are drug-resistant, and lead to progressive brain dysfunction such as intellectual disability, severe psychomotor developmental delay and early death in patients.

Method: A complete clinical and paraclinical examination has been done by expert specialists and clinical geneticist. Genomic DNA was extracted and evaluated through Whole Exome Sequencing analysis. We performed Sanger sequencing for segregation of EIEE related variants in order to confirm in the affected individuals and heterozygous state in the healthy parents.

Result: we identified 16 types of EIEE related variants in 26 families referred to our lab for genetic testing. All affected individuals had seizures with age onset of under 1 year of age, and Global Developmental Delay (GDD).

Conclusion: we identified 16 genes related Early Infantile Epileptic Encephalopathy from 26 families in a three-year cohort study. We expect to see more well-organized clinical trials based on a comprehensive knowledge about novel treatment approaches of EIEEs and our further studies would be following up the current treatments of our cases.

Keywords: Infantile Epilepsy, Neurogenetic disorders, EIEE syndromes, Epileptic Encephalopathy

Genetic study of more than 2000 patients affected with various genetic diseases, By Whole Exome Sequencing (WES) technique in Isfahan, Iran

Mansoor Salehi1,2,*, Zoya Najafi1,3
1- Cellular, Molecular and Genetics Research Center, 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 Biology, Science and Research branch, Islamic Azad University, Tehran, Iran.
Email: m_salehi@med.mui.ac.ir, zoyanajafi@srbiau.ac.ir

Background and Objectives: As it was predicted, Whole Exome Sequencing (WES) is now well established as a routine diagnostic technique in clinical genetic laboratories, not only for disease diagnosis, but also for determining prognosis and even for the treatment of the disease. The aim of this study was to summarize results obtained in application of WES on more than 2000 patients, in Isfahan Iran.

Methods: Genomic DNA were isolated from the patient's specimen and quantified. WES was performed using an Illumina NextSeq500. First, libraries were generated by Topomize DNA LT Library Prep Kit. The hybridization capture of DNA libraries was performed with xGen Lockdown panels. Sequenced reads were aligned to UCSC hg19 human reference genome downloaded from the GATK website. Alignment of the sequence reads, indexing of the reference genome, variant calling, and annotation were performed with a pipeline based on Burrows-Wheeler Alignment using BaseSpace Onsite. Variants were annotated using Alamut-HT and visualized on Alamut Viewer 2.2. After read mapping, the output alignment file was sorted using the Genome Analysis Tool Kit (GATK). Then, after variant annotation, low-quality variants were removed. The potential pathogenicity of the detected variants were evaluated using the American College of Medical Genetics and Genomics, in silico prediction tools, current literature, as well as compatibility with the known phenotypes and inheritance patterns.

Results: Majority of the detected variants were in genes with autosomal recessive pattern of inheritance (about 61%), which is much higher than autosomal dominant causing genes (5%) and X-linked (5%). Although, the majority of the disease-causing variants located in nuclear genome, analyzing of mitochondrial genome variants in the WES data yielded 15 variants in MT-DNA genes. Interestingly 20 patients had copy number variants (CNV) more than 5 kb.
Interestingly only about 28% of the samples failed to produce acceptable genetic diagnosis results, and of course this diagnostic rate was more or less dependent to the family history and clinical findings of the patients.

Conclusion: This study shows the distribution of causative genetic variants in our patients, so that these data would serve as a reference for future genetic screening and/or diagnosis in this population. Also, although cost effectiveness of the WES, makes this technology attractive, but there are still a number of problems with it, including commentary of unknown significant variants, incidental findings, and novel variants.

Keywords: whole exome sequencing, Patients, Genetic disease

A novel metabolic disorder in the degradation pathway of endogenous methanol due to a mutation in the gene of alcohol dehydrogenase

Maryam Razzaghy-Azar a,b, Mitra Nourbakhsh c,*, Mehdi Vafadar a, Mona Nourbakhsh a, Saeed Talebi d, Ali Sharifi-Zarchi e, Elham Salehi Siavashani f, Masoud Garshasbi g
a Hazrat Aliasghar Children's Hospital, Iran University of Medical Sciences, Tehran, Iran
b Metabolic Disorders Research Center, Endocrinology and Metabolism Molecular-Cellular Sciences Institute, Tehran University of Medical Sciences, Tehran, Iran
c Department of Biochemistry, School of Medicine, Iran University of Medical Sciences, Tehran, Iran
d Department of Medical Genetics and Molecular Biology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
e Computer Engineering Department, Sharif University of Technology, Tehran, Iran
f PardisGene Company, Tehran, Iran
g Department of Medical Genetics, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran
correspondent: mrazar_md@yahoo.com nourbakhsh.m@iums.ac.ir mehvaf@yahoo.com dr.mnrbh@gmail.com
talebi.s@iums.ac.ir sharifi@sharif.edu elham.salehi@pardisgene.com masoud.garshasbi@modares.ac.ir

Background: A small amount of methanol is produced endogenously in the human body but it is efficiently metabolized by alcohol dehydrogenase (ADH) and other enzymes. The products are eliminated without harm. In this study, we present a new entity of inborn error of metabolism in methanol degradation due to a mutation in the ADH1C gene coding for the γ subunit that is part of several ADH isoenzymes.

Method: Acylcarnitine profile (C0–C18.2) was measured by tandem mass spectrometry [MS/MS], urine organic acid by Gas Chromatography-Mass Spectrometry [GC/MS]), amino acid levels by high performance liquid chromatography (HPLC), urine amino acids, carbohydrates and succinylacetone by thin layer chromatography (TLC). Blood level of methanol was measured by spectrophotometry (Milton Roy, US Colorimetry), based on a colorimetric method. Laboratory assays were for ethanol by an enzymatic method by ADH, formic acid by a colorimetric method and formaldehyde by the method of Bricker and Johnson. Complete blood count (CBC), measurement of serum levels of urea, creatinine, sodium, potassium, calcium, phosphate, chloride, as well as, serum cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, AST, ALT, LDH, CPK, aldolase were done by routine laboratory assays. A trio whole-exome sequencing (WES) test and then whole-genome sequencing were performed by Centogen in Germany and raw data were evaluated by geneticists in Iran.

Results: This disorder was discovered in an 11.58-year-old boy. During 9 month of his hospital admission, he had periods of 1to 4 days coma, and between these periods he had ataxia, dysarthria and sometimes was verbose and euphoric. Following hemodialysis, he became conscious and appeared healthy for 3-4 days. Clinical and Para clinical organ evaluations, laboratory tests for all of the known hereditary metabolic disorders and routine biochemical profile were normal. Finally toxicological evaluation of his blood showed a high methanol level: 12.2 mg/dL (3.8 mmol/L) [normal range up to 3.5 mg/dL (1.09 mmol/L)] while the formaldehyde level was undetectable.

The findings of normal size of the liver and normal liver function tests coupled with normal eye examination with elevated blood methanol level and undetectable formaldehyde level, suggested ADH insufficiency. Adding zinc to the drug regimen 15 mg/day dramatically reduced the patient's methanol level and alleviated abnormal signs and symptoms. When zinc supplementation was discontinued, the patient relapsed into coma and hemodialysis was required once again. The genetic study showed a homozygous mutation in ADH1C gene located in exon 3, and both parents were heterozygous for this mutation.

Conclusion: Genetic defect in alcohol dehydrogenase due to mutation in ADH1C results in accumulation of methanol, drunken state, ataxia, and leads to coma. This condition can be successfully treated with zinc supplementation as the cofactor of ADH.

Keywords: Methanol, Alcohol dehydrogenase, Intoxicated behavior, Alcohol metabolism

Functional Evidence for ITSN1 Involvement in Development of Intellectual Disabilities

Mohammad Haddadi*
Department of Biology, Faculty of Basic Sciences, University of Zabol, Zabol, Iran
Correspondent : m.haddadi@uoz.ac.ir, hadadimohmad@gmail.com

Background: Intellectual disability (ID) is a heterogeneous and complicated neurological disorder with a relatively high prevalence in consanguine sub-populations. In Iran a GWAS on a Zaboli family reported an association for a novel genetic mutation in the *ITSN1* gene. In order to put light on the functional role of this gene, *in vivo* modelling needs to be examined. *Drosophila melanogaster* is a unique and well-known insect model organism suitable for such investigations as its genome has an ortholog of 75% of disease-causing human genes. Moreover, *Drosophila's* tiny brain resembles a high level of neurogenetics and neurofunctional identities to the human brain.

Materials and Methods: The RNAi-mediated post transcriptional gene silencing was undertaken to down regulate the *dap160* gene which is fly ortholog to *ITSN1* gene. For this purpose the Gal4/UAS system has been employed. Indeed, Gal4/UAS is a highly efficient binary genetic tool in *Drosophila* that offers manipulation in gene expression in a time- and tissue-specific manner. Down-regulation of the target gene was limited to central nervous system of the flies and then evaluated and confirmed via qPCR. The pathogenic effects of *dap160* down regulation were assessed through brain IHC and microscopy, and well-established and reliable behavioural assays including olfactory conditioning, and courtship conditioning learning and memories.

Results: Based on the massive functional resemblance between the *ITSN1* gene and its orthologs in *Drosophila melanogaster* synaptic vesicle recycling, down-regulation of the *dap160* resulted in neuronal dysfunction which was evident by remarkable decline in total fluorescent signal of mushroom bodies and impairment of olfactory and courtship conditioning memories which are noteworthy to be considered as some sort of ID-like symptoms in flies.

Conclusions: The transgenic *Drosophila* underwent brain-restricted down-regulation of *dap160* display malformation and malfunction in mushroom bodies (MBs) structures. These defects can be considered as proof for the declaration on the association of observed genetic alteration and familial ID in the Iranian population.

Keywords: Intellectual disability, *ITSN1* gene, Functional assay, *Drosophila melanogaster*, *dap160* gene

The clinical and exome sequencing data of patients with familial Parkinson Disease in Iran

Mohammad Soudyab2 , Mohammad Shariati 4 & 3 , Reza Jafarzadeh Esfehani 2 , Neda Shalaie3,, Shabnam Vafadar3, Vahid Nouri 4 , Michael Zech 5 & 6, Julianne Winkelmann 5&6&7&8, Ali shoeibi 3 and Ariane Sadr-Nabavi 1&2&3&4&5&6

1 Medical Genetic Research Center (MGRC), School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

2 Department of Medical Genetics, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

3 Department of Neurology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

4 Academic Center for Education, Culture, and Research (ACECR)-Khorasan Razavi, Mashhad, Iran.

5 Institute of Neurogenomics, Helmholtz Zentrum München, Munich, Germany.

6 Institut für Humangenetik, Technische Universität München, Munich, Germany.

7 Lehrstuhl für Neurogenetik, Technische Universität München, Munich, Germany.

8 Munich Cluster for Systems Neurology, SyNergy, Munich, Germany.

Correspondent: sadrnabavia@mums.ac.ir

Introduction: Parkinson's disease (PD) is a common progressive neurodegenerative disorder with both motor and nonmotor symptoms. Recent studies have demonstrated various susceptibility loci and candidate genes for familial forms of the disease. However, the genetic basis of the familial form of PD is not widely studied in Iranian population. Therefore, the present study aimed to investigate the possible causative genetic variants responsible for development of familial Parkinson disease among Iranian patients.

Methods: Total number of 500 clinical records of Iranian patients with clinical diagnosis of Parkinson's disease was evaluated and 29 families with at least two affected individuals with early onset PD were chosen to enroll in the present study. Selected families mainly were from eastern parts of Iran. They belonged to different ethnicities. An expert group of Neurologists examined these families and confirmed the clinical diagnosis of Parkinson's disease. Whole exome sequencing (WES) was performed for every patient and the possible causative genetic variants related to development of PD were reported.

Results: Among the patients with familial early onset PD (EOPD), most of the patients enrolled in present study were male (18 patients) and the mean age was 39.62 years. In the study population, eight patients had new genetic variants in *TH, DCTN1, LRRK2* and *MAPT* genes. All the genetic variants were heterozygotes. Only one patient was compound heterozygote for a pathogenic variant (c.686C>T; p. Arg229His) and a likely pathogenic variant (c.1264G>A; p. Gly422Arg) in *TH* gene. Most of the new genetic variants were in *LRRK2* gene. All the four genetic variants in *LRRK2* gene were pathogenic variants. A likely pathogenic variant in *LRRK2* gene (c.1512_1513del; p.Leu505SerfsTer24) was seen in another member of the same family. The

second common gene bearing likely pathogenic variants for Parkinson's disease was *MAPT* gene. One patient had a likely pathogenic variant in *DCTN1* gene (c.1712T>G; p. p.Met571Arg).

Conclusion: The present research revealed some novel pathogenic variants for EOPD among Iranian population. Further functional studies are warranted to confirm the pathogenicity of these novel variants and establish their clinical application for early diagnosis of EOPD.

Keywords: Parkinson's disease, Whole exome sequencing, Genotype

Report of a novel CFTR gene frame shift variant causing cystic fibrosis in a large Iranian family

Mohammadreza Dehghani 1, 2, Kimia Monshizadeh 3, Zahra Farshchian Yazdi 3
Mohammadhossein fakherbaheri 3, Mohammad Yahya Vahidi Mehrjardi1, 2.
1. Medical Genetics Research Center, Shahid sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
3. Student Research Committee, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
dehghani.dr@gmail.com,kimia.monshizadeh@yahoo.com, farshchiyanzahra@yahoo.com, mh.fakherbaheri@gmail.com, mmvahidi@gmail.com.

Background and Aim: Cystic fibrosis (CF) is a disease that involves the cells producing sweat and mucous causing disorders in the respiratory system. Symptoms of this disease include recurrent respiratory infections and lack of proper weight gain in newborns. About 70,000 patients with CF have been identified worldwide and 1,000 new cases are reported annually. It is an autosomal recessive disease caused by a mutation in the CFTR (the cystic fibrosis transmembrane conductance regulator) gene. The most common cause of this disease is p.F508del. However, more than 2,000 different mutations in the CFTR gene have now been observed. Here we aim to find genetic variants' influence on CF.

Method: We studied a large consanguineous Iranian family having three young sons with similar clinical characterization to CF. NGS was performed for the older child. After detecting the mutation, Sanger sequencing was done for other members in the family.

Results: NGS identified a homozygous deletion in 2 Nucleotides of CFTR in exone 4.

Conclusion: According to the prevalence and importance of CF disease, molecular studies that lead to the identification and diagnosis of new variants in the population are very important and practical and can reduce the rate of the CF disease in the population.

Keywords: Cystic fibrosis, CFTR, NGS

A 26.92 Mb interstitial deletion at 7q32.3q36 in an Iranian patient with multiple anomalies

Mohammad Reza Abbaszadegan1,2,4*, Reza Ebrahimzadeh-Vesal 3, Sousan Hosseini 3, Maliheh Alimardani1,2
1Medical Genetics Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;
2Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;
3 Pardis Pathobiology and Genetics laboratory, Mashhad, Iran
4 Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran 5 Pardis Pathobiology and Genetics laboratory, Mashhad, Iran
*Corresponding author
Abbaszadeganmr@mums.ac.ir ; rzebrahimzadeh@gmail.com ; su.hosseini.2@gmail.com ; Alimardanim981@mums.ac.ir

Background: The partial deletion of 7q is a rare chromosomal anomaly. Individuals with larger deletions in this region have also been characterized by dysmorphic features, developmental delay, speech and language disorder. Moreover, the phenotypes of patient with 7q31 microdeletion were reported as mental retardation, simian crease, facial abnormality. The chromosomal region of 7q32.3-q35 comprised several protein-coding genes, a part of the RAS/MAPK signaling pathway, and plays an important role in human development; hence, its dysregulation leads to severe congenital anomalies. Moreover, other genes located on 7q31q36 region including CNTNAP2, associated with speech delay, CPA4 and MKLN1 that controls the closure of cranial sutures and several aspects of brain growth, and mode of cognition. As a result, this region is known as a hot spot for the evolution of human-specific communication abilities.

Case presentation: The index is a 9-year-old Iranian girl referred to our clinic due to an initial diagnosis of multiple anomalies including cleft lip and palate, scoliosis, hypotonia, speech problem, ectrodactyly, microcephaly, megacolon, hearing problem, and anomaly in toes. The parents were non-consanguineous and referred to the Pardis Genetic Center in 1398.

Results & Conclusion: At first, tandem mass spectrometry (MS/MS) were ordered and normal result was seen. Additionally, whole exome sequencing were ordered which showed no pathogenic mutation related to this patient's anomalies. Then, pathogenic loss of 26.92 Mb on 7q32.3q36 was detected using CGH Array (figure 3). The karyotypes of parents showed normal 46,XX and 46,XY(figure 4) and excluded this chromosomal aberration in both healthy parents. The right choice of diagnostic test in this case considering multiple anomalies in this patient is a lesson to be learned. Moreover, it is important to perform genetic counseling and prenatal diagnosis (PND) for next pregnancy to prevent the birth of another affected child.

Keywords: multiple anomaly, deletion, dysmorphic features.

Report of Chromosome Abnormalities in individuals with Consanguineous marriage referred to Sarem Women's ' Hospital, Tehran, Iran

Farkhondeh Behjati1,2,3* , Mahdieh Pashaei1,3 ,Akram Abdi1,2 , , Fahimeh Mousavi1,2 , Iman Bagherizadeh1,2 , Atefeh Dokhanchi1,2 , Ensieh Ghadami1,2 , Kamran Bahadory1 , Abootaleb Saremi1,2

Sarem Fertility & Infertility Research Center (SAFIR), Sarem Women's Hospital, Iran University of Medical Sciences (IUMS), Tehran, Iran.
Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Tehran, Iran
Genetics Research Centre, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

* Corresponding Author: Professor Farkhondeh Behjati

Email Addresses:
Professor Farkhondeh Behjati (PhD): fbehjati@gmail.com & f_behjati@uswr.ac.ir
Dr. Mahdieh Pashaei (PhD): mahdieh_pashaei@yahoo.com
Ms. Akram Abdi (M.Sc): ak.abdi1362@gmail.com
Mr. Iman Bagherizadeh (M.Sc): imanimoni@gmail.com
Ms. Fahimeh Mousavi (M.Sc): f.mousavi.uswr.@gmail.com
Ms. Ensieh Ghadami (M.Sc): ghadamiensi@gmail.com
Dr. Kamran Bahadory (MD): drbahadory@gmail.com
Dr. Abootaleb Sarem (MD): saremiat@yahoo.com

Background and Aim: Consanguinity is defined as the marriage between close relatives. The deleterious effects associated with consanguinity are mostly due to the expression of rare recessive genes inherited from common ancestors. Consanguineous marriage is significantly higher in many genetic diseases leading to prenatal, neonatal, child morbidity or mortality. Consanguineous marriage is a common practice in the Middle East including Iran with a rate of 30-85%. The present study was undertaken to analyze incidence of chromosomal abnormality (CA) in patients with consanguineous marriage, seeking genetic counselling, in Sarem Women's Hospital, Tehran, Iran.

Materials & Methods: Standard Cytogenetics techniques were carried out on the peripheral blood of 1055 patients with consanguineous marriage. The patients were referred to the Cytogenetics laboratory of Sarem Women's hospital during 2006-2021. Chromosome analysis was carried out using GTG-banding technique. 969 patients were due to genetic counseling for consanguineous marriage and 86 patients with a referral of consanguineous marriage and other reasons comprising of the previous history of recurrent abortions, infertility, intellectual disability, expired child, stillbirth, and a child with chromosome abnormality.

Results: Chromosomal abnormalities were found in 34 patients (3.22%) out of a total of 1055 patients. Most of the abnormalities were structural (97 %).

Chromosomal abnormalities were found in 15 females (44.1%) and 19 males (55.9%). Inversions were the most common chromosomal abnormalities (64.7 %) diagnosed in this study. Pericentric inversion around the centromere of chromosome 9 was observed in 18 cases (1.70 %). Chromosomal inversions were found in the heterochromatin region of chromosome 1 in one case and chromosome 2 in one other case. Paracentric inversion of chromosome 14 was found in one case. One patient had a pericentric inversion of large size in one chromosome 9. Robertsonian translocation was found in three patients. Five Patients had reciprocal translocation between two chromosomes. One patient had insertion of an unknown chromosome material within the short arm of chromosome 3. Amongst patients with consanguineous marriage and other reasons, only those with recurrent abortions (3 abnormals) and infertility (1 abnormal) had chromosome abnormalities. Chromosome abnormality rate in individuals referred for consanguineous marriage genetic counseling was 3.09% compared to 4.65% in those referred for consanguineous marriage and recurrent abortions or infertility.

Conclusion: Compared to the rate of chromosome abnormality in the general population of less than 1%, the 3.09% of chromosomal abnormalities rate in consanguineous marriages in this study is high. However, this rate is even higher in individuals with consanguineous marriage and a history of recurrent abortion or infertility, with 4.65%. Due to the high rate of consanguineous marriages in the Iranian population, genetic counseling and chromosome analysis in couples with consanguineous marriage is highly recommended.

Keywords: Consanguineous Marriage; Genetics Counseling; Chromosomal Abnormality; GTG-Banding; Recurrent Abortions; Infertility.

Case report of translocation (21; 14) in relation to infertility and birth of a girl with karyotype 46, xx, der (21) add (14) (q13) del (21) (q11.2) In a family

Authors: Mohadeseh Khoshandam (PhD) 1, Naser kallhor (MSc)2, Leila naserpoor (PhD)3
1-Department of Reproductive Biology, Academic Center for Education, Culture, and Research (ACECR), Qom branch, Iran.
2- Department of Mesenchymal Stem Cells, the Academic Centre for Education, Culture and Research, Qom Branch, Qom, Iran
3-Department of Reproductive Biology, Academic Center for Education, Culture, and Research (ACECR), Qom branch, Iran.
Email: mohi_khosh70@yahoo.com
Email: leilanasery48@gmail.com
Email: naserkalhor@gmail.com

Background and Purpose: Infertility affects about 15% of couples, with approximately 205 million pregnancies occurring each year worldwide, with recurrent miscarriages of 15-20% Includes diagnosed pregnancies.

There are several causes for recurrent miscarriage and infertility, the most important of which are physical problems, endocrine diseases, thrombophilia, immune factors, antiphospholipid syndrome, infection and chromosomal abnormalities. Cytogenetic analysis has become increasingly important to describe a number of causes of infertility. Male infertility has been reported to be associated with chromosomal aberrations, which usually include sex and autosomal chromosomes. The most common cause of recurrent miscarriage is chromosomal abnormalities in the fetus. More than 80% of them occur in the first trimester. In general, balanced chromosomal translocation occurs in 4-6% of the normal population. On the other hand, the prevalence of chromosomal balanced translocation in couples experiencing recurrent miscarriages has been reported to be 20 times that of the normal population. Acrocentric chromosome translocation is the most common type of chromosomal balance in couples with a history of recurrent miscarriage and infertility due to similar sequences in rRNA, especially 21 to 14 translocation.

Research Methods: We report a 26-year-old woman with 4 years of infertility and 2 times of unsuccessful IVF. Peripheral blood lymphocytes were obtained for karyotype and metaphase was examined by standard trypsin GTG method.

Findings: The mother is healthy with karyotype 46, XX, t (14, 21) and the father is healthy with 46, XY. Their daughter has 46, XX, der (21) add (14) (q13) del (21) (q11. 2) and also the sister's sister has the karyotype 46, XX, t (14, 21). The couple is a cousin. They had one abortion at 16 weeks due to an unbalanced karyotype, two failed IVFs, and one spontaneous abortion.

Conclusion: In 2 members of this family, chromosomal translocation with karyotype 46, XX, t (14, 21) was observed, which confirms the results of similar studies in this field, which show the risk of infertility and childbirth with mental problems in displaced couples. Chromosomes are larger than normal populations. According to the results of this study, it seems that cytogenetic studies in families with a history of infertility and unsuccessful IVF are necessary to search for translocation.

Keywords: infertility, chromosomal displacement, chromosomal abnormalities.

Inherited deletion of 9p24.3p22.3 and duplication of 18p11.32p11.31 associated with neurodevelopmental delay/intellectual disability: characterization of involved genes and phenotypic matching

Naser Ajami1,2, Mohammad Amin Kerachian1,2, Mehran Beiraghi Toosi3, Farah Ashrafzadeh4, Reza Jafarzadeh-Esfehani5, Susan Hosseini6, Mohammad Reza Abbaszadegan1,7
1. Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2. Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
3. Department of Pediatric Neurology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
4. Department of Neurology, Mashhad University of Medical Science, Mashhad, Iran.
5. Academic Center for Education, Culture and Research (ACECR), Khorasan Razavi, Mashhad, Iran
6. Pardis Pathobiology and Genetics Laboratory, Mashhad, Iran
7. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
ajamin951@mums.ac.ir, kerachianMA@mums.ac.ir, BeiraghiTM@mums.ac.ir, ashrafzadehf@mums.ac.ir, JafarzadehR951@mums.ac.ir, AbbaszadeganMR@mums.ac.ir

Background and Aim: Neurodevelopmental disorders (NDDs) are a heterogeneous class of conditions that impact brain development and affect several domains of daily functioning. It has been estimated that at least 30% of NDDs are caused by genetic factors. In the general population, CNVs are not only a prevalent source of genomic variation but are also a major cause of NDDs and related disorders. Here, we present a 3.5-year-old Iranian female child and her affected 10-month-old brother affected with derivative chromosome 9, originating from a balanced translocation t (9;18) (p22;p11.31) present in the mother. We characterized also the postnatally detected rearrangement by aCGH.

Method: Karyotyping of both children and the parents were performed on routinely cultured peripheral blood lymphocytes. A total of 20 metaphase spreads were analyzed in each case according to the GTG banding technique. aCGH was also performed for characterization of detected rearrangement. For comparing the CNVs, the DECIPHER database was searched separately for individuals who had deletions of chromosome 9p and duplication of 18p which overlapped with the deletion and duplication identified in our case. For aligning the genes affected by detected CNVs to clinical and functional phenotypic features, we used PhenogramViz software.

Results and discussion: We describe a 3.5-year-old Iranian female child and his affected 10-month-old brother with maternally inherited derivative chromosome 9 [der(9)]. The postnatally detected rearrangement has been finely characterized by aCGH analysis. aCGH analysis revealed a 15.056Mb deletion of 9p24.3p22.3 encompassing 14 OMIM morbid genes, and gain of 3.309 Mb on 18p11.32p11.31. Moreover, we matched the patient's clinical manifestations and the

regions involved in the rearrangement by comparison to the clinical presentation and the 9p and 18p genomic imbalances of other reported cases using DECIPHER and PhenogramViz. In the 9p24.3p22.3 region, approximately 80 pathogenic/likely pathogenic/uncertain CNVs were in the DECIPHER that harbored pure overlapped size with CNV detected in our case. The size of these CNVs ranged from 12.01 kb to 18.45 Mb and 52 CNVs were smaller than 1 Mb in size affecting 10 OMIM morbid genes. The 18p11.32p11.31 region had 19 overlapped CNVs in the DECIPHER with the size ranging from 23.42 kb to 1.82 Mb. These CNVs affect 8 intolerant genes. For phenotypic matching, the patient's phenotype and CNVs data were provided and interred to PhenogramViz. For 9p deletion CNV, 53 affected genes were identified and 20 of them were matched to 24 HPO terms describing the patient's phenotypes. Also, for CNV of 18p duplication, 22 affected genes were identified and 5 of them were matched to 12 phenotypes. In conclusion, major clinical features of chromosome distal 9p deletion syndrome were seen in patients harboring deleted CNVs smaller than 1 Mb in size in the 9p24.3 region.

Keywords: Intellectual Disability, 9p Deletion Syndrome, Duplication of 18p, DECIPHER, PhenogramViz.

Is sperm telomere length altered in teratospermic infertile men?

Mohammad Hasan Sheikhha 1*, Maryam Fattahi 1, Mohadese Maghsudlu 2
1-Clinical and Research Center for Infertility, Yazd Reproductive Sciences Institute, Yazd Iran
2- Genetics Department, Tehran University of Medical Sciences, Tehran, Iran

Correspondent:
sheikhha@yahoo.com

***Background*:** Fertility problem as one of the major challenges in the modern era afflicts almost 8 to 15% of couples worldwide with even higher prevalence in industrialized countries. Male infertility is responsible for 40-50% of these cases. Male infertility is known as a multifactorial defect, and among different classifications, teratospermia ot teratozoospermia is determined by the existence of over 85% morphologically abnormal spermatozoa in semen. One of the most novel issues in genetic alterations studies is variation of sperm telomere lengths (STL) and its collaboration to male infertility. To our knowledge, changes of telomere length in teratospermia had not been separately evaluated before, thus the present study has been focused on STL alterations.
Objectives: Investigation of any difference in telomere length of teratospermia specimens and normal sperms.

Material and methods: Overall 60 men referred to Arak Fertility Clinic, were included which were categorized in teratospermia and normozoospermic groups. Sperm genomic DNA extraction was conducted and STL was evaluated by the use of qPCR. In order to evaluate the relative telomere length the T/S ratio was calculated for each specimen by the use of $2^{-\Delta\Delta Ct}$ formula. Data analysis was performed with the help of SPSS statistical software.

Results*:* Statistical evaluation of relative telomere length was calculated by the ratio of telomere to single copy gene for teratospermia and normal specimens. The average amount for teratozoospermia samples was 0.0033 while it was 0.011 for normal specimens. This results significantly demonstrated that relative telomere length in teratospermia samples are nearly three times shorter than this in normal samples ($p<0.05$).

***Conclusions*:** Telomeres length is one of the issues which has been highlighted in the field of different biological factors that affect semen characteristics lately. Our results represent the reduction of telomeres length in teratospermia and suggest that this alteration might be one of the factors contributing to incompetency of this kind of specimens. However defining relevant molecular processes requires further detailed investigations.

Keywords: Telomere Length, Teratospermia, Sperm, Male infertility

Expression and Clinicopathological Significances of lncRNAs: Could ARA and ZEB2NAT be the Potential Breast Cancer-Related Biomarkers?

Asaad Azarnezhad, Liver and Digestive Research Center, Research Institute for Health Development, Kurdistan University of Medical Sciences, Sanandaj, Iran
Correspondent: Azarnezhad@gmail.com

Background and Aim: Pieces of evidence have shown that a significant proportion of cancer-prone factors are not attributed to alterations in protein-coding sequences. Adriamycin resistance-related *(ARA)* and natural antisense of ZEB2 (*ZEB2NAT)* long non-coding RNAs (lncRNAs) have been indicated with oncogenic properties by regulating various signaling pathways and epithelial-to-mesenchymal transition (EMT), which may have diagnostic and prognostic potential as a novel group of biomarkers. The current study aimed to evaluate the expression status of *ARA* and *ZEB2NAT* lncRNAs and their clinicopathological significance in a population with breast cancer (BC).

Materials and Methods: Total RNA was extracted from 60 tumor samples and their normal adjacent tissues (NATs). The lncRNA expressions were measured using quantitative reverse transcription PCR (RT-qPCR) and statistical analyses were performed by SPSS version 25.

Results: Our data showed a significant upregulation of *ARA* and *ZEB2NAT* lncRNAs in tumor tissues compared to NATs (p <0.001; p = 0.021, respectively). *ARA* and *ZEB2NAT* expression were observed to be significantly associated with tumor grade, nuclear grade, tumor stages, and lymph node metastasis (p <0.05). Additionally, *ARA* expression was significantly correlated with breastfeeding status (p = 0.027).

Conclusion: Our data revealed that *ARA* and *ZEB2NAT* lncRNAs were overexpressed in BC. Furthermore, the selected lncRNAs were found to might be the potential biomarkers for BC diagnosis and prognosis. However, the findings of the current research are required to be replicated in other studies with larger sample sizes.

Key Words*: Breast cancer, Long non-coding RNA, Biomarker,* ARA, ZEB2NAT

SMARTDX: A NGS Data Analysis Platform for Clinical Laboratory

Toktam Dehghani1*, Reza Jafarzadeh Esfehani2, Maliheh Alimardani345, Maryam Gholizadeh1, Sepideh Hassibian7, Ariane Sadr-Nabavi4, Mohammad Reza Abbaszadegan346, Saeid Eslami1*

1 Department of Medical Informatics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
2 Blood Borne Infections Research Center, Academic Center for Education, Culture and Research (ACECR)- Khorasan Razavi, Mashhad, Iran.
3 Medical Genetics Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
4 Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
5 Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
6 Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
7 Faculty of Life Sciences and Biotechnology, Shahid Beheshti University, Tehran, Iran.

dehghanit982@mums.ac.ir; JafarzadehR951@mums.ac.ir; alimardanim981@mums.ac.ir; GholizadehM991@mums.ac.ir; s.hassibian@mail.sbu.ac.ir; sadrnabavia@mums.ac.ir; abbaszadeganmr@mums.ac.ir; eslamis@mums.ac.ir

Background: In the recent decade, a significant increase has been observed in the usage of Next-Generation Sequencing (NGS) for gene amplicons in biological and medical research. Moreover, a large number of investigations in environmental medical diagnostics are performed by the NGS data analysis. NGS is a rapid, high-throughput, and cost-effective approach for performing genetic tests and it becomes the most popular technology for sequencing the whole exome and genome of patients. Despite the growth of NGS utilization in clinical genetic laboratories, many challenges remain in the registration and analysis of this data, such as (i) dealing with the massive amounts of data, (ii) requirement of high-performance computational systems and programming skills, (iii) variety of bioinformatics tools, and (iv) low quality of visualization of results and reports. Thus, further improvements in the current algorithms and workflow are essential.

Results*:* We introduce SmartDXCloud, an online automatic platform for registering and analyzing NGS data. The main goal of this platform is to simplify and straightforward the procedure of analyzing NGS data. Four objectives are pursued in this platform. First, the presented platform is user-friendly and fully automated and it reduces the need for human resources. Second, it achieves reliability and accuracy through performing standard analysis, e.g. minimal workflows, gene panels, incidental finding, and carrier screening analysis. Third, traceability and reproducibility are considered by storing and reporting intermediate results via the website, which could be

practical for re-analyzing data. Forth, all results are graphically displayed and could be shared with collaborators or publications.

Conclusion: SmartDXCloud would facilitate the registration, analysis, visualization, and sharing of NGS data. It has the potential to improve the decision-making process for diagnosing and treating genetic disorders. The platform is currently available at https://www.SmartDXCloud.ir.

Keywords*:* Automatic Platform, Pipeline, Genetic Variants, Next Generation Sequencing (NGS), Whole-Exome Sequencing (WES), Personalized Genomic Medicine, User-friendly Graphical Interface.

Autosomal recessive polycystic kidney disease: late- onset renal enlargement and proteinuria with rare PKHD1 mutation.

Tina Zeraati1,2, Maliheh Lotfi1,3, Mohammad Reza Abbaszadegan3,4, Anoush Azarfar2, Ehsan Ghayoor Karimiani5, Abbas Ali Zeraati2*

1. Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
2. Kidney Transplantation Complications Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
3. Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

4. Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

5. Genetics Research Centre, Molecular and Clinical Sciences Institute, St George's, University of London, Cranmer Terrace, London SW17 0RE, UK

Tina Zeraati: zeraatit4@mums.ac.ir

Maliheh Lotfi: Lotfim951@mums.ac.ir

Mohammad Reza Abbaszadegan: AbbaszadeganMR@mums.ac.ir

Anoush Azarfar: Azarfara@mums.ac.ir

Ehsan Ghayoor Karimiani: eghayoor@gmail.com

Abbas Ali Zeraati: zeraatia@mums.ac.ir (corresponding author)

Background: Autosomal recessive polycystic kidney disease (ARPKD) is a genetically inherited pediatric kidney disease. It is caused by a mutation in the PKHD1 gene located on chromosome 6. The predominant phenotype is characterized by early-onset bilateral enlarged kidneys, in addition to fibrocystic changes in both kidney and liver. Fetuses or infants usually present with potter syndrome, and they are more likely to develop severe renal insufficiency. Patients usually die perinatally or in infancy. Liver involvement has been reported in adults with ARPKD who have survived the neonatal period and childhood. However, renal involvement is often not expected in adulthood. We hereby describe a 33-year-old female case of adult-onset proteinuria and nephromegaly. She was confirmed to be affected by a rare homozygous mutation of the *PKHD1* gene with autosomal recessive inheritance inherited from both her consanguineous heterozygote parents.

Methods: Proteinuria was incidentally discovered based on the patient`s laboratory tests. She. The patient was referred to the nephrology department. A thorough physical examination, along with kidney ultrasound was performed. Finally, she was diagnosed with adult polycystic kidney disease. The patient was referred to the genetic department. DNA was extracted from whole blood. Human Whole-exome enrichment was performed using Agilent SureSelect V6 Target Enrichment Kit, followed by Next-Generation Sequencing (NGS) using Illumina HiSeq4000 platform to yield an average coverage depth of 100X. The DNA sequence was mapped to, and analyzed in comparison with, the published human genome build (UCSC hg19 reference sequence). In order to verify causative variant, carrier testing for the patient`s parents and her mild symptomatic sister was performed using Sanger sequencing.

Results: A 33-year-old female patient with no remarkable past medical history was referred due to new-onset severe proteinuria. The urinary protein concentration was 430 mg/dl. On ultrasound, bilateral kidney enlargement and variable-sized renal cysts were observed. Similarly, her older sister had a history of renal cysts. She was diagnosed with adult polycystic kidney disease. Considering the age of onset and the absence of hepatic involvement, an autosomal dominant mutation in the PKHD gene was expected. Surprisingly, a homozygous VUS variant was identified in exon 38 of the PKHD1 gene c.6283A>C (p.Thr2095Pro) according to ACMG method. This gene has been reported in association with Polycystic kidney disease 4 (Phenotype MIM number 263200) with autosomal recessive inheritance (OMIM#606702). The same heterozygous variant was confirmed for her parents and sister according to the Carrier testing results.

Conclusion: Adult polycystic kidney disease manifested with renal symptoms is rarely associated with an autosomal recessive inheritance. ARPKD in adults appears to be a challenging issue that is encountered regarding genetic counseling. Considering the poor prognosis of ARPKD in childhood, genetic assessment using NGS methods seems essential for the PKD patients, thereby preventing the consequences in the next generation.

Keywords: ARPKD, Polycystic Kidney, whole-exome sequencing, proteinuria

Early-infantile onset epilepsy and developmental delay caused by bi-allelic GAD1 variants

Atieh Eslahi 1,2, Farzaneh Mirzaei 1, Farzaneh Alizadeh 1, Shima Farrokhi 1, Yasamin Yousefi 1, Majid Mojarrad *1,3,4

Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Student Research Committee, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Genetic Center of Khorasan Razavi, Mashhad, Iran

Correspondent: mojaradm@mums.ac.ir

Atiye.eslahi69@gmail.com

Mirzaei.farzane@gmail.com

alizadeh7029@gmail.com

yasaminsmartgirl@gmail.com

shimafarrokh_21@yahoo.com

Introduction: Gamma-aminobutyric acid (GABA) and glutamate are the most abundant amino acid neurotransmitters in the brain. GABA, an inhibitory neurotransmitter, is synthesized by glutamic acid decarboxylase (GAD). Its predominant isoform GAD67, contributes up to 90% of base-level GABA in the CNS, and is encoded by the GAD1 gene. Disruption of GAD1 results in an imbalance of inhibitory and excitatory neurotransmitters, and as Gad1–/– mice die neonatally of severe cleft palate, it has not been possible to determine any potential neurological dysfunction. Furthermore, little is known about the consequence of GAD1 disruption in humans

Methods: Six patients from six unrelated families were identified through GeneMatcher and enrolled in this study. Genetic testing through whole exome sequencing (WES) was carried out in different research centres after informed consent was obtained from the parents or legal guardians of the studied subjects.

Results: All affected individuals carried ultrarare GAD1 variants, which were predicted to result in impaired protein function. Homozygous variants were identified in five families (Families A, B, D, E and F), whereas compound heterozygous variants were found in Family C

Conclusion: Our findings highlight an important role for GAD1 in seizure induction, neuronal and extraneuronal development, and introduce GAD1 as a new gene associated with developmental and epileptic encephalopathy

Keywords: Gamma-aminobutyric acid (GABA), GAD1 gene, whole exome sequencing (WES), developmental and epileptic encephalopathy

Recurrent familial hydatidiform mole as a rare clinical problem

Fereidoon Abdolmaleki1,2, Arash Pooladi1,2, Seyedeh Reyhaneh Yousefi Sharami1, Esmail Rahimi2, Ronak Shahsavari2.

1- Faculty of Medicine, Kurdistan University of Medical Sciences, Sanandaj, Iran

2-MAAD Genetics Polyclinic & Lab., Sanandaj, Kurdistan, Iran

Email:

f.abdolmaleki@gmail.com,arash.pooldai@gmail.com,yousefi805@yahoo.com, rahimi.esmaeil88@gmail.com, ronak.shahsavari90@gmail.com

Background and Objectives: Abnormal hyperproliferation of trophoblastic cells are the cause of a group of diseases called Gestational Trophoblastic Disease (GTD) including Hydatidiform mole which is derived from the trophectoderm. A rare kind of the mole is Recurrent Hydatidiform Mole (RHM) that is a hereditary form of moles. Two maternal-effect genes, *NLRP7* and *KHDC3L*, are responsible for this condition which is inherited in an autosomal recessive pattern (by both of these genes). *NLRP7* is mutated in 48–80% of patients with Recurrent HYDM. *KHDC3L* (KH domain containing 3-like is also known as C6ORF221) is located on chromosome 6q13 as a new recessive gene, and is involved in 10–14% of recurrent HYDM cases with no *NLRP7* mutations.

Material and methods: The Proband is a 29 years old woman with 5 molar pregnancies terminated with D&C (G_5 P_0 Ab_5 L_0). Her parents are double first cousins (consanguineous marriage, F=1.56%). She also has a sister with 4 missed abortions with molar pregnancies. Her younger brother has an osteomalacia and gate developmental delay.

Results: As the first step in the genetic study, Cytogenetic investigation was carried out and her karyotype was normal (46,XX). The proband was therefore a candidate for Whole Exome Sequencing and a Homozygote c.1A>G mutation in *KHDC3L* gene was detected (with rs606231235, p.Met1Val as translation start site or translation initiation codon mutation). This mutation was confirmed by Sanger sequencing and segregated in both her parents and her affected sister.

Discussion & Conclusion:RHM is a condition that can seriously affect a women reproductive health. Some of these women with *KHDC3L* gene pathogenic mutations can never get pregnant with a healthy fetus. There is not any report in the literature for the healthy pregnancy for women with *KHDC3L* gene pathogenic mutations.

Keywords:GTD, Hydatidiform mole, HYDM, Recurrent familial hydatidiform mole, RHM, *KHDC3L* gene, c.1A>G, Autosomal Recessive.

A novel ARV1 mutation in an Iranian family with developmental and epileptic encephalopathy-38

Sahar Bayat, Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Email:bayatsahar40@gmail.com

Background: Developmental and epileptic encephalopathy-38 (DEE38) is an autosomal recessive neurodegenerative disorder categorized by the onset of various types of seizures typically between about 4 and 7 months of age. Death may occur in early childhood because of aspiration or intractable epilepsy. Its prevalence is unknown. Among the several genes involved in epilepsy, *ARV1* (MIM 611647) is the most clinically associated gene with reported mutations identified in patients suffering from DEE38. It is located on chromosome 1q42.2. The coding-mRNAs encods a lipid transporter in the endoplasmic reticulum (ER), which is involved in modulating the fatty acid homeostasis process. The aims of the study were mutational screening of a consanguineous family from Iran, whose proband showed developmental and epileptic encephalopathy-38.

Methods: The Iranian family with DEE38 comprised of two affected members from a consanguineous marriage. Extracted genomic DNA from blood sample was used to perform whole exome sequencing in the affected member of the family with DEE38. Finally, Sanger sequencing was done for variant validation and segregation analysis,.

Results: The proband (IV-2), was born to healthy Iranian consanguineous parents originating from Kurdish ethnicity and presented with clinical indications of seizures, microcephaly and abnormal MRI findings. A novel homozygous two base pair deletion in exon 4 of the *ARV1* gene (chr1:231131650_231131651delTT; c.593_594delTT) was identified that results in a frameshift mutation and protein premature truncation (p.Ile198MetfsTer4; ENST00000310256). The detected variant has not been described in the 1000 genomes, ExAC and Iranome databases. Due to the in silico prediction tools and InterVar classifying system, the variant was found to be damaging. The parents were heterozygote carriers for the deletion and did not present any sign of the disease. Based on the above evidence, ARV1 variant is classified as likely pathogenic.

Discussion: Whole exome sequencing (WES) is extremely powerful and affordable. It is becoming more labor-and cost-efficient and is preferred to other means of screening, in particular in rare diseases with a recessive pattern. Using WES of the known *ARV1* gene in a DEE38 patient, we found a new nonsense variant (p.Ile198MetfsTer4). We recommend that *ARV1* should be included in panels for early onset neurocognitive impairment and epileptic encephalopathy.

Keywords: Whole Exome Sequencing, *ARV1* gene, Mutation, Developmental and epileptic encephalopathy-38.

Introduction of a Five-lncRNA Signature as a diagnostic biomarker in Gastric Cancer based on TCGA Data

Mahmoud Ghanei1,2, Arash Poursheikhani1,2, Azadeh Aarabi3, Negin Taghehchian4, Mohammad Reza Abbaszadegan1,2*

Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Department of Chemistry, Faculty of Science, Ferdowsi University of Mashhad, Mashhad, Iran

Corresponding author: abbaszadeganmr@mums.ac.ir

Arash7p@yahoo.com

Aarabia1@mums.ac.ir

GhaneiM4@mums.ac.ir

n_taghehchian@yahoo.com

BACKGROUND: Late diagnosis of gastric cancer in advance stages as well as its poor prognosis, necessitates finding of specific early diagnostic and prognostic biomarkers. **OBJECTIVE:** In this study, using the TCGA RNAseq data of gastric cancer patients, we evaluated the diagnostic value of lncRNAs that had differential expression**.** **METHODS:** P-value, FDR, log fold change for whole transcripts in theTCGA database were evaluated. Then, the RNAseq gene names were compared with total known lncRNA names in the biomart repository, for the identification of differentially expressed lncRNAs. Furthermore, the specificity and sensitivity of lncRNAs were also determined. Additionally, we predicted target genes using GO and KEGG signaling pathway analysis. **RESULTS:** Five lncRNAs, *PART1*, *UCA1*, *DIRC3*, *HOTAIR*, and *HOXA11AS* had proper sensitivity and specificity and had target genes involved in gastric cancer-related signaling pathways.

CONCLUSIONS: The results of our study demonstrated that the five-lncRNAs *PART1*, *UCA1*, *DIRC3*, *HOTAIR*, and *HOXA11AS* could be used as a potential panel of diagnostic biomarkers in gastric cancer.

Keywords: lncRNA, gastric cancer, TCGA, Signature, diagnostic biomarker

Evaluating the Frequency of FLT3, NPM1, and CEBPA Mutations in Patients with Acute Myeloid Leukemia in Northeastern Iran

Mohammad Parsa-kondelaji1, Hossein Ayatollahi2, Mohammad Hadi Sadeghian2*

1Department of Hematology and Blood Banking, Faculty of Medical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran

2Pathology Department of Hematology and Blood Banking, Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

*Corresponding Author: sadeghianmh@mums.ac.ir

mohammadparsa7410@gmail.com, AyatollahiH@mums.ac.ir

Background: Acute myeloid leukemia (AML) is one of the malignant blood diseases with high prevalence and death cases worldwide. Since AML is a highly heterogeneous genetic disorder caused by changes in the natural mechanisms of blood cell proliferation and differentiation, it can be diagnosed based on cytogenetic and molecular abnormalities. Many genes are frequently mutated in AML such as fms-like tyrosine kinase 3 (FLT3), Nucleophosmin 1 (NPM1), and CCAAT/enhancer binding protein-α (CEBPA) with a different prognosis. This study aimed to investigate the frequency of FLT3 and NPM1 mutations in AML patients in northeastern Iran.

Methods: This cross-sectional study was performed on 120 patients with AML from February 2017 to February 2020. These 120 patients included 100 requests for FLT3 mutation, 49 requests for NPM1 mutation, and 52 requests for CEBPA mutation. To detect mutations, peripheral blood samples and bone marrow aspiration were taken from all participants. Also, DNA extraction, polymerase chain reaction (PCR), and sequencing were performed.

Results: While M2 and M1 were the highest AML types detected, M6 and M7 had the lowest frequency. Also, FLT3, NPM1, and CEBPA mutations had a prevalence of 34%, 52%, and 12.2%, respectively. The prevalence of AML was not significantly correlated with sex and age and no relationship was seen between AML and blood indices (HB, RBC, WBC, and HB).

Conclusion: FLT3, NPM1, and CEBPA mutations are very common in AML. Interestingly, we observed the abundance of FLT3 mutations in M2 and M1subgroups, a high rate of NPM1 mutation in M2 subgroup, and a high rate of CEBPA mutations in M2 and M3 subgroups.

Keywords: FLT3, NPM1, CEBPA, Mutation, Acute myeloid leukemia

Genetics of vision impairment in eastern Iran (a 10-year report)

Masoome Alerasool1, Majid Mojarrad1*

Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Correspondent: Mojaradm@mums.ac.ir,

Alerasoulm991@mums.ac.ir

Background: Visual impairment is a major health problem worldwide with important socio-economic consequences. The number of people affected by the common causes of vision loss has increased because of population ageing. Globally, the main causes of blindness are cataract, glaucoma and age-related macular degeneration (AMD). Other eye diseases consist of inherited retinal disorders (IRD) such as retinitis pigmentosa (RP), and conditions associated with other diseases or syndromes. IRDs are a large group of clinically and genetically heterogeneous disorders, with variable age of onset and degree of severity. The estimated prevalence is about 1/ 2.000–1/3.000. Advances in genetic characterization allowed identification of over 270 causative mutations associated with inherited retinal disorders.

Material and methods: The current study is a single-center, retrospective study at Genetic Foundation of Khorasan Razavi (Mashhad, Iran) from 2013 to 2021. We analyzed 106 affected cases with syndromic and non-syndromic visual impairment. The next generation sequencing (NGS)-Targeted panel or whole exome sequencing (WES) was performed to detect genetic variations. Demographic characterization such as age of onset, ethnicity, familial history, and consanguinity were evaluated.

Results: According to the genetic test results, genetic etiology in 75.5% of patients has been detected. The majority cause of eye disorders consists of different form of LCA (12%) and RP (10%). Ten genes play role in cause of RP and the prominent one was TULP1. AIPL1, GUCY2D and RDH12 were detected more prevalently in LCA cases. The only involved gene in Stargardt and glaucoma was ABCA4 and CYP1B1 respectively. CRYGC, CRYGD and HSF4 genes were identified in non-syndromic cataract and CBS and RAB3GAP2 in syndromic ones. The Usher, Bardet-Biedl and Alport syndromes consist approximately 15% of syndromic eye disorders.

Interestingly, we observed rare syndromic cases of Marfan Syndrome, Jalili syndrome, Peroxisome biogenesis disorder, Cornelia de-Lange syndrome, Albinism, and neurofibromatosis who referred as visual impairment.

Eighty-one percent of index cases were result of consanguinity marriage. Forty-five percent from non-consanguineous families harboring pathogenic mutations in homozygous state which could be due to founder effect and 25% of them had heterozygous mutation with autosomal dominant mode of inheritance, and 10% with hemizygous x-linked recessive mutation.

Conclusions: Our study supports clinical and genetic heterogenicity of eye disorders in eastern of Iran. Knowing the genetic causes and founder effect of eye disorders improves understanding of prognosis for individual patients and facilitates the identification of relatives at risk. An appropriate genetic diagnosis also allows reproductive choices with either a preimplantation or prenatal genetic diagnosis.

Key words: Eye disorder, Whole exome sequencing, Founder effect, Genetics

SPG4 and SPG11 are the most common types of hereditary spastic paraplegia (HSP) in Iran

Afagh Alavi (Ph.D)[1]*, Aida Ghasemi (MSc.)[1], Mohammad Rohani (MD)[2], Fardad DanaeeFard (MSc.)[1], Atefeh Davarzani (MSc.)[1], Reza Hajati (MSc.)[1], Shahriar Nafissi (MD)[1], Farzad Fatehi (MD)[1]

[1] Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran
[2]Department of Neurology, Iran University of Medical Sciences, Hazrat Rasool Hospital, Tehran, Iran
[3]Department of Neurology, Neuromuscular Research Center, Shariati Hospital, Tehran University of Medical Sciences, Tehran, Iran

Correspondent: afaghalavi@gmail.com; af.alavi@uswr.ac.ir

Background: Hereditary spastic paraplegia (HSP) is a clinically and genetically heterogeneous group of neurodegenerative diseases, characterized by progressive lower-limb spasticity, and weakness. To date, >84 genetic types of HSP with different modes of inheritance have been defined. Among them, SPG4 and SPG11, caused by *SPAST* and *SPG11* mutations, are the most frequent types of autosomal dominant-HSP (AD-HSPs) and autosomal recessive-HSP (AR-HSPs), respectively. As, SPG4 accounts for ~40% of all patients and ~60% of AD-HSPs and SPG11, is responsible for ~3%-5% of all patients, 19%-31% of AR-HSPs, and ~75% of all HSP-patients who have radiologic signs of thin corpus-callosum (TCC). Despite using whole-exome sequencing (WES) method, ~50% of the HSP causative-genes, especially AR-HSPs genes remained unknown. Herein, we present the result of genetic analysis and prevalence of different HSP subtypes among a relatively large cohort of Iranian HSP affected individuals.

Methods: WES was performed on DNA of 74 unrelated-Iranian HSP probands. Variants that did not affect amino-acid chain or splicing were filtered out. Thereafter, SNPs with a minimal allele frequency >0.01 for AR-HSPs and >0.001 for AD-HSPs in the public databases were removed. The remaining variants were evaluated based on the American College of Medical Genetics (ACMG) criteria. Candidate variants were confirmed in the probands, subsequently screened in the family members to co-segregation analysis. NGS-based copy number variation (CNV) analysis is also currently in progress in the patients who have not reached a genetic diagnosis.

Results: The pathogenic/likely pathogenic variants were identified in 42/74 families. These variants had been located in 22 disease-causing genes, including *SPG11* (10 families), *SPAST* (6 families), *ATL1*, *SPG7*, *COQ7*, *CAPN1*, *KIF5A*, and *GJC2* (each in two families), *ERLIN1*, *ERLIN2*, *ENTPD1*, *CYP7B1*, *ZFYVE26*, *GCH1*, *CYP2U1*, *MFN2*, *KIF1B*, *ALS2*, *TUBB4A*, *C19orf12, PRDM8 and ATAD3A* genes (each in one family).

Conclusion: As shown in other studies, in our project SPG4 and SPG11 were the most common types of AD-HSP and AR-HSP, respectively. Nonetheless, it seems there is a significant difference between SPG4 and SPG11 prevalence in the Iranian (~8% and ~13%, respectively) than Western populations (40% and 3-5%%, respectively). This difference was also observed in the other common HSP-causative genes such as *ZFYVE26* and *REEP1*. Variants in some ultra-rare HSP-causative genes, *CAPN1*, *COQ7*, *PRDM8*, *ATAD3A* and *ENTPD1*, were also identified in our cohort, so that some of those were identified as the second case in the world.

Altogether, the disease-causing variants were found in ~56% of probands that it specifies the power of WES in the diagnosis of genetic causes of heterogeneous diseases like HSP. Also, a strong genotype-phenotype correlation was observed in some HSP types; TCC and genetic anticipation (the earlier age at onset in the successive generations) were observed in the magnetic resonance imaging (MRI) of all SPG11 and SPG4 cases, respectively.

Key words: Hereditary spastic paraplegia (HSP); SPG4; SPG11; Whole-exome sequencing (WES)

A prenatal diagnosis of frame shift mutation in *CEP135* gene associated with primary microcephaly in an Iranian family

Zahra Nasrpour Navaei[1], Susan Hosseini[2], Mohammadreza Abbaszadegan[3]

1 MSc student of human genetics, Department of medical genetics and molecular medicine, Faculty of medicine, Mashhad university of medical sciences, Mashhad, Iran

2 MSc of molecular genetics, Pardis Pathobiology and Genetics Laboratory, Mashhad, Iran

3 Professor of medical genetics, Medical Genetics Research Center & Department of medical genetics and molecular medicine, Mashhad University of Medical Sciences, Mashhad, Iran

Correspondent: abbaszadeganmr@mums.ac.ir

Nasrpournz981@mums.ac.ir

Su.hosseini.2@gmail.com

Background: Autosomal recessive primary microcephaly is a rare neurodevelopmental disease that is characterized by decreased size of cerebral cortex, receding architecture and mild to moderate intellectual disability. The frequency of MCPH in consanguineous population is 1 in 10000. To date there are 25 genes associated with MCPH. Most important of them are *MCPH1*, *WDR62*, *CASC5*, *ASPM* and *CEP135*. The related genes have roles in cell cycle regulation, cell division, and microtubule organization at centrosome. Mutations affect their functions and cause abnormal cell division and cortical development.

Materials and methods: The present case is a 29-year-old gravid female with history of an affected child with microcephaly. She and her husband are related. DNA was extracted from whole blood. Human whole exome enrichment was performed with Agilent kit and then sequenced using Illumina HiSeq4000 platform. All exons and flanking 10bp were sequenced and analyzed. Detected variations include: single point mutations, small Indels (within 20bp).

Results: Heterozygous c.1392dupC variant on CEP135 gene has been detected. This novel variant has been reported for its pathogenicity and most likely to be disease-causing. *CEP135* gene is associated with primary autosomal recessive microcephaly. [OMIM: 614673]. Sanger sequencing of mentioned variant has been performed for her husband and affected child. The mentioned variant was validated in heterozygous and homozygous forms in father and their son respectively. Prenatal genetic testing was performed in fetus and heterozygous c.1392dupC was found.

Conclusion: WES is an important diagnostic tool and it would enable appropriate genetic counseling and decision making for reproductive choices. In this case, prenatal diagnosis of primary microcephaly and also appropriate genetic counseling for the couple had become possible.

keyword: MCPH, WES, *CEP135*, microcephaly

A homozygous nonsense variant in *RAB33B* is responsible for rare familiar cases of Smith McCort dysplasia 2 in Khorasan Razavi

Zahra Chekini[1], Alireza Pasdar [1,2*]

1 Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

2 Division of Applied Medicine, Medical School, University of Aberdeen, Aberdeen, UK.

Correspondent: PasdarA@mums.ac.ir

z.chekini@gmail.com

Background: Smith–McCort dysplasia 2 (SMC2; MIM#615222) was first defined in 1958. It is a rare autosomal recessive inherited spondylo-epiphyseal-metaphyseal dysplasia caused by biallelic *RAB33B* variants. *RAB33B* (NM_031296.2) is located on chromosome 4q31.1 with two exons. RAB33B, a member RAS oncogene family, belongs to small GTPase superfamily which have a critical role in Golgi transport (from Golgi to ER) of proteins such as exocytosis and endocytosis. It is also involved in the formation of autophagosomes. To date, only 11 variants have been reported in 19 patients with SMC2 from nine families with different ethnic backgrounds. The current case report study describes the findings of a large consanguineous family with six affected patients with SMC2.

Methods: A family, who had several cases of skeletal abnormalities, after getting consent, was chosen for this case report. Genomic DNA was extracted from whole blood samples. Whole exome sequencing (WES) was performed to look for causative variants. Sanger sequencing method was performed for segregation analysis and carrier testing.

Result: A 40 year old man who manifested progressive difficulty in walking and skeletal dysplasia since the age of 5 years, he presented with a short trunk dwarfism, short stature, and genu varum. He had normal intellectual development and intelligence quotient (IQ) as well as school performance was excellent. WES for patient identified a biallelic variant c.211 C>T (p.R71*) in exon one of the *RAB33B* gene. This pathogenic nonsense mutation resulting in premature termination of translation at residues 71 subsequently loss of function. Sequencing of the *RAB33B* gene for the patient's brother, who was referred for pre-marriage counseling revealed heterozygous

of c.211 C>T. His 30 years old sister had manifested similar features. In this family, two related first cousins from consanguineous marriage and one-second cousin with 15, 20, and 10 years old had variable degrees of SMC2 syndrome. In addition, his second cousin from consanguineous marriage had a short stature with kyphosis.

Conclusion: This report warrants considering this rare syndrome as differential diagnosis when approaching patients with musculoskeletal dysplasia. Although it is a rare syndrome, however, founder effect of *RAB33B* gene in this geographic region is likely.

Key words: Smith-McCort dysplasia; Skeletal dysplasia; *RAB33B*; Pathogenic variation

Clinical evaluation of immunogenic adjuvant therapy with dendritic cells loaded with recombinant chimeric antigens in patients with gastric cancer

Dr. Mohammad Reza Abbaszadegan

Medical Genetics Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;

Department of Medical Genetics and Molecular Medicine, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran;

Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Email: abbaszadeganmr@mums.ac.ir

Abstract: Gastric cancer (GC) is the fourth most common cancer in the world with 19.3 million new cases and second leading cause of cancer death with 10 million cancer deaths in 2020. Therapeutic approaches for GC include surgery, radiotherapy and chemotherapy, which are usually unsuccessful and lead to low survival rate, high recurrence rate and short term response to chemotherapy. Today, immunotherapy, as targeted therapy, is proposed as a novel efficient therapeutic for cancer treatment with good prognosis and least damage to the patient and minimized adverse effects on normal cells. In several studies, antigen-based DCs immunotherapy has been reported to induce effective immunity against cancer. In general, DC-based vaccination is well tolerated with few side effects such as flu-like symptoms and fatigue. In recent years, our research team focused on the ability of DCs loaded with tumor RNA or specific Antigens in stimulation of the immune system against the cancer cells. For example, we evaluated ex vivo immune response of ESCC patients against our newly designed chimeric construct consisting of highly immunogenic cancer-testis antigens. After confirming effective expression of the in vitro transcribed chimeric mRNA in ex vivo electroporated dendritic cells (DCs) of the ESCC patients, the patients' CTLs were primed by DCs and cytotoxicity assay was performed to evaluate how the primed CTLs can recognize and target the chimeric mRNA-loaded cells. The chimeric protein was strongly expressed relative to the housekeeping gene expression in electroporated cells (fiqure1). The cytotoxicity of the CTLs was significantly higher in DCs loaded with chimeric mRNAs compared to mock DCs ($p>0.05$) in all of the tested ESCC patients. We are introducing a novel construct that our functional study showed stimulation and induction of an effective immune response in ESCC patients. The designed chimeric mRNA-loaded DCs are capable of priming CTLs effectively and induce cytotoxicity against tumor. Moreover, we observed cytokine-matured DCs loaded with mRNA of sphere-forming cells from GC patients were able to induce *IFN-γ* gene expression in T-lymphocytes. DCs loaded with mRNA of sphere-forming cells that elicit effectively specific T cell-mediated immune responses *in vitro*, may be considered as a promising therapeutic vaccination in GC patients in future. Therefore, the aim of this study was clinical evaluation of DCs immunotherapy loaded with recombinant chimeric antigens in patients with gastric cancer and introduction of a safe and efficient immunotherapy approach for GC treatment.

Keywords: gastric cancer, immunotherapy, Dendritic cells, chimeric antigens.

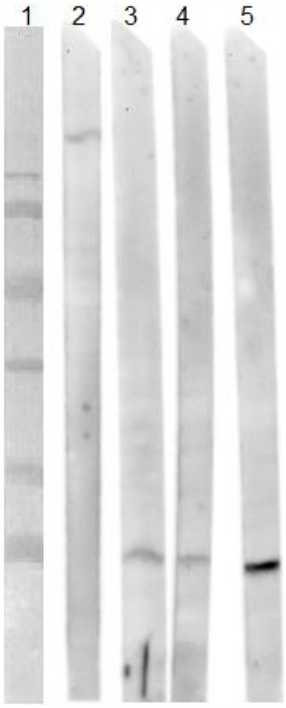

Figure1: western blot results for confirmation of recombinant protein (1: protein ladder, 2: electroporated DCs cells with medium, 3: electroporated K562 cells with chimeric mRNA, 4: electroporated KYSE cells with chimeric mRNA, 5: electroporated DCs cells with chimeric mRNA.

Biomarker Discovery Based on Aptamers

Aida Gholoobi, [1] Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran; [2] Metabolic Syndrome Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Email: gholoubiad@mums.ac.ir

Exploration and determination of human diseases biomarkers lead to early detection, accurate therapy, and prognosis as well as response to therapy. Efficient and sensitive methods revealing clinical biomarkers are limited due to various technical problems facing the current technologies. Aptamers are single-stranded oligonucleotides, which can selectively and specifically bind to a wide range of targets with high affinity. Besides, aptamers compared to their counterparts, antibodies, have advantages including ease of synthesis, flexible chemical modification, stability in various conditions, and low immunogenicity. Recently, aptamer-based strategies have revolutionized biomarker discovery, such as Cell-SELEX and SOMAScan technology. Through Cell-SELEX, a range of nucleic acid aptamers can be identified to recognize cell surface biomarkers of various cells. Using SOMAScan technology thousands of proteins of diverse biological specimens can be analyzed to become a multiplexed proteomics platform for biomarker discovery. A brief review will be presented to introduce technologies based on aptamers in the field of biomarker discovery.

Key words: Disease biomarker, Aptamer, SOMAScan, Cell-SELEX

Application of stem cells in the repair of cardiovascular disease: A review of the author's two academic books

Ehsan Saburi

Assistant Professor of Molecular Medicine, Mashhad University of Medical Sciences

Correspondent: sabourie@mums.ac.ir

Cardiovascular diseases are known as the most common causes of death in different countries, for which there are different treatment strategies with different effectiveness. Given that the success of each of these methods is not accurate, novel approaches have led to combination therapies with new technologies. Stem cells are undifferentiated cells that, in addition to being able to multiply symmetrically and asymmetrically, can differentiate into different types of cells. This high potential has provided new fields in the repair of tissue lesions caused by various diseases. Despite the many moral restrictions on the use of embryonic stem cells, the use of stem cells isolated from other sources, such as bone marrow, adipose tissue, teeth and placenta, etc. is becoming more widespread. Inverse differentiation has also been widely used to produce induced stem cells from somatic cells using the four Yamanaka factors (KLF4, Sox2, Oct4, c-myc) as well as the recent use of chemical products, miRNA, and so on.

The use of stem cells in the treatment of cardiovascular disease is focused considering the following four areas; IPSC production and differentiation into myocardial and neuronal cells by various methods, cell delivery processes to cardiac tissue, and application of myocardial cell secretion in tissue repair, vascular repair and regeneration. Today, with the passage of the first generation (based on differentiated cell injection) and the second generation (based on stem cell isolation, differentiation and transplantation) cell therapy, the third generation of cell therapy based on transdifferentiating / partial induced PSC methods in the substrate of synthetic polymer scaffolds or natural scaffolds free of cells (cell free scaffold) and differentiation in the presence of excipients (secretome, miRNA, etc) is proposed. However, today it has been proven that the high potential of stem cells located in the cardiac compartment allows direct regeneration, albeit slowly, which has highlighted the need to use auxiliary factors to accelerate this process.

key words; Stem cells, cardiovascular disease, cell scaffold, iPSC, Secretome

The Effects of Adipose Derived Mesenchymal Stem Cells (AT-MSCs) on Secondary Progressive Multiple Sclerosis

Fahimeh Lavi Arab[a], Forouzan Yousefi[a], Hojjat Naderi-Meshkin[b, c], Mahdi Mirahmadi[b, d], Karim Nikkhah[e], Houshang Amiri[f, g], Marjan Erfani[e], Fatemeh Ayoobi[h], Nafiseh Sadat Tabasi[a], Ali Nikkhah [i] and Mahmoud Mahmoudi[j*]

a Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

b Stem Cell and Regenerative Medicine Research Group, Academic Center for Education, Culture, and Research (ACECR), Khorasan Razavi Branch, Mashhad, Iran

c Wellcome-Wolfson Institute for Experimental Medicine, Belfast, U.K.

d Biotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

e Department of Neurology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

f Neurology Research Center, Kerman University of Medical Sciences, Kerman, Iran

g Department of Radiology and Nuclear Medicine, VU University Medical Center, Amsterdam, The Netherlands

h Occupational Safety and Health Research Center, NICICO, World Safety Organization and Rafsanjan University of Medical Sciences, Rafsanjan, Iran

i National Forensic Mental Health Services, Dublin, Ireland

j Department of Immunology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Correspondent:

Fahime.lavi@gmail.com

Background: Multiple sclerosis (MS) is a chronic demyelinating disease of central nerves system (CNS) with gross pathological symptoms in affected people and pronounced socioeconomic expenditure.

Currently available treatments for secondary progressive multiple sclerosis (SPMS) have limited efficacy and/or safety concerns. Mesenchymal stem cells (MSCs) due to their immune-modulatory, neurogenerative and self-renewal traits have achieved fascinating prospects in regenerative medicine. Adipose-mesenchymal derived stem cells (AdMSCs) represent a promising option and can be readily obtained using minimally invasive procedures. To date, MSCs utilized for curing of MS have been almost derived from bone marrow and infused cryopreserved at low doses (1-2×10^6 cell/Kg).

Methods: Here, we explored safety and functionality of fresh and high dose (4.66×10^6 cell/Kg) autologous AT-MSCs in 10 female patients with secondary progressive MS (SPMS, EDS=4-6). To this end, we assessed adverse events after a one-year follow-up in patients. Furthermore, we evaluated expanded disability status scale (EDSS). Also, immunomodulatory effect of MSCs was investigated by evaluating gene expression of inflammatory and anti-inflammatory cytokines besides proportion of peripheral blood T regulatory cells as important modifier cells in hemostasis of self-tolerance in autoimmune diseases like MS.

Reaults: In our study, high doses of non-cryopreserved AT-MSCs were successfully administrated into 10 SPMS patients without any serious side effects after one year Furthermore, we noted efficacy of AT-MSCs in terms of reduction of EDSS. Moreover, immunomodulatory effects of AT-MSCs was confirmed by enhancing of Treg population and anti-inflammatory cytokines as well lowering inflammatory cytokines in patients.

Conclusion: non-cryopreserved AT-MSCs because of their immunomodulatory properties can be a safe and feasible for inhibiting of multiple sclerosis progression or even disease improvement.

Keywords: Non-cryopreserved adipose tissue mesenchymal stem cells, Secondary progressive multiple sclerosis

Duchenne Muscular dystrophy gene therapy in Iran: present and prospective

Atieh Eslahi [1], Yasamin Yousefi [2], Farzaneh Alizadeh [1], Shima Farrokhi [1], Majid Mojarrad [1*]
1- Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2- Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
Email Addresses:
*Corresponding author
Alizadehf951@mums.ac.ir
Yasaminsmartgirl@gmail.com
Eslahia951@mums.ac.ir
Farrokhish961@mums.ac.ir
majidmojarrad@gmail.com

Duchenne muscular dystrophy (DMD) is the most common lethal muscular dystrophy in the world, which causes morbidity and death of patients before the end of the second decade of life. Efforts to genetically treatment the disease have been going on for more than three decades.

In recent decades, the emerging of CRISPR technology has led to a dramatic increase in the success of DMD gene therapy studies.

In this presentation, we will introduce the studies conducted in this field in the last decade at Mashhad University of Medical Sciences and explain the future perspective of this field.

We have succeeded in producing different cellular models with different mutations of dystrophin exon deletions, and we are currently working on a knockout mouse model with dystrophin exon 51 deletion. These cellular and animal models can pave the way for therapeutic studies using various genetic modification tools.

Progressive protein aggregation in retinitis pigmentosa type 11 patient iPSC-derived retinal pigment epithelium and its reversal through activation of autophagy.

Maria Georgiou, Chunbo Yang, Robert Atkinson, Kuan-Ting Pan , Adriana Buskin, Sebastian E. J. Ludwig, Sushma Nagaraja-Grellscheid, Colin Johnson, Robin Ali, Henning Urlaub,

Majlinda Lako, Sina Mozaffari-Jovin

Email: smozaffmp@gmail.com

***Background*:** Mutations in pre-mRNA processing factor 31 (PRPF31), a core protein of the spliceosomal tri-snRNP complex, cause autosomal-dominant retinitis pigmentosa type 11(RP11). It has remained an enigma why mutations in ubiquitously expressed tri-snRNP proteins result in retina-specific disorders, and so far, the underlying mechanism of splicing factors-related RP is poorly understood. Here, we used iPSC technology to generate retinal organoids and RPE models from three patients with severe and very severe RP11, normal individuals and a CRISPR/Cas9-corrected isogenic control.

Method: To fully assess the impacts of *PRPF31* mutations, quantitative proteomics analyses of retinal organoids and RPE cells was carried out showing RNA splicing, autophagy and lysosome, unfolded protein response (UPR) and visual cycle-related pathways to be significantly affected.

Result*:* Strikingly, the patient-derived RPE and retinal cells were characterized by the presence of large amounts of cytoplasmic aggregates containing the mutant PRPF31 and misfolded, ubiquitin-conjugated proteins including key visual cycle proteins, which accumulated progressively with time. Mutant PRPF31 variant was not incorporated into splicing complexes, but reduction of PRPF31 wildtype levels led to tri-snRNP assembly defects in Cajal bodies of RP11 patient retinal cells with reduced U4/U6 snRNPs and accumulation of U5, smaller nuclear speckles and reduced formation of active spliceosomes giving rise to global splicing dysregulation. Moreover, the impaired waste disposal mechanisms further exacerbated aggregate formation, and targeting these by activating the autophagy pathway using Rapamycin resulted in reduction of cytoplasmic aggregates and improved cell survival.

Conclusion: Our data demonstrate that it is the progressive aggregate accumulation that overburdens the waste disposal machinery rather than direct PRPF31-initiated mis-splicing, and thus relieving the RPE cells from insoluble cytoplasmic aggregates presents a novel therapeutic strategy that can be combined with gene therapy studies to fully restore RPE and retinal cell function in RP11 patients.

Keywords: Pre-mRNA splicing, Retinitis pigmentosa, PRPF31

Correction the point mutations in DMD patients with PRIME editing technique

MohammadReza Mirinezhad

University of Laval, Quebec, Canada

reza.mirinezhad@crchudequebec.ulaval.ca

Email: reza.mirinezhad@crchudequebec.ulaval.ca

Introduction:Duchenne muscular dystrophy (DMD) is one of the most common and severe muscular dystrophies, affecting about 1 in 3,500 boys that kills the patient at the early decades of life. Around 15-30% of patients have point mutation in their genes.

In this, I am trying to implement a new gene therapy plan based on CRISPR technique named PRIME editing by working on 45 DMD patients with different point mutations. The phase. I of this project is being run with 5 patients (2 Canadian and 3 Iranian patients).

Methods: Briefly PRIME editing contains two functional components:

1. Cas protein or other modified Cas systems, which are derived from CRISPR technique and cuts the target DNA.

2. pegRNA, which contains the identifying sequence (spacer) for patient's DNA and determines the site of cut, as well as an oligonucleotide scaffold that binds to the Cas-cleavage protein. At the end of region 3' of this RNA is another sequence that includes PBS sequences for primer binding site and RT template (Reverse transcriptase domain) that contains of normal base of the sequence. After cutting and removing the sequence containing the mutant base, an identical sequence with the normal base will be replaced in the patient's DNA, which restores the function of the gene and leads to the production of a normal dystrophin protein.

pegRNAs, are designed with pegFinder, Benchling and the DMD genome reference site (www.dmd.nl) was used. This pegRNAs were constructed using Golden Gate technique in the plasmids like plasmid pCMV-PE2 for the reverse transcriptase fused with Cas9 nickase and pU6-pegRNA-GG-acceptor and new plasmid, pCMV-PEmax-P2A-hMLH1dn for carrying pegRNA cargo.

The function of these plasmids will be evaluated in HEK293 cells and subsequently in myeloblast cell of the patients that we have collected. The length of each component in pegRNA affects on sensitivity and specificity of the system. In this project, I have designed and tested 12 different lengths of pegRNA components (RTT and PBS) for each mutation, the most efficient one will eventually be selected

The results will be evaluated by extracting DNA from the cell, PCR amplifying and deep sequencing with Illumina technology.

The next step in the project will be carried out in vivo on mdx mice model. In this step plasmids containing PRIME editors are injected into the anterior tibial muscle by electroporation method.

Immunohistochemistry will be used with a cryostat and fixation technique after sacrificing the mice to determine the expression of Dystrophin protein.

To continue the dual modified adeno-associated virus 9 (AAV9) containing PRIME editors are injected into the mice intravenously.

Results:At the end the results will be comprehensively evaluated to know if it is satisfactory enough to be used on Human clinical trials.

Key words: Gene therapy, Gene editing, CRISPR, PRIME editing, DMD

Inactivation of HPV18-E6 by CRISPR /Cas9 system mediated by Adeno associated virus in human cervical cancer cells

Zahra noroozi[1], Mehdi Shamsara[2], Elahe Motevaseli[1*], Mohammad Hossein Modarressi[3*]

[1]Department of Molecular Medicine, School of Advanced Technologies in Medicine, Tehran University of Medical Sciences, Tehran, Iran.
[2]Department of Animal Biotechnology, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
[3]Department of Medical Genetics, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran.
Correspondent: modaresi@tums.ac.ir
Co-corresponding author: e_motevaseli@tums.ac.ir

Background and Objectives: Cervical cancer is the most prevalent cancers in females. Human papillomavirus (HPV) infections are linked with most of cervical cancers. The E6 proto-oncogene of the virus interacts with the cellular suppressor proteins, like p53 and inhibits their action. The objective of this research was investigation of the effects of E6 gene disruption on cell proliferation and apoptosis of the cervical cancer-derived Hela cells**.**

Material and methods: Two sgRNAs were designed over the HPV18 E6 gene and cloned into an AAV-CRISPR-based vector. The AAV-E6-CRISPR/Cas9 virions were prepared in the Hek293t cells and tittered. Experimental subjects were divided into the control, mock, AAV-E6-sgRNA1/Cas9, AAV-E6-sgRNA2/Cas9 and AAV-E6-sgRNA1+2/Cas9 groups. The E6 cleavage after the virus transduction was detected by T7E1 digestion assay. Cell cycle, apoptosis induction and cell proliferation were assayed in the AAV-E6-CRISPR/Cas9 infected cells. The p53 protein level was also quantified in western blot.

Results: Our data showed that The E6 gene disruption resulted in apoptosis and proliferation inhibition and cell cycle arrest in the AAV-E6-CRISPR/Cas9 infected cells. The protein level of p53 was also upregulated in the E6-disrupted cells. However, these changes were not observed in the control and mock groups.

Discussion & Conclusion: These findings proved that AAV-mediated delivery of CRISPR/Cas9 is able to reverse the phenotype of HPV-infected cells and this approach might be applied locally against the HPV-related cervical cancer.

Keywords: Adeno Associated Virus; CRISPR/Cas9; E6; cervical cancer; Human Papillomavirus

Treatment of 5 severe CVD-19 cases admitted to the intensive care unit (ICU) with allogeneic mesenchymal stem cells

Hamid Reza Rahimi[1,] *, Meysam Gachpazan[2], Jalil Tavakkol-Afshari[3], Amir Adhami[4]

1.Medical Genetics Research Centre, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

2.Medical Genetics Research Centre, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

3.Immunology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

4.Medical Genetics Research Centre, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Correspondent: RahimiHR@mums.ac.ir, Gachpazanm961@mums.ac.ir, TavakolAJ@mums.ac.ir

Introduction: In the last two years, a coronavirus disease known as COVID-19 made a pandemic that has engaged the whole world since late November 2019. Several organs are at risk in this viral disorder, such as respiratory. Several clinical studies demonstrated the cytokine storm through inflammatory mediators' release when infected with the virus. Various researchers suggested cell-based therapy to struggle against COVID-19 because of the number of positive cases and the total death.

Method: In this research, 5 hospitalized patients in the ICU had been selected which therapeutic medicine had no effects on them. The allogeneic graft of human umbilical cord mesenchymal stem cells was grafted into these patients through the venous infusion. Furthermore, the interleukin-6 and TNF-a were measured as inflammatory cytokines were don in all steps.

Results: 5 patients were inserted in this study with the level of 80% of lung involvement in paraclinical detection infection. After the venous infusion, we find a decreasing rate of inflammatory cytokines (IL-6-TNF-a). While 4 of 5 patients were discharged from the ICU and were released from the hospital after ten days, one died.

Discussion: After just 10 days of venous infusion, 4 of 5 patients were discharged from the hospital. Thus, we showed the advantages of cell therapy in this study and demonstrated the immune system modulatory as an immune suppressor.

Cloning of designed cassette for HBB gene editing and Analysis of its efficiency using Green fluorescent Assay in HEK293 cell line

Maliheh Lotfi[1,2], Atefeh Ashouri[1,2], Sina Mozaffari Jovin[2], Majid Mojarrad[2], Maryam Hashemi[3], Mohammad Reza Abbaszadegan[1,2]

1. Medical Genetics Research Center, Mashhad University of Medical Sciences,

2. Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

3. Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Correspondent: AbbaszadeganMR@mums.ac.ir

Lotfim951@mums.ac.ir, ashouria961@mums.ac.ir, mozaffaris@mums.ac.ir, mojaradm@mums.ac.ir, HashemiM@mums.ac.ir

Background and Aim: Beta thalassemia is one of the most common monogenic inherited disorders worldwide. More than 50,000 affected children are born annually. Despite a great effort to prevent and treat these patients, lack of an effective and permanent cure has always been one of concerns in the field of treatment. Development of genome editing technology including CRISPR/Cas9 over the last decade allow manipulation of DNA sequence. CRISPR/Cas9 system is able to correct mutation in the presence of donor template by using a single guide RNA. Herein, we aim to assess the efficacy of CRISPR/Cas9 system as a proof of concept to target the *HBB* locus in HEK293 cell line.

Method: In order to target *HBB* locus several sgRNAs were designed on exon1 and Inron1 of *HBB* gene. In accordance with sgRNAs cut sites two donor template constructs called intronic and exonic donor template were designed and cloned, respectively. Then, the efficiency of on target mutagenesis of sgRNAs were evaluated by T7 endonuclease I assay. Following preparation of eSpCas9 (1.1) plasmid containing sgRNA and donor template plasmid, they were co-transfected into HEK293 cell line using Lipofectamin 3000 kit. HDR positive cells were isolated through neomycin resistance. In-out PCR was performed to confirm HDR in selected colonies. Finally, PCR product was evaluated for final verification of recombinant cells by Sanger sequencing.

Results: eSpCas9 (1.1) plasmid containing sgRNA, intronic and exonic donor template plasmids were confirmed by PCR, restriction enzyme digestion, and sequencing. Approximately, 40% transfection efficiency was demonstrated by co-transfection of eSpCas9 (1.1)-sgRNA5 plasmid and intronic donor template plasmid in HEK293 cell line. HDR efficiency was calculated to be about 37.5 percent by examination of the selected colonies using In-out PCR.

Conclusion: The outcome of this study demonstrates this strategy could successfully target *HBB* locus by homologous recombination in HEK293 cell line.

Keywords: beta thalassemia, homology directed repair, T7 endonuclease I, genome editing, HEK 293 cell line

GJB2 mutations in Iranian Azeri population with autosomal recessive nonsyndromic hearing loss (ARNSHL): First report of c.238 C>A mutation in Iran

Ehsan Abbaspour Rodbaneh[1], Mohammad Panahi[2], Bahareh Rahimi[3], Haleh Mokabber[1], Reza Farajollahi[1,4], Behzad Davarnia[1]

1 Medical Genetics and Pathology, Ardabil University of Medical Sciences, Ardabil, Iran

2 Department of Medical Biotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

3 Department of Medical Biotechnology, Faculty of Allied Medical Sciences, Iran University of Medical Sciences, Tehran, Iran

4 Ardabil Welfare Organization, Ardabil, Iran

Correspondence Behzad Davarnia, Medical Genetics and Pathology, Ardabil University of Medical Sciences, Ardabil, Iran. Email: b.davarnia@gmail.com

Objective: Autosomal-recessive nonsyndromic hearing loss (ARNSHL) is a heterogeneous genetic disorder. Mutations in the gap junction protein beta 2 (GJB2) gene, encoding connexin 26, are a significant cause of ARNSHL in different ethnic groups. This study aimed to identify the frequency and type of GJB2 mutations in the Iranian Azeri population.

Methods: Fifty unrelated families presenting ARNSHL in Ardabil Province, the northwest of Iran, were studied to determine the frequency and type of GJB2 mutations leading to ARNSHL. ARMS-PCR screened all DNA samples to detect c.35delG; p. Gly12Val mutation. In addition, normal samples for c.35delG; p. Gly12Val were analyzed by direct sequencing for other GJB2 mutations.

Result: Of the fifty families, 13 (26%) showed a GJB2 gene mutation, with c.35delG; p. Gly12Val mutation was the most prevalent one that occurred in eight (61.5%) out of the 13 families. Of the families, two were homozygous for c.358-360delGAC; p. Glu120del mutation, and one was homozygous for c.290dupA; p. Tyr97Ter and c.299–300delAT; p. His100Arg mutations. Also, we detected a novel mutation, c.238C>A; p. Gln80lys, in one of the families.

Conclusion: Our findings are comparable to previous studies, indicating c.35d3lG; p. Gly12Val mutation in the GJB2 gene is the most common cause of GJB2-related hearing loss in the Iranian Azeri population. Furthermore, our study highlights the significance of ARNSHL screening programs of live births based on local population data in Iran.

چکیده

پوسترها

Posters

Abstracts

Evolutionary Era of Genome Editing by CRISPR for Immunodeficiencies; A Dream or principles?

Pourandokht Farhangian [a], Nazila Bahmaie [b*]

[a] MSc of Medical Genetic, Department of Medical Genetics and Molecular Medicine, Faculty of Medicine, Zanjan University of Medical Sciences, Zanjan, Iran.
[b*] PhD of Allergy and Immunology, Department of Allergy and Immunology, Faculty of Medicine, Graduate School of Health Science, Near East University (NEU), Nicosia, Northern Cyprus, Cyprus.

Email: shirin.farhangian60@gmail.com

nazila69bahmaieimmunology@gmail.com

Background and Aim: According to the unsuccessfulness in the results of previous approaches recruited for cancer therapy, there is a long-cherished dream of targeted genome modification through programmable nucleases. In this study, authors aimed to evaluate those nucleases for targeted editing of the human genome) in order to introduce the most recently-known therapeutic approaches for patients with primary immunodeficiencies (PIDs).

Results: Despite various classes of designer nucleases for targeted editing of the human genome (including zinc finger nucleases (ZFNs), transcription activator-like effector (TALE) nucleases (TALENs), development of CRISPR/Cas9 has led to an unprecedented development of gene editing procedures, cleaving DNA at predetermined sites in the human genome, and activating the DNA repair through non-homologous end-joining (NHEJ) or homology-directed repair (HDR) machinery of the cell with high nuclease activity and specificity in various preclinical disease models. In case of PIDs, allogeneic hematopoietic stem cell transplantation (HSCT) are not accessible for all of patients due to the lack of HLA-matched donor, and a risk for suffering from graft-versus-host (GVHD) disease. Alternatively, development of gene-based therapies on the transplantation of genetically-corrected autologous HSCs using the modified viral vector in the X-linked severe combined immunodeficiency (X-SCID) patients was not too successful due to un-regulated transgene expression from viral vector. Then, it is the exact time for the evolutionary era of genome editing by engineered nucleases. For Chronic Granulomatous Disease (CGD) which is caused by mutations in $gp91^{phox}$, $p22^{phox}$, $p40^{phox}$, $p47^{phox}$, $p67^{phox}$ of the nicotinamide adenine dinucleotide phosphate oxidase (NADPH) enzyme complex, optimized ZFN and CRISPR/Cas9 systems co-delivered with either an AAV6 vector or single-strand oligo DNA (ssODN) as donor templates was successful. For Wiskott-Aldrich Syndrome (WAS) which is caused by mutation in the WAS gene encoding the WAS protein (WASp), CRISPR/Cas9 significantly restored the functional and phenotypic defects in macrophages, platelets, T, and B cells derived from corrected HSPCs, revealed the preserved in vivo differentiation potentials, with evidence of corrected WASp expression being preserved in long-term repopulating cells and lack of any noticeable toxicity. For X-linked hyper-IgM syndrome (XHIM) which is caused by mutations in the CD40 Ligand (CD40L), primary T cells and HSPCs suggest that a small number

of edited patient-derived XHIM-HSPCs suffice to be differentiated into gene-corrected T cells for restoring IgG class switching and ameliorate the disease phenotype.

Conclusion: Gene editing will represent a further step to provide a correction in the defective genes at their genomic locus, maintaining appropriate regulatory control of gene expression and reducing the risk of genotoxicity through ectopic vector insertion. Among them, CRISPR nucleases are all being developed to create highly specific gene targeting, necessitating an esteemed scientific collaboration between geneticist and immunologists.

Application of karyotyping and FISH methods for the Study of chromosomal rearrangements in leukemia patients.

Zoya Najafi[1, 2, 3], Valiollah Mehrzad[4], Alireza Sadeghi[4], Zhaleh Asadi Fakhr[5], Farinaz tabibi[1, 2], Mansoor Salehi[1, 2, 5, *]

1.Cellular, Molecular and Genetics Research Center, Isfahan, University of Medical Sciences. Isfahan, Iran

2.Medical Genetics Research Center of Genome, Isfahan University of Medical Science, Isfahan, Iran

3.Department of Biology, Science and Research branch, Islamic Azad University, Tehran, Iran.

4.Department of Hematology and Oncology, Medical School, Omid Hospital, Isfahan University of Medical Sciences, Isfahan, Iran.

5.Department of Genetics and Molecular Biology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran.

Email: correspondent: m_salehi@med.mui.ac.ir

zoyanajafi@srbiau.ac.ir, v_mehrzad@med.mui.ac.ir, sadeghi_hem@yahoo.com, jale.asadi@gmail.com, farinaztabibi65@gmail.com

Background: The early detection and assessment of leukemia disease with thorough differentiation of malignant leukocytes in the primary stages, is a significant problem in disease diagnosis. Cytogenetics chromosomal abnormalities have been reported in cancer cells such as acute leukemia. To investigate chromosomal abnormalities, karyotyping and fluorescence in Situ hybridization (FISH) methods were performed on referred bone marrow (BM) samples in Isfahan medical Genetic center of Genome.

Methods: Conforming to standard protocol, bone marrow was cultured with special culture media (Marromax), prepared slides were G-banded GTGbanded and analyzed. Fluorescence in situ hybridization (FISH) testing were performed by using a panel of Leukemia-associated probes from Metasystem company, to detect t(8;21), t(9;22), 20qter, 20q12, 5p15, 5q31 (EGR1 gene and CDC25C gene), 5q33 (RPS14) and chromosome 7 and 8 centromeres.

Results: Karyotype analysis on 163 patients' diagnosed leukemia were performed. From these numbers 36 cases had chromosomal abnormalities. Among this abnormalities 12 cases had Translocations; t(8;21, t(9;22), t(2;10), t(15;17)), 1 case deletion (Del 7q), 15 cases Complex karyotype, 6 cases Aneuploidy and other cases such as MAR?????. In 13 cases culture failed**.** FISH analysis performed on 48 samples and 14 of these cases had chromosomal abnormalities**.**

Conclusion: Use of karyotype and FISH methods are tools for the detection of chromosomal abnormalities for early detection of cancer. However not all would help with the diagnosis of leukemia as chromosomal abnormality are absent in some. But molecular tests such as NPM1, FLT3, CEPA genes and related panels such as CML, AML, CLL, can help with the diagnosis.

Although, bone marrow karyotype is beneficial for diagnosis, using the FISH method, could be useful for clinical monitoring. On the whole, It helps with primary diagnosis and appropriate therapy process and eventually selection of suitable treatment for the patients.

Keywords: Bone marrow, Chromosome abnormality, Karyotype, Fluorescence in situ hybridization (FISH)

Evaluation of expression panel in urine for non-invasive screening of bladder cancer

Azam Rasti[1], Fatemeh Movahedi Motlagh[1], Samira Sabbagh Nejad Yazd[2], Mohammad Hossein Modarressi[1]*

1.Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

2.Research and Clinical Centre for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

*Correspondence Mohammad Hossein Modarressi, Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

Email: Rasti_azam@yahoo.com, movahedi_fatemeh85@yahoo.com, sabbagh4704@gmail.com, modaresi@tums.ac.ir

Background: Human bladder cancer (BCa) is one of the most common cancer types in humans. It is the 6th most common malignancy in men and the 10th most common tumor type worldwide. BCa is the cause of a high number of mortalities, and it is related to high healthcare costs. Gold standard for the detection of bladder cancer is based on cystoscopy. However, this is invasive, bears a certain health risk and relatively expensive. Alternatively, cytological evaluation is non-invasive and provides specific information, but it seems has low accuracy in low-grade BCa. Several non-invasive molecular tumor tests have been developed to improve the sensitivity of UC but none of these markers have been accepted for diagnosis or follow-up in routine practice or clinical guidelines.

OBJECT: To overcome the limitations of mentioned techniques, it is desirable to design a non-invasive test based on the expression profile of genes in urine for screening BCa in humans.

METHODS: Urinary exfoliated cells (UECs) were collected from eleven patients with BCa and ten healthy people. RNA extraction and synthesis of cDNA were carried out. Real-time PCR for nine genes was performed.

RESULTS: Five genes were expressed in both the BCa and healthy groups. One gene was not expressed in either group. Three genes were expressed in the BCa group and not in the healthy group.

CONCLUSIONS: This study identified three of the nine examined genes as potential biomarkers for the detection of BCa. Of course, this issue needs to be evaluated with further healthy and BCa individuals.

Key words: Bladder Cancer, Non-Invasive Tests, Expression Profile

Circulating miR-21: a potential biomarker in diabetic individuals with a positive family history of type 2 diabetes mellitus

Zakieh Yazdanpanah [1], Nasrin Kazemipour [1], Seyed Mehdi Kalantar [2*]
1-Biochemistry Division, Department of Basic Science, School of Veterinary Medicine, Shiraz University, Shiraz, Iran.
2- Department of Medical Genetic, Medical School, Shahid Sadoughi University of Medical Science, Yazd, Iran.
*-Corresponding author: Seyed Mehdi Kalantar, Department of Medical Genetic, Medical School, Shahid Sadoughi University of Medical Science, Yazd.
Email Addresses: smkyzd@gmail.com

Background and Objectives: The recognition of ideal biomarkers for the early detection of Type 2 diabetes mellitus (T2DM) is a significant priority in various researches. Diabetes-related long-term complications are associated with damage and impairment of multiple organs. Therefore, it is necessary to identify susceptible individuals in the prediabetic phase with a family history of type 2 diabetes (FHD) to devise prevention programs aimed at dealing with factors related to the progression of the disease. In the present study, we examined whether the expression of circulating miR-21 is associated with prediabetic and T2DH. Also, whether this molecule can be used as a potential blood-based biomarker to identify individuals with positive family history of T2DM in the prediabetic stage.

Methods: Plasma miR-21 expression in three groups, including control (n=29), pre- T2DM (n=29) and T2DM (n=24) subjects were measured. Quantitative real-time PCR (qRT-PCR) was applied to detect the relative expression of miR-21. Biochemistry and clinical parameters including FBS, HbA1c, TG, TC, SBP, DBP, LDL-C and, HDL were assessed with standard laboratory measurements. Pearson and Multiple linear regression analysis were employed to conduct correlation analysis. Furthermore, the receiver operating characteristic (ROC) curve was used to analyze the diagnostic value of miR-21 in pre-T2DM and T2DM.

Results: The expression of miR-21 was significantly upregulated in plasma samples of pre-T2DM and T2DM in comparison with control subjects ($P<0.001$), while there was no significant difference in pre-T2D individuals relative to the control group ($P<0.05$). ROC curves demonstrated an approximately equal level of diagnostic performance for both pre-T2DM and T2DM groups with the healthy group with AUC of 0.77 (95% CI 0.65 to 0.90; p=0.0004) and AUC of 0.78 (95% CI 0. 0.64 to 0.92; p=0.0042), respectively. miR-21 expression was positively associated with HbA1c (r=0.456), FBS (r=0.412), and TG (r=278) and might have diagnostic value for T2DM and pre-T2DM. The regression analysis corroborates that HbA1c was the significant factor correlated with the plasma miR-21 expression (R=0.507).

Conclusion: This study indicates that the plasma expression of miR-21 can be considered as a non-invasive and fast indicative tool for distinguishing individuals' pre-T2DH and T2DH from healthy counterparts with the genetic predisposition.

Keywords: family history of type 2 diabetes, pre-diabetes, microRNA-21, microRNA-126, biomarker.

Abbreviations: T2DM, type 2 diabetes mellitus; FBS, fasting blood sugar; HDL-C, high-density lipoprotein cholesterol; TG, Triglyceride; TC, Total cholesterol; LDL-C, low-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; SBP, systolic blood pressure; DBP, diastolic blood pressure.

NGS Applications in Microbiome; Ocular Metagenomics Sequencing

Sara Taghizadeh[a], Mahdieh Hassani[b], Seyed Farzad Mohammadi[a]*

a.Translational Ophthalmology Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences

b.Medical Genetics Department, School of Medicine, Ilam University of Medical sciences

*Correspondent: sfmohammadi@tums.ac.ir,

sfmohammadi@sina.tums.ac.ir

Background and Objectives: Insights in the ocular microbiome are still at an early stage and numerous more questions stay unanswered compared with other human-associated microbial communities. The current information on the human microbiome changed our viewpoint on microscopic organisms and human health and altogether improved our understanding of human pathophysiology. Moreover ocular medicine, microbiome research might impact treatment. Metagenomic next-generation sequencing could be a capable strategy for pathogen discovery that combines progressed genome sequencing innovation with cutting-edge bioinformatics to analyze microbial populations. Metagenomic next-generation sequencing has the potential to recognize uncommon, unculturable, and indeed already unidentified pathogens from a clinical isolate. Of particular interest to ophthalmology, this strong information extraction can happen from exceptionally little volume clinical samples. Here we discuss the opportunities and limitations of this technique and its current and future application to ophthalmic diagnostics.

Methods: Review of the peer-reviewed literature on conventional and advanced methods as applied to the diagnosis of infectious diseases of the eye.

Conclusion: NGS is a novel technology for identifying the pathogens responsible for ocular infections with the potential to improve the accuracy and speed of diagnosis and hasten the selection of the best therapy. NGS has proved to have the potential to dramatically improve the detection of infectious organisms and treatment. NGS can be used to address multiple questions regarding different pathogens. Unbiased metagenomics evaluation directly based on clinical samples remains prohibitively expensive. So, the cost of the testing needs to be further reduced to make NGS as acceptable as a routine test in the clinical microbiological laboratory. In addition to the cost, Time delays are critical for most ocular infectious diseases, especially for those with a high likelihood of causing blindness. This delay must be decreased if management and outcomes are to be advanced. More clinical data will have to be collected for further evaluation of NGS applications in ocular infections. Perhaps many unknown pathogens highlighted by NGS studies that are not listed in the database library may become clinically relevant as new discoveries and guidelines become available.

Keywords: Next-generation sequencing (NGS), microbial whole-genome sequencing (MWGS), ocular infection, metagenomics

cfDNA Technology in Cancer Research; Diagnosis, Prognosis, and Treatment Monitoring of Retinoblastoma

Sara Taghizadeh[a], Fariba Ghassemi[a], Seyed Farzad Mohammadi[a]*

a.Translational Ophthalmology Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences

* Corresponding author fariba.ghassemi@gmail.com

Background and Objectives: Retinoblastoma (RB) is the most common intraocular malignancy afflicting children. The estimated incidence varies through countries from 3.4 to 42.6 cases per million live births.

The retinoblastoma susceptibility gene RB1 is a tumor-suppressor that encodes a protein with a regulatory role in the cellular growth cycle at the G1 checkpoint. It is located on subband 13q14.2. Both alleles of the retinoblastoma gene have to be inactivated in both germline and somatic types for tumor development.

The distinction between germline and somatic mutations is vital, as germline cases need close monitoring with short-term risk of new ocular tumors and long-term risk of second systemic cancers. The risks of both are extensively reduced if there is evidence to indicate a constitutional mutation is particularly unlikely. Furthermore, there are essential implications for screening examinations in the wider pedigrees (siblings and offspring). Confirming the non-heritable nature of the retinoblastoma can keep away from screening examinations in many family members, with extensive financial savings and keep away from plausible morbidity in unaffected family members, formers screened unnecessarily.

Methods: This review searches for the medical utility of aqueous humor (AH) liquid biopsy for RB at diagnosis and longitudinally during therapy for its diagnostic value, prognostic significance, and possible future application to a precision oncology model to direct personalized management of RB. This novel method ultimately allows for the identification of tumor-derived molecular biomarkers in RB eyes without invasive tumor biopsy or enucleation. The AH liquid biopsy opens the door to apply a long time of knowledge about RB genomics in an impactful clinical application and to better understand intra-tumoral dynamics during therapy.

Conclusions: This review demonstrates the feasibility of testing AH samples in non-enucleated eyes, with AH cell-free DNA (cfDNA) enabling detection of somatic variants in patients undergoing intravitreal chemotherapy (IVC) where the tumor is not available. Capture-based technology was used to identify previously undetected RB1 gene mutations and loss of heterozygote (LOH) alterations in AH cfDNA. Our gathered data also suggests that the majority of cfDNA in the AH is from the tumor due to the compartmentalized nature of ocular fluids, a factor that facilitates this test. Among the enucleated eyes tested, higher amounts of cfDNA were seen in eyes undergoing primary enucleation. Adequate but lower DNA levels were present in eyes treated with systemic chemotherapy and local treatment such as laser and cryotherapy. This would suggest the cfDNA load varies with the tumor load.

Keywords: retinoblastoma (RB), aqueous humor (AH), cell-free DNA (cfDNA), intravitreal chemotherapy (IVC)

A report of a novel pathogenic variant in *SGCG* gene leading to Limb-girdle muscular dystrophy type 2C

Samanian, Sara (Ph.D.) [1]; Afkary, Banafsheh (M.Sc)[1]; Mahjoubi, Frouzandeh(Ph.D.) [1, 2]

1.Iranian Genetic Foundation, Solaleh Genetic Laboratory, Tehran, Iran

2.Medical Biotechnology Department, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

Correspondent: Frouz@nigeb.ac.ir, Frouz2@yahoo.com

Sara.samanina@yahoo.com, Frouz@nigeb.ac.ir, b.afkari.1992@gmail.com

Background: Limb-girdle muscular dystrophies (LGMD) are one of the most common types of muscular dystrophy. There are at least thirty types of LGMD (2), and as of today, more than 50 genetic loci have been identified for this disease some cause autosomal dominant and some autosomal recessive inheritance mode of this disease. LGMD2C is caused by mutations in the gamma-sarcoglycan gene (SGCG; 608896) on chromosome 13q12 and is inherited in an autosomal recessive manner. The onset age of the disease vary from 1-12 years and symptoms are progressive proximal muscle involvement, Muscle atrophy, contracture of the Achilles tendon, shoulder girdle weakness, unstable gait, gowers sign, loss of independent ambulation around age 12 years, scoliosis, restrictive lung disease, heart problems and wheelchair use by 10-30 years.

Objective: In this study, we report a novel mutation in exon 2 of SGCG gene found in three affected siblings of a family by NGS. To the best of our knowledge, this variant (c.177dupA) has never been reported previously and based on the family segregation plus population studied we believed this variant may be assigned as a novel pathogenic variant.

Method: we analyzed the patient and family members through whole Exome Sequencing (WES) and segregation and population analysis.

Result: We found a novel homozygous mutation in exon 2 of SGCG gene (c.177dupA) never been reported previously which made the diagnosis of the patients as Limb-girdle muscular dystrophies type 2C (LGMD2C).

Conclusion: Based on the family segregation plus population studies we believed this variant may be assigned as a novel pathogenic variant.

Keywords: Limb-girdle muscular dystrophy, LGMD2C, autosomal recessive, whole Exome Sequencing, segregation study

Evaluation of the Frequency, Prognosis and Survival of RUNX1 and ASXL1 Mutations in Patients with Acute Myeloid Leukemia in Northeastern Iran

Mohammad Parsa-kondelaji1, Hossein Ayatollahi2, Mohammad Hadi Sadeghian2*

1.Department of Hematology and Blood Banking, Faculty of Medical Sciences, Mashhad University of Medical Sciences, Mashhad, Iran

2.Pathology Department of Hematology and Blood Banking, Cancer Molecular Pathology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

Correspondent: sadeghianmh@mums.ac.ir

Email: mohammadparsa7410@gmail.com

Email: AyatollahiH@mums.ac.ir

Background: One of the malignant blood diseases with high prevalence and mortality worldwide is acute myeloid leukemia (AML). This disease can be diagnosed based on cytogenetic and molecular abnormalities because it is a highly heterogeneous genetic disorder caused by changes in the natural mechanisms of blood cell proliferation and differentiation. Many genes such as runt-related transcription factor 1 (RUNX1) and additional sex combs like-1 (ASXL1) are frequently mutated in AML with a different prognosis. This study was conducted to evaluate the frequency and prognosis of RUNX1 and ASXL1 mutations in AML patients in northeastern Iran.

Methods: This cross-sectional study was performed on 40 patients with AML from February 2015 to February 2018. All patients were followed up for 36 months. We evaluated the frequency and survival rate of RUNX1 and ASXL1 mutations in patients. To detect mutations, peripheral blood samples and bone marrow aspiration were taken from all participants. Also, DNA extraction, polymerase chain reaction (PCR), and sequencing techniques were used.

Results: One male patient (2.5%) had RUNX1 mutations and four cases (10%; 3 females vs. 1 male) had ASXL1 mutations. The survival rates of AML patients after 1, 3, 6, 9, 12, 24, and 36 months were 98%, 90%, 77%, 62%, 52%, 27%, and 20%, respectively. There was a significant relationship between the occurrence of ASXL1 mutations and the survival of patients with AML (P=0.027). Also, there was a significant relationship between the incidence of death and hemoglobin levels in patients with AML (P=0.045). Thus, with an increase of one unit in patients' hemoglobin level, the risk of death was reduced by 16.6%.

Conclusion: According to our results, patients with AML had a high mortality rate, poor therapy outcome, and low survival rate. ASXL1 and RUNX1 mutations are associated with worse prognosis in patients with newly diagnosed AML. Also, we found that the prevalence of ASXL1 and RUNX1 mutations was higher in northeastern Iran compared to other regions. Since these two genes are involved in hematopoiesis and leukogenesis, it is recommended that further studies be conducted to evaluate them.

Keywords: RUNX1, ASXL1, Mutation, Acute Myeloid Leukemia, Prognosis, Survival

Role Of Gut Microbiome in The Pathogenesis of Multiple Sclerosis

Mahdieh Hassani*[1], Sara Taghizadeh[2]

1.Department of Genetics, Faculty of Medicine, Ilam University of Medical sciences

2.Translational Ophthalmology Research Center, Farabi Eye Hospital, Tehran University of Medical Sciences

correspondent: Mahdieh68hasani@gmail.com

staghizadeh@sina.tums.ac.ir

Introduction: Multiple sclerosis (MS) is an insidious autoimmune disease that affects the central nervous system. Recent studies clarified the role of the gut microbiome in this disorder. The gut microbiota is made of an enormous number of prokaryotic and eukaryotic microorganisms which educate our immune system.

Method: This study examined the various information sources that have shown the gut microbiome association with MS pathogenesis and used this information to theorize the role of this factor in the treatment and prevention of MS.

Results: Major influential factors involved in MS etiology are genetic, environmental factors, immune system dysfunction, and a stressful lifestyle. The gut microbiota is involved in a variety of disorders, especially neurological ones. It's affected by environmental factors and lifestyle. Animal studies have demonstrated the role of the gut microbiome in MS pathology; Based on this, scientists proposed the concept of the "microbiota-gut-brain axis". A stressful lifestyle and an unhealthy diet are prevalent problems in human life.

Conclusion: Based on our literature review improving the dietary program of the patients and the evaluation of gut microbiome during follow-up of treatment can be a good approach to MS prevention and treatment.

Key words: Multiple sclerosis, gut microbiome, microbiota-gut-brain axis

Reliance on CRISPR-Cas rapid sensitive detection technology in prenatal diagnosis of genetic disease

Farzaneh Iravani [1], Setare Adibzadeh[2]

1. Genetic Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

2. Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran

Correspondent:

setareadibzade@gmail.com

Background: Congenital malformations remain in the top of 10 leading causes of infant death and Prenatal diagnosis to identify women with high risk pregnancies of chromosomal abnormalities and other monogenic fetal disorders is still the most reliable important solution, Although There are many considerable reports in the literature highlighting the efficacy of prenatal diagnosis test such as chorionic villus sampling or amniocentesis but also they have some pitfalls like enlarge anxiety, risk of over- or underdiagnoses of anueploidy for the target chromosomes and the most important is the risks of miscarriage and baby loss. Considering these risks growing research has been focused on identifying a non-invasive approach, concentrated on the isolation of fetal cells in maternal blood during pregnancy.

Result & conclusion: Nucleic acid detection is a necessary part of medical diagnosis and CRISPR-Cas (clustered regularly-interspaced short palindromic repeats-associated system) is an emerging technology for molecular detection. CRISPR-Cas ۹ is well known in genetic engineering for effective targeted DNA editing and discover precise molecular mechanisms of disease or cell drug resistance mechanism. CRISPR-Cas13 is a variant of CRISPR-Cas9 where Cas13 detect RNA instead of DNA and CRISPR-based nucleic-acid detection method such as SHERLOCK (specific high-sensitivity enzymatic reporter unlocking) have been recently attracting attention in medical diagnosis. Cas13a specifically recognizes and cleaves only RNA, rather than DNA. The amplified RNA fragments in sample are mixed with Cas13 protein crRNA and fluorescent RNA probes. If the target molecules are present in the sample, Cas13 recognizes them via crRNA and indiscriminately cleaves (by collateral activity) fluorescent RNA probes, disrupting the interaction between the fluorophore and the quencher. The presence and intensity of the fluorescent signal thus indicate the amount of the target in the biological sample. It can cleave the specific sequence of mutant RNA at target sequence of genetic condition. Make it development in fetal cell isolated from maternal liquid to prenatal diagnosis of genetic mutation.

Key words: CRISPR-Cas, Prenatal diagnosis, Genetic disease

Report of Mutation Detection and Accurate Sizing of FMR1 CGG Repeats in Two Iranian women with Primary Ovarian Insufficiency (POI) and Idiopathic Female Infertility

Akram abdi[1,2], MSc, Tamouchin Moharrami[2], PhD, Reza Mahdian[1,3], MD, PhD, Abootaleb Saremi[2], MD

1. Department of Civil- Danesh Alborz University, Abyek, Qazvin.

2. Sarem Fertility & Infertility Research Center (SAFIR) & Sarem Cell Research Center (SCRC), Sarem Women's Hospital, Iran University of Medical Science (IUMS), Tehran, Iran.

3. Genetic & Metabolism Group. Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran.

Correspondent: Dr.Reza Mahdian : Biotechnology Research Center, Pasteur Institute of Iran, Tehran, Iran, Mobile: 09127988388

Email Address: dr.reza.mahdian@gmail.com, Ak.abdi1362@gmail.com,Tamochinmoh@gmail.com, Dr.saremi@sarem.org

Background and Objective: The fragile X mental retardation 1 (FMR1) gene contains a CGG repeat sequence within its 5'UTR. Its expansions can result in both neurological and reproductive disorders. Repeats more than 200 is defined as a full mutation and causes fragile X syndrome. The repeat numbers between 55 and 200 CGG are defined as premutation and can cause premature ovarian insufficiency (POI) and diminished ovarian reserve (DOR). Normal alleles have 5 to 44 and intermediate 45 to 54 CGG repeats. POI affects ~ 1% of women before the age of 40 and 0.1% before the age of 30. The aim of this study is to report the mutation detection and size of the FMR1 CGG repeats using TRP PCR in two Iranian women with the age of 29 and 30 years.

Materials and Methods: The Two Patients with premature ovarian insufficiency (POI) and idiopathic infertility after genetic counseling were referred to Molecular Laboratory in the Department of Medical Genetic, Sarem Women's Hospital. DNA of whole blood samples were extracted using salting out method. Amplification of FMR1 gene of each patient performed by TRP PCR using automated capillary electrophoresis conducted for detection of expended FMR1 alleles.

Findings: CGG repeats sizing using TRP-PCR for the patients identified heterozygote PM allele in one and heterozygote IM allele for the other patient.

Conclusion: In this study the FMR1 gene PM allele and also IM allele were associated with infertility. It is recommended that women with POI and Infertility of unknown etiology, and particularly those with a family history of POI, should be considered for CGG repeats.

Keywords: FMR1 gene, CGG repeats, premature ovarian insufficiency (POI), Infertility, TRP PCR

Spastic Paraplegia type 11 with a consistent clinical phenotype (case report)

Parisa Kadkhodaei Elyaderani, Sharareh Salmanizadeh, Elham Abbasi, Mansour Salehi*

1.Cellular, Molecular and Genetics Research Center, Isfahan University of Medical Sciences, Isfahan, Iran.

2.Medical Genetics Research Center of Genome, Isfahan University of Medical Sciences, Isfahan, Iran.

Kadkhodai23@gmail.com, Sharare.salmanizade@yahoo.com, Elham_abbasi69@yahoo.com, Genome.res.cent@gmail.com

Background and Objectives: HSP, Hereditary Spastic Paralysis, is a heterogeneous neurodegenerative disorder most commonly seen during childhood or early adolescence. Patients with HSP often have slow-progressing spastic paralysis, mental impairments, progressive spasticity, and weakness in the lower limbs due to loss of upper motor neurons, Stiffness while walking, and the development of a thin corpus callosum (TCC). Over 70 different SPG loci and 60 corresponding genes have been discovered, according to recent studies. Among the main subtypes of autosomal recessive inheritance, *SPG11* (OMIM 604360) mutations were the most frequent in every population ranging from 15% to 21%.

Material and methods: In this case, we describe a 19-year-old female who was referred because of progressive lower limb spasticity and weakness, ataxia, muscle atrophy and loss weight approximately 16 kg, ankle clonus, pain, kyphosis, and mild vision loss. Prior to the age of twelve, all developmental stages (motor, social, and linguistic) were normal. Her doctor's clinical diagnosis was related to spastic paraplegia disease, which Next-generation sequencing (NGS) was used to clarify the diagnosis.

Results: Based on the results of NGS, the likely pathogenic homozygous variant in the *SPG11* gene (NM_001160227): c.5454dupA; p.Glu1819Argfs Ter11 was identified as autosomal recessive.

Discussion & Conclusion: Hereditary Spastic Paraplegia disease can manifest with a variety of symptoms. Due to the difficulty in the investigations of patients with HSP symptoms, NGS became a valuable screening tool in HSP studies and differential diagnoses. As a result, not only the clinical and radiological findings (MRI), molecular evidence also fully confirm the diagnosis.

Keywords: *SPG11*, Autosomal Hereditary Spastic Paraplegia, Muscle spasticity and weakness, Corpus callosum dysgenesis, Next-generation sequencing

miR-219 overexpressed human Adipose-derived stem cells encapsulated in Alginate hydrogel: a new approach for spinal cord injury repair

Hamideh Babaloo[1], Somaye Fallahnezhad[1]

1- Department of Anatomy and Cell Biology, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran

hbabaloo92@gmail.com

fallahnezhads@mums.ac.ir

Background: After a spinal cord injury, oligodendrocyte loss and demyelination occur (SCI). Transplantation of myelinating cells to stimulate remyelination may be useful in enhancing function. The selection of an appropriate cell and maintaining cell proliferation when cells are delivered directly to the site of injury are critical for the repair method to be successful. Alginate hydrogel was used as an appropriate tissue engineering scaffold for this purpose, in addition to determining the type of cell.

Material and methods: Overexpression of miR219 converted human Adipose derived Stem Cells (hAdSCs) into oligodendrocyte progenitor cells (OPCs) in order to examine the link between myelination and functional improvement. SCI was induced in adult female Wistar rats using a compression technique, and they were randomly assigned to one of four experimental groups: SCI, Vehicle, hAdSCs, or OPC. MiR219 overexpressed hAdSC generated OPCs encapsulated in Alginate hydrogel, as an injectable scaffold, were administered to the injury site ten days after the injury. The BassoBeattieBresnahan test was used to evaluate the recovery of motor function every week for 10 weeks in this study, with an emphasis on boosting functional recovery after SCI. The histology assay was subsequently done.

Results: The rate of motor function recovery in the OPC group was significantly higher than in the SCI and vehicle groups, but there were no significant difference between the OPC and hAdSCs groups, despite the fact that the rate of myelination in the OPC group was significantly higher than the other groups.

Conclusion: These findings showed that remyelination was not the cause of motor function recovery.

Keywords: human adipose derived stem cells, demyelination , remyelination, Alginate hydrogel, spinal cord injury, miR-219

The use of buffered alcohol-based fixatives in minimizing cellular nucleic acids fragmentation in a cell suspension solution

Ghazal Sedaghat Shayegan[1], Mohammad Hossein Modaresi[2]

1,2 Tehran University of Medical Sciences, Department of Medical Genetics

gh-shayegan@razi.tums.ac.ir

modaresi@tums.ac.ir

Background: Nucleic acids are highly informative biomolecules, which are in particular useful in noninvasive diagnosis and prognosis. The interpretation and the accuracy of a given molecular diagnostic test result depends to a great extent on the quality of the sample at both the storage and processing times. The principle objective of the presented study was to establish a composition for minimizing nucleic acids fragmentation under storage conditions and to ascertain the relative effect of various buffer compositions on protecting cellular DNA and RNA constituents against degradation during storage time.

Methods: The overall protective effect of Ethanol in similar concentrations of Phosphate, Citrate, Tris and Acetate buffered solutions were determined by evaluating cell lysis prohibition and visualizing integrity of extracted genomic DNA and total RNA on 1% agarose gel. The concentration of genomic DNA and total RNA were assessed by NanoDrop Spectrophotometer and the density of representative bands on agarose gel were compared to its initial state for each of aforementioned buffer compositions. The amplification of several short and medium sized fragments was evaluated by PCR reaction. Different cell suspensions were made by distributing equal density of HeLa cells among various fixative solutions and these cells were counted afterwards manually to monitor cell lysis.

Results: Fixed Hela cells in each of buffered Ethanol solutions exhibited fine cellular morphology. There was no alteration in DNA band intensity and concentration of extracted genomic DNA from stored samples in any one of the Citrate, Acetate or Phosphate buffered fixatives even though DNA integrity reduced by half in case of fixing specimen in 50% Ethanol containing 50mM Tris. Both 28s and 18s rRNA bands on agarose gel stayed on the basal level of the time point zero after 7 days storing in a mixture of 50mM aqueous Citrate or Acetate buffer and 50% Ethanol at room temperature.

Discussion: The final preparations revealed Phosphate buffer made a more adequate preservation of genomic DNA in comparison with Tris buffer. Concerning fixation with alcoholic fluid, it became apparent that the acidic PH such as that of Acetate and Citrate buffers exerted marked effect on RNA preservation potency of liquid fixative.

keyword: Alcoholic fixative, nucleic acid fragmentation, sample storage, DNA integrity, RNA integrity

Association of a genetic variant in the ATP-binding cassette sub-family B member 1 with risk of cervical cancer

Fatemeh Khojasteh-Leylakoohi [a], Sara Malekzade [a,*], Samira Armanpour [a,*], Masoumeh Gharib [c], Mehrane Mehramiz [a], Negin Alavi [a], Gordon A Ferns [e], Habibollah Esmaily [f,#], Malihe Hasanzadeh [b,#] Amir Avan [d,g,#]

a- Metabolic syndrome Research center, Mashhad University of Medical Sciences, Mashhad, Iran.
b- Department of Gynecology Oncology, Woman Health Research Center, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
c- Department of Pathology, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.
d- Recombinant Proteins Research Group, The Research Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran.
e- Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex, BN1 9PH, UK.
f- Department of Biostatistics, School of Health, Mashhad University of Medical Sciences, Mashhad, Iran.
g- Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.
Email Addresses:
Fatemeh.khojasteh27@gmail.com
Malekzadeh.sa@gmail.com
Dr.p.mohamadi@gmail.com
m.mehramiz@ecu.edu.au
neginalavi@yahoo.com
HasanzadeMofradM@mums.ac.ir
Gharibm@mums.ac.ir
G.Ferns@bsms.ac.uk
Esmailyh@mums.ac.ir
avana@mums.ac.ir

Background and Aim: Cervical cancer (CC) is the fourth most prevalent cancer among women in the world that It is among the leading cause of cancer death among women, after breast and colorectal cancer. DNA mutations, chromosomal rearrangements, apoptotic markers, expression of oncogenes and tumor inhibitor genes are suggested as potential markers for the diagnosis and prognosis of cervical cancer. The ATP- binding cassette sub-family B member1 (ABCB1) gene encodes a membrane-associated protein involved in the transportation of various molecules across extra- and intra-cellular membranes, while several studies have shown the role of Fc gamma receptors (FCGR) polymorphisms, in disease susceptibility. Here we have investigated the association of genetic variants, rs1128503, in ABCB1 and rs396991 in FCGR3A genes in patients with cervical cancer.

Methods: Genotyping of 213 control and 51 cases of cervical cancer (CC) was performed using TaqMan real-time PCR. Logistic regression was used to assess the relationship between cervical cancer risk and genotypes. The significant prognostic variables in the univariate analysis were included in multivariate analyses.

Results: The frequencies of the GG, AG, and AA genotypes at the ABCB1 locus were: 21.5%,62.7%,15.6% in the patient group and 14.6%, 52.8%, 32.5% in the healthy group respectively. The rs1128503 polymorphism was associated with an increased risk of cancer in recessive and additive genetic models. In particular, cervical cancer patients with GG genotype had a significantly increased odds ratio (OR) of 2.6 (95%CI: 1.1-5.8, p=0.02) under a recessive model after adjustment. No association was detected for FCGR3A with cervical cancer risk.

Conclusions: Our findings demonstrated an association of a genetic variant in the ABCB1 gene with increased risk of developing cervical cancer. It is necessary to carry out further studies in a larger, multi-center and multinational setting to explore the value of emerging marker for the assessment of cervical cancer risk.

Keywords: Cervical cancer, ABCB1, rs1128503, rs396991, FCGR3A

Inhibition of Wnt/b-catenin pathway via PNU-74654 reduces tumor growth in colorectal cancer

Forouzan Amerizadeh [a,b,c,*], Fatemeh Khojasteh-Leylakoohi [a], Farzad Rahmani [d,*], Seyedeh-Najibeh Nasiri [a,*], Seyed Mahdi Hassanian [a,d] Soodabeh Shahidsales [c], Mona Joudi-Mashhad [c], Majid Ghayour-Mobarhan [a], Gordon A Ferns[e], Majid Khazaei [a,#], Amir Avan [a,c,f,#]

a- Metabolic syndrome Research center, Mashhad University of Medical Sciences, Mashhad, Iran.

b- Department of Modern Sciences and Technologies, and Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

c- Cancer Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

d-Department of Medical Biochemistry, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

e- Brighton & Sussex Medical School, Division of Medical Education, Falmer, Brighton, Sussex BN1 9PH, UK.

f- Medical Genetics Research Center, Mashhad University of Medical Sciences, Mashhad, Iran.

Email Addresses:

Amerizadeh951@mums.ac.ir

shahidsaless@mums.ac.ir

JoudiM@mums.ac.ir

Fatemeh.khojasteh27@gmail.com

Nasirin3@mums.ac.ir

ghayourm@mums.ac.ir

Khazaeim@mums.ac.ir

RahmaniF981@mums.ac.ir

HasanianMehrM@mums.ac.ir

G.Ferns@bsms.ac.uk

avana@mums.ac.ir

Background and Aim: Colorectal-cancer (CRC) is amongst the most lethal-cancers, mainly due to its metastatic spread and drug chemoresistance. Hence there is a need for new approaches to either increase the efficacy of current therapy or introduce new therapies that have a better activity. Increasing evidence suggests that dysregulation of WNT-signaling-pathway plays am essential role in development and prognosis of CRC. Here we have investigated the therapeutic potential of targeting WNT/b-catenin pathway using a novel Wnt/b-catenin inhibitor, PNU-74654, in combination with 5-FU in CRC.

Methods: The anti-proliferative-effect of PNU-74654 was evaluated in two-/three-dimensional cell models. The activity of agents on cell growth, migration, invasion, cell cycle and apoptosis

was evaluated by MTT, wound healing assay, invasion, FACS, annexin V staning, respectively. The oxidant/antioxidant levels were also assessed by determining the level of MDA, SOD, as well as by DCFH-DA assay. We used a xenograft model of CRC to investigate PNU-74654 activity alone and in combination with 5-FU follow by histological staining and biochemical and gene expression analyses by RT-PCR and western blot.

Results: PNU-74654 inhibited cell-growth and synergistically affected the anti-tumor properties of 5-FU via modulation of Cyclin D1 and survivin. This agent inhibited the migration/invasion of colorectal cancer cells via perturbation of E-cadherin. Furthermore, PNU-74654 inhibited the tumor growth, which was more pronounced using the PNU-74654 plus 5-FU combination via induction of reactive oxygen species, down-regulation of SOD and modulation of MCP-1, P53, TNF-α.

Conclusions: Our finding demonstrated that PNU-74654 can target Wnt-pathway, interfere with cell-proliferation, induced-cell death, reduced-migration and interact with 5-FU, supporting further investigations on this therapeutic-approach for colorectal cancer.

Keywords: colorectal cancer; PNU-74654; anti-tumor effect , Wnt pathway, 5-FU combination

Cloning of gRNAs designed for edition of BCL11A erythroid enhancer into PX458, PX459 plasmids

Fatemeh Movahedi Motlagh[1], Azam Rasti[1], Hamid Reza Soleimanpour-Lichaei[2], Mohammad Hossein Modarressi[1]*

1.Department of Medical Genetics, School of Medicine, Tehran University of Medical Sciences, Tehran, Iran

2.Department of Stem Cells and Regenerative Medicine, National Institute of Genetic Engineering and Biotechnology, Tehran, Iran

*Correspondent: modaresi@tums.ac.ir

Email: movahedi_f@razi.tums.ac.ir, Rasti_azam@yahoo.com, hrs@nigeb.ac.ir

Background: One of the most common inherited hemoglobin disorders is thalassemia. Beta thalassemia patients depend on long term treatment and are still associated with morbidity and mortality. By far, the most definitive treatment of these patients is bone marrow (BM) transplantation. However, there are many problems associated with transplantation including limited appropriate donors with the complete similarity of HLA. Donors may encounter complications such as transplant rejection, and Immunoglobulin reaction of host cells (GVHD). Therefore, gene therapy of patients' own hematopoietic stem cells is a better approach. Autologous gene edited HSC is a definite cure, and can overcome the problems of finding BM compatible donor, and GVHD. To editHSCs, One of approaches is to increase HbF expression using crispr/cas9 system. Increased HbF may ameliorate hemoglobinopathies symptoms. Decreased HbF suppressor, BCL11A, is one of methods to increase HbF expression. The aim of this study is cloning of gRNAs designed for editing BCL11A erythroid enhancer into px-458, px-459 plasmids.

Methods: After anealing of sgRNA oligos, they were ligated into plasmids. Ligated plasmids were transformed into chemically competent bacterial cells and were plated on agar plates containing the Ampicillin. Clones were picked after 24 hours, inoculated into 5 ml LB broth medium supplemented with Ampicillin and were cultured overnight. The plasmids were extracted. The presence of the inserts were confirmed by PCR and sequencing of PCR products.

Results: The sequencing data were analyzed using snapGene software. The results show that cloning of gRNAs into px-458, and px-459 plasmids were successfully performed.

Key words: gene editing, crispr/cas9, HbF, BCL11A

Determination of the success rate of IVF in patients with polycystic ovary syndrome by investigation of candidate microRNAs relative expression

Jafari M[1]., Montazeri F[2], Haddadi F[3]., Kalantar S.M[4].

1 Ms.c student of Genetics, Department of biology, Faculty of Sciences, University of Zabol, Zabol, Iran

Email Addresses:

1-Jafarimahboobe991006302@gmail.com,

2-marjan.montazeri@gmail.com,

3-fatemeh.haddadi@uoz.ac.ir,

4-smkyzd@gmail.com

Background: MicroRNAs (miRNAs) stand out as promising biomarkers to determine the quality of sperm, oocytes, embryos, and endometrium that could be used to predict implantation outcome. miRNAs are small RNA molecules that modulate post-transcriptional gene regulation. Secreted or extracellular miRs are highly stable in body fluids, reflect disease states, and are easily detectable in a short time frame making hem highly suitable for biomarker detection. However, their role in responding to different treatments for PCOS in IVF cycles is still unknown.

Objective: Detection of biomarkers for predicting IVF out come

Methods: We performed a systematic review of controlled observational studies. Then selected the candidate miRNAs . This retrospective study was designed to evaluate the expression profiles of extracellular miRNAs in serum and endometrial tissue to evaluate the results of IVF rate in people with polycystic ovary syndrome.

Results: Successful embryo implantation requires the establishment of a receptive endometrium. Poor endometrial receptivity has generally been considered as a major cause of an IVF failure. Protein glycosylation is associated with many physiological and pathological processes. The fucosylation is catalyzed by the specific fucosyl transferases. Fucosyl transferase IV (FUT4) is the key enzyme for the biosynthesis of 1,3-fucosylated glycans carried by glycoproteins, and the previous studies showed FUT4expression changed dynamically during preimplantation.

Conclusion: MicroRNAs (miRNAs) are known to regulate specific gene expression. However, the relationship between specific miRNA and FUT4, as well as the role of miRNA/FUT4 in the of uterine receptivity remains elusive. In the current study, we reported that the levels of miR-200 family members were significantly increased in serum from IVF failures and IVF success.

Keywords: MicroRNAs, PCOS, IVF, Fucosyltransferase IV, miR-200 family

Decreased expression of methyl guanine methyltransferase by CRISPR method for better efficacy of Temozolomide in Hek293T cell line

Yasamin Yousefi[1], Farzaneh Alizadeh[2], Atiyeh Eslahi[2], Shima Farrokhi[2], Ahmad Asoodeh[1], Mehran Gholamin[3], Majid Mojarrad[*, 2]
1-Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
2- Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3-Division of Human Genetics, Immunology Research Center, Bu-Ali Research Institute, MUMS, Mashhad, Iran
Email Addresses:
yasaminsmartgirl@gmail.com
Alizadehf951@mums.ac.ir
Eslahia951@mums.ac.ir
Shimafarrokh_21@yahoo.com
Asoodeh@um.ac.ir
GholaminM@mums.ac.ir
mojaradm@mums.ac.ir

Keywords: CRISPR, MGMT, TMZ

Background and Objectives: Glioblastoma (GBM) is the most aggressive and invasive glioma which have poor prognosis. TMZ is a DNA alkylating drug used to treat glioblastoma. However due to primarily over-expression of O^6-methylguanine methyltransferase (MGMT) TMZ treated patients do not respond to TMZ. Reprograming of MGMT promotor and enhancer can result in complete attenuation of MGMT expression and subsequently increase the sensitization of tumor cells to TMZ toxicity effects. Here, we used CRISPR-dCas9 system to target methylation across the entire CpG Island at MGMT promoter or enhancer.

Material and method: We computationally designed sgRNAs to target specific regulatory locus of MGMT. GFP cassette were cloned into plasmid expressing dCas9 fusion with DNMT3A effector domain and then designed sgRNAs were cloned in DNMT3A expressing. The HEK293T cells were transfected with DNMT3A-GFP-sg vectors using Lipofectamin 3000. Cells were treated with 1µg/ml Puromycin growth medium to enrich persistent transfected cells. Total RNA was extracted and then were reverse-transcribed for quantitative PCR. Real time PCR was performed in triplicate and gene expression analysis was performed with *GAPDH* as a reference gene. Then cell viability were evaluate by MTT assay to examine the efficacy of TMZ in transfected cells.

Results: Analysis of MGMT expression by RT-qPCR in dcas9-dnmt3A-sgRNAs targeting promotor and enhancer region indicated respectively 99% and 84% reduction of mRNA expression for promotor and enhancer. MTT assay results indicate that cells with low level of MGMT expression were more sensitive to TMZ induced cell death.

Conclusion: Collectively, MGMT expression suppression using CRISPR based methylation modification can be a promising tool as new adjuvant therapy drug in treatment of glioblastoma.

Computational prediction of the effect of S477N mutation on the RBD binding affinity and structural characteristic, a molecular dynamics study

Mozhgan Mondeali[1], Mohammad Hossein Modarressi[1], Khabat Barkhordari[1], Ali Etemadi[1]

1.Tehran University of Medical Sciences

mondealitums@gmail.com

modaresi@tums.ac.ir

barkhordarikhabat@gmail.com

etemadih.uw@gmail.com

Background: The COVID-19 pandemic, caused by SARS-CoV-2, has led to significant concerns in worldwide due to its catastrophic effects on public health. The SARS-CoV-2 infection is initiated with the binding of the receptor-binding domain (RBD) in its spike protein to the receptor angiotensin converting enzyme 2 (ACE2) in the host cell membrane. owing to the error-prone entity of the viral RNA-dependent polymerase complex, the virus genome including the coding region for the RBD gains new mutations, leading to the appearance of multiple variants. These variants can potentially impact transmission, virulence, antigenicity and evasive immune properties. S477N mutation located in the RBD is observed in the SARS-CoV-2 omicron (B.1.1.529) variant and there is a lot of focus on it.

Methods: In this study, we investigated the consequences of S477N mutation at the molecular level using computational approaches such as molecular dynamics simulation, protein-protein interaction analysis, immunoinformatics and free energy computation. The Gibbs free energy change were used to analyze the impact of the RBD mutations on the binding affinity of SARS-CoV-2 variants with the ACE2.

Results: The results showed that displacement of Ser with Asn increases the affinity of the spike protein to ACE2 receptor and thus enhances the transmission potential of the virus. This mutation changes the stability, folding and secondary structure of the spike protein. Also, it reduces antibody neutralization, raising concern about re-infection, vaccine breakthrough and therapeutic values.

Conclusions: we provide new insights into the mechanisms underlying the infectivity of SARS-CoV-2 and display S477N mutation impacts the WT RBD structure and conformation as well as increases the binding affinity to angiotensin-converting enzyme receptor.

Keywords: S477N, COVID_19, molecular dynamic, SARS-COV2 mutations

Identification of a Novel Heterozygous Frameshift Deletion in the SOX2 gene Due to Gonadal Mosaicism using targeted next-generation sequencing

Pooneh nikuei, Hamed Farazy, Fouzieh Hajizadeh

Social Welfare Organization,BandarAbbas, Iran

Bayangene Genetic Center, Shiraz., Iran

Email:poonehnikuei@gmail.com

Background: Syndromic microphthalmia-3 (MCOPS3) is caused by heterozygous mutation in the SOX2 gene on chromosome 3q26 and is characterized by clinical anophthalmia or microphthalmia with or without defects of the optic nerve, optic chiasm, and optic tract. Extraocular abnormalities include brain anomalies, seizures, motor disability, neurocognitive delays, sensorineural hearing loss, and esophageal atresia.

Method: We report on a family of six members: unaffected parents, two affected sons, one healthy son and an affected granddaughter. Next generation sequencing was carried out in the proband. Variants were then identified and confirmed using Sanger sequencing. Prenatal diagnosis of the detected variant was then performed in the family later.

Result:A novel heterozygous frameshift deletion in the SOX2 was identified and it was confirmed in proband and also in his affected brother with milder phenotype. However, this mutation was not found in their parents, indicating gonadal mosaicism.

Conclusion: This report demonstrates the importance of genetic counseling and these type of events must be taken into consideration in the genetic counselling of families

Generation of DMD cell models with most common DMD mutations by using CRISPR-Cas9 genome editing system

Farzaneh Alizadeh [1], Yasamin Yousefi [2], Atieh Eslahi [1], Shima Farrokhi [1], Majid Mojarrad [1*]

1- Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
2- Department of Chemistry, Faculty of Sciences, Ferdowsi University of Mashhad, Mashhad, Iran
Email Addresses:
*Corresponding author
Alizadehf951@mums.ac.ir
Yasaminsmartgirl@gmail.com
Eslahia951@mums.ac.ir
Farrokhish961@mums.ac.ir
majidmojarrad@gmail.com

Background and Objectives: Duchenne Muscular Dystrophy (DMD) is a progressive muscle wasting disorder caused by loss of function mutations in the dystrophin gene. Although, there is no definitive cure so far, extensive efforts have been made to introduce effective therapeutic strategies. In-depth study of DMD pathology, improvement of therapeutic strategies and screening of effective drugs require the upgrading of various experimental models. Genome editing approach particularly CRISPR/Cas9 technology represent a very efficient and flexible genome-engineering tool, introducing various DNA alteration in different models. Immortalized cell lines as an efficient in vitro models are essential for biological research; however, there are only a few immortalized DMD cell lines. Due to the wide spectrum of DMD mutations and the complications to obtain DMD patient muscle biopsies, we optimized a CRISPR/Cas9 gene editing approach to model third common DMD mutations that encompassed approximately 8 % of patients.

Methods: By using online bioinformatics CRISPR design tools the best two guide RNAs that targeted exons 53 flanking regions in DMD gene were selected. In the cloning procedure PX458 plasmids contain both sgRNAs targeted exon 53 produced through restriction enzyme based cloning method. To generate immortalized DMD cell models, this plasmid were transfected into immortalized C2C12 cells by using lipofectamin 3000 reagent and the recombinant cells were enriched by FACS. Exon deletion and genome-editing efficiency was examined through GAP-PCR, sequencing and qPCR at the genomic level. The differentiation of C2C12 myoblasts to form protein-rich multinucleated myotubes was optimized. These assays was down through RT-PCR, sequencing and western blotting in single clones that obtain by limiting dilution method.

Results: We successfully produce recombinant plasmid, both sgRNAs related to exon 53. We generate DMDΔ53 cell line through transient transfection. GAP-PCR and sequencing results show the efficiency of the CRISPR-Cas9 system to efficient deletion of mentioned exon and region in length of 13 kb in the DMD gene. By using limiting dilution method, we obtain singles clone with homozygote and heterozygote deletion of exon 53 that confirmed by q-PCR. Producing truncated transcript due to the targeted deletion showed by RT-PCR and sequencing. Finally, lack of dystrophin expression in homozygote DMDΔ53 in comparison with heterozygote DMDΔ53 and normal C2C12 cells confirmed through western blotting.

Conclusion: We showed the efficacy of CRISPR-Cas9 system to generate of DMD cell models with the targeted deletions of dystrophin gene.

Keywords: Duchenne muscular dystrophy, Dystrophin, Gene therapy, In-vitro models, Gene editing, CRISPR/Cas9