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[OP-01]

A Case of Rubinstein-Taybi Syndrome Presenting with Novel Mutation Detected in the *CREBBP* Gene and Severe Dysphagia

Özlem Anlaş¹, Tamer Çelik², Didem Gülcü Taşkın³

¹Çiğli Training and Research Hospital, Clinic of Medical Genetics, İzmir, Türkiye

²University of Health Sciences Türkiye, Adana City Training and Research Hospital, Clinic of Pediatric Neurology, Adana, Türkiye

³University of Health Sciences Türkiye, Adana City Training and Research Hospital, Clinic of Pediatric Gastroenterology, Adana, Türkiye

Abstract

Rubinstein-Taybi syndrome is an autosomal dominant multiple congenital anomaly syndrome characterized by a characteristic facial appearance and wide fingers and toes. Here, we present an 8-month-old case with a c.2095_2096insGG(p.Gln699Argfs*4) heterozygous novel mutation in the *CREBBP* gene and dysphagia.

Keywords: Rubinstein-Taybi syndrome, novel mutation, broad thumb, dysphagia

Introduction

Rubinstein-Taybi syndrome (RTS) is an autosomal dominant multiple congenital anomaly syndrome characterized by a characteristic facial appearance and wide fingers and toes. *De novo* mutations in the *CREBBP* gene are responsible for approximately 50-60% of the cases and in the EP300 gene for 3-8%. The etiological cause is unknown in 30% of patients (1). Here, an 8-month-old case with a novel mutation in the *CREBBP* gene is presented.

Case Report

An 8-month-old girl patient was referred to us due to dysmorphic appearance and microcephaly. Birth weight, height and head circumference were 2500 g (10p), 48 cm (10-50p) and 33 cm (10-59p), respectively. As in seen Figure 1, physical examination revealed narrow forehead, hirsutism on the forehead, mild blepharophimosis, beak nose, drooping nose tip, short philtrum, thin upper lip, mild retromicrognathia, short neck, thick fingers and toes, and genital examination was normal. With the current findings, RTS was considered in the patient. The c.2095_2096insGG(p.Gln699Argfs*4) heterozygous mutation was detected in *CREBBP*. This mutation is a pathogenic novel mutation that causes a frameshift and creates a premature stop codon. Parental mutation analysis results were found to be normal.

Discussion

The incidence of RTS is 1/100,000-125,000. It has been observed that patients sit up at the age of 1.5 on average, walk at the age of 2.5-3, and say their first words at the age of 2 (2). Our patient started walking at the age of 2 years and 8 months and said his first meaningful word at the age of 1.5. In two studies, the average IQ level was found to be between 36-51. Short attention span, autism-like behavior, and hyperactivity may be observed (3). Our patient's developmental tests revealed developmental delay at all stages. Strabismus, cataract, glaucoma, refractive errors, nystagmus, and retinal anomalies can be seen in older ages (4). Our patient started using glasses at the age of 4. Nutritional problems such as gastroesophageal reflux disease (GERD) are observed in approximately 68% of patients. Dysphagia is also a symptom of this disease that should be emphasized (5). Scott et al. (6) reported that a 7-year-old male patient, diagnosed at the age of 3, had a complaint of dysphagia for 2-3 years. Dilation was performed upon detection of a post-cricoid web in endoscopic examination. At the ages of 8 and 9, dilation was performed 2 more times. They evaluated this finding, which is generally associated with iron deficiency and is very rare in children, as congenital in this case diagnosed with RTS (6). Kumar and Thota (7) detected Barrett's esophagus (BE) in a 26-year-old male patient diagnosed with RTS and complaining of dysphagia. They learned from the patient's history that he had GERD for 15 years. Periodic dilations were required during follow-up of the patient who underwent endoscopic balloon dilation. Twelve years after the diagnosis of BE, low-grade dysplasia developed in the BE segment and radiofrequency ablation was planned. Dysphagia recurred in the patient 2 years later (7). Noble et al. (8) reported 2 cases diagnosed with RTS with a history of dysphagia. The first is a 12-year-old male patient who was diagnosed in infancy. The patient, who had a history of GERD and aspiration pneumonia, had a history of dysphagia since the time of RTS diagnosis. The patient, who underwent endoscopy and dilatation many times until the age of 12, never had an esophageal biopsy. As a result of an esophageal biopsy performed at the age of 12, eosinophilic esophagitis and gastritis were detected, and his symptoms regressed with diet and corticosteroid treatment. The other case reported by Noble et al. (8) is a 10-year-old male patient with a history of dysphagia for 5 years. Following the recurrence of dysphagia after endoscopy and dilatation at the age of 9, the esophageal biopsy result performed at the age of 10 was found to be compatible with eosinophilic esophagitis. His symptoms regressed with the diet and corticosteroid treatment given (8). As seen in the literature review, it has been reported that dysphagia recurs as a result of symptomatic and interventional treatments unless the etiological cause is determined. At the age of 4, our patient was still unable to eat any food other than liquid due to dysphagia. No pathology was detected on OMD radiography. Endoscopy could not be performed because the family did not agree. At the age of 5.5, dysphagia began to regress and a follow-up decision was made.

Conclusion

Dysphagia is an important sign in RTS and it recurs as a result of symptomatic and interventional treatments unless the etiological cause is determined. This case, in which a novel mutation was detected, is presented to contribute to the literature.

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Figure 1. The patient

[OP-02]

Custom WES Panel Results in Patients with PCOS

Yunus Arıkan¹, Taylan Onat²

¹Yozgat Bozok University Faculty of Medicine, Department of Medical Genetics, Yozgat, Türkiye

²İnönü University Faculty of Medicine, Department of Obstetrics and Gynecology, Malatya, Türkiye

Abstract

Introduction: Polycystic ovary syndrome (PCOS) is a very heterogeneous endocrinopathy that can be grouped in multifactorial diseases. Studies conducted in different populations using three different diagnostic criteria have shown that its prevalence in the world is up to 20%. The potential of different variations in different genes to explain different phenotypes of PCOS has taken its place in the literature.

Methods: In our study, we evaluated 16 PCOS patients by applying androgen excess PCOS criteria and evaluated candidate genes that may be responsible for PCOS in a double-blind manner with a custom exome panel. We evaluated the patients' germline mutations and variants and whole exome analysis of 8 different genes involved in TGF-beta, WNT, MAPK, insulin signaling and steroid biosynthesis in the ION AmpliSeq Custom panel. We tried to explain the genotype-phenotype relationships of the mutations we found with clinical and biochemical laboratory data.

Results: After filtering steps, we detected 431 variations, 85 of which were different. We revealed 3 different mutations, one of which was novel, in two different patients. We described the PCOS phenotype related to the mutations we found in the *RUNX2* and *MAPK14* (*p38 alpha*) genes. We have demonstrated for the first time in our population a missense variation in the *SRD5A2* gene that may affect total testosterone levels in PCOS.

Conclusion: After the segregation analyzes of the novel mutation that is located in the *RUNX2* gene and in the *MAPK14* gene are performed, it will be possible to introduce the candidate gene(s) responsible for PCOS into the literature.

Keywords: PCOS, candidate gene, exome sequencing, *RUNX2*, *MAPK14*

[OP-03]

Contributions of NGS to Transplantation Genetics

Mustafa Gökhan Ertoşun

Akdeniz University Faculty of Medicine, Department of Medical Genetics, Antalya, Türkiye

Abstract

The development of next generation sequencing has led to a major breakthrough in the field of medical genetics. Next generation sequencing is also very important for transplantation genetics in the field of medical genetics. With the widespread use of next generation sequencing, innovations in transplantation genetics touch the lives of many patients in this field.

Keywords: Transplantation, genetics, next generation sequencing, NGS

Introduction

Transplantation is a life-saving treatment for end-stage organ failure. Organ and tissue transplantation is a vital procedure, but it can be associated with problems such as incompatibility between donor and recipient, tissue rejection and post-transplant diseases. These can be prevented by the use of advanced immunosuppressant agents and the necessary genetic testing.

Transplantation genetics is a field of research, investigation and application used in important areas such as evaluation of disease risk after organ or tissue transplantation, determination of the compatibility of donors and recipients and post-transplantation follow-up (1). Transplantation genetics, which was limited by traditional methods, has undergone a significant evolution with the development and application of next generation sequencing technology.

1. Use of Genetic Methods in the Pre-transplant Process

a) Investigation of the Factors Causing End-stage Organ Failure

End-stage organ failure is a health condition in which organs are so severely damaged that they cannot function normally. Organ failure can affect the function of important organs such as the heart, kidneys, lungs or liver. Such failures can have many causes and genetic diseases constitute an important subgroup of organ failures (2).

- **Kidney Failure and Genetic Diseases:** Renal failure is a condition in which the kidneys cannot fulfill their normal functions and genetic diseases can be an important cause of renal failure (2,3). For example, polycystic kidney disease, which is seen clinically after a loss of function in the *PKD1* and *PKD2* genes, leads to the formation of cystic masses in the kidneys and may cause kidney failure over time. Alport syndrome, which is associated with genes such as *COL4A3*, *COL4A4* and *COL4A5*, is associated with kidney damage, hearing loss and eye abnormalities and is usually caused by a genetic mutation (4). Fabry disease (related to *GLA* gene) is one of the lysosomal storage disorders and may lead to kidney damage (5). Such genetic diseases, which can be observed especially in pediatric patients, increase the risk of renal failure and may ultimately lead to the need for kidney transplantation (6).

- **Liver Failure and Genetic Diseases:** Liver failure is a condition in which the liver is damaged to the state it cannot fulfill its normal functions. For example, Wilson's disease, which is associated with the *ATP7B* gene, is an inherited disease in which copper accumulates more than normal and causes liver damage (7). Alpha-1 antitrypsin deficiency, caused by a mutation or loss of function in the *AT1* gene, is an inherited disease that causes abnormalities in the lung and liver (8). These and similar genetically based diseases may lead to liver failure by affecting the normal functions of the liver and may necessitate liver transplantation.

- **Heart Failure and Genetic Diseases:** Heart failure is a condition in which the heart cannot pump enough blood to the body. Cardiomyopathies are among some genetic diseases that increase the risk of heart failure. Cardiomyopathy is a genetic disease that causes abnormal growth and dysfunction of the heart muscle. Another example is the susceptibility to coronary artery disease caused by high cholesterol levels and low-density lipoprotein receptor mutations.

Genetic diseases can be one of the underlying causes of organ failure and can lead to the need for serious treatments such as organ transplantation. Early diagnosis, genetic testing and genetic counseling play an important role in preventing or managing genetic diseases and end-stage organ failure. While conventional methods investigate target genes or even some of the exons of the targeted genes, the next generation sequencing method, which has the important advantage of high data throughput, can analyze patients from a wide perspective and observe changes in multiple genes. As recently observed in our unit, multi-genic changes underlie the disease pathogenesis of many patients. For these reasons, next generation sequencing seems to be more advantageous than targeted Sanger sequencing in the diagnosis of such multigenic diseases.

b) HLA Typing and Tissue Compatibility Analysis

The HLA class I and class II loci are the most polymorphic genes in the human genome (9). Moreover, almost half of the genes in the MHC region have paralogous copies on chromosomes 1, 9 and 19, which may have enabled the rapid evolution of new functions (10). Allelic variations in *MHC* genes form the basis of histocompatibility in transplantation. Successful matching of HLA-A, HLA-B and HLA-DR is considered the most important factor. In case of HLA mismatch, the success of transplantation and the survival of the transplanted organ decrease depending on the rate of mismatch (11). Therefore, HLA typing is vital to identify mismatches and to ensure appropriate matching of donor-recipient pairs.

For effective *HLA* gene typing, many methods with low margin of error that can generate high data output have been developed (12,13). These methods, including next generation sequencing, have recently started to be applied in the field of solid-organ transplantation. The YNS method, which enables the detection of rare sequence variants in individual samples with a large number of sequence reads (300,000-400,000) in a single run, has become routinely used in the clinical setting in the last few years. This routine use is partly due to the fact that the cost of sequencing technologies has decreased over time to a level comparable to Sanger sequencing (14,15).

Even patients with HLA-compatible transplants may experience acute or chronic rejection processes, suggesting a role for non-HLA factors in alloimmunity (16). Studies have shown that graft loss in organ transplant recipients within 10 years is caused by non-HLA factors in 38% of cases, only 18% by HLA-related factors and 43% by non-HLA immunologic factors (e.g., surgical complications, drug toxicity or infections) (17,18). When non-HLA mediators are mentioned, killer cell immunoglobulin-like receptor (KIRs), MHC class 1 polypeptide-associated sequence A (MICA) and minor histocompatibility antigen (miHAs) come to mind firstly. Donor-recipient mismatches of these potential non-HLA mediators may trigger an alloimmune response through antigen-receptor interactions or polymorphic genes encoding non-specific peptides (19).

While only HLA compatibility of the patients was investigated with a single data during the transplantation process with conventional methods, the use of methods that allow obtaining multiple data such as YNS has enabled the examination of not only HLA compatibility of the patients but also immunologically important non-HLA loci whose effect on organ rejection has been scientifically proven. This allows genetic tests performed before transplantation to be maximized and have wider clinical implications due to the high data output that can be obtained.

c) Evaluation of Recipient-Donor Suitability for Transplantation by Genotype/Phenotype Relationship

In the conventional transplantation process, it is sufficient that the donor's general health condition and the condition of the kidney to be transplanted have been healthy until then and have HLA compatibility with the recipient. Nowadays, we know that some genetic diseases can occur with a late-onset manner, that is, at an advanced age. What needs to be considered especially in living transplant donors, is the possibility of a potential organ failure in the future due to genetic variations/mutations even if the donor is observed to be healthy during the pre-transplantation process. Therefore, instead of focusing only on the recipient, it is necessary to make a decision considering the health of the organ donor as well. There is a very important Latin saying in medical education: "Primum Non-Nocere", meaning "First Do No Harm". When treating our patient with organ failure, it is possible to accelerate the process of organ failure by selecting an individual as an organ donor who may have a possible organ failure in their later years as a result of genetic variations. In addition, the transplanted organ may have a late-onset genetic disease, making it highly likely that the transplanted organ will fail again in the future.

It is known that the percentage of development of pediatric organ failure on the basis of genetic factors is quite high (20). It is also quite common in Türkiye that the potential organ donors of this patient group are recipients parents. Considering these conditions, it is very likely that the mutation causing organ failure in the child is present in the parents too. Even if the carrier status of the parents for diseases with autosomal recessive inheritance pattern is a condition that requires long planning in the transplantation process, the main problem is the mutations found in genes associated with diseases with two different inheritance patterns such as COL4A3. Autosomal Recessive (MIM:203708) and Autosomal Dominant (MIM:104200) inheritance patterns associated with COL4A3 and Alport syndrome, which may progress with end-stage renal failure, have been described in the literature.

For these reasons briefly mentioned above, instead of examining only current clinical findings, genetic examination of both the recipient and the donor during the transplantation process will increase the success rate in the transplantation process. In this way, the transplanted organ will have a longer survival outcome, and the donor candidate will be prevented from becoming a transplant candidate as a result of a possible organ failure in the future.

2. Using Genetic Methods in the Post-Transplant Process

a) Rejection Monitoring

Post-transplant tissue rejection is a condition in which the recipient rejects the transplanted tissue or organ (21). Post-transplant genetic analysis can be used to provide early diagnosis of tissue rejection. Genetic material in the recipient is analyzed and immune responses can be detected. In this way, tissue rejection can be detected at an early stage and appropriate treatment can be initiated.

Analysis of circulating free DNA (cfDNA) in serum allows early diagnosis of organ rejection and increases patient comfort as it is performed with a non-invasive method (22). cfDNA in serum is DNA fragments circulating in the blood as a result of aging and death of cells. In case of organ rejection, rejection of the transplanted organ by the recipient leads to destruction of organ cells and circulation of donor-derived free circulating DNA (dd-cfDNA). Analysis of (cfDNA) in serum is based on the detection of dd-cfDNA in the bloodstream and can be performed non-invasively to help detect organ rejection at an early stage.

In rejection monitoring, blood samples are taken at regular intervals and cfDNA levels are monitored. If an abnormal increase or change in cfDNA level is observed, the risk of organ rejection is assessed and necessary measures are taken. This helps to better monitor organ survival and transplant outcome. When the tests performed in our center are examined, the destruction of the transplanted organ is recognized before it is reflected in the clinic and taking the necessary measures not only positively affects the survival of the transplanted tissue, but also contributes greatly to minimizing the loss of function.

b) Microchimerism Monitoring

Next generation sequencing is also used in microchimerism analysis. Detection of small amounts of donor cells remaining in the recipient after transplantation can increase transplantation success (23,24). High-resolution YNS facilitates the detection of microchimerism and helps to determine treatment strategies for the recipient by confirming the presence of donor cells. Especially in transplants of tissues with bone and bone marrow content such as composite tissue transplants, monitoring of microchimerisms is very important in terms of organ rejection (24).

c) Drug Level Monitoring

Next generation sequencing technology also plays an important role in pharmacogenetic analysis. Pharmacogenetics is a field that studies the effects of drugs according to an individual's genetic profile. Tacrolimus drug level monitoring is also considered in this context. Tacrolimus helps to prevent rejection of the donor organ by recipients by suppressing the immune system (25). Tacrolimus drug administered to the recipient after transplantation surgery reduces the immune response by inhibiting the activation of T-cells. This is important to ensure acceptance of the donor organ by the recipient and long-term success. Next generation sequencing analyzes the recipient's genetic profile to obtain information about their response to tacrolimus and drug metabolism. This enables more personalized and effective treatment planning for recipients.

The cytochrome P450 3A family, particularly CYP3A4 and CYP3A5, are among the best-characterized and best-studied determinants of the variability in blood levels of immunosuppressant drugs. Genetic variations in the genes coding for these cytochromes may affect the pharmacokinetics of tacrolimus and mTOR inhibitors (26,27). Many allelic variants of CYP3A4 and CCYP3A5 coding genes have been identified and shown to significantly alter the metabolism and clearance of tacrolimus, affecting the blood levels of the drug. The vast majority of these variants cause a loss of function of the enzyme and therefore significantly increase blood/serum tacrolimus levels. For example, CYP3A5*1 encodes a functional enzyme. Any mutation in this allele leads to the formation of dysfunctional proteins (28,29). For CYP3A4 in the same cytochrome family, it has also been reported in the literature that some CYP3A4*22 allelic variants cause decreased enzymatic activity (30). It should of course be noted that the frequencies of these alleles may vary widely between different population groups.

d) Prevention of Side Effects (Infection and Cancer)

By adjusting the dose of immunosuppressant agents in a patient-specific manner in line with the genetic data we will obtain individually, we have the opportunity to minimize side effects such as secondary infections and cancer caused by these agents.

It has also been shown in the literature that some genes such as *LINC00882*, *CACNA1D* and *CSMD1* and changes in these genes are associated with cancers seen after transplantation (31). Pre-evaluations of these genes have a very important place in the planning of the whole post-transplant process, starting from the selection of immunosuppressant agents to be used after transplantation.

e) Non-invasive Prenatal Diagnosis

Because of the teratogenic effects of high doses of immunosuppressive agents and some pharmacologic drugs taken during the transplantation process, the pregnancy process of transplanted individuals should be carefully monitored and some drugs should be replaced with other agents in case of pregnancy (32). Detection of fetal anomalies during pregnancy of transplant recipients is important in this respect. Therefore, prenatal diagnosis gains importance in these cases. Non-invasive diagnostic methods, which have recently gained popularity, can also be used in this patient group. Non-invasive prenatal diagnosis (NIPD) aims to detect fetal-related genetic disorders before birth, with the potential to reduce the risk of fetal birth defects by detecting markers in the peripheral blood of pregnant women.

Prenatal diagnosis by preimplantation genetic diagnosis is as important in Uterus transplants as it is in solid organ transplants. In uterine transplantation, which aims to provide prospective parents with a healthy child, it is very important to follow up the individual who has undergone uterine transplantation in terms of both maternal and infant anomalies throughout the pregnancy process. Postnatal karyotyping and NIPD tests were found to be compatible and it was shown that NIPD tests can also be applied in this patient group.

In conclusion; innovative genetic approaches increase the success of the transplantation process and positively affect the quality of life of patients. Thanks to all these scientific approaches, the use of genetic tests in the field of transplantation and the integration of next generation sequencing technology play an important role in increasing the success and efficiency of transplantation processes.

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[OP-04]

Molecular Diagnosis of Lethal Multiple Pterygium Syndrome in a Fetus Presenting with Fetal Akinesia Deformation Sequence

Tarık Çetin¹, Hatice Mutlu^{2,3}, Duygu Duman³, Türker Duman³

¹Ankara University Faculty of Medicine, Ankara, Türkiye

²Ankara University Faculty of Medicine, Department of Pediatric Genetics, Ankara, Türkiye

³The Rare Diseases Application and Research Center of Ankara University (NADİR), Ankara, Türkiye

Introduction

Fetal akinesia deformation sequence (FADS) is a rare disorder characterized by decreased fetal movement in utero, which leads to various joint contractures and other developmental abnormalities in the affected fetus. The genetic basis of FADS is complex and heterogeneous. Here, we present a patient presenting with FADS who has been diagnosed as lethal multiple pterygium syndrome (LMPS).

Case Report

A male patient who died in first hour following the birth was referred to pediatric genetic clinic. Hydropic appearance, flattened nasal bridge, micrognathia, low set ears, short neck, cystic hygroma, bilateral flexion contractures at elbows, fingers, hips, knees and bilateral rocker bottom feet were noticed during the postmortem examination. Antenatal ultrasonography revealed reduced fetal movement, multiple arthrogryposis, clenched hand, hydrops and pulmonary hypoplasia. His parents were consanguineous and had a history of 2 termination of pregnancy with similiar phenotype. The homozygous *CHRNA10*:c.753_754del(p.Val253AlaTer44) variant associated with LMPS was detected in the patient who underwent WES with a prediagnosis of FADS.

Conclusion

Mutations in the *CHRNA10* gene have been reported to cause recurrent pregnancy loss and LMPS. Genetic testing, including whole genome sequencing, can help identify specific genetic mutations responsible for FADS in individual cases. However, due to the genetic heterogeneity of FADS, a precise diagnosis might not always be achievable. It's important to work closely with medical geneticists to determine the underlying cause of FADS in specific individuals. Genetic counseling is crucial to understand the inheritance pattern, recurrence risks, and potential treatment options for affected families.

Imagawa-Matsumoto Syndrome: The First Case from Türkiye

Zeliha Yücel¹, Emine Berrin Yüksel², Altuğ Koç³

¹Karaman Training and Research Hospital, Clinic of Neurology, Karaman, Türkiye

²Karamanoğlu Mehmetbey University Faculty of Medicine, Department of Medical Genetics, Karaman, Türkiye

³Gentan Genetic Diseases Evaluation Center, İzmir, Türkiye

Abstract

Imagawa-Matsumoto syndrome (IMMAS; MIM #618786) is an autosomal dominant syndrome characterized by overgrowth, dysmorphic features, musculoskeletal abnormalities, developmental delay, and intellectual disability. The first case was reported in 2017 and has subsequently been diagnosed in only another 12 patients. We also present the first IMMAS patient from Türkiye. A 19-year-old female was admitted to the neurology outpatient clinic due to behavioral disorder and intellectual disability. Her physical examination revealed macrocephaly and dysmorphic features like a round face, broad forehead, hypertelorism, and variable skeletal anomalies such as flat feet, clinodactyly, and macrocephaly. Cranial magnetic resonance imaging showed agenesis of the corpus callosum and polymicrogyria. Chromosomal analysis results were consistent with a normal constitutional female karyotype and microarray analysis showed a *de novo* 1.5-MB size deletion on the long arm of chromosome 17; band q11.2 encompassing the Polycomb Repressive Complex 2 Subunit (*SUZ12* gene, MIM *606245). This report will contribute to the limited information in the literature.

Keywords: Behavioral disorder, Imagawa-Matsumoto syndrome, Intellectual disability, Macrocephaly, *SUZ12*

Introduction

Imagawa-Matsumoto syndrome (IMMAS; MIM #618786) is characterized by overgrowth, development delay, intellectual disability, and dysmorphic features. Imagawa et al. (1) were the first to report such a case with mutation in the *SUZ12* (Polycomb Repressive Complex 2 Subunit; MIM *606245) gene -an 11-year-old female (with a dizygotic twin) born to non-consanguineous Japanese parents, that had postnatal overgrowth, intellectual disability, such dysmorphic features as hypertelorism, downslanting palpebral fissures, and a prominent forehead, musculoskeletal and structural brain anomalies, in 2017. These findings were initially interpreted as Weaver syndrome (WS; MIM #277590), which is characterized by prenatal or postnatal overgrowth, accelerated osseous maturation, cranial dysmorphism, intellectual impairment, and limb anomalies; however, the patient did not fulfill all the criteria for WS. As such, the patient was diagnosed as 'Weaver-like'syndrome. Subsequently, in 2018, two new patients from unrelated families were described with postnatal overgrowth and development delay, and novel *SUZ12* mutations as Weaver-like syndrome by the same group, the clinical characteristics of these patients were very similar to the first reported patient (2). In 2019, Cyrus et al. (3) reported another 10 patients with an overgrowth phenotype, physical abnormalities, delayed developmental stages, and rare heterozygous *SUZ12* variants. Considering these ten patients in addition to the previous three patients, the clinical framework of pathogenic *SUZ12* variants has settled, which mostly causes overgrowth, distinctive facial features, limb anomalies, and intellectual disability. It later began to be called "Imagawa-Matsumoto syndrome".

Case Report

A 19-year-old female presented to our neurology outpatient clinic with a history of intellectual disability and behavioral disorder since childhood. The patient has non-consanguineous Turkish parents and was born via spontaneous vaginal delivery after an uneventful full-term pregnancy. She had a low birth weight (<2500 g, <3 percentile) and was hospitalized in the newborn intensive care unit for 2 months due to neonatal pneumonia. The parents reported a history of mild motor developmental delay; the patient was able to sit at age 12 months and walk at age 2 years. Notably, her speech and language skills development were delayed; her first word was spoken at age 3 years.

Upon physical examination the first remarkable feature was a dysmorphic phenotype, including a prominent forehead, round face, broad nasal ridge, and macrocephaly. The patient's head circumference was 58 cm (+2 SD), while her weight and height were within the normal range. Musculoskeletal system assessment showed flat feet, large hands, camptodactyly, clinodactyly. Her neurological examination was normal, except for cognitive impairment. In the genitourinary system examination, it was learned that she had irregular menstrual periods. She applied to the gynecologist with this symptom; subsequent suprapubic pelvic and urinary ultrasonography performed by a gynecologist revealed an ectopically located left kidney in the pelvis, which also had a rotation anomaly.

Routine blood tests were within normal limits. Cranial magnetic resonance imaging showed agenesis of the corpus callosum and polymicrogyria (Figure 1), which was followed by chromosomal analysis. Chromosomal analysis results were consistent with a normal constitutional female karyotype. Chromosomal microarray was the next step of the genetic testing algorithm and showed a copy number loss (1 copy) of 1.5 MB (195 markers) size deletion on the long arm of chromosome 17; band q11.2 encompassing the *SUZ12* gene (Figure 2). The deletion was reported *de novo* because the change in the index could not be detected in the parents.

Discussion

IMMAS is characterized by overgrowth, prominent facial dysmorphism, developmental delay, intellectual disability, and variable skeletal abnormalities. Our patient had corpus callosum agenesis and polymicrogyria; therefore, it is important to include neuroimaging in the diagnostic algorithm of this syndrome to detect structural brain abnormalities. On the other hand, our patient is the first female IMMAS patient with a genitourinary abnormality and renal anomalies, including renal ectopia which also had a rotation anomaly. In this respect, our case adds a new clinical component to the syndromic profile of IMMAS.

Conclusion

Herein we presented the first IMMAS patient from Türkiye-the 14th known case worldwide. As such, the present case report will help improve the syndromic profile of IMMAS and expand the limited IMMAS database.

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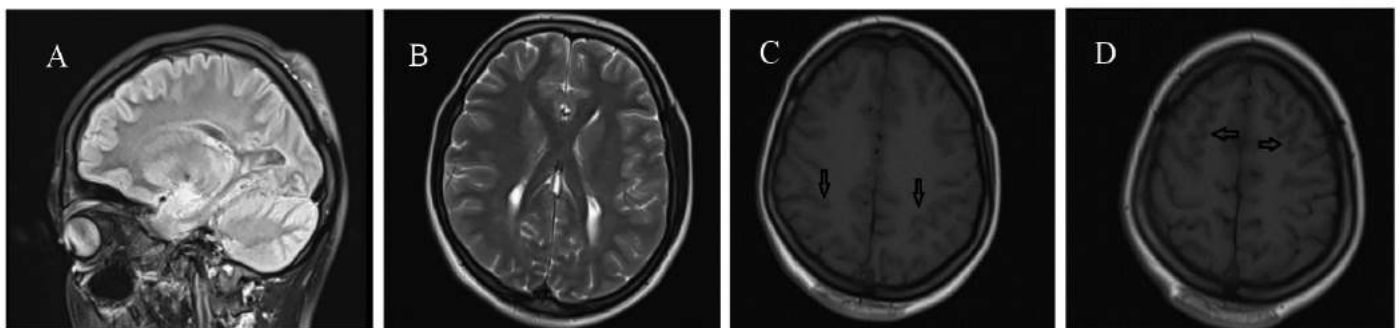


Figure 1. MRI imaging of the patient's structural brain abnormalities. MRI imaging demonstrated at sagittal (A) and axial (B) fluid attenuation inversion recovery (FLAIR) sequence agenesis of the corpus callosum and axial (C, D) T1-weighted (T1W) sequence bilaterally frontotemporoparietal polymicrogyria
MRI: Magnetic resonance imaging

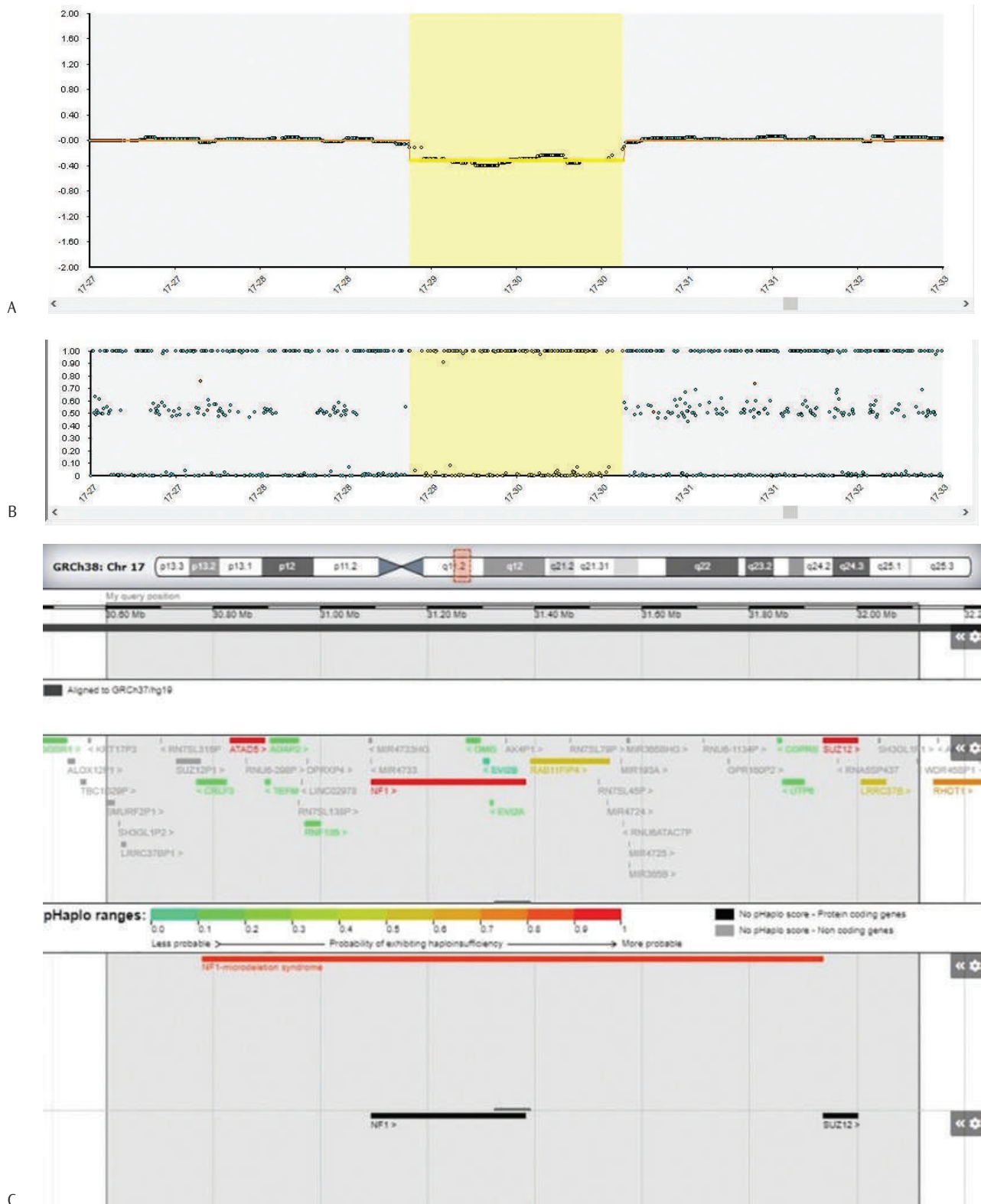


Figure 2. Microarray analysis of the patient. The log R chart (A) showed deletion of the region and the B-allele chart (B) revealed the absence of heterozygous allele peak in deleted region. The deletion (gray shaded area) included the “NF1-microdeletion syndrome region” and *NF1*, *SUZ12* genes which is shown by Decipher Genome Browser (C) (<https://www.deciphergenomics.org/browser>).

[OP-06]

Genetics of Charcot-Marie-Tooth Disease: Novel Variants and Rare Inheritances

Müşerref Başdemirci

Konya City Hospital, Konya, Türkiye

Abstract

Introduction: Charcot-Marie-Tooth disease (CMT) constitutes a heterogeneous group affecting motor and sensory neurons in the peripheral nervous system. The clinical features of CMT is variable due to genetic heterogeneity. To date, more than 80 causative genes have been reported. Autosomal dominant inheritance is common, while autosomal recessive inheritance and X-linked inheritance is rare.

Methods: We evaluated the genetic results of 5 cases who presented with a pre-diagnosis of CMT. NGS panel containing 55 genes was performed in four cases. WES was performed on a case with cognitive retardation in addition to CMT.

Results: Hemizygous pathogenic variants in *GJB1* gene was detected in two male cases. One variant [c.223C>T (R75W)] was a previously known variant, while the other [c.379A>C (I127L)] was novel variant. Both cases were evaluated as CMTX. A novel homozygous pathogenic c.2T>G(M1R) variant in the *NEFL* gene was detected in a male patient. The case was evaluated as the third case in the literature as autosomal recessive CMT1F. One of the two female patients had a known heterozygous pathogenic c.280C>T (R94W) variant in the *MFN2* gene, and the other had a novel heterozygous likely pathogenic c.455_456insG (D153Rfs*2) variant in the *GNB4* gene. These cases were evaluated as CMT2A2A and CMTDIF, respectively.

Conclusion: Genetic studies in CMT provide a wide range of benefits, from the identification of novel variants to the establishment of genotype-phenotype correlations. This study found three novel variants as the underlying causes of rare types of CMT with uncommon inheritance.

[OP-07]

Effectiveness of MediSom in Identifying Critical Fusions in Tumor Genome Profiling

Hüseyin Köseoğlu¹, Fatma Seca Giriş¹, Elçin Yüksel¹, Bilsay Metin Ekiz¹, Akif Ayaz^{1,2}, Ömür Gökmen Sevindik³

¹Istanbul Medipol University, Genetic Diseases Evaluation Center, İstanbul, Türkiye

²Istanbul Medipol University, Faculty of Medicine, Department of Medical Genetics, İstanbul, Türkiye

³Istanbul Medipol University Faculty of Medicine, Department of Hematology, İstanbul, Türkiye

Abstract

Introduction: Accurate and efficient detection of gene fusions is critical for the proper management of cancer patient's diagnosis and treatment processes. This detection is often performed using bioinformatics software. The Istanbul Medipol University Center for Genetic Diseases Assessment Center aims to make significant contributions to this field with the MediSom Gene Fusion and SNP Detection pipeline. The capabilities of MediSom have been compared to internationally recognized bioinformatics programs in the detection of critical fusions in tumor genome profiles.

Methods: In this study, a comparative analysis was conducted on data from 17 cancer patients through the use of international bioinformatics software. Out of the 17 patient data, 3 were diagnosed with Sarcoma, and 14 were diagnosed with Lymphoma. Genetic data of patients were analyzed using the MediSom Gene Fusion and SNP Detection pipeline, and the results were compared with the results of 5 FISH studies.

Discussion: The study results demonstrated the successful detection of gene fusions in 15 patients using online software and MediSom. Among the fusion genes identified in Lymphoma patients were 5 cases of BCR-ABL1, 4 cases of ETV6-RUNX1, 2 cases of TCF3-BBX1, and 1 case of P2RY8-CRLF2. In Sarcoma patients, the detected fusions included EWSR1-FLI1, EWSR1-ERG, and PAX3-FOXO1 fusions. Additionally, FISH studies validated the results of MediSom in terms of fusion allele frequency, a critical measure reflecting the accuracy of detection algorithms.

Results: The MediSom Gene Fusion and SNP Detection pipeline have been observed as a valuable tool in the effective detection of gene fusions. When compared to international online software, a high level of concordance was observed. FISH studies further supported the reliability of MediSom. This study could provide valuable contributions to genetic diagnosis and treatment processes. In the future, a more detailed investigation into how MediSom performs in a broader range of patient groups and various genetic variations could shape advancements in this field.

[OP-08]

CGG Repeat Profile and Clinical Characteristics of Fragile X Full Mutant, Premutant and Gray Zone Patients

Hacer Ukba Kına¹, Engin Altundağ¹, Ayşegül Yılmaz², Ummet Abur¹

¹Ondokuz Mayıs University Faculty of Medicine, Department of Medical Genetics, Samsun, Türkiye

²Ondokuz Mayıs University Faculty of Medicine, Department of Pediatric Genetic Diseases, Samsun, Türkiye

Abstract

Introduction: Fragile-X Syndrome (FXS) is the most common cause of heritable intellectual disability (ID). It shows an X-linked inheritance pattern and more common in males than females. In addition to ID in full mutant (FM) patients, premutation carriers are at increased risk for diseases such as premature ovarian failure (POF) and Fragile-X Associated Tremor-Ataxia Syndrome (FXTAS). The aim of this study was to evaluate CGG in the *FMR1* gene genotype and phenotype profile of patients with repeat number 55 and above.

Methods: Ninety-two patients were included in the study. Genomic DNA was isolated from patient samples. CGG numbers with TP PCR test were determined. Patients were evaluated according to the ACMG guidelines for FM, premutant (PM) and gray zone.

Results: Forty-one patients were male and 51 were female. Thirty-four patients FM, 43 patients PM and 15 in the gray zone. Forty-four patients had size mosaicism. Of these patients, 16 had FM and 28 had PM. Expansion was generally found in CGG transmissions from mother to son. Contraction was found in father to daughter transmissions. Twenty-seven patients had attention deficit hyperactivity disorder and 22 patients had autism-spectrum disorder. The majority of these patients had FM. Eighteen patients had POF. Forty-four patients had a family history of early menopause and 93% of these patients were FM and PM. Two patients had findings compatible with FXTAS. In this disease caused by CGG repeat numbers with mitotic and meiotic instability, generational transmission and follow-up in terms of comorbidities are very important.

Keywords: Intellectual disability, Fragile X, premature menopause

[OP-09]

Evaluation of Germline CHEK2 Variants and Challenges in Genetic Counseling

Ömer Salih Akar¹, Güzin Demirağ², Ayfer Kamalı Polat³, İbrahim Yalçın⁴, Mehmet Kefeli⁵, Bahiddin Yılmaz², Sultan Çalıışkan⁵, Ümmet Abur¹, Engin Altundağ¹

¹Ondokuz Mayıs University Faculty of Medicine, Department of Medical Genetics, Samsun, Türkiye

²Ondokuz Mayıs University Faculty of Medicine, Department of Internal Medicine, Division of Medical Oncology, Samsun, Türkiye

³Ondokuz Mayıs University Faculty of Medicine, Department of General Surgery, Samsun, Türkiye

⁴Ondokuz Mayıs University Faculty of Medicine, Department of Obstetric and Gynecology, Division of Gynecologic Oncology, Samsun, Türkiye

⁵Ondokuz Mayıs University Faculty of Medicine, Department of Medical Patology, Samsun, Türkiye

Abstract

Introduction: Germline variants in *CHEK2* gene have been associated with susceptibility to various cancers, especially breast and colon cancer. Although, it is known to be a gene with a moderate effective, it has been found a relationship between the type of mutations and cancer risk. For breast cancer, the odds ratio for truncating variants (eg. c.1100del, c.444+1G>A, ex8_9del) was found to be around 2.5, while for missense variants (eg. I157T, R117G, S428F, T476M) this ratio was around 1.5. Therefore, this situation should be taken into consideration when providing genetic counseling.

Methods: A custom-designed germline panel was applied to 580 patients with suspected familial cancer syndromes and results were evaluated with Qiagen QCI Interpret. All *CHEK2* gene variants detected in patients were collected and evaluated for mutation type and clinical significance based on Clinvar and Varsome data.

Discussion: A total of 18 different variants were detected in 53 patients in the cohort (9%). The E84E variant, which is a benign variant, was the most frequently observed variant with 37 patients 6.3%. Among the truncating mutations, c.444+1G>A (1 patient) and c.592+3A>T (3 patients) were detected, while various missense variants were detected in other cases. Of these, R180C, L183F, I157T, T476M were among the variants with pathogenic/likely pathogenic records in the Clinvar database. W93R, E478Q, D438Y, K141T, H371T, K373T, R519L, R137Q, S356L, E450K mutations were registered as of uncertain clinical significance in Clinvar. E84E and T532T are benign variants detected.

Conclusion: Various variants in the *CHEK2* gene are frequently encountered in daily cancer genetics practice. There is no clarity in the recommendations to be made in case of detection of some variants, especially missense variants, that have been detected throughout the gene and have previously been identified as disease-causing in databases such as Clinvar and in the literature, and this makes genetic counseling quite challenging.

Keywords: CHEK2, Truncating mutation, Familial cancer syndromes, Genetic counselling, Variant of unknown significance

Introduction

The *CHEK2* gene was originally described in 1998 as an effector kinase in cell cycle regulation and ATM-CHK2-p53 DNA damage response pathway. Initial reports denoted the CHEK2 c.1100del variant could be causative in patients with TP53-wt Li-Fraumeni syndromes but following larger studies reported a high frequency (>1%) of this variant in healthy controls and lack of evidence for association to Li-Fraumeni syndrome (1).

Germline variants in *CHEK2* gene have been associated with susceptibility to various cancers, especially breast, colon and prostate cancers (2). Although it is considered that the *CHEK2* gene is a moderate risk gene for cancer predisposition, the risk is associated with the mutation type, family history, and modifying risk factors (3). Due to this uncertainty there is a big challenge for management of patients with CHEK2 germline pathogenic variants (GPV). Also with the emerging sequencing technologies, lots of variants with unknown significance were described among the genes (4).

Here, we report the distribution of all *CHEK2* gene variants in our cohort with tumor predisposition.

Materials and Methods

Patients referred for genetic analyses for familial cancer syndromes were enrolled in this study. DNA was extracted from peripheral blood with QIAamp® DNA Mini kit. Library preparation was achieved with a custom-design panel including all coding regions for *CHEK2* gene. High-throughput sequencing were done with Illumina Mi-Seq and Next-Seq platforms. Raw data were analyzed with QIAGEN CLC Workbench Premium and variants were interpreted with QIAGEN Digital Insights. All *CHEK2* gene variants detected in patients were collected and evaluated for mutation type and clinical significance based on Clinvar and Varsome data.

Results

A total of 580 patients with clinical suspicion of hereditary cancer syndromes including breast, ovarian and endometrium cancers were enrolled in this study. All the variants detected regardless of clinical significance were recruited. A total of 18 different variants were detected in 53 patients in the cohort (9%). A well-known benign variant, E84E, was the most frequently observed variant with 37 patients (6.3%). Also a benign variant T532T was detected in two patients (0.35%).

Two different truncating and 14 different missense variants were detected among the patients. Truncating mutations, c.444+1G>A was detected in a patient and c.592+3A>T was detected in 3 patients. Missense variants included R180C, L183F, I157T, T476M, W93R, E478Q, D438Y, K141T, H371T, K373T, R519L, R137Q, S356L, E450K. Among these missense variants, for R180C, L183F, I157T, T476M, there were both pathogenic/likely pathogenic and unknown significance citations in the Clinvar database. W93R, E478Q, D438Y, K141T, H371T, K373T, R519L, R137Q, S356L, E450K variants were registered as of uncertain clinical significance in Clinvar.

Discussion

Various variants in the *CHEK2* gene are frequently encountered in daily cancer genetics practice. There is no clarity in the recommendations to be made in case of detection of some variants, especially missense variants, that have been detected throughout the gene and have previously been identified as disease-causing in databases such as Clinvar and in the literature, and this makes genetic counseling challenging. Also there are many emerging new association efforts with various cancers (5). There have been a big challenge for genetic counseling in these patients. Clinical recommendation guidelines like “NCCN Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic Version: 2.2024” and “Genetic/Familial High-Risk Assessment: Colorectal Version: 1.2023” include parts concerning *CHEK2* gene. In these guidelines, it is described that primary breast cancer has 20-40% absolute risk and annual mammogram and breast magnetic resonance imaging (MRI) should be recommended. Also colorectal and prostate cancer risks are determined for CHEK2 variants. Also it is denoted that this risk is based only frameshift mutations and missense variants like I157T have lower risk ratios. So genetic counseling is unclear for missense variants (6).

A new practice resource have been published by “American College of Medical Genetics and Genomics”. According to this paper, truncating variants were associated with the breast, colorectal and prostate cancers moderately. In the truncating mutations, mammogram and breast MRI from age 40 years for female breast cancer, self-examination for males breast cancer, measuring PSA levels for prostate cancers with family history have been recommended. For breast and colon cancer, missense variants are not clinically actionable in isolation and surveillance should be based on family history. For prostate cancer, it is not clear both the risk scores and management (7).

Conclusion

Lots of variants in *CHEK2* gene have been reported in the daily clinical genetic practice and there are lots of challenges about the genetic counseling. The latest published report is a candidate to remove the confusion on this issue. However, radical methods are not currently recommended for any of the CHEK2 variants.

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[OP-10]

Importance of SNP-Based Microarray Technology in Disorders of Sexual Development Work-up; Lessons Learned from a Male Case with 46,XX Karyotype

Ali Torabi, Talha Laçın, Burak Aktaş, Ali Çiçekli, Özkan Bağcı, Ebru Marzioğlu Özdemir, Nadir Koçak, Tülin Cora, Muammer Büyükinan, Muhammed Emin Sarı, Gülsüm Türkoğlu

Selçuk University Faculty of Medicine, Department of Medical Genetics, Konya, Türkiye

Abstract

A 3-year-old male with 46,XX karyotype was referred to the medical genetics department. The post-partum manifestation was normal except micropenis, mid-penile hypospadias, and bilateral cryptorchidism findings. Thirty-seven weeks cesarean sectioned our patient's weight, height and body mass index percentile had been measured at -0.65 SDS, -1.01 SDS, and 0.2 SDS respectively. Ultrasonographic imaging confirms the testicular position without any atrophic or parenchymal abnormality with the right inguinal hernia. Normal Sertoli cell functions were revealed after serial anti-Mullerian hormone testing. A gonadal single dose stimulation test with 250 mcg/1.73 m² dosage of Ovidrel® revealed normal testosterone production as well. FISH-SRY revealed not any positive signals in 150 interphases and 5 metaphases first. SNP-CMA (>700K SNP probe) test was done utilizing GenomeStudio v2.0.5 software that confirms XX pattern in IGV. By using the chromosomal browser tab in the software, proximal abnormal patterns in the Y chromosome were detected. This pattern was different from other Y chromosomal patterns in the normal females with XX karyotype. Further SNP-Probe analysis shows the positiveness of the RPS4Y, ZFY, and SRY-associated SNP-Probes. By smooth filtering of the LOGR ratio, the very low mosaic pattern was revealed in the mentioned probes, which was later confirmed by the high metaphase FISH-SRY study. This case is showing the importance of gene(probe)-based approach analysis as well rather than the chromosomal or copy number variation-based approach only. On the other hand, it also clarifies the importance of the comparative (case-control) analysis approaches in the SNP microarrays technique.

[OP-11]

Investigation of the Effects of Conditioned Medium Derived from Amniotic Membrane on Proliferation in Cancer and Normal Cell Lines

Zeynep Betül Sarı^{1,2}, Muhammed Emin Sarı¹, Ayten Ertuğrul¹, Aybike Pekin³, Tülin Cora¹

¹*Selçuk University Faculty of Medicine, Department of Medical Genetics, Konya, Türkiye*

²*Selçuk University Advanced Technology Research and Application Center, Konya, Türkiye*

³*Selçuk University Faculty of Medicine, Department of Obstetrics and Gynecology, Konya, Türkiye*

Abstract

Introduction: Human amniotic membrane (hAM), obtained after birth, is used in the treatment of various diseases due to its anti-angiogenic, anti-microbial and anti-proliferative properties. In recent years, researches have been focused on the anti-cancer properties of the amniotic membrane. However, reports on this subject are limited. In this study, we aimed to evaluate the anti-cancer effect of the conditioned medium (CM) derived from hAM on various cancer cell lines *in vitro*.

Methods: After cesarean section, placentas of healthy donor mothers were taken and amniotic membrane was obtained. hAM-CM was obtained by incubating amniotic membrane pieces with DMEM with 10% FBS and 1% PSA for 24 hours. HEK293, HUVEC and BV2 for normal cell groups; HEp2, PANC, MDA, A549 for cancer cell groups were cultured. To evaluate the cell viability of hAM-CM in normal and cancer cell lines, XTT test was performed by treating with 75% hAM-CM for 48 hours and the effects on the viability of cancer and normal cells compared to control cells were evaluated.

Results: According to the XTT results, after AM-CM treatment, the viability of normal cells decreased by an average of 13% ($p < 0.05$), while there was also a decrease in the viability of cancer cells (HEp2, PANC, MDA, A549) by 4% (ns), 12% ($p < 0.01$), 35% (ns), 18% ($p < 0.001$), respectively.

Conclusion: Our results showed that the CM produced from hAM inhibited the proliferation of some cancer cells, while it would not be effective in others. hAM application for breast and lung cancer may be a new hope.

Keywords: Amniotic membrane, cancer, conditioned media, cytotoxicity, proliferation

Introduction

Cancer is one of the leading causes of death in the world (1). Despite conventional treatments such as surgery, chemotherapy and radiotherapy, cancer still has significant morbidity and mortality. Over the last decades many research studies have shifted their interests from conventional therapies to new therapies (2). Human amniotic membrane (AM) has recently attracted attention as an alternative treatment against cancer. The inner layer of the placenta is called amniotic membrane. AM, a biological barrier that supports the fetus, consists of amniotic epithelial cells, amniotic mesenchymal stem cells and fibroblasts (3). Due to its important biological properties, the AM has been used in medicine, especially in the field of ophthalmology and dermatology, for more than a century (4). In 1910, Davis used AM as a wound dressing for burn patients (5). Currently, AM derivatives are used in almost every field of medicine such as ophthalmology, plastic surgery, dermatology, cardiology, neurology, urology, diabetology, nephrology, pneumology, hepatology, transplantation, dental surgery, gynecology, orthopedic surgery and ear, nose and throat. There are more than 180 ongoing or completed clinical trials on this topic registered worldwide (6).

Different properties of AM, such as proapoptotic, anti-angiogenesis, cell cycle arrest, and immune regulatory, have made AM a suitable candidate for cancer-related research. In oncology, there is little research on AM, but it is anticipated that it may have more potential to be explored with further research (6). Seo et al. (7) first put forward a hypothesis on the potential of AM's anti-carcinogenic properties in 2008. Studies conducted so far have revealed the anti-cancer properties of AM through the secretion of different proteins and cytokines. Studies on the anti-tumoral effects of AM and cells in a limited number of cancer models indicate a potential anti-cancer effect of AM (8-10).

The anti-cancer mechanism of AM has not been clarified yet. Therefore, further studies are needed to elucidate the molecular mechanisms of the amniotic membrane, determine the factors involved in its anti-cancer effects, and translate amniotic cancer treatment into clinical practice. Because it is predicted that AM can be used as a new, safe and inexpensive substance with fewer side effects for cancer treatment in the future, due to its anti-inflammatory, anti-fibrotic, pro-apoptotic and anti-angiogenic effects. Thus, in this study we planned a pilot study aiming to investigate the anti-cancer effects of the human AM conditioned medium (hAM-CM) on normal and cancer cell lines. Our results may provide new perspectives about cancer treatment.

Materials and Methods

Placental Tissue Collection and Preparation of Amniotic Membran Condition Medium:

After obtaining informed written consent from all subjects according to the guidelines set by the Ethical Committee of Selçuk University of Medical Sciences, placentas were collected from Selçuk University Faculty of Medicine, Department of Obstetrics and Gynecology. Placentas (40 weeks) from healthy donor mothers ($n=6$) after caesarean section from normal term pregnancies were harvested, suspended in cold PBS and transported to the laboratory. The AM was manually stripped and cut from the chorionic membrane and placed in sterile physiological solution. Bloody or torn pieces of the AM was washed two or three times to completely remove them and then cut into 4 equal pieces. Each piece will be sterilised in a laminar flow by continuous washes in PBS containing 100 U/mL penicillin and 100 µg/mL streptomycin. Amnion pieces were placed in 10 cm plates and incubated with DMEM with 0% FBS for 24 hours. After removing the AM fragments, the remaining medium is centrifuged at 1,200 rpm for 5 min to remove cellular debris, after which the cell pellet was discarded; the remaining medium is termed human conditioned medium (hAM-CM) and was pooled from 10 different placenta donors and stored at -20°C until used in experiments.

Cells and Cell Culture

HEK293 (human embryonic kidney cells), HUVEC (human umbilical vein endothelial cells), BV2 (mouse microglial cells), HEp2 (human larynx cancer cell), PANC (human pancreatic cancer cells), MDA (human breast cancer cells), A549 (human lung cancer cells) were purchased from the American Type Culture Collection (ATCC). Both of the cells were maintained in Dulbecco's Modified Eagle's Medium (DMEM) (Gibco, USA) supplemented with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin/streptomycin (Gibco, USA) in a humidified 5% CO_2 incubator at 37°C .

Cell Proliferation Assay

Cells were seeded into 96-well plates (1×10^4 cells per well). After overnight attachment, the medium was replaced with hAM-CM diluted in a ratio of 3:4 (75%) with a complete medium for 48 h. An untreated group of cancer and normal cells that incubated with a complete medium was considered as a control group. Cell viability was assessed at 48 h. The XTT kit (BI, USA) was used according to the manufacturer's instructions. Results were obtained by detecting absorbance at a wavelength of 540 nm for formazan and 650 nm for background with the microplate reader (Biotek-Epoch, USA). Three replicate wells were used for each analysis. The percentage of cell viability (%) compared to the untreated cells was determined at each concentration using the formula provided below:

$$\text{Cell viability (\%)} = \frac{\text{OD of sample}_{540} - \text{OD of sample}_{650}}{\text{OD of control}_{540} - \text{OD of control}_{650}} \times 100$$

Morphology Analysis

To determine the effect of co-culture on cell morphology, treated/untreated normal and cancer cells were examined under an inverted light microscope (Nikon) after 48 h before XTT assay.

Statistical Analysis

Data were presented as the mean \pm standard deviation of three independent assays, and data were analyzed using the GraphPad Prism software version 6.00 for Windows (GraphPad Software, USA). The student's t-test was used to perform statistical analysis. Differences were considered significant at $p < 0.05$.

Results

Effects of hAM-CM on Cell Morphology

In AM-CM-treated cell groups, only the morphology of microglia cells (BV2) differed compared to control cells. The connection between cells was broken after treatment. On the other hand, no significant changes were observed in the morphology of other cells (Figure 1A). There is no significant morphological differentiation in the cancer group compared to the control groups, except for breast cancer cells. However, breast cancer cells have severe morphological differentiation as if under stress (Figure 2A).

Effects of hAM-CM on Cell Proliferation

According to the XTT cell viability assay results, there was a decrease in cell viability in both healthy and cancer cells when compared to untreated control cells. In normal cells (HEK293, HUVEC, BV2), there was a decrease of 13% (ns), 14% ($p < 0.01$), and 11% ($p < 0.01$), respectively (Figure 1B). In cancer cells (HEp2, PANC, MDA, A549), there was a decrease of 4% (ns), 12% ($p < 0.01$), 35% (ns), and 18% ($p < 0.001$), respectively (Figure 2B).

Discussion

Using hAM has shown encouraging results in the treatment of various diseases; but, their impact on cancers is contradictory and insufficient. Thus, we investigated the anti-cancer properties of the conditioned medium derived from the human AM on cancer cells to determine whether AM produce paracrine mediators that affect cancer cell culture growth.

To be sure whether the effect of AM-CM on cancer cells is specific or not, we also evaluated its effect on normal cells. For this reason, we studied laryngeal, pancreatic, breast and lung cancer cells, as well as epithelial (HEK), endothelial (HUVEC) and microglia (BV2) cells. As a result, the viability rate of normal cells was 87% on average, which was lower than the viability rates of MDA (65%) and A549 (82%) cells compared to cancer cells. In the study of Rolfo et al. (11), AM did not inhibit the proliferation of normal prostate cells. Based on these results, we can conclude that the anti-cancer activity of AM is cell dependent. Therefore, by treating AM in different cell lines, the cells in which AM is most effective can be investigated. Mamede et al. (3) treated prostate, breast, colon, lung, pancreatic, biliary, endometrial, bladder, esophagus, liver, bone and skin cancer cells with proteins derived from AM (AMPE). Accordingly, they proved that some cancer cell lines are much more sensitive to AMPE. This result is in accordance with our data that the inhibition of metabolic activity by AMPE may differ even between different cell lines of the same cancer type, depending on the specific genetic profile of each cell line (3). Therefore, it is important to study the individual response of each cell line.

Jafari et al. (10), in their study, demonstrated inhibition of cell proliferation in breast cancer cell lines treated with AM-CM. According to our data, among the four cancers we studied, the inhibitory effect of AM-CM was highest in breast cancer.

Rolfo et al. (11) studied with CM which was produced by incubating mesencimal cells derived from mesencimal cells (hAMSC) with DMEM medium for 3 days and 1 week. Their data indicated that hAMSC microenvironment inhibited prostate cancer cells growth. In our work, during the production of AM-CM, amniotic fragments were incubated in the medium for 24 hours. If this period is increased to 48 or 72 hours or more hours, it will be clear whether the activity of AM-CM on cancer cells will increase or not.

In the literature, different forms of AM such as whole membrane, fresh, dried or freeze-stored, and AM-derived epithelial cells and mesenchymal cells have also been used (12-14). In this study, we used conditioned media obtained from fresh whole membrane. In future studies, the effect of these forms of AM on cancer cells can also be evaluated.

AM has been reported to have many functions such as anti-angiogenic, anti-fibroblastic, proapoptotic, anti-microbial, immunomodulatory activities as well as enhancing cell migration and growth (15-18). In our study, we investigated the effect of AM on cell growth. We aim to investigate other effects such as migration, metastasis, apoptosis in future studies.

Conclusion

Our data report that conditioned media delivered from human AM probably contains soluble factors responsible for triggering an anti-tumor response. But its effect is cell dependent.

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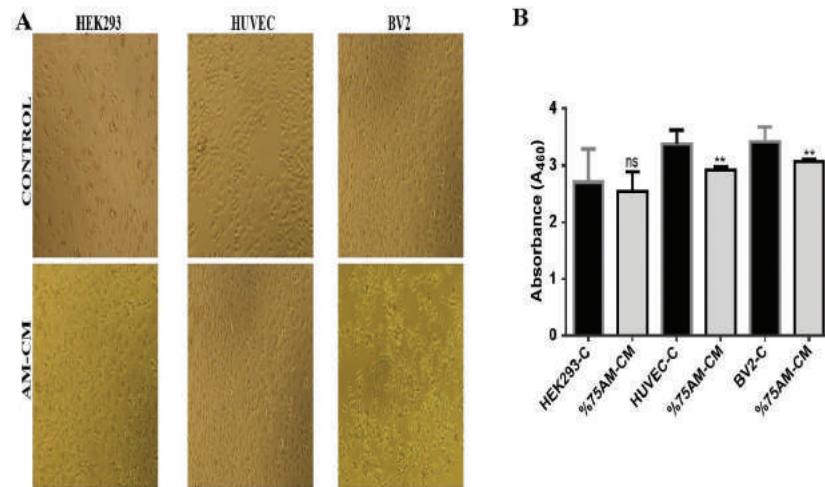


Figure 1. The effect of hAM-CM treatment on cell morphology and proliferation in normal cells. (A) The morphology in control, and treated groups of HEK293, HUVEC and BV2 cells. (B) hAM-CM decreases cell proliferation in HUVEC and BV2 cells (**p<0.01).

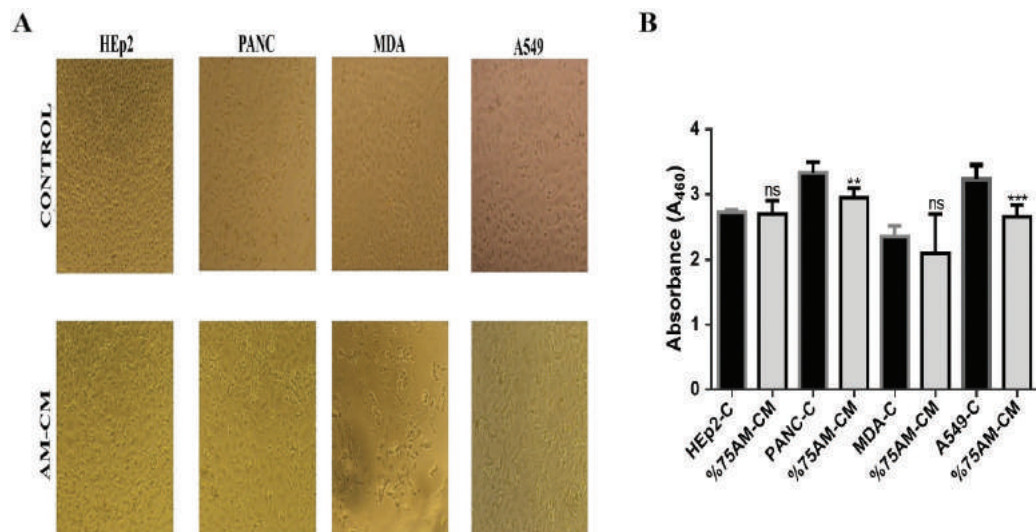


Figure 2. The effect of hAM-CM treatment on cell morphology and proliferation in cancer cells. (A) The morphology in control, and treated groups of HEp2, PANC, MDA and A549 cells. (B) hAM-CM decreases cell proliferation in PANC, MDA and A549 cells (**p<0.01; ns and ***p<0.001).

[OP-12]

Molecular Diagnosis in Spinocerebellar Ataxias

Fatma Demir¹, Büşranur Çavdarlı¹, Gülay Güleç Ceylan^{1,2}, C. Nur Semerci Gündüz^{1,2}

¹Ankara City Hospital, Clinic of Medical Genetics, Ankara, Türkiye

²Ankara Yıldırım Beyazıt University Faculty of Medicine, Department of Medical Genetics, Ankara, Türkiye

Abstract

Introduction: Spinocerebellar ataxias (SCA) constitute a genetically and phenotypically heterogeneous group of progressive neurodegenerative diseases inherited in an autosomal dominant. Mutations causing the disease often result from the expansion of tandem repeats within the disease gene, leading to abnormal elongation of polyglutamine. Among the disease mechanisms of SCAs are toxic RNA gain-of-function, mitochondrial dysfunction, channelopathies, autophagy, and transcriptional dysregulation. In addition, among the autosomal recessively inherited cerebellar ataxias, there are SCA affecting extraneurologic systems with onset of symptoms especially in early childhood.

Methods: A total of 160 patients who presented to our clinic with a preliminary diagnosis of SCA between 2019 and 2023 were included in the study. A panel including SCA 1, 2, 3, 6, 7, and 8 were studied through short tandem repeat analysis. Whole exome sequencing (WES) was performed on two patients with normal results.

Results: Among the 160 patients evaluated with SCA symptoms (female/male: 77/83, average age: 36.4), an increase in repeat numbers explaining clinical findings was detected in 31. Of these patients, 25 were diagnosed with SCA type 1, 5 with type 2, and 1 with type 8. Two patients who were found to have normal results and underwent WES were diagnosed with SCA type 8 (MIM: 610743) and Spastic Ataxia, Charlevoix-Saguenay (MIM: 270550).

Discussion: In this study, the most common types were diagnosed in 19.3% of patients with a prediagnosis of SCA. While types 3, 2, 6, and 1 are most commonly reported in the literature, our study observed types 1 and 2 as the most frequent. Evaluating family history and additional systemic involvement in patients with symptoms starting early is crucial for determining tests for diagnosing other SCA types. Currently, there is no cure for SCAs, but knowing the subtype can assist in managing symptoms, determining treatment strategies to improve quality of life, and accessing preimplantation/prenatal diagnosis options to prevent the disease.

Keywords: Spinocerebellar ataxia, short tandem repeat analysis, whole exome sequencing

[OP-13]

Change in Lipid Metabolism Gene Expressions during TGF- β -Induced Epithelial-Mesenchymal Transition Process

Zeynep Dođru^{1,2}, Akif Ayaz^{2,3}, Abdullah Hüseyin Köseođlu², Erdal Fırat Caralan², Türkan Yiđitbaşı^{1,2}

¹Medipol University Faculty of Medicine, Department of Biochemistry, Istanbul

²Istanbul Medipol University, Genetic Diseases Assessment Center, Istanbul

³Istanbul Medipol University Faculty of Medicine, Department of Medical Genetics, Istanbul

Abstract

Introduction: Epithelial-mesenchymal transition (EMT) is a biological process where cells lose their epithelial characteristics and acquire mesenchymal features. EMT plays a critical role in normal development and cancer metastasis. During EMT, lipid metabolism meets cellular energy demands, contributes to structural changes, and supports metastasis. Transforming growth factor-beta (TGF- β) signaling pathways initiate EMT and regulate the transcription of genes influencing lipid metabolism. This study aims to examine changes in lipid metabolism gene expressions during TGF- β -induced EMT and identify candidate genes that may play a role in this process.

Methods: In this study, Py2T breast cancer cells derived from MMTV-Py2T transgenic mice were cultured to induce a mesenchymal phenotype with TGF- β . RNA isolation was performed from Py2T and Py2T-TGF β cells, followed by a targeted RNA sequence analysis. The study focused on 180 genes involving adipogenesis, lipogenesis, lipid storage, β -oxidation, MEK/ERK pathways, and also examined interleukin gene expressions. Gene pathway analysis was conducted using various bioinformatics tools.

Discussion: These results demonstrate the complexity of lipid metabolism gene expressions during TGF- β -induced EMT and the significance of cellular phenotypes in this process. Additionally, genes such as Trib3, Ceacam1, Bmp2, and Itgb3 may be associated with EMT and could impact cancer prognosis. Future studies could further support these findings by examining the functions of these genes in more detail.

Results: According to the bioinformatics analysis results, significant changes in expression levels were detected in a total of 29 genes, including important genes like Fasn, Cebpa, and Egr1. In comparison to cancer epithelial cells, cancer mesenchymal cells showed a decrease in Trib3 (-2.7-fold) and Ceacam1 (-3.7-fold) gene expression levels and an increase in Bmp2 (5-fold) and Itgb3 (4.4-fold) gene expression levels ($p < 0.05$).

Keywords: Breast cancer, EMT, lipid metabolism, TGF- β

[OP-14]

Evaluation of Genetic Heterogeneity in Joubert Syndrome Case Series

Nigar Shirinova¹, Muhammed Burak Bereketoğlu¹, Özge Sönmezler², Çağla Rencüzoğulları², Sevcan Tuğ Bozdoğan^{1,2}, Ibrahim Boğa^{1,2}, Atıl Bişgin^{1,2}

¹Çukurova University Faculty of Medicine, Department of Medical Genetics, Adana, Türkiye

²Çukurova University AGENTEM (Adana Genetic Diseases Diagnosis and Treatment Center), Adana, Türkiye

Abstract

Introduction: Joubert syndrome is a primary ciliopathy disorder which is inherited as autosomal recessive and characterized with cerebellar and brainstem malformations. In addition to being a clinically heterogeneous disease with multisystemic involvement, it is also genetically heterogeneous as more than 39 genes can be involved in the etiology. Molar teeth sign due to cerebellar vermis hypoplasia on brain magnetic resonance imaging is pathognomonic; and hypotonia, developmental delay, intellectual disability are among the diagnostic criteria. In this study, the results of patients who were referred to our center with a preliminary diagnosis of Joubert syndrome and underwent molecular genetic examinations were presented and evaluated in terms of genotype-phenotype correlation. It was aimed to raise awareness about Joubert syndrome, which is relatively common among rare diseases.

Methods: Among the patients included in the study, in 7 of them targeted genes (*CPLANE1*, *NPHP1*, *TMEM237*, *KIAA0586*, *TMEM67*, *CC2D2A*, *HYDIN*, *KIF7*, *AHI1*, *ARMC9*) were sequenced via Next Generation Sequencing Method and Whole Exome Sequencing was performed in the other 16 patients.

Results: As a result of the studies, homozygous mutations were detected in 7 patients, compound heterozygous mutations in 7 patients, and heterozygous mutations in 9 patients. A total of 21 different variants were detected in 10 different genes. As a result of the evaluation according to ACMG criteria, 15 variants were classified as pathogenic, 5 variants of unknown clinical significance, and the other 1 variant was not previously identified and was classified as possible pathogenic as a result of *in silico* analyses. The c.1466+2T>A variant in the *CC2D2A* gene is the novel mutation to be introduced into the literature. The gene with the most frequent mutation is revealed as *KIAA0586* gene.

Discussion: By revealing the etiopathogenesis of rare diseases, the possibility of early diagnosis, prenatal and pre-implantation genetic diagnosis increases, and preventive medicine can come to the fore. With the increase in similar studies, it will be possible to create national databases and develop new algorithms for diagnosis and treatment.

Keywords: Rare disease, Joubert syndrome, mutation, whole exome sequencing

[OP-15]

A Case of Angelman Syndrome with c.2570_2571delAA Variant in *UBE3A* Gene, and Unusual Findings

Hatice Koçak Eker

Konya City Hospital, Genetic Diseases Evaluation Center, Konya, Türkiye

Introduction

Angelman syndrome is a neurodevelopmental disorder characterized by severe developmental delay or intellectual disability, profound speech impairment, movement or balance disorder, typical abnormal behaviors with an apparent happy demeanor that includes frequent laughing, smiling, and excitability. Microcephaly and seizures are also common. Four known genetic mechanisms may cause Angelman syndrome; deletion of the 15q11.2-q13 critical region (60-75%), paternal uniparental disomy (2-5%), imprinting defect (2-5%) and mutation in the *UBE3A* gene (10%). In a group representing 5-26% of patients, the genetic defect remains unidentified. Here, we present a case with Angelman syndrome with unusual clinical findings, and likely pathogenic variant in *UBE3A* gene.

Case Report

Seven-years-old male patient was evaluated for developmental delay. He could not speak, his 13 teeth were extracted because of caries, he slept all day, and he could stay awake with treatment, he had sensory integration defect. On examination, he had microcephaly, deep set eyes, macrostomi, scoliosis, tremor on right hand, and wide-based gait. His karyotype, microarray, 15q11.2-q13 methylation analysis were normal. Thereafter we have performed the sanger sequencing analysis of *UBE3A* gene. We found a heterozygous likely pathogenic frameshift c.2570_2571delAA(K857Tfs*2) variant (NM_000462.5). The parents had normal sequencing for the same position.

Discussion

Despite the sleep problem was contrary to the literature, electroencephalogram was normal, hypopigmentation and happy puppet appearance and typical position were absent, the diagnosis was made with some remarkable clinical features initially. This rare case is presented to emphasize the importance of proceeding with clinical findings, and to contribute to the phenotype-genotype correlation.

Rare Syndrome Caused by a Novel Splice Variant in *UBE2A*: A Case Report

Esma Kayılıoğlu¹, Fatma Demir¹, Ahmet Cevdet Ceylan^{1,2}

¹Ankara Bilkent City Hospital, Clinic of Medical Genetics, Ankara, Türkiye

²Ankara Yıldırım Beyazıt University, Department of Medical Genetics, Ankara, Türkiye

Introduction

X-linked intellectual disabilities are responsible for approximately 10-12% of intellectual disability cases in males. Nascimento type X-linked syndromic cognitive developmental disorder (MRXSN) is one of these conditions. Mutations in the *UBE2A* gene and large Xq24 deletions encompassing *UBE2A* are responsible for this condition. The *UBE2A* gene encodes the E2 enzyme (ubiquitin-conjugating enzyme) involved in attaching ubiquitin to proteins. In MRXSN, in addition to intellectual disability, individuals may exhibit seizures, speech difficulties, dysmorphic facial features, skin abnormalities, and urogenital system anomalies.

Case Report

A 6-year-old male patient, who is being followed up for diagnoses of epilepsy and global developmental delay at the Neurology Department, is being consulted. He has been experiencing seizures since the age of 11 months, occurring approximately once a year. These seizures are characterized by the patient staring at a single point and experiencing atonia in the body. The patient was born to healthy, non-consanguineous parents after an uncomplicated 38-week pregnancy. Sitting without support started at age 1.5 and walking in the form of steps was achieved at the age of 4. The child has never developed speech and only produces non-sensical sounds. He understands simple commands. In physical examination; obese appearance, broad face, synophrys, deep-set almond-shaped eyes, wide mouth, short neck, broad and flat nasal bridge, widely spaced nipples, micropenis, onychodystrophy in toenails, and hirsutism on the back and arms were observed. Additionally, bilateral frontal paroxysmal findings were detected in electroencephalography (EEG). Due to the syndromic appearance, microarray analysis was performed on the patient, which yielded normal results. In the whole exome analysis, the hemizygous c.241+5G>A variant was detected in the *UBE2A* gene and was interpreted as likely pathogenic. This variant was also identified as heterozygous in his mother. Segregation analysis of other family members is ongoing.

Discussion

MRXSN, initially described in 2006 by Nascimento and colleagues, was identified in three individuals diagnosed with cognitive impairment, where a non-sense mutation in *UBE2A* was detected. In addition to that, in cases reported so far, large and small deletions, various missense mutations, an insertion, and two splicing variants have been identified. These splicing variants were identified in a 5-year-old Chinese patient and two Italian brothers aged 26 and 36. In the case of the 5-year-old patient, cognitive impairment, speech disorder, and white matter anomalies were observed. However, due to the absence of dysmorphic facial features, seizures, skin anomalies or, urogenital system abnormalities, this case was considered mild. In contrast, dysmorphic facial features are evident in Italian siblings. In addition to the neurodevelopmental features of the syndrome, skin and urogenital system anomalies, serious behavioral disorders have been observed.

Conclusion

The distinctive dysmorphic facial features, skin, nail, and urogenital anomalies, intellectual disability and neurodevelopmental delay observed in MRXSN patients suggest that this syndrome presents clinically recognizable characteristics. In cases of undiagnosed intellectual disability in male patients, differential diagnosis may consider *UBE2A*-related MRXSN based on a detailed medical history, physical examination, and family history. With this rare case, which has been identified in 20 families in the literature so far, it is aimed to better understand the characteristics of the syndrome and demonstrate the impact of the observed novel variant on the phenotype.

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[OP-17]

Neuromuscular Diseases, Genetic Etiology and Next Generation Sequence Analysis

Makbule Nihan Somuncu, Emine Gökteş, Ayşe Gül Zamani, Mahmut Selman Yıldırım

Necmettin Erbakan University, Meram Faculty of Medicine, Department of Medical Genetics, Konya, Türkiye

Abstract

Introduction: Neuromuscular diseases (NMD) are characterized by the deterioration of muscles nerves building blocks of the movement system. Non-congenital acquired NMD may occur due to environmental factors, infections, side effects of various pharmacological treatments. On the other hand, gene variants that affect muscle-nerve structure function have a major role, which has a genetic or hereditary etiology.

Methods: This study aims to investigate NMD using the next generation sequence (NGS) analysis method from patients who applied to the Department of Medical Genetics with the prediagnosis of neuromuscular involvement between the years 2020-2023. In our study, more than 200 NMD-related gene NGS methods were analyzed in twenty-four pre-diagnosed patients with NMD.

Discussion: NMD is classified as anterior horn, peripheral nerve, neuromuscular junction and muscle diseases. In the classification, spinal muscular atrophy, amyotrophic lateral sclerosis, Guillain Barre syndrome, Charcot-Marie-Tooth, congenital myasthenic syndromes, muscular dystrophies are among the prominent ones. DNA sequence analysis is extremely important of NMD diagnosis algorithm of patients. Therefore, it becomes possible to perform NMH typing in such a wide spectrum and to target gene therapy with the detection of the variant, as in Duchenne muscular dystrophy. The novel variants will contribute to the development of differential diagnosis and treatment strategies, combination of clinic and genetic diagnosis will reveal the definitive diagnosis.

Conclusion: NMH-related VUS, pathogenic variant was detected in patients in the study, a clinically compatible genetic diagnosis of the patients was determined. For variant zygosity and novel detected variants, segregation analysis was performed, genetic counseling was given for risky individuals and carriers in the family.

[OP-18]

Genetic Epilepsy and Molecular Diagnosis

Makbule Nihan Somuncu, Emine Gökteş, Mahmut Selman Yıldırım, Ayşe Gül Zamani

Necmettin Erbakan University, Meram Faculty of Medicine, Department of Medical Genetics, Konya, Türkiye

Abstract

Introduction: Epilepsy is a neurodegenerative disease with phenotypic and genotypic heterogeneity. While a single gene mutation may be responsible for etiopathology, epilepsy types with familial inheritance or predisposition may also show complex inheritance together with environmental factors. In parallel with the development of molecular genetic techniques, epilepsy-related gene variants can be identified with next generation sequence (NGS), the inheritance of the disease can be revealed and genetic counseling can be given with risk assessment.

Methods: This study aims to investigate the genetics of epilepsy using the NGS analysis method from patients who applied to the Department of Medical Genetics with a prediagnosis of epilepsy between the years 2020-2023. Early infantile epileptic/encephalopathy panels were studied through Minieq sequencing system. The pathogenicity classification of the detected variants over a hundred thirty patients was determined together with the clinical diagnosis. The segregation analyses were performed according to the zygosity, in line with their pedigrees.

Discussion: Rapid advances in NGS have evolved to exciting results in epilepsy genetics. The combination of epilepsy-related gene panels and clinical tests has become more common, leading to more effective diagnoses, especially in early-onset epilepsy. This diagnostic algorithm sheds light on the pathophysiology of both early-onset benign and epileptic syndromes and devastating developmental and epileptic encephalopathies.

Conclusion: Among the patients included in the study, variant classification was made according to pathogenicity scoring according to NGS results, and segregation was determined in patients with novel or homozygous variants. In line with the results, molecular genetic diagnosis and genetic epilepsy determination of the patients were revealed.

5-Aza-2'-Deoxycytidine May Suppress Prostate Cancer Cell Proliferation by Altering The Expression of miRNAsİlknur Suer^{1,2}, Murat Kaya²¹Istanbul University, Istanbul Faculty of Medicine, Department of Medical Genetics, Istanbul, Türkiye²Istanbul University, Istanbul Faculty of Medicine, Department of Internal Medicine, Division of Medical Genetics, Istanbul, Türkiye**Abstract**

Introduction: Prostate cancer (PCa) is one of the most common and lethal malignant tumors. 5-Aza-2'-deoxycytidine (Aza) is a methylation inhibitor and an essential drug used in the treatment of different malignancies by modifying the expression of several genes. In our study, the effects of Aza on cellular processes and expression of miR-15a-5p, miR-16-5p, and miR-7-5p in PCa cells were investigated.

Methods: DU145 and PC3 PCa cells were treated with 10 µM Aza (Aza+), and control cells were treated with 10 µM DMSO (Aza-). The effect of Aza on PCa cell proliferation was determined using the WST-8 method, and the effect on apoptosis was determined using the Caspase-3 assay kit. examined. The migration potential of cells was evaluated using a scratch assay. cDNA was then synthesized from the total RNA samples obtained from Aza(+) and Aza(-) cells. MiR-15a-5p, miR-16-5p, and miR-7-5p were quantified by qRT-PCR using the TaqMan primer-probe assay.

Results: In DU145/Aza(+) and PC3/Aza(+) cell lines, proliferation, and migration were significantly decreased and apoptosis was increased in Aza(+) cells compared to Aza(-) cells. In addition, decreased expression of miR-15a-5p and miR-16-5p was detected in DU145/Aza(+) and PC3/Aza(+) cells compared to the control group, while increased miR-7-5p expression was detected.

Discussion: Azacitidine suppressed the proliferation and migration of PCa cells and induced apoptosis. Azacitidine may have affected these cellular processes by directly miRNA hypomethylation or indirectly by changing the expression of miR-15a-5p, miR-16-5p, and miR-7-5p through different target genes.

Keywords: Prostate cancer, DU145, PC3, 5-Aza-2'-deoxycytidine, miRNA

Introduction

Prostate cancer (PCa) is the second most common cancer among men. It has been reported that the combined application of 5-Aza with various chemotherapeutics (such as docetaxel) in the treatment of PCa has a positive contribution to the increase in treatment sensitivity, especially in chemotherapy-resistant patients (1). MicroRNAs (miRNAs) are small molecules. Although non-coding, they are crucial molecules because they act as regulators of the coding genes. miRNAs targeting oncogenes are called tumor suppressor miRNAs (Ts-miRs), while those targeting tumor suppressor genes are called Onco-miRs (2). 5-Azacitidine is an effective but toxic methylation inhibitor. 5-aza-2'-deoxycytidine (also known as 5-Aza or decitabine) was synthesized by reducing the toxicity of this molecule. This agent is used to demethylate methylated genes and non-coding RNAs that regulate gene expression for therapeutic purposes in cancer (3). It has been suggested that miR-15a and miR-16-1, which are detected at reduced levels in the tumor tissue and serum of patients with PCA, may be non-invasive biomarkers for the diagnosis and prognosis of PCa (4-6). In addition, miR-7 expression has been reported to decrease in drug-resistant PCa tissues (7). In our study, the effects of Aza on cellular processes and the expression of miR-15a-5p, miR-16-5p, and miR-7-5p in PCa cells were investigated.

Materials and Methods

Culture of DU145 and PC3 cells and treatment with 5-Aza-2'-deoxycytidine: DU145 and PC3 cells were cultured in RPMI1640 medium containing 10% FBS and 1% antibiotics at 37°C and 5% CO₂. The 50% inhibitory concentration (IC₅₀) of 5-Aza in DU145 and PC3 cells was determined as 10µM. DU145 and PC3 PCa cells were treated with 10µM Aza (Aza+), and control cells were treated with 10µM DMSO (Aza-). The effect of 5-Aza-2'-deoxycytidine on PCa cell proliferation, apoptosis, and migration: For the proliferation assay, cells were seeded in triplicate in a 96-well plate. On the other hand, cells were seeded on a 6-well plate for apoptosis and migration assays. The effect of Aza on PCa cell proliferation was investigated using the WST-8 method (Ecotech Biotech), and the effect on apoptosis was determined using the Caspase 3 Human Instant ELISA Kit (Invitrogen) according to the manufacturer's protocol. The effect of Aza on PCa cell migration was examined using the scratch assay. PCa cells were seeded in 6-well plates (4 × 10⁵ cells/well). Scratches were performed using a 100 µL pipette tip when the cells reached 100% confluency. After 48 h, the scratch closure potentials of the Aza (+) and Aza(-) cells were compared under an inverted microscope. cDNA synthesis and expression analysis: cDNA was synthesized from 30ng/µl total RNA samples obtained from Aza(+) and Aza(-) cells. MiR-15a-5p, miR-16-5p, and miR-7-5p were quantified by qRT-PCR using a TaqMan primer-probe assay (Thermo Fisher).

Statistical Analysis

The student's t-test and the 2-ΔΔCt method were used for statistical data analysis. P value less than 0.05 was considered significant. GraphPad Prism 9.5.1 program was used to create the figures, and values are indicated as p≤0.05 (*), p≤0.01 (**), and p≤0.001 (***)

Results

When the effect of 5-Aza on cellular processes in DU145/Aza(+) and PC3/Aza(+) cells was observed; it was detected that proliferation (Figure 1) and migration (Figure 2) were significantly decreased and apoptosis (Figure 3) increased in Aza(+) cells compared to Aza(-) cells.

In addition, decreased miR-15a-5p ($p=0.013$; $p=0.003$, respectively) and miR-16-5p ($p=0.003$; $p=0.011$, respectively) expressions were detected in DU145/Aza(+) and PC3/Aza(+) cells compared to the control group, while increased miR-7-5p ($p=0.002$; $p=0.0012$, respectively) expression was detected (Figure 4).

Discussion

It has been understood that 5-Aza-2'-deoxycytidine suppresses the proliferation and migration of PCa cells and induces apoptosis. This may have affected these cellular processes by direct miRNA hypomethylation or indirectly by altering the expression of miR-15a-5p, miR-16-5p, and miR-7-5p through different target genes.

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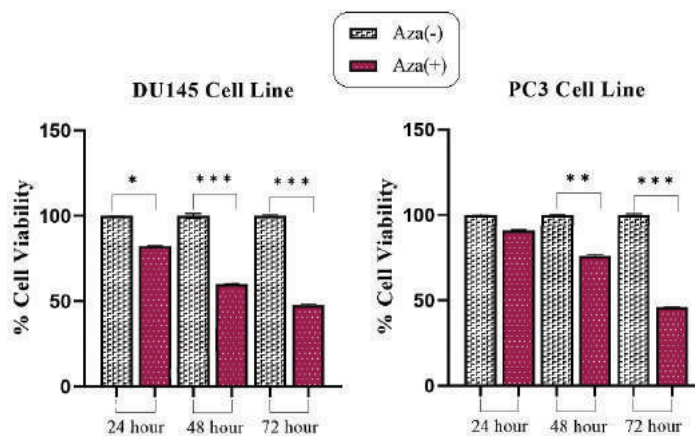


Figure 1. The effect of 5-Aza-2'-Deoxycytidine on PCa cell proliferation

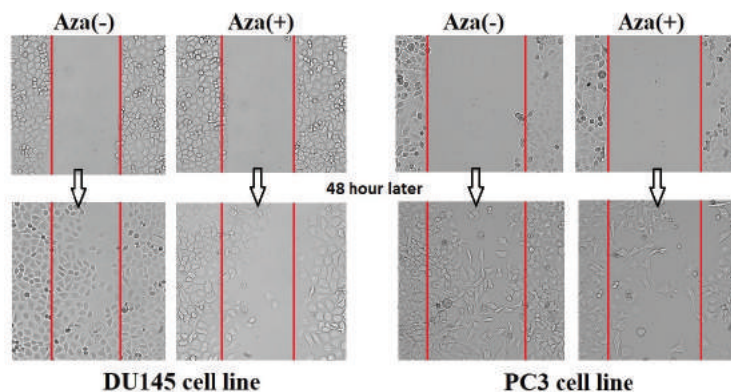


Figure 2. Cell migration (scratch) assay of PCa cells treated with 5-Aza

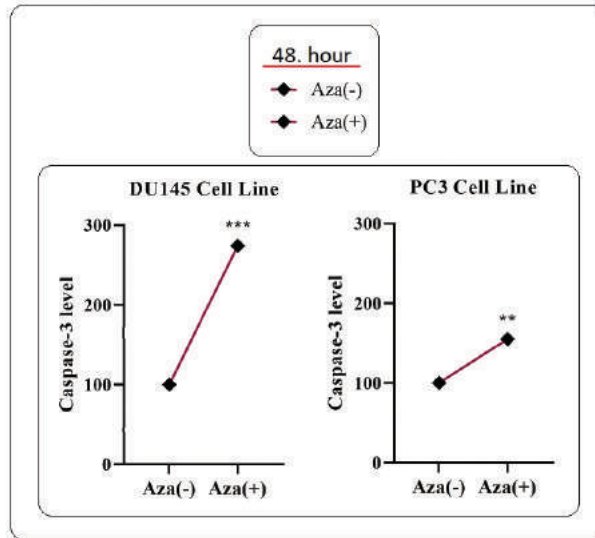


Figure 3. Apoptosis assay result for DU145/Aza(+) (p=0.00017) and PC3/Aza(+) (p=0.002) cell lines

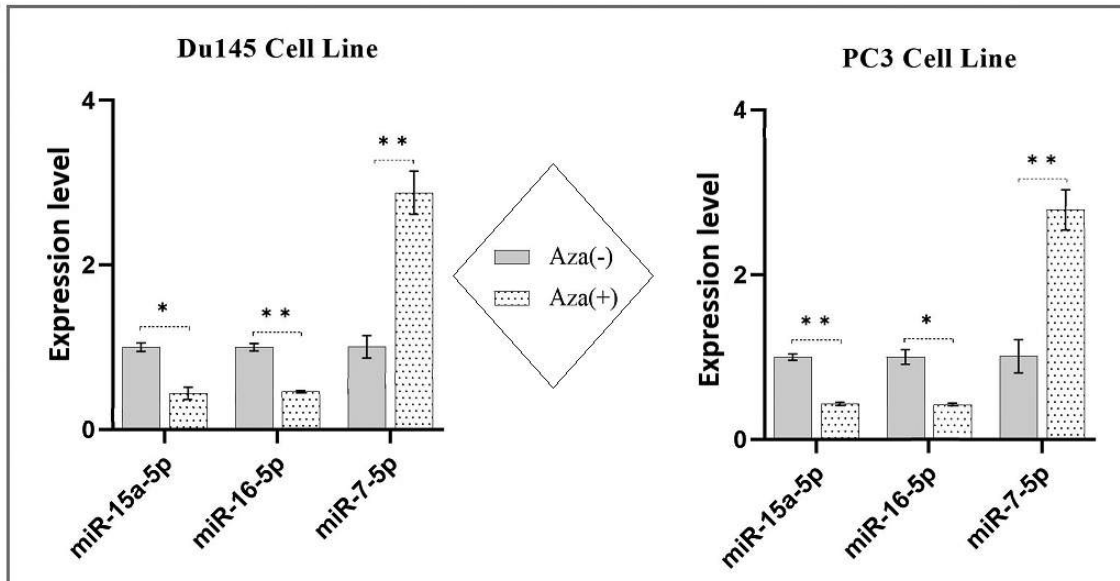


Figure 4. Relative expression level of miR-15a-5p, miR-16-5p and miR-7-5p in Aza(+) and Aza(-) cell

[OP-20]

Investigation of *NF-κB* Gene Polymorphisms in Individuals Infected with COVID-19 Virus

Nil Özbilüm Şahin¹, Burcu Bayyurt², Sevgi Baltacı³, Serdal Arslan⁴, Mehmet Bakır³

¹Sivas Cumhuriyet University Faculty of Science, Department of Molecular Biology and Genetics, Sivas, Türkiye

²Sivas Cumhuriyet University Faculty of Medicine, Department of Medical Biology, Sivas, Türkiye

³Sivas Cumhuriyet University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology, Sivas, Türkiye

⁴Mersin University Faculty of Medicine, Department of Medical Biology, Mersin, Türkiye

Abstract

Introduction: Coronavirus disease-2019 (COVID-19) is a disease caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) coronavirus that emerged in Wuhan, China. SARS-CoV-2 is an enveloped, positive-sense, single-stranded RNA virus belonging to the betacoronavirus genus. NF-κB is a family of protein transcription factors that can orchestrate many inflammatory processes. NF-κB genes have been associated with the development of many viral diseases and cancer. Therefore, this study aimed to investigate COVID-19 disease and NF-κB1-94 Ins/Del ATG, NF-κB1A 3'UTR A/G, NF-κB1A -826 C/T polymorphisms.

Methods: The patient group was composed of 150 individuals who were definitively diagnosed with COVID-19 disease by Sivas Cumhuriyet University Faculty of Medicine, Department of Infectious Diseases. Approval was received by Sivas Cumhuriyet University Clinical Research Ethics Committee (decision no: 2021-02/07). The control group consisted of 150 healthy individuals whose blood was taken during the period when there was no COVID-19 epidemic (Ethics Committee decision no: 2009-02/5). Total DNA was isolated from the blood samples taken, and then PCR-RFLP study was performed to determine polymorphisms. The data were uploaded to the SPSS (Ver: 23.0) program and the chi-square test was used for evaluation.

Results: A statistically significant difference was found in the D allele distribution for the NF-κB1 -94 ins/del polymorphism. Additionally, a statistically significant result was found in the WD and DD genotype distribution. For NF-κB1A 3'UTR A/G polymorphism, there was a statistically significant difference in the distribution of the G allele of patients with COVID-19. Additionally, CT and TT genotype distributions were found to be statistically significant. The T allele distribution for the NFκ-B1A -826 C/T polymorphism has a statistically significant difference, and the CT and TT genotype distributions were also found to be statistically significant.

Conclusion: There is a significant relationship between COVID-19 disease and NF-κB genes, but further studies are needed for this gene.

Keywords: NF-κB, COVID-19, polymorphism

Acknowledgments: This study was supported by CUBAP project no: F-2021-635.

[OP-21]

Mosaic Paternal Uniparental Disomy of chromosome 11 in a Patient with Beckwith-Wiedemann Syndrome

Büşra Eser Çavdarıtepe

Konya City Hospital, Clinic of Medical Genetics, Konya, Türkiye

Abstract

Beckwith-Wiedemann syndrome (BWS) is an imprinting disorder that leads to overgrowth and increased risk of embryonic tumors. It is associated with genetic and epigenetic changes on the chromosome 11p15 region, which includes imprinted genes that regulate fetal and postnatal growth. BWS is considered a spectrum (BWSp) ranging from classic BWS to isolated lateralized overgrowth. A 5-year-old girl patient was referred to us from the neurology outpatient clinic due to speech retardation. There was no problem in the prenatal, natal and postnatal periods. It was learned that she was diagnosed with atypical autism. On examination, straight eyebrows, mild prognathism, cafe-au-lait spots were noticed on the trunk. Brain MRI and EEG were normal. The patient's hearing test was normal. It was planned to study karyotype and microarray from the patient. Karyotype result was 46,XX. As a result of the SNP-array, loss of heterozygosity (LOH) was detected in the 11p15.5p15.4 chromosomal region with a size of 9.4Mb and an estimated mosaicism of ~10%. As a result of the SNP-array study from the parents, the mosaic-UPD in the patient was found to be paternal UPD. With this result, the patient was diagnosed with BWS. Paternal UPD of chromosome 11 accounts for up to 20% of molecularly confirmed cases of BWSp. Upd(11)pat is usually the product of mitotic recombination and therefore appears as mosaic. It is therefore a challenge for diagnosis and can affect only parts of the body or organ. This disease is underdiagnosed because of low level mosaicism. In cases with normal results from blood, further examination with tissue biopsies is required. Upd(11)pat belongs to the BWSp subgroup with the second highest tumor risk and therefore requires special awareness in diagnosis and clinical management.

[OP-22]

Rare Case: Nablus Mask-Like Facial Syndrome

Mert Polat, Mustafa Bakırtaş, Yunus Kasım Terzi, Zerrin Yılmaz Çelik

Başkent University Faculty of Medicine, Department of Medical Genetics, Ankara, Türkiye

Introduction

Nablus mask-like facial syndrome is a rare microdeletion syndrome associated with a distinctive facial appearance. The first case was identified in 2000 by Teebi, who observed a unique facial feature in a child from the Palestinian city of Nablus. Individuals with this syndrome typically exhibit features such as blepharophimosis, shiny and taut facial skin, sparse eyebrows, a bulbous nose, a protruding columella, and ear anomalies. Additionally, individuals with the syndrome may experience mental retardation and developmental delays. Nablus mask-like facial syndrome is exceptionally rare, with only 13 cases published to date. In this study, we will evaluate the genotype and phenotypic findings of our patient who presented to our clinic with speech delay and dysmorphic facial features and was subsequently diagnosed with Nablus mask-like facial syndrome.

Case Report

The patient is a 2.5-year-old boy born at 35 weeks of gestation. His parents are cousins. There were no prenatal or natal issues or concerns. There was no known occurrence of a similar condition within the family. It was learned that there was no similar patient in the family, sitting without support in the postnatal period occurred at 6-7 months, walking without support at 11 months and he could not speak yet. Body weight was 12 kg (10-25p) and height was 88 cm (10-25p). Physical examination revealed raised anterior hair, sparse eyebrows, bulbous nose, tense and shiny facial skin, microretrognathia, high palate, and retractile testis. Conventional karyotyping and molecular karyotyping with Affymetrix, CytoScan 315K (Affymetrix CytoScan Optima®) were performed on a peripheral blood sample obtained from our patient. The results were analyzed using the Chromosome Analysis Suite (ChAS) software. The conventional karyotyping result is 46XY, Molecular karyotyping showed a 1.5 MB deletion in the 8q22.1 region, spanning from 92,997,020 to 95,562,991, when compared to the GRCh38 reference genome.

Discussion

8q22.1 deletion syndrome, also recognized as Nablus mask-like facial syndrome, is an exceedingly rare condition. In our patient, the phenotypic characteristics and the 8q22.1 deletion identified through molecular karyotyping align with the features of Nablus mask-like facial syndrome. However, it is noteworthy to mention that there are documented cases in the literature where individuals with an 8q22.1 deletion exhibit mental retardation, developmental delays, and dysmorphic features, but they lack the typical facial findings of Nablus mask-like facial syndrome. Currently, the precise relationship between genotype and phenotype in this syndrome remains partially understood. Therefore, conducting a comparative analysis of the deletion region and the genes associated with similar cases may offer valuable insights into this matter.

[OP-23]

Analysis of Parental Chromosome and QF-PCR from Abortus Materials in our Genetic Diagnosis Center: Assessment of Results in a Retrospective Manner

Dilek Çelebi¹, Ayşe Gül Zamani², Mahmut Selman Yıldırım²

¹Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

²Necmettin Erbakan University, Meram Medical Faculty Hospital, Department of Medical Genetics, Konya, Türkiye

Abstract

Introduction: The aim of this study aims to determine as retrospectively analyze the results of the parental chromosomal analysis of the couples who pregnancy loss with QF-PCR analysis in patients who received abortion materials.

Methods: In this study, 1,237 parents who karyotyping analyzed and QF-PCR analysis of 624 abortus materials at the department Meram Medical Faculty Medical Genetics Laboratory of Necmettin Erbakan University were evaluated between 2014-2018 retrospectively.

Results: According to the results of our study; chromosomes anomaly was found in 11.8% of the QF-PCR analyzes. In these anomalies were 58.1% trisomy, 22.9% polyploidy, and 18.9% monozomy X. In family chromosome analysis findings, 97.1% of the parents have normal chromosomal organization, while 2.9% of them were abnormal. In the couples with chromosomal abnormalities; 0.6% variant, translocation carriers, 0.2% Turner syndrome, 0.2% Klinefelter syndrome (KS), 0.1% mosaicism, and 0.1% (Y) were detected.

Conclusion: Genetic anomalies are important in the etiology of abortion and the trisomies are the most common anomalies in our study as compatible with the literature studies. On the other hand 3 cases were encountered that is KS in the data that have the the parental chromosome analysis. We believe that the results obtained make a significant contribution to current information will help to especially in the population preventive medicine applications and studies to be done for genetic counseling.

Keywords: Abortion, Klinefelter syndrome, prenatal diagnosis

[OP-24]

Clinical Exome Sequencing in Syndromic Epilepsy Patients Evaluation of Analysis Results

Hamza Polat¹, Bilgen Bilge Geçkinli²

¹University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital, Clinic of Medical Genetics, Diyarbakır, Türkiye

²Marmara University Faculty of Medicine, Department of Medical Genetics, İstanbul, Türkiye

Abstract

Introduction: Complex inheritance mostly plays a role in the development of epilepsy. In complex inheritance, the interaction of genes and environmental factors is seen. In our study, it was aimed to reveal clinical features of syndromic epilepsy patients, advantages-disadvantages of the clinical exome sequencing (CES), and diagnostic availability, to evaluate the relationship between the obtained molecular data and clinical features, and to provide genetic counseling to the families with the case whose molecular etiology was solved and the disease and variant.

Methods: A total of 146 patients, 70 men, and 76 women, who were diagnosed with epilepsy and had additional clinical findings such as syndromic appearance and mental retardation, were included in the study. Genes associated with syndromic epilepsy were analyzed using the CES kit containing 4,496 genes, using current databases with next generation sequencing (NGS) method.

Results: In our study, 17 (12%) pathogenic variants (P) with known clinical significance, 16 (11%) potentially pathogenic (LP) and 6 (3%) variant unknown significance (VUS) variants were detected in 38 (27%) individuals. Of the variants, 8 (5%) were in genes associated with encephalopathies, 6 (3%) were in genes associated with neurocutaneous diseases, 4 (2%) were in genes associated with metabolic pathways, and 21 (14%) variants are in genes that may cause rare syndromic epilepsy. In our study, 18 variants that can be classified as P, LP, or VUS were not reported in databases and were brought to the literature.

Conclusion: Genetic etiology was clarified and genotype-phenotype correlation was discussed in 38 patients with syndromic epilepsy. All these findings revealed the importance of NGS applications in elucidating the genetic mechanisms involved in the formation of epilepsy.

Keywords: Epilepsy, epilepsy genetics, clinical exome sequencing (CES)

[OP-25]

The Power of MLPA-NGS Coexistence in the Management of Charcot Marie Tooth Patients

Emine Göktaş, Makbule Nihan Somuncu, Ayşe Gül Zamani, Mahmut Selman Yıldırım

Necmettin Erbakan University, Meram Medical Faculty Hospital, Department of Medical Genetics, Konya, Türkiye

Abstract

Charcot Marie tooth (CMT) disease is an inherited motor and sensory neuropathies. Its prevalence is about 1/2500. Distal muscle weakness, muscle atrophy, loss of deep tendon reflexes, and foot deformities are expected findings. The findings are symmetrical, slowly progressive, and usually begin in the first decade. CMT can be inherited in an autosomal dominant, autosomal recessive, or X-linked manner. Duplications in the *PMP22* gene are responsible for the etiology in more than half of CMT patients. Nearly 80 genes are responsible for the other half, the most common being *PMP22*, *GJB1*, *MPZ*, *MFN2*, *GDAP1*, *HINT1*, *SH3CT2*, *SORD* genes. Eight patients aged between 3-55 years were admitted to our center with complaints such as difficulty in walking, electromyography abnormalities, loss of sensation in the hands and feet. MLPA analysis and next generation sequencing (NGS) analysis were performed for the etiology of the patients. Unlike the literature, no duplication in the *PMP22* gene was observed in our patients in MLPA; variants were detected in the gene panels of 4 of our patients. The variants are in the *GJB1*, *MPZ*, and *ARHGEF10* genes. The c.397A>C:1127L homozygous variant in *GJB1* gene detected in a 17-year-old male patient who presented with gait disturbance and motor and sensory polyneuropathy is novel. Although it is known that *PMP22* gene duplications are frequently responsible for the etiology of CMT, all of our patients had normal MLPA and found responsible variants by NGS method; it emphasizes the importance of the technical combination of MLPA and NGS in the approach to CMT patients.

Keywords: Charcot Marie tooth, MLPA, next generation sequencing

[OP-26]

Waardenburg Syndrome to PCWH Syndrome: Clinical Heterogeneity in the *SOX10* Gene

Muhammed Burak Bereketoglu¹, Ceren Deniz Ceylan¹, Cem Müjde², Sevcan Tuğ Bozdoğan²

¹Çukurova University Faculty of Medicine, Department of Medical Genetics, Adana, Türkiye

²Çukurova University Balcalı Hospital Health Practice and Research Hospital; Çukurova University AGENTEM (Adana Genetic Diseases Diagnosis and Treatment Center), Department of Medical Genetics, Adana, Türkiye

Introduction

SOX10 is a transcription factor that plays a role in the differentiation of melanocyte vestibular and Schwann cells. Mutations in the *SOX10* gene cause Waardenburg syndrome, which includes hearing loss, iris heterochromia, and piebaldism. It can present with a broad clinical spectrum, from PCWH syndrome to which neurological findings such as polyneuropathy and Hirschprung's disease are also added. In our study, we presented the clinical heterogeneity shown by the pathogenic variant in the *SOX10* gene in the family.

Case Report

The patient who applied to our medical genetics outpatient clinic was a 6-year-old boy with a total colectomy due to Hirschprung disease. Their parents were 1st-degree cousins. On physical examination, satyr ears, bright blue tint, ptosis in the eyes, dental anomalies, and proportionate short stature were observed. In his neurological examination, deep tendon reflexes were weak, and muscle strength in his extremities was evaluated as 3/5. In addition, the patient had ataxia, anosmia, mental retardation, and bilateral sensorineural hearing loss (SNHL). Vestibular magnetic resonance imaging revealed posterior semicircular canal atresia, bilateral vestibular dysgenesis and vestibular aqueduct agenesis. Electromyography revealed sensorineural polyneuropathy with axonal destruction. Additionally, fundus examination revealed peripapillary atrophy and fundus albinous. The patient's father and brother had bilateral SNHL, iris heterochromia, anosmia, and large incisive teeth. Piebaldism was observed only in the father. As a result of clinical evaluations, *SOX10* whole gene analysis was planned with the preliminary diagnosis of index PCWH syndrome and paternal and maternal Waardenburg syndrome type 4C. *SOX10* whole gene sequence analysis was performed by the Sanger sequencing method. Bioinformatic analyses were classified in line with the "ACMG" guide. Detailed counseling was given before/after the test.

Conclusion

As a result of the analyses, a heterozygous p.Ser13Asnfs*19 (c.38_39delGcinsA) variant was detected in the index, father, and mother. Although this variant is a new variant that has not been previously reported in the literature, it was evaluated as pathogenic *in silico* analyses. It is thought that the mutation shows variable expressivity and clinical heterogeneity in the family and may contribute to the clinical evaluation of Waardenburg disease.

[OP-27]

A Family Diagnosed with Progressive Spastic Tetraplegia and Axial Hypotonia and Amyotrophic Lateral Sclerosis with a Pathogenic Variant in the *SOD1* Gene

Hamza Polat

University of Health Sciences Türkiye, Gazi Yaşargil Training and Research Hospital, Clinic of Medical Genetics, Diyarbakır, Türkiye

Introduction

Progressive spastic tetraplegia and axial hypotonia (PSTAH) is an autosomal recessive neurological disease characterized by the onset of severe and progressive motor dysfunction in the first year of life. Affected individuals have severe axial hypotonia with spastic tetraplegia and hyperekplexia reflecting upper motor neuron involvement. Pathogenic variants in the biallelic *SOD1* gene have been associated with PSTAH syndrome. Monoallelic pathogenic variants in the *SOD1* gene have been associated with ALS. In our study, we aimed to evaluate the connection between molecular data and clinical features and to provide genetic counseling regarding the disease and variant to the cases with determined molecular etiology and their families. After DNA isolation from the peripheral blood of the cases, genes associated with spasticity were analyzed using the gene-containing CES kit and the next generation sequencing method using current databases. Sanger sequencing method was used for confirmation and segregation analysis of the variants detected in the cases.

Case Report

A three-year-old boy whose parents were cousins was followed up due to hypotonia and loss of gross and fine motor functions. In the neurological examination of the patient, severe, progressive spastic tetraparesis with extensor plantar responses was observed. Some fasciculations were observed without other definitive electromyography signs of lower motor neuron involvement. His cognitive development was appropriate for his age, although he was non-verbal. Brain imaging was consistent with mild frontoparietal atrophy. In the genetic analysis of the case, a homozygous c.247dup p.(Asp84Argfs*8) possible pathogenic variant was detected in the *SOD1* gene. In the segregation analysis, the variant was detected as heterozygous in both parents. It was learned that the mother of the case was followed up with a preliminary diagnosis of ALS due to muscle weakness and fasciculation.

[OP-28]

Adaptor Protein Complex 4-associated Hereditary Spastic Paraplegia: A Case Series of Seven Patients

Büşra Özgüç Çalışkan¹, Mehmet Canpolat², Hakan Gümüş², Hüseyin Per², Fatih Kardaş³, Munis Dündar¹

¹Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

²Erciyes University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, Kayseri, Türkiye

³Erciyes University Faculty of Medicine, Department of Pediatrics, Division of Nutrition and Metabolism, Kayseri, Türkiye

Abstract

AP-4 is a heterotetrameric protein complex belonging to the adapter proteins (AP-1-AP-5) family, which facilitates the selective uptake of transmembrane cargo proteins into vesicles and assists their intracellular exchange. Biallelic pathogenic variants in one of four genes (*AP4B1*, *-E1*, *-M1*, *-S1*) encoding subunits of the AP-4 complex ($\beta 4$, $\epsilon 4$, $\mu 4$, $\sigma 4$, respectively) result in AP-4-associated hereditary spastic paraplegia (HSP). Its prevalence is estimated to be $<1/1000000$. In this study, we present a case series of 7 patients with AP-4-related HSP from 5 different families. Patients referred to our department due to spasticity between 2019-2022 were retrospectively reviewed. Seven cases diagnosed with AP-4-related HSP were included in the study. By clinical exome sequencing, homozygous mutations were detected in *AP4M1* in 6 patients and in *AP4B1* in 1 patient. The most common findings in the patients were spasticity (7/7), neurodevelopmental delay (7/7), microcephaly (6/7), and epilepsy (5/7). When we evaluated the variant spectrum, there were 3 different variants in the *AP4M1* gene. The c.1012C>T (p.Arg338*) variant, which is frequently mutated in Turkish population and thought to have a founder effect, was the most common change in our cohort (3/6). In addition, a homozygous c.975-25_975-22delGACT (rs767251230) variant was detected in a girl from a consanguineous Afghan family. This is a rare variant (gnomaAD frequency 0.0008%) that has not been previously reported in patients. In summary, we aim to contribute to the literature with 7 patients diagnosed with AP-4-related HSP, which constitutes a rare part of HSP, and we present the homozygous c.975-25_975-22delGACT variant in *AP4M1*, which has not been reported in any patient before. In addition, this study contributes to the claims that the c.1012C>T (p.Arg338*) variant, which is detected at a high rate despite the small number of patients, may have a founder effect in the Turkish population.

Keywords: Hereditary spastic paraplegia, *AP4M1*, *AP4B1*

[OP-29]

A Rare Syndrome with New Phenotypic Features and Mutation: Weiss Kruzskas Syndrome

Rashad Abdullayev¹, M. Burak Bereketoğlu¹, Derya Ufuk Altıntaş², İbrahim Boğa^{1,3}, Atıl Bişgin^{1,3}, Sevcan Tuğ Bozdoğan^{1,3}

¹Çukurova University Faculty of Medicine, Department of Medical Genetics, Adana, Türkiye

²Çukurova University Faculty of Medicine, Department of Pediatrics, Division of Allergy and Immunology, Adana, Türkiye

³Çukurova University Balcalı Hospital Health Practice and Research Hospital; Çukurova University AGENTEM (Adana Genetic Diseases Diagnosis and Treatment Center), Department of Medical Genetics, Adana, Türkiye

Introduction

Weiss Kruzskas syndrome is a rare disease accompanied by multiple congenital anomalies. It manifests with growth and developmental delays and branchiofacial anomalies. Affected individuals experience hypotonia, feeding problems, and corpus callosum agenesis, as observed in brain magnetic resonance images. The *ZNF462* gene encodes a transcription factor that plays a crucial role in embryonic development and chromatin remodeling. Pathogenic variants causing loss of function are implicated in the etiology of Weiss Kruzskas syndrome, which is inherited in an autosomal dominant manner.

Case Report

A 5-year-old male patient was admitted to our outpatient clinic in the department of medical genetics due to growth and developmental retardation, frequent infections, and a dysmorphic facial appearance. The physical examination revealed skin tags in bilateral ears, pits in the right ear, fistula, pads in the fingers, an epidermoid cyst in the right eye, mane neck, pectus excavatum, and laryngomalacia. ECHO examination showed ASD, while laboratory tests indicated low immunoglobulin, and CD levels. Intravenous immunoglobulin treatment was initiated. Additionally, the patient's father had a similar history of immunodeficiency, and his dysmorphic features coincided with those of the patient.

Whole exome sequencing was performed using the next-generation sequencing method. Bioinformatic analyses were conducted following the "ACMG" guidelines via QCI-Analyse and QCI-Interpret. The analyses revealed a heterozygous p.R451*(c.1351C>T) variant in the *ZNF462* gene. This novel, previously unidentified variant was deemed pathogenic in *in silico* analyses. The variant detected in our index case was also examined in the parents, and the same variant was identified in the phenotypically similar father.

Conclusion

Immunodeficiency has not been identified to date in Weiss Kruzka syndrome, which includes developmental delays and non-specific dysmorphic findings. Its clinical features, overlapping with many syndromes, are quite heterogeneous. Usually diagnosed through whole exome sequencing, Weiss Kruzka syndrome is a rare condition that should be considered in the differential diagnosis.

[OP-30]

Coffin Siris Syndrome with Corpus Callosum Agenesis

Vedat Yüce, Şenol Demir, Onur Hanoğlu, Esra Hilal Ceylan, Bilgen Bilge Geçkinli

Marmara University, Pendik Training and Research Hospital, Clinic of Medical Genetics, İstanbul, Türkiye

Introduction

Coffin-Siris syndrome (CSS) is a rare genetic disease with multisystem involvement characterized by intellectual disability. Fifth finger distal phalanx/nail aplasia or hypoplasia, developmental delay, facial dysmorphism, hypotonia, hirsutism/hypertrichosis and nervous system involvement are among the components of the CSS. We aim to make a genotype-phenotype comparison of the current clinical findings of CSS and to contribute to the literature with the new clinical findings detected in our patient.

Case Report

7-year-old female patient had corpus callosum agenesis, seizure, strabismus, learning difficulties, hyperlaxity, and cutis laxa. In the pedigree analysis there was a 3rd degree consanguinity between the parents and no similar history was found in the family. It was determined that the patient had a retardation in the developmental stages. Physical examination revealed facial dysmorphism and hypoplasia of the nails.

Results

Chromosome analysis from the patient's peripheral blood was found to be 46,XX and no deletion-duplication was detected in the microarray result. Molecular analysis of clinical exome sequencing method revealed a heterozygous c.5704A>T p.Lys1902* in the *ARID1B* gene (NM:001374828). This variant was not reported on Clinvar but according to ACMG criteria it was evaluated as likely pathogenic and segregation analyzed was planned.

Discussion

There are 12 genes related to CSS and the clinical findings are quite heterogeneous. So it is difficult to make genotype-phenotype correlation of CSS. In conclusion, this study reveals an unreported clinical finding in the literature and contributes to the genotype-phenotype correlation of CSS.

[OP-31]

A Rare Syndrome with a Rare Complication: Schimke Immunoosseous Dysplasia and Cerebral Hemorrhage

Rüveyda Zeynep Kazan¹, Hatice Mutlu¹, Merve Havan², Tanıl Kendirli², Ceyda Kırsaçlıoğlu³, Zariye Kuloğlu³, Aydan Kansu³

¹Ankara University Faculty of Medicine, Department of Child Genetics, Ankara, Türkiye

²Ankara University Faculty of Medicine, Department of Pediatric Intensive Care, Ankara, Türkiye

³Ankara University Faculty of Medicine, Department of Pediatric Gastroenterology, Ankara, Türkiye

Introduction

Schimke immunoosseous dysplasia (SIOD) is an autosomal recessive disorder characterized by spondyloepiphyseal dysplasia, cellular immune deficiency, and nephropathy. Biallelic mutations in SWI/SNF-related matrix-associated actin-dependent regulator of chromatin subfamily A-like protein (*SMARCL1*) gene is the only gene responsible of the phenotype. The symptoms associated with cranium more likely present as ischemia, internal carotid artery atherosclerosis and moyamoya phenomenon.

Case Report

Here, we describe a 20-months-old girl who admitted to hospital for failure to thrive. She was the only child of a consanguineous family. Intrauterine growth deficiency was noted. On examination her weight, height and occipitofrontal diameter were below -6 SD. She had large anterior fontanel, relative macrocephaly, bitemporal narrowing, thick eyelashes, prominent nasal tip, short trunk, and lumbar kyphosis. Although she had developmental delay she could not neither sit nor hold her head steady for the last one week. On X-ray, osteopenia, small capital femoral epiphyses, hypoplasia of the basilar portions of the ilia were detected. Brain imaging showed wide hypodensity on the left frontotemporal and right temporoparietal areas depicting parenchymal hemorrhage. *SMARCL1*:c.1687C>T homozygous pathogenic variant was identified. During the follow-up, the patient's clinical condition deteriorated rapidly and deceased.

Conclusion

Reduced blood flow to the brain (cerebral ischemia) is one of the rare complications of SIOD. However, sudden death secondary to cerebral hemorrhage and ischemia in infancy has not been reported before. It should be kept in mind in regular neurological examination and cranial imaging should be performed from the early infancy.

[OP-32]

A Rare Case Report of Two Siblings: A Novel GRIN1 Variant

Çekdar Kapazan, Vedat Yüce, Şenol Demir, Bilgen Bilge Geçkinli, Pınar Ata, Ahmet Arman

Marmara University, Pendik Training and Research Hospital, Clinic of Medical Genetics, İstanbul, Türkiye

Introduction

Neurodevelopmental disorder with or without hyperkinetic movements and seizures (NHDMS) (OMIM:138249) is a rare genetic disease characterized by psychomotor developmental delay, autism, and dysmorphic facial features. Monoallelic or biallelic mutations in the *GRIN1* gene which encodes NMDA channel subunit 1 have been associated with the NHDMS. Our aim is to present the clinical findings of two siblings with homozygous variants in the *GRIN1* gene and discuss the phenotype- genotype correlation.

Case Report

Two siblings, a 5-year-old girl and an 11-year-old boy were referred to our clinic due to global neurodevelopmental delay. Their parents are first-degree cousins, and there is no similar history in the family. In the detailed anamnesis and physical examination, the boy had hypotonia, autism, involuntary movements, speech delay, and self-injurious tendencies, and dysmorphic facial features. Also, the sister had dystonia, gait and speech delay, strabismus, and dysmorphic facial features. The siblings had normal results of metabolic tests, cranial magnetic resonance, abdominal ultrasound, electroencephalogram, and echocardiogram.

Results

Molecular analysis of the whole-exome sequencing method revealed a homozygous c.1640C>T (p.Pro547Leu) in the *GRIN1* gene (NM:007327). This variant was evaluated as likely pathogenic according to ACMG criteria. Segregation analysis revealed that the parents were heterozygous while the sister was homozygous for this mutation.

Discussion

The reporting of new cases is important for a better understanding of the characteristics of the rare genetic diseases. To conclude this study reveals a novel pathogenic variation to the literature and contributes to the phenotype-genotype correlation for NHDMS.

[OP-33]

Co-occurrence of Williams-Beuren Syndrome and Mosaic Turner Syndrome

Levent Şimşek, Fahrettin Duymuş

Konya City Hospital, Clinic of Medical Genetics, Konya, Türkiye

Introduction

Turner's syndrome (TS) is a form of gonadal dysgenesis with an incidence of 1:2000 to 1:5000 in live female births. The karyotype 45,X/46,XX is the most frequent mosaic type of this disease (36%). Williams-Beuren syndrome (WBS) is a microdeletion syndrome with a frequency of 1/20,000 caused by del7q11.23. Here, we report co-occurrence of WBS and mosaic TS, which has not been previously reported in the literature.

Case Report

Three years old female patient was admitted to our outpatient clinic with complaints of growth and neuromotor developmental retardation. She was born to a 22 year-old mother; her birth weight was 2700 g. On physical examination, her height was 96 cm (-1.18 SDS) and her weight was 14.8 kg (-0.46 SDS). The patient's findings include long philtrum, down-sloping shoulders, microdontia, hypertelorism, strabismus, large ears, thin upper lip, thick lower lip vermilion, wide nasal tip, anteverted nares, high forehead, wide mouth, and periorbital fullness. Laboratory examinations revealed hypothyroidism; and echocardiography showed supravalvular aortic stenosis. Karyotype and FISH analysis for WBS were planned in the light of the clinical findings. Chromosome analysis of lymphocytes showed mosaicism for TS (45,X[8]/46,XX[42]) and FISH analysis revealed del7q11.23 in all metaphases and interphases examined.

Conclusion

Co-occurrence of two distinct chromosomal abnormalities is an extremely rare phenomenon. In literature, there is only one report of co-occurring WBS and TS without mosaicism. This is the first report of a patient with both WBS and mosaic TS to our knowledge.

[OP-34]

PGT-M for Neurogenetic Diseases with Monogenic Inheritance: Applications and Experiences

Mustafa Bakırtaş, Selin Akad Dinçer, Yunus Kasım Terzi, Zerrin Yılmaz Çelik

Başkent University, Ankara Hospital, Department of Medical Genetics, Ankara, Türkiye

Abstract

Introduction: PGT-M can be recommended to all monogenic diseases where the disease causing loci are well defined. The most common indications for PGT-M are cystic fibrosis hemoglobinopathies for autosomal recessive disorders, myotonic dystrophy type 1, neurofibromatosis, Huntington's disease and hereditary cancer syndromes for autosomal dominant disorders. PGT for X linked disorders primarily performed for Duchenne muscular dystrophy (DMD), hemophilia, and fragile X syndrome.

Methods: This study discusses materials and methods of PGT-M studies for neurodevelopmental disorders performed in our department including DMD, spinal muscular atrophy, carbamoyl phosphate synthetase 1 deficiency, I-cell disease (mucopolidosis type 2), spastic paraplegia type 15, intellectual developmental disorder autosomal recessive 72, and asparagine synthetase deficiency. Families who sought to become pregnant by selecting healthy embryos and had identified pathogenic variants associated with single-gene diseases were included in this study.

Results: Twenty two trophectoderm embryo biopsies were sent to our institution for PGT-M studies. Whole genome amplification could not be achieved 7 out of the 22 embryos. Among the 15 embryos that were evaluated, 4 were found to be wild-type, 4 were carriers, 3 were homozygous mutants, and 1 was hemizygous. Four clinical pregnancy observed after 8 embryo were transferred from 6 different families.

Discussion: The choice of method in PGT studies can vary depending on the inheritance pattern of diseases and mutation types. The reason for application may differ, such as selecting healthy embryos or HLA-compatible embryos. During the PGT-M study process, families seeking to have children should be informed about potential situations that may arise (failed implantation, multiple pregnancies, inability to find a transferrable embryo) through genetic counseling. Technical and clinical experiences during this study will serve as a guide for future studies.

[OP-35]

Blended Phenotype in a Case with Brain Malformation, Neurodevelopmental Disorder and Epilepsy

Emine Karataş¹, Aslıhan Kiraz¹, Zehra Filiz Karaman³, Hüseyin Per², Munis Dündar¹

¹Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

²Erciyes University Faculty of Medicine, Department of Pediatric Neurology, Kayseri, Türkiye

³Erciyes University Faculty of Medicine, Department of Radiology, Kayseri, Türkiye

Abstract

One of the important causes of severe neuromotor developmental delay and epilepsy is malformations of cortical development (MCD) such as pachygyria, lissencephaly. Genes involved in neuronal cell proliferation, migration and post-migration cortical organization have been implicated in MCD. The *CAMPAS1* gene, which provides microtubule and spectrin binding activity, is one of the rare causes that have been recently identified. Another rare cause of severe neuromotor developmental delay and epilepsy is the *NBEA* gene. It encodes the neurobeachin protein, a neuro-specific structural protein that plays a role in vesicle traffic and synaptic structure. In this study, we present a case with loss of function variant in these two rare genes. A proband 7-year-old female patient, only child of consanguineous parents, microcephaly, seizure, infantile spasm, neurodevelopmental retardation, hypotonia, pachygyria, corpus callosum agenesis, hypoplasia of the brain stem, spasticity in the extremities, nutrition problems, recurrent respiratory tract infection findings. Whole exome analysis of the patient revealed c.1153C>T p.Gln385* non-sense homozygous likely pathogenic variant in *CAMPAS1* gene and *de novo* c.6867G>A p.Trp2289* non-sense heterozygous pathogenic variant in *NBEA* gene. We considered it as a blended phenotype. In the literature, the case of these two genes together has not been reported before. There are limited case reports with these two genes. For this reason, it is thought that it will contribute to the literature. In this study, we emphasize that in the presence of complex and severe clinical findings, two or more genes may be responsible and further investigation may be required.

[OP-36]

Evaluation of Genetic Disorders Associated with Epilepsy in Children

Zhanara Anarbaeva¹, Ayşe Pelin Toker¹, Mert Polat¹, Yunus Kasım Terzi¹, İlknur Erol², Taner Sezer², Zerrin Yılmaz Çelik¹

¹Başkent University Faculty of Medicine, Department of Medical Genetics, Ankara, Türkiye

²Başkent University Faculty of Medicine, Department of Pediatric Neurology, Ankara, Türkiye

Abstract

Introduction: Epilepsy is a neurological disorder characterized by recurrent seizures due to increased neuronal and electrical activity. According to the World Health Organization, approximately 5 million people are diagnosed with epilepsy for the first time each year. While idiopathic epilepsy has no identifiable underlying cause, genetic factors play a role in many cases. Advances in genetic science have led to the identification of many genetic syndromes and single-gene mutations associated with epilepsy. Many syndromes associated with or caused by chromosomal aneuploidies, copy number variations, and single-gene mutations have been reported in association with epilepsy. This study aimed to evaluate the genetic syndromes detected in patients with epilepsy.

Methods: We retrospectively analyzed the genetic test results of 230 patients with epilepsy who presented to our department between January 2016 and January 2023.

Results: Abnormalities were detected in 30 patients (13%). Chromosomal anomalies were found in four patients, microdeletion syndrome in 14 patients, and single-gene mutations in 12 patients. The most common single-gene mutations were in the *ADGRV1*, *ARSA*, *ATN1*, *COL4A1*, *CPA6*, *KCNQ*, *MACF1*, *PACS1*, *PIGQ*, *PRRT2*, *SLC19A2*, and *STXBP1* genes.

Conclusion: Genetic factors play a significant role in the development of epilepsy. Genetic testing can be helpful in identifying the underlying genetic cause in syndromic and non-syndromic cases, which can aid in diagnosis, prognosis, and counseling.

Keywords: Epilepsy, genetics, diagnosis

[OP-37]

A Case of 48,XXYY Syndrome Presenting with ADHD

Ege Baltacı¹, Mert Polat¹, Ayşe Pelin Toker¹, Zhanara Anarbaeva¹, Zerrin Yılmaz Çelik¹, İlknur Erol²

¹Başkent University, Ankara Hospital, Clinic of Medical Genetics, Ankara, Türkiye

²Başkent University, Adana Dr. Turgut Noyan Research and Application Center, Department of Pediatric Neurology, Ankara, Türkiye

Introduction

Attention deficit hyperactivity disorder (ADHD) which is characterized by increased movement, decreased impulsivity control and attention span is the most common neurobehavioral condition in childhood (1). Research suggests that ADHD is one of the psychiatric disorders with the highest association with genetic abnormalities. Mutations in genes such as *5HTT*, *DAT1*, *DRD4*, *DRD5*, *HTR1B*, *SNAP25*, *BAIAP2*, *SLC6A3* have been implicated as well as copy number changes (2). Determining the causes related to chromosomal abnormalities in neurodevelopmental disorders such as ADHD, which exhibit genetic heterogeneity, is important in terms of clinical approach and assessing genetic risks. 48,XXYY syndrome is a syndrome that occurs in one out of every 18,000-50,000 male infants and was previously classified as a variant of Klinefelter syndrome. However, as more cases were reported, its unique psychiatric spectrum and set of findings led the medical community to recognize 48,XXYY syndrome as a separate condition. It is characterized by delayed speech and motor skills, hypotonia and feeding problems. Additionally, epilepsy (15%), tremors (60%), asthma and allergies (60%), and dental problems (90%) can also be present (3). ADHD, among other psychiatric conditions, has also been reported by some studies (4).

Case Report

We present a 7-year-old male patient who was referred to pediatric neurology for difficulty in learning and behavioral issues. There was no family history of any mental illness or consanguinity. He was later diagnosed with ADHD. His physical examination was normal except for mild hypertelorism. He had a history of cryptorchidism. He also had a history of epilepsy and was seizure-free with anti-epileptics. His magnetic resonance imaging was normal except for non-specific T₂ hyperintensities in the white matter. Karyotyping was performed from peripheral blood and 48,XXYY was recognized on all thirty metaphases analyzed.

Discussion

Neuropsychiatric disorders are multifactorial with a strong genetic component. This genetic component is usually on a molecular level (5). However, if there are certain dysmorphic findings and no family history, starting the genetic workup with a basic karyotype can be both timesaving and cost-

effective. Finally, due to its multiple long-term complications (neuropsychiatric problems, hypogonadism, inguinal hernias, vascular conditions, dental issues, asthma, type 2 diabetes, congenital cardiac anomalies, etc.), 48,XXYY syndrome should be considered as a distinct clinical and genetic entity and not just a variant of Klinefelter syndrome. For this distinction, it is important to provide genetic counseling to the family and create awareness for their future medical care needs.

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[OP-38]

A Rare Variant in the *CDH2* Gene: The Second ACOGS Case from Türkiye

Gökçen Özbek¹, Şeyma Aktaş Paskal¹, Mehmet Canpolat², Munis Dündar¹

¹Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

²Erciyes University Faculty of Medicine, Department of Pediatrics, Division of Neurology, Kayseri, Türkiye

Introduction

Agenesis of the corpus callosum, cardiac, ocular, and genital syndrome (ACOGS) (OMIM: 618929) is a rare genetic disorder caused by heterozygous mutations in the *CDH2* gene. The *CDH2* gene located at the 18q12 locus encodes N (neuronal)-cadherin, which plays a crucial role in early brain morphogenesis. ACOGS is a neurodevelopmental disorder characterized by developmental delay and/or intellectual disability, corpus callosum agenesis/hypoplasia, craniofacial dysmorphism, and ocular, cardiac, and genital anomalies.

Case Report

A 2-year-old male patient was genetically evaluated due to craniofacial dysmorphic features, strabismus, limited upward gaze, epilepsy, intellectual disability, speech delay, sexual development disorder, and cranial magnetic resonance findings of corpus callosum agenesis and scaphocephaly. The patient's echocardiogram showed patent ductus arteriosus, patent foramen ovale, left ventricular non-compaction. A cystoscopy revealed scrotal hypoplasia, hypoplastic phallus, absence of the right gonad, and atrophic testicular structure on the left. Whole exome sequencing analysis identified a heterozygous intronic variant in the *CDH2* gene (c.1344+5G>A).

Conclusion

Although this variant in the *CDH2* gene is listed in the ClinVar database, it has not been reported in the literature before. Additionally, this is the second reported ACOGS case from Türkiye. The discovery of non-compaction cardiomyopathy in our patient may represent a new phenotype associated with ACOGS.

[OP-39]

An Interesting Family: A Patient with Blended Phenotype with Sexual Development Disorder and Coenzyme Q10 Deficiency and His Sibling Diagnosed with Joubert

Rümeysa Atasay¹, Leyla Nur Yılmaz¹, Ayten Güleç², Hüseyin Per², Mehmet Canpolat², Aslıhan Kiraz¹, Munis Dündar¹

¹Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

²Erciyes University Faculty of Medicine, Department of Pediatric Neurology, Kayseri, Türkiye

Abstract

Consanguineous marriages cause rare blended phenotypes in common geographies and the emergence of different homozygous diseases in the same family. In this article, a family in which different rare mechanisms come together as a result of consanguineous marriage is presented. A 2-year-old proband with a blended phenotype with sex development disorder and coenzyme Q10 deficiency has psychomotor retardation, micropenis,

undescended testicles, dysmorphic findings, hypotonia, and the gonads have a bilateral testicular appearance and are located in the inguinal canal. In the patient's brother, who previously died due to hydrocephalus, a non-sense homozygous variant c.1051C>T p.(Arg351*) was detected in the 7th exon of the NM_001134830 transcript of the *AHI1* gene. The patient's cytogenetic analysis result is: 46,XX,ish,der(X)t(X;Y)(p22.3;p11.2)(SRY+). In the molecular karyotyping analysis performed due to hypotonia, psychomotor retardation and dysmorphic findings; a change of 2.1 Mb in size was detected in the Xp22.33p22.32 region and 3.1 Mb in size in the Yp11.2 region. This change is 46,XX sex reversal 1; it has been associated with SRXX1 (OMIM number:#400045) disease. In the subsequent whole exome analysis, a c.437T>G (Phe146Cys) missense homozygous probable pathogenic variant was detected in the NM_016035 transcript in the 5th exon of the *COQ4* gene, which explains other clinical findings. In primary deficiency of coenzyme Q10 (OMIM number: #616276), which is a lipid component of the mitochondrial respiratory chain, symptoms vary; hypotonia, decreased coQ levels in muscle tissue, regression in psychomotor development, seizures, and increased serum lactate levels are observed. It is generally lethal in the first years of life, and in this respect, it is thought that the clinical findings of the proband will contribute to the literature with their milder course. To our knowledge, there is no other case in the literature reporting a sexual development anomaly or primary CoQ10 deficiency.

Keywords: SRY, gender development disorder, hypotonia, coenzyme Q10, COQ4

[OP-40]

Diagnosis of Townes-Brocks Syndrome in a Turkish Adolescent with End-Stage Renal Failure

Mustafa Yakubi¹, İsmail Dursun², Munis Dündar¹

¹Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

²Erciyes University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Nephrology, Kayseri, Türkiye

Abstract

Introduction: Townes-Brocks syndrome (TBS) is identified by a triad of imperforate anus, malformed ears, and thumb abnormalities. Additional associated features include renal complications, congenital heart abnormalities, foot abnormalities, and genitourinary malformations, and intellectual disability. Less common characteristics encompass iris coloboma, Duane anomaly, Arnold-Chiari malformation type 1, and developmental delay. Here we report a 15-year-old male patient with congenital hearing loss, global developmental delay, and the incidental discovery of chronic kidney disease. The patient's physical examination indicated TBS. However, clinical exon sequencing (CES) genetic testing revealed a pathogenic mutation in the *SALL1* gene and confirmed the diagnosis.

Methods: We took the patient for clinical exome sequencing.

Results: CES analysis revealed a novel heterozygous likely pathogenic variant in *SALL1* gene (NM_002968/3 c.1336_1370del p.(Phe446Leufs4)). We also detected a hemizygous likely pathogenic variant in the *POU3F4* gene (NM_000307/5 c.478C>T p.(Gln160)), which could similarly lead to the patient's congenital deafness (OMIM #304400 Deafness, X-linked 2). This suspicion arose because the patient's cousin also has congenital hearing loss without any other anomalies. Thus, we have arranged for further genetic examination of the cousin.

Conclusion: Our patient had a frameshift mutation in the hotspot region, leading to premature truncation. This resulted in a severe TBS phenotype with severe dental issues, which led to the extraction of teeth during childhood due to significant mouth deformities and functional problems. Recent studies have also emphasized that premature *SALL1* protein truncation results in a more severe TBS phenotype due to a dominant negative effect.

[OP-41]

Detection of Somatic Variant in *PIK3R2* Gene in a Patient Followed with Galactosemia

Nurana Mammadova¹, Fırat Özçelik¹, Mehmet Canpolat², Munis Dündar¹

¹Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

²Erciyes University Faculty of Medicine, Department of Pediatric Neurology, Kayseri, Türkiye

Introduction

Megalencephaly-polymicrogyria-polydactyly-hydrocephalus (MPPH) syndrome is a rare developmental brain disorder characterized by brain enlargement, bilateral perisylvian polymicrogyria (BPP) and variable ventriculomegaly, and occurs as a result of pathogenic mutations in the *AKT3*, *CCND2* or *PIK3R2* genes. Variable degrees of ventriculomegaly are observed in almost all children with MPPH syndrome. In this case report, we present a case referred to our clinic due to hemimagalencephaly, BPP and galactosemia. In the patient we underwent clinical exome sequencing (CES), we detected possible pathogenic and pathogenic variants in the *PIK3R2* gene (20% variant fraction) and *GALT* gene, respectively. The *PIK3R2*

gene regulates the activity of an enzyme called phosphatidylinositol 3-kinase (PI3K). The PI3K-AKT-mTOR pathway is essential for the normal development of many parts of the body, including the brain. Mutations in the *PIK3R2* gene can cause excessive activation of the PI3K/Akt/mTOR pathway, resulting in overgrowth of neurons to form developmental brain disorder syndrome, both MPPH and BPP, characterized by seizures and other clinical features.

Galactosemia is an autosomal recessive inherited condition that affects galactose metabolism, and is caused by the deficiency of the enzyme galactose-1-phosphate uridyltransferase (GALT). Classical galactosemia is divided into three types: Clinical variant galactosemia and biochemical variant galactosemia, the most common and most severe form being the classical one.

Case Report

In this study, we present a case diagnosed with galactosemia, which we associate with hemimegalencephaly and BPP. Our case is a 4-year-old girl patient. As a result of CES performed on the patient, c.329-2A>C splice acceptor, pathogenic variant was detected in the *GALT* gene and c.1669G>T p.(Asp557Tyr) possible pathogenic variant was detected in the *PIK3R2* gene (20% variant fraction).

Result

The variants detected in the patient were evaluated as blend phenotype. This study contributed to the importance of next-generation sequencing in detecting somatic mutations. It should be noted that somatic variants may not be of blood origin, so analysis of a second tissue such as saliva or skin should be considered. It should be noted that somatic variants cannot be detected in blood, so analysis of a second tissue such as saliva or skin should be considered.

Keywords: Galactosemia, *GALT*, BPP, *PIK3R2*, hemimegalencephaly

[OP-42]

A Novel Homozygous Variant in *TBC1D24* Gene: A Case Report

Seyma Aktaş Paskal¹, Ayten Güleç², Mehmet Canpolat², Munis Dünder¹

¹Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

²Erciyes University Faculty of Medicine, Department of Pediatrics, Division of Pediatric Neurology, Kayseri, Türkiye

Introduction

DOORS syndrome (deafness, onychodystrophy, osteodystrophy, mental retardation and seizures; MIM 220500), caused by variants in *TBC1D24* gene (TBC1 domain family member 24, MIM 613577), is a rare autosomal recessive genetic neurometabolic disorder. DOORS syndrome has five universal and prominent symptoms, which included deafness, absent or hypoplastic finger and/or toenails, finger deformities, mental retardation, and seizures. In addition, ophthalmologic anomalies, peripheral neuropathy, and characteristic coarse facial features such as a large nose with a wide nasal bridge, bulbous tip and anteverted nares, a long prominent philtrum, and downturned corners of the mouth can be observed. Less common findings included dental, kidney, and cardiac anomalies.

Case Report

A 1-year-old male patient, the child of a non-consanguineous couple, was genetically evaluated with infantile resistant epilepsy, hypotonia, nail dystrophy, digit anomaly, hearing loss, and dysmorphic facial findings. Karyotype and microarray analysis were 46,XY. Whole exome sequencing revealed a homozygous missense c.463G>C (Ala155Pro) variant in *TBC1D24* gene.

Discussion

In this study, we report a novel variants in DOORS syndrome and contribute to the clinical and molecular spectrum of the disorder.

[OP-43]

Deletion of Multiple Exons of KIF1C Detected by Next Generation Sequencing Associated with Spastic Ataxia 2

Ebru Tunçez¹, Oğuzhan Bahadır²

¹Konya City Hospital, Clinic of Medical Genetics, Konya, Türkiye

²DiagnoSeq Genetic Diseases Assessment Center, Ankara, Türkiye

Abstract

Introduction: Spastic ataxia 2 (SPAX2) is a rare, complex subtype of hereditary spastic paraplegia characterized by onset in the first two decades of cerebellar ataxia, associated with gait disturbances, dysarthria, and variable spasticity of the lower limbs. Autosomal recessive SPAX2 is caused by homozygous mutation in the *KIF1C* gene on chromosome 17p13. In 2014 Novarino et al. identified a homozygous deletion of exons 14-18 of the *KIF1C* gene in patients with SPAX2.

Methods: A 15-year-old male was referred with frequent falls, tremor, dysarthria, lower limb spasticity, hyperreflexia and specific learning disability. Echocardiography showed patent foramen ovale but no abnormal findings were found by brain magnetic resonance imaging, magnetic resonance spectroscopy, electroencephalography, electromyography and metabolic tests. In a NGS panel of 63 genes targeted for hereditary spastic paraplegia, all exonic regions and exon-intron junctions were analyzed by Qiagen Clinical Insight Interpret software after DNA isolation from peripheral blood of the patient. Microarray analyzes were performed with Illumina iScan System, 700K.

Results: No pathogenic mutation was detected in targeted NGS panel analysis, but homozygous deletion was suspected because exons 19-23 of the *KIF1C* gene could not be sequenced. Microarray analysis revealed a ~14-kb homozygous deletion of *KIF1C* and *SLC52A1* (arr[GRCh38]17p13.2(5020462_5034482)x0).

Conclusion: To the best of our knowledge, there is no previous report of SPAX2 with deletion of exons 19-23 of the *KIF1C* gene. We aimed to contribute to the literature about this rare form of spastic ataxia with the findings of this patient.

[OP-44]

Confirmation of *PATL1* Gene as Neurodevelopmental Disease Gene by Fruit Fly Model

Berfin Dağ¹, Mert Tufan¹, Abdullah Sezer², Arzu Çelik Fuss^{1,3}

¹Boğaziçi University Faculty of Medicine, Department of Molecular Biology and Genetics, Istanbul, Türkiye

²Ankara Etlik City Hospital, Clinic of Medical Genetics, Ankara, Türkiye

³Boğaziçi University Faculty of Medicine, Center for Life Sciences and Technologies, Istanbul, Türkiye

Abstract

Neurodevelopmental disorders are characterized by limitations in social behavior and intellectual disability (ID). To understand the genetic causes of ID and examine the biological pathways underlying ID risk genes reported by next-generation sequencing studies, the fruit fly *Drosophila melanogaster* is an ideal model, allowing screening of candidate genes with simple analyses. *PATL1* gene variants detected in two independent patients diagnosed with ID provided strong evidence that the *PATL1* gene is a candidate for ID. *PATL1* protein localizes in cytoplasmic p bodies and regulates mRNA metabolism. *In vivo* studies revealed that when mutated, HPAT, the *PATL1* ortholog in the fruit fly, causes synaptic defects at the neuromuscular junctions, and HPAT is vital for plasticity at synapses (Pradhan et al., 2012). We aimed to determine the genetic and neuromorphological consequences of the variations in the *PATL1* gene by performing functional and morphological analyses with mutant lines of the *HPAT* gene. We showed that knock-down of HPAT in the whole body causes lethality, and pan-neuronal knockdown of HPAT causes partial lethality with severe defects in motor functions. As a result of morphological analysis, we showed that HPAT is required for axon adhesion and guidance in the mushroom body in the fly brain, which is accepted as the analog of the human hippocampus. Also, we showed that HPAT has a critical role in the laminar organization of the ellipsoid body, the analog of the human brain basal ganglia in the fly brain central complex, which governs target-oriented navigation and motor control. All our findings highlight the critical role of HPAT in the central nervous system and synaptic functioning. This work is supported by TÜBİTAK project number: 122Z319.

Keywords: Neurodevelopmental disease, animal models, *Drosophila melanogaster*, p bodies, *PATL1*

[OP-45]

Insensitivity to Pain, Congenital, with Anhidrosis (CIPA): Presentation of 2 Siblings

Onur Hanoğlu, Ahmet Arman, Pınar Ata, Bilgen Bilge Geçkinli, Şenol Demir, Ayşenur Ersoy

Marmara University Faculty of Medicine, Department of Medical Genetics, İstanbul, Türkiye

Introduction

Hereditary Sensory and Autonomic Neuropathy (HSAN) is a rare syndrome characterized by genetic heterogeneity. Insensitivity to pain and autonomic dysfunction are common features in affected patients. Homozygous or compound heterozygous mutations in the *NTRK1* gene have been associated with HSAN type 4 (CIPA), which is accompanied by anhidrosis among the common symptoms. The aim of our study is to discuss the clinical characteristics of two siblings in whom we detected mutations in the *NTRK1* gene within the context of genotype-phenotype relationship.

Case Report

Two Syrian-origin sisters were referred to our clinic due to recurrent fever episodes, anhidrosis, pain and temperature insensitivity, delayed wound healing, and self-mutilating behaviours. There were no specific prenatal, natal, or postnatal history features for the patients. However, both siblings had been hospitalized multiple times due to recurrent fever episodes. The siblings, whose parents had a first-cousin marriage, did not have a similar history in their family. During the physical examinations of the patients, common findings included bite marks on the tongue, amputations in the distal phalanges of their fingers due to self-biting, and non-healing wounds on their bodies. In the Whole Exome Sequencing analysis of the older sibling, a homozygous c.200delA (p.N67Tfs*2) frameshift variant in the *NTRK1* (NM_002529.3) gene was detected, which is not defined in ClinVar but evaluated as a potential pathogenic variant according to ACMG criteria. Segregation analysis in family members identified the parents as heterozygous carriers of the same pathogenic variant, while the affected sibling was homozygous for it.

Conclusion

Despite the rarity of CIPA as a genetic disease, a molecular diagnosis plays a significant role in screening at-risk individuals within the family, as well as in providing appropriate genetic counselling, conducting prenatal diagnosis, and following up with patients.

[OP-46]

A Case of Char Syndrome with a Novel TFAP2B Variant

Fırat Özçelik¹, Aslıhan Kiraz¹, Süleyman Sunkak², Ali Şahin³, Yusuf Özkul¹, Munis Dündar¹

¹*Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye*

²*Kayseri City Hospital, Clinic of Pediatrics, Division of Pediatric Cardiology, Kayseri, Türkiye*

³*Erciyes University Faculty of Medicine, Department of Neurosurgery, Kayseri, Türkiye*

Introduction

Char syndrome (OMIM:169100) is a rare disorder characterized by dysmorphic facial features, patent ductus arteriosus, and hand anomalies. Its prevalence is unknown. The most common facial features are depressed nasal bridge, broad nasal tip, downslanted palpebral fissures, hypertelorism, short philtrum, and triangular mouth. The characteristic hand anomaly is aplasia or hypoplasia of the middle phalanges of the fifth fingers. Other congenital heart and extremity anomalies are also encountered. More rarely craniosynostosis, short stature, and other findings affecting organs such as teeth, eyes, and ears can also be seen. This autosomal dominantly inherited syndrome is caused by heterozygous disease-causing variants in *TFAP2B*. *TFAP2B* belongs to the AP-2 gene family and encodes the transcription factor AP-2 beta. It is thought to have functions in cell proliferation and embryonic development.

Case Report

A 4-month-old female patient was consulted to our department with dysmorphic facial features and craniosynostosis. Hypertelorism, depressed nasal bridge, upslanted palpebral fissures, hypoplasia of the middle phalanges of the fifth fingers, and metopic synostosis were detected in the clinical examination. An echocardiogram revealed patent ductus arteriosus. Molecular testing with clinical exome sequencing revealed a novel heterozygous frameshift variant c.142del p.(Gln48Lysfs*48) in exon 2 of *TFAP2B* (NM_003221).

Discussion

Both haploinsufficiency and dominant-negative mechanisms have been reported in this syndrome, our case is likely caused by haploinsufficiency. Our report broadens the mutational spectrum of *TFAP2B* gene causing Char syndrome and highlights the importance of detailed physical examination and molecular testing in diagnosing rare syndromes.

[OP-47]

The Impact of Musical Performance on Cognitive Functions and Its Relationship with BDNF val66met and COMT val158met Polymorphisms

Sayeste Çağıl İnal^{1,2}, Emel Güneş¹

¹Ankara University Faculty of Medicine, Department of Physiology, Ankara, Türkiye

²Ankara University Institute of Health Sciences, Ankara, Türkiye

Introduction: Musical training alters cognitive skills. The genetic contribution to these skills remained less explored. *BDNF* and *COMT* genes are related to brain plasticity. Drawing on a previous studies suggested that brain plasticity may be higher among musician we aimed to investigate the effect of musical training by using some tasks, which evaluate visuospatial and verbal skills, along with genetic profiling of *BDNF* and *COMT* polymorphisms.

Methods: Participants were 30 musicians and 30 control subjects with right-hand dominance. Cognitive assessments were done using Corsi block tapping (CBT), mental rotation (MRT), and verbal fluency tasks (VFT). CBT and MRT are visuospatial tasks performed separately by both hands. VFT has phonetic and semantic components. Genotype samples were taken from the participants using buccal brushes.

Results: CBT and VFT showed significant differences between the groups. Left-hand scores of the CBT were higher in musicians. Results show a positive change in the visuospatial abilities of musicians while using the non-dominant hand, which suggests that there is a relation between motor skills and visuospatial abilities. In the VFT, a significant decrease in errors made by musicians was found. Fewer errors made by musicians indicate that executive inhibition in musicians is better. Genotypes showed differences in some cognitive scores. Based on the results of our study, environmental variables, such as musical training, might be interacting with the *COMT* val158met polymorphism, specifically val allele, and thus give rise to differing cognitive phenotypes.

Conclusion: The study supports the idea that gene-environment interaction should be assessed together. Indicated polymorphisms seem to be candidate genes for determining the genetic mechanism.

[OP-48]

Examining the Effect of Sugar Diet on miR-126-3p Expression Level in Autistic Mouse Model

Nuriye Gökçe¹, Ecma Güvenilir², Zeynep Yılmaz Şükranlı², Samed Yora², Neslihan Başgöz², Serpil Taheri^{2,3}, Minoou Rassoulzadegan^{3,4}, Yusuf Özkul^{1,3}

¹Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

²Erciyes University Faculty of Medicine, Department of Medical Biology and Genetics, Kayseri, Türkiye

³Erciyes University, Betül-Ziya Eren Genome and Stem Cell Center, Kayseri, Türkiye

⁴Université Côte d'Azur, CNRS, Inserm, France

Abstract

Autism is a multifactorial disorder that affects the central nervous system, with an etiology that has not been fully elucidated. Epigenetics refers to the study of changes that can directly affect gene expression without any alterations in DNA sequence. Epigenetic mechanisms can bring about changes in a gene's activity at transcriptional, post-transcriptional, translational, and post-translational levels. MicroRNAs (miRNAs), a subset of small non-coding RNAs, are among the best-characterized epigenetic mechanisms. MiRNAs play critical roles in processes such as development, protein secretion, and gene regulation. Studies in the literature have shown that different diet programs applied to autism patients can lead to changes in the severity of their behaviors. Additionally, diets used in various diseases have been shown to alter the expression levels of related genes. According to information from the literature, it has been found that the expression levels of six miRNAs are low in children with autism and their families. Therefore, valproic acid (VPA) mouse models, which exhibited autism-like behavior (confirmed through behavioral and molecular tests) were used. In the current study, newborn mice were fed diets containing 20%, 30%, and 10% (normal diet) sugar immediately after birth, and behavioral tests were conducted when they reached 2 months of age. The effects on the expression levels of autism-related marker miR-126-3p in the hippocampus, sperm, and blood tissues were examined. The results of our study showed significant differences in the hippocampus tissue of the VPA group compared to the group fed a 20% sugar diet with saline injection in behavioral experiments. Furthermore, there was a significant increase in sperm tissue in the group fed a 30% sugar diet compared to the control group. Our study results indicate that diets applied in autism can potentially alter both behavioral and molecular phenotypes. Additionally, the changes in sperm miRNA expression profiles due to sugar diet suggest that dietary habits may significantly impact subsequent generations.

Keyword: Autism, VPA, sugar diet, miR-126-3p

[OP-49]

Investigation of the Effects of Human REG3A Antimicrobial Protein on CD4+ T and Dendritic Cells *Ex Vivo*

Zehra Büsra Azizoğlu^{1,2}, Ahmet Eken^{1,2}

¹Erciyes University Faculty of Medicine, Department of Medical Biology, Kayseri, Türkiye

²Genome and Stem Cell Center, Department of Molecular Biology and Genetics, Kayseri, Türkiye

Introduction: Regenerative islet-derived protein 3-alpha (REG3A), also called hepatocarcinoma-intestinal-pancreas or pancreas-associated protein, functions as an antimicrobial protein in the intestine. In this study, we investigated the immunomodulatory effects of REG3A, a member of the Reg family, on primary T-cells such with respect to polarization, cytokine production, proliferation, and on dendritic cell (DC) maturation.

Methods: Proliferation, apoptosis and IL-2 production by PBMCs obtained from blood taken from healthy volunteers using the Ficoll-Hypaque density gradient were examined. Naive CD4+ human T-cells were differentiated into selected Th1, Th2, Th17 and T-cells. CD14+ monocytes were differentiated into DCs. Select doses of recombinant human REG3A proteins were added to the culture. Flow cytometric analysis results were examined using FlowJo and Graphpad 9.

Discussion: Picogram doses of REG3A on T-cells did not cause statistically significant difference in terms of proliferation, apoptosis and IL-2 production. A dose-dependent decrease in FOXP3+ T-cells was seen but was not significant. REG3A exposure did not result in a significant difference in the expression of checkpoint inhibitors such as KLRG, PD-1, and CTLA4 by FOXP3+ T-cells. On the other hand, REG3A reduced Th17 cell-derived IL-17 and IL-22 production. Apoptosis experiments have shown that this reduction was due to cytotoxic effect of REG3A on Th17 cell cultures. Th2 cell-derived IL-4 production was not affected by REG3A exposure. When DCs were examined, REG3A exposure increased the surface expressions of ICOS and MHCII by mature DCs.

Conclusion: These results indicate that human REG3A has more pronounced effects on the Th17 lineage and may affect DC maturation. This project is part of thesis at ERÜ Health Sciences Institute, Department of Medical Biology a supported by the ERÜ BAP project with code TDK-2020 10779.

Keywords: Antigen presenting cell, CD4+ T-cell, REG3A

[OP-50]

Investigation of the Effect of Schisandrin B on Cytokine and Chemokine Production in Human Peripheral Blood Cells

Şerife Erdem^{1,2}, Ahmet Eken^{1,2}, Hamiyet Altuntaş¹, Mustafa Çakır³

¹Erciyes University Faculty of Medicine, Department of Medical Biology, Kayseri, Türkiye

²Erciyes University, Genome and Stem Cell Center, Kayseri, Türkiye

³Yüzüncü Yıl University Faculty of Medicine, Department of Medical Biology, Van, Türkiye

Introduction: The aim of this study is to characterize the effects of different doses of Sch-B on human immune cells, particularly T-cell subtypes, through *ex vivo* experiments at the molecular level.

Methods: To characterize the effects of Sch-B on human immune cells, lymphocytes isolated from peripheral blood of healthy donors were cultured with different doses of Sch-B for 24 hours, and the released chemokines and cytokines were evaluated using multiplex ELISA (the experiment was performed in quadruplicate). Lymphocytes cultured in Sch-B medium were stimulated with CD3/CD28 and curdlan. After stimulation bilegend multiplex ELISA was performed, followed by analysis and quantification using flow cytometry.

Results: Depending on the dose of Sch-B, there was a significant decrease in cytokine levels of interleukin (IL)-5, IL-13, IL-2, IL-6, IL-9, IL-10, and interferon- γ , while the levels of tumor necrosis factor- α , IL-17A, IL-17F, IL-4, and IL-22 cytokines significantly increased. Additionally, under conditions of Curdlan stimulation, there was a significant increase in IL-8 chemokine release with dose-dependent Sch-B, while the release of MIP-1 α , MCP-1, ENA-78, and GRO- α chemokines showed a significant decrease. There were no significant changes in the release of IP-10, TARC, RANTES, MIP-3 α , and MIP-1 β chemokines between groups. In CD3/CD28-stimulated groups, there was a significant increase in IP-10, TARC, RANTES, and MIG chemokines, a significant decrease in MIP-1 α , MCP-1, MIP-1 β , ENA-78, MIP-3 α , and GRO- α chemokines, while there was no change in IL-8 chemokine release.

Conclusion: Sch-B exhibits immunomodulatory properties, affecting the cytokines and chemokines released from human lymphocytes under *in vitro* conditions.

Keywords: Multiple sclerosis, lymphocytes, Th17 cell, treg cell, cell culture, multiplex ELISA

[OP-51]

Evaluation of the Effectiveness of Combined Treatment with the PD-L1 Inhibitor Atezolizumab and the c-MET Inhibitor Crizotinib in MCF-7 Breast Cancer Cells

Neslihan Haklıgur¹, Ahsen Güler^{1,2}, Mevlüde İnanç³

¹Erciyes University, Betül Ziya Eren Genome and Stem Cell Center, Kayseri, Türkiye

²Erciyes University Faculty of Medicine, Department of Medical Biology, Kayseri, Türkiye

³Erciyes University Faculty of Medicine, Department of Medical Oncology, Kayseri, Türkiye

Abstract

Introduction: Atezolizumab is a PD-L1 inhibitor. PD-L1 immune checkpoint inhibitors are used in the treatment of breast cancer. However, response rates as monotherapy are limited. Additionally, most patients are resistant to treatment. c-MET has been reported to be associated with drug resistance in various cancer cells. Additionally, in studies showing the relationship between c-MET and immunotherapy, it has been reported that c-MET inhibitors increase the effectiveness of immunotherapies. Therefore, in our study, we evaluated the possible effectiveness of atezolizumab and c-MET inhibitor crizotinib combined treatment in MCF-7 breast cancer cells.

Methods: Some MCF-7 breast cancer cells were treated with atezolizumab, some cells were treated with crizotinib, and some cells were treated with atezolizumab + crizotinib. Additionally, some breast cancer cells were co-cultured with peripheral mononuclear blood cells (PMBC). Then, some of co-cultured cells were treated with atezolizumab, some with crizotinib, and some with atezolizumab + crizotinib. Then, cell viability (MTS analysis), colony formation (clonogenic detection) and apoptosis rates (annexin V analysis) of all these cells were analyzed.

Results: According to the results, it was found that atezolizumab had no effect on cell viability, colony formation and apoptosis rate in MCF-7 cells in the presence and absence of PMBC cells. It was found that crizotinib dose-dependently decreased cell viability and colony formation in MCF-7 cells and significantly increased the rate of apoptosis. It was found that the combination of atezolizumab + crizotinib in the presence of PMBC cells reduced cell viability and colony formation and increased the rate of apoptosis of cancer cells. However, it was found that these effects were similar to those on cells treated with crizotinib alone, so combined application did not increase the effectiveness of atezolizumab.

Conclusion: These findings show that atezolizumab has no effect on MCF-7 cells and crizotinib has a cytotoxic effect on breast cancer cells.

Keywords: Breast cancer, atezolizumab, PD-L1, crizotinib, c-MET

[OP-52]

A Metabolic Disease in which Neurological Findings are Prominent: Arginase Deficiency

Özgür Balasar

Konya City Hospital, Konya, Türkiye

Abstract

Arginase deficiency (MIM#207800) is an autosomal recessive disease caused by compound heterozygous and homozygous mutations in the *ARG1* gene. It is an inborn error of metabolism resulting from a defect in the hydrolysis of arginine into urea and ornithine, in the last step of the urea cycle. It is characterized by episodic hyperammonemia, spasticity, loss of ambulation, seizures and severe intellectual disability in untreated individuals. Rarely, it can be life-threatening and cause death. A 10-year-old male patient was referred to us because of walking disorder. The patient's parents were sixth degree relatives. His siblings were healthy, but his mother's cousin's son also had difficulty walking. On physical examination, the patient had spasticity and ataxic gait. The patient had gait disorder for 5 years. Additionally, the patient had hypothyroidism, learning disability, and abnormal EEG findings. For these reasons, chromosome analysis and molecular karyotyping tests performed on the patient were normal. Also, when the *SPAST* and *ATP7B* gene analysis and the triplet repeats of *FXN* gene were found to be normal, whole exome sequencing (WES) test was performed on the patient. In the WES analysis, the c.702_703insAGACT (p.G235fs*20) variant in *ARG1* gene (NM_000045.4), which explains the patient's clinic, was detected as homozygous. This variant is a likely pathogenic variant causing loss of function. Comprehensive analysis such as WES analysis enable accurate diagnosis and appropriate clinical management. We also thought that we could contribute to the literature by presenting a new case about this rare disease.

Relationship between *GSTP1*, *XRCC1*, *ERCC1*, *MTHFR* *TSER* and *DPYD* Gene Polymorphisms and Progression-Free Survival in Colorectal Cancer Patients Received FOLFOX Treatment

Ege Rıza Karagür¹, Atike Gökçen Demiray², Hakan Akça¹

¹Pamukkale University Faculty of Medicine, Department of Medical Genetic, Denizli, Türkiye

²Pamukkale University Faculty of Medicine, Department of Medical Oncology, Denizli, Türkiye

Abstract

Introduction: In this study, we compared the genetic polymorphisms associated with 5-fluorouracil and oxaliplatin chemotherapy drugs and the progression-free survival times of stage IV and metastatic colon cancer patients who were treated only with the FOLFOX chemotherapy regimen. In this retrospective study, national and international ethical rules were complied with. This study approved Pamukkale University Ethic Committee with number of E-60116787-020-443991.

Methods: *GSTP1*-313A>G, *XRCC1*-G28152A, *ERCC1*-8092C>A, *ERCC1*-19007T>C, *MTHFR*-677C>T, *MTHFR* 1298A>C, *DPYD* IVS 14+1 G>A, single nucleotide polymorphisms in *GSTP1*, *XRCC1*, *ERCC1*, *MTHFR* *TSER* and *DPYD* genes were sequenced by pyrosequencing method. The polymorphism and progression-free survival times of each patient were subjected to Cox analysis to investigate the response to treatment after FOLFOX treatment and the effect of the relevant polymorphism.

Results: The progression-free survival times evaluated in the wild type (wt) and heterozygous (het) subgroups were found to be statistically significant in the *XRCC1* G28152A (log-rank p=0.0113), and *ERCC1* C8062A (log-rank p<0.000.1) polymorphisms.

Keywords: Colorectal cancer, SNP, FOLFOX, polymorphism, PFS

Introduction

Colorectal cancer is a common and deadly cancer worldwide. It is the third leading cause of cancer-related deaths (1). Almost one-third of patients have locoregional lymph node metastases at the time of diagnosis and are diagnosed at stage III. When treated with surgery alone, 40-50% of patients with locoregional disease develop recurrence or distant metastases due to micrometastases (2,3). Adjuvant chemotherapy aims to eliminate these micrometastases and ensure cure. For the first time in the literature, Moertel et al. (4) showed that 12 months of 5-fluorouracil (5-FU) and levamisole combination for stage III lymph node-positive colon cancer led to a 33% reduction in mortality rates. Following the demonstration of the survival advantage of the combination of 5-FU and leucovorin (LV) for 6 months, adjuvant chemotherapy based on 5-FU/LV for 6 months has become the standard treatment for stage III colon cancer (5). The Multicenter International Study of Oxaliplatin/5-FU/LV in Adjuvant Treatment of Colon Cancer trial found that adding oxaliplatin to 5-FU/LV resulted in a 7.5% increase in 5-year disease-free survival (DFS) and a 4.2% increase in 6-year overall survival, and recommended oxaliplatin-based chemotherapy as standard postoperative treatment for these patients (6). Gene polymorphisms and mutations reduce the efficiency of chemotherapy regimens by affecting the metabolism of drugs. Specific polymorphisms enhance the efficacy of some drugs, while others greatly increase the toxicity of these drugs. Therefore, they are thought to be effective in predicting clinical response to chemotherapy or chemotherapy-related toxicity in many cancer types. 5-FU acts via fluorodeoxyuridine monophosphate (FdUMP), a molecule that inhibits thymidylate synthase (TS). It increases the risk of various types of cancer by acting on functional polymorphisms in the *TS* gene. It enables nucleotide synthesis by altering the activity of the enzyme. Dual (2R) and triple (3R) repeating polymorphisms in the thymidylate synthase promoter end region (*TSER*) are described. *In vitro* and *in vivo* studies have shown that *TS* expression is overexpressed by the 3R allele of the *TSER* genotype. Thus, several epidemiologic studies on colorectal cancer risk and *TSER* 2R/3R polymorphism affecting Folate metabolism have been conducted but the results are inconsistent (7). Reduced 5-FU clearance and increased 5-FU toxicity have been observed in some colorectal cancer patients with *DPD* gene containing IVS14+1G>A polymorphism. When methylenetetrahydrofolate reductase (*MTHFR*) enzyme activity decreases, intracellular 5,10-methylenetetrahydrofolate (5,10-MTHF) levels increase and FU cytotoxicity may be increased due to this increase (8). Two types of single nucleotide polymorphisms (SNPs) frequently develop on this enzyme: C677T (rs1801133) and A1298C (rs1801131) (9). Alanine and valine change their positions as a result of the C677T transition, thus forming a more thermally unstable protein that reduces enzyme activity (10). The A1298C variant (Glu429Ala) can also reduce *MTHFR* enzymatic activity by causing a missense mutation. In C677T homozygous (TT) or heterozygous (CT) genotypic conditions, *MTHFR* enzyme activity is reduced and blood homocysteine levels are increased. When the A1298C enzyme is converted from the normal (AA) to the homozygous (CC) or heterozygous (AC) form, a decrease in *MTHFR* activity is observed, but no thermo labile protein is formed. Reduced enzyme activity results in high levels of 5,10-MTHF and thymidine, leading to increased DNA synthesis and repair. Therefore, *MTHFR* polymorphisms are thought to be protective against tumorigenesis (11,12). Oxaliplatin, a diamminocyclohexane derivative of cisplatin with higher water solubility and fewer toxic side effects, is actively used in colon cancer combination chemotherapy regimens (13,14). Oxaliplatin inhibits DNA replication and transcription by making covalent bonds to DNA. Oxaliplatin's mechanism of action and gene polymorphisms, families, Excision Repair Cross-Complementing group 1 (*ERCC1*), X-Ray Repair Cross-Complementing group 1 (*XRCC1*), glutathione Stransferases P1 (*GSTP1*) SNPs.

Methods

Patient Group

Patients with pathologically diagnosed colorectal cancer, stage IV according to WHO classification, who were admitted to Pamukkale University Faculty of Medicine, Department of Medical Oncology between 2014-2018 were included.

Genetic Evaluation

i. DNA Isolation

Blood samples of the patients included in the study group were taken 2-3 mL into hemogram blood tubes. DNA isolations were performed on Qiagen EZ1 advanced XL device using 200 μ L of patient blood.

ii. Mutation Analysis

Polymorphism analysis of the patients' DNA was performed simultaneously with amplicon generation using Corbet 5Plex RT-PCR to monitor the number of amplicons. PCR products were then analyzed for polymorphism by pyrosequencing method using Qiagen Pyromark Q24 device. The SNPs included in the study were GSTP1-313A>G, XRCC1-G28152A, ERCC1- 8092C>A, ERCC1-19007T>C, MTHFR- 677C>T, MTHFR 1298A>C, DPYD IVS 14+1 G>A.

In this retrospective study, national and international ethical rules were complied with. This study approved Pamukkale University Ethic Committee with number of E-60116787-020-443991.

Statistical Analysis

COX analysis was used to investigate the effect of genetic mutations on patients' responses to treatment. Statistical analysis was performed with SPSS-21 and Graphpad programs. Results were evaluated at 95% confidence interval. $P < 0.05$ was considered statistically significant.

Results and Discussion

We studied 38 patients, 27 men (71%) and 11 women (28%), with advanced and metastatic stage (stage IV) colorectal cancer who received 5-FU-LV-oxaliplatin (FOLFOX) chemotherapy regimens. In our study, the mean age of the patients was 68.94 ± 8.83 years and the median age was 68.5 years. In this context, 71.05% of the study group consisted of male patients and in this context, the study group is consistent with the literature in terms of both age and gender distribution (15). Demographic and genetic characteristics of the patients are given in Table 1-3.

According to GSTP1 A313G, XRCC1 G28152A, ERCC1 C8062A, ERCC1 T19007C, MTHFR C677T, MTHFR A1298C, DPYD IVS 14+1 G>A, TSER polymorphism; in progression-free survival analyses performed in mutated (mut), wild-type (wt) and heterozygous (het) subgroups, statistically significant differences were found for XRCC1 G28152A, ERCC1 C8062A polymorphisms.

According to XRCC1 G28152A polymorphism, a statistically significant difference was found in the survival analysis between patients with G/G genotype and patients with G/A and A/A genotypes. The median DFS time was calculated as 18.09 months for patients with G/G genotype, 12.11 and 31.7 months for patients with G/A and A/A genotypes (log-rank $p = 0.0113$) (Figure 1). When we consulted the literature, Huang et al.'s (16) studies on patients with metastatic colorectal cancer who received FOLFOX treatment showed a correlation between progression-free survival times in patients with wild type, heterozygous and mutant genotypes.

According to ERCC1 C8062A polymorphism, a statistically significant difference was found in the survival analysis between patients with C/C genotype and patients with C/A and A/A genotypes. The mean DFS time was calculated as 18.45 months for patients with C/C genotype and 21.54 and 3.42 months for patients with C/A and A/A genotypes (Log Rank $p < 0.000,1$) (Figure 1). There are many studies on ERCC1 C8062A polymorphism and non-small cell lung cancer, colorectal cancer, nasopharyngeal cancer on both survival and progression-free survival. The data obtained in our study support each other with the literature data (17-19). This concordance suggests that the ERCC1 C8062A polymorphism is an important predictor of survival on treatment.

Although GSTP1 A313G, ERCC1 T19007C, MTHFR C677T, MTHFR A1298C polymorphisms did not show statistical significance in COX analysis for progression-free survival, it is clear that patients with wild-type genotype showed a later progression compared to mutant and heterozygous patients in accordance with the literature (Figure 1) (20,21).

This study suggests that treatment options for patients with advanced colorectal cancer should be tailored according to GSTP1 A313G, XRCC1 G28152A, ERCC1 C8062A, ERCC1 T19007C, MTHFR C677T, MTHFR A1298C, DPYD IVS 14+1 G>A, TSER polymorphisms. Patients with the mutant variant are recommended to prefer therapies other than FOLFOX for better survival. The limitation of this study is the patient population. This study could be strengthened with a larger number of participants.

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Table 1. Demographic characteristics of the patients

Characters	Patient (n=38) (%)
Age	
≤62	12 (33.3%)
>62	26 (66.6%)
Sex	
Male	27 (71.05%)
Female	11 (28.95%)
Smoking habit	
Smoker	34 (89.47%)
Non-smoker	4 (10.53%)

Table 2. Gene polymorphism analysis results of the patients (oxaliplatin)

Single nucleotide polymorphism	Metastatic, (n=38)
GSTP1 A313G	
Wild type	18
Heterozygous	14
Mutant	6
XRCC1 G28152A	
Wild type	22
Heterozygous	9
Mutant	7
ERCC1 C8062A	
Wild type	19
Heterozygous	10
Mutant	9
ERCC1 T19007C	
Wild type	14
Heterozygous	11
Mutant	13

Table 3. Gene polymorphism analysis results of the patients (5-FU)

Single nucleotide polymorphism	Metastatic, (n=38)
MTHFR C677T	
Wild type	18
Heterozygous	12
Mutant	8
MTHFR A1298C	
Wild type	18
Heterozygous	8
Mutant	12
DPYD IVS 14+1 G>A	
Wild Type	38
Heterozygous	-
Mutant	-
TSER	
Wild type	14
Heterozygous	14
Mutant	10

5-FU: 5-Fluorourasil

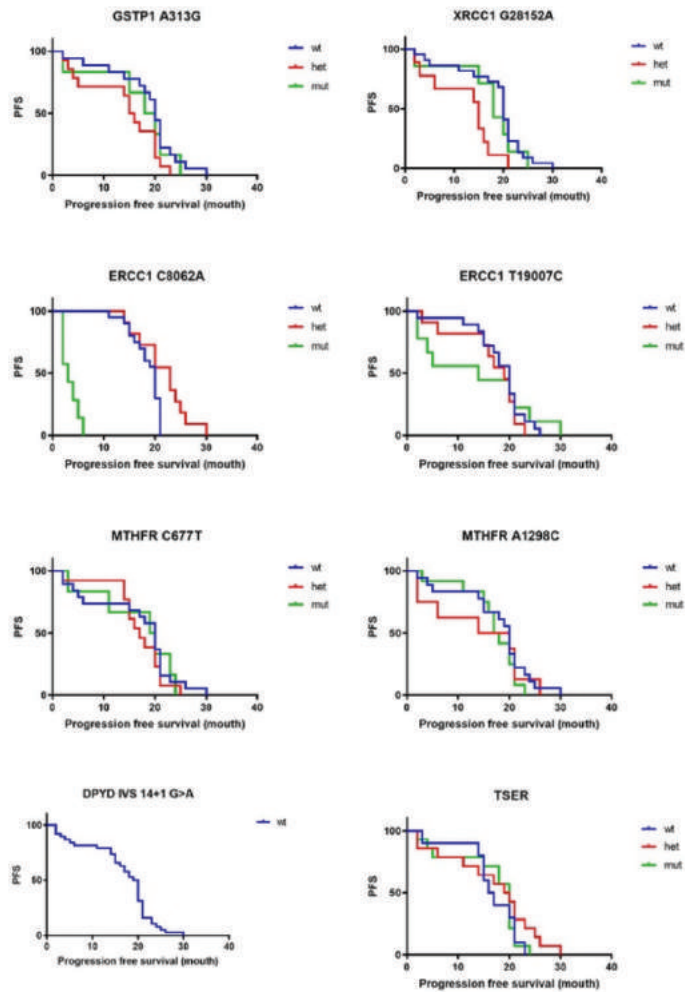


Figure 1. Progression-free survival curves according to GSTP1 A313G, XRCC1 G28152A, ERCC1 C8062A, ERCC1 T19007C, MTHFR C677T, MTHFR A1298C, DPYD IVS 14+1 G>A, TSER polymorphisms in advanced stage patients (wild type: wt, heterozygous: het and mutant: mut)

Attention to Hypotonic Infant: Zellweger Family with Pathogenic Compound Heterozygous c.1804-2A>G and c.3693_3696delGTCA(p.Gln1231Hisfs)rs769836601 Variation in the *PEX1* Gene

Recep Eröz¹, Fatih Kurt², Hilal Akalın³

¹Aksaray University Faculty of Medicine, Department of Medical Genetics, Aksaray, Türkiye

²Düzce University Faculty of Medicine, Department of Child Health and Diseases, Düzce, Türkiye

³Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

Introduction

Zellweger syndrome (ZS) is a rare autosomal recessive disease manifested by dysfunction of peroxisomes necessary for the oxidation of very long chain fatty acids (1). ZS, also known as cerebrotendinous degeneration, is a hereditary disease that affects many systems with serious neurological involvement. Hypotonia, seizures, jaundice, deterioration in liver function tests, malnutrition, and bone anomalies occur in the neonatal period. They have typical facial appearances; they have a high forehead, wide anterior fontanelle, epicanthus, upslanted palpebral fissure, and a hypoplastic supraorbital appearance. Retinopathy, cataracts, kidney cysts, adrenal insufficiency, and hearing impairment are other clinical features that may be observed. They usually die within the first year of life (2). Its incidence varies between 1/50,000 and 1/100,000. While the Canadian province of Quebec is the region with the highest incidence, Japan is the country with the lowest incidence. ZS has been associated with 13 different mutations in the *PEX* gene. *PEX1* and *PEX6* gene mutations were detected in 65% of the patients. *PEX* genes code for proteins called peroxins. Peroxins are necessary for the biogenesis of peroxisomes and ensure the transfer of proteins to the peroxisomal matrix and membrane. The absence of functional peroxisomes results in the absence of β -oxidation of very long-chain fatty acids and the accumulation of peroxisomal substrates. Tissue disorders occur in many organs (3). Next generation sequencing (NGS) is the process of multiplying, sequencing and accurately detecting the variations in large numbers of DNA fragments formed by cutting DNA through enzymatic reactions (4). In this study, we aimed to contribute to the literature by presenting the patient, who was diagnosed with ZS as a result of NGS, together with her family members.

Case Report

A 10-day-old baby was applied because of not being able to feed, bruising while feeding, and little movement. He was born at an external center at 38 weeks, G2P1Y1, weighing 3240 g. He cried as soon as he was born and was given to his mother. His mother stated that the baby had not been able to breastfeed well since birth, was crying slowly and was moving little. In his family history, it was learned that the first baby of the family died at 20 weeks of intrauterine period, and that the mother and father were their maternal uncle's children. In the physical examination of the patient, weight: 2700 g, height: 49 cm and head circumference: 34 cm were measured. Dysmorphic facial findings (protruding forehead, wide anterior fontanelle, flattened nasal root, epicanthus, upslanted palpebral fissure, small chin) were detected in the patient, whose vital signs were normal. Her respiratory system was normal, her respiratory rate was 50/min, and she had no gasping or groaning respirations. Circulatory system examinations were normal, heart rate was 130/minute, and no additional sounds or murmurs were heard. Externally, she appeared to be a girl, and the genitourinary system examination was normal. Abdominal examination was normal and no hepatomegaly or splenomegaly was detected. The patient was hypotonic on neurological examination and neonatal reflexes were absent. Blood hemogram, biochemical tests, CRP, thyroid function tests and kidney function tests were normal. From liver function tests: AST: 262 IU/L, ALT: 146 IU/L, GGT: 80 IU/L, PT: 28 sec, aPTT: 95 sec, INR: 2.3, T. dil: 16.3 mg/dL, D. bil: 6.8 mg/dL was detected. The patient was taken to the neonatal service with preliminary diagnoses of neonatal sepsis, metabolic disease, and biliary atresia. A blood culture was taken and the antibiotic combination ampicillin and gentamicin was started. There was no growth in blood culture. Although the patient was administered vitamin K, there was no decrease in aPTT value. Abdominal USG revealed no hepatomegaly or splenomegaly, but renal cortical cysts were detected. MR cholangiography was performed for the differential diagnosis of biliary atresia, and it was learned that the bile ducts were open. The factor 8, 9, 11 and the Von Willebrand factor levels were found to be normal. Brain MRI revealed pachygyria, perisylvian polymicrogyria, and corpus callosum agenesis. The chromosome analysis result of the peripheral blood sample taken from the patient was normal. Considering these findings, the patient's long-chain fatty acid level was sent with the preliminary diagnosis of ZS. It was determined that the C26 (hexacanoic acid) level increased, the C22 (docosanoic acid) level decreased, and the C26/C22 and C24/C22 ratio increased. As a result, to clarify the diagnosis, blood with EDTA was taken from the patient and the *PEX1* gene was scanned with NGS.

In the genetic examination, autosomal recessive (OR) class 2 pathogenic c.1804-2A>G mutations in the intronic region of the *PEX1* gene and pathogenic c.3693_3696delGTCA(p.Gln1231Hisfs)rs769836601 variation in the exon 23 of *PEX1* gene were detected and a diagnosis of ZS was made. Thereupon, segregation analysis was performed on the family and the pathogenic heterozygous c.3693_3696delGTCA(p.Gln1231Hisfs)rs769836601 variation in the father and the pathogenic novel c.1804-2A>G variation in mother were detected in the *PEX1* gene (Figure 1). The patient died in the 6th month of his life.

Methods

Physical examination, laboratory tests and imaging: The patient's physical and neurological examination were performed. Hemogram, biochemical tests, CRP, thyroid function tests, kidney function tests, liver function tests, long chain fatty acid level, factor 8, 9, 11 and Von Willebrand factor levels, and blood culture were performed from the blood. Abdominal USG, MR cholangiography, and brain MR imaging were performed.

Karyotype analysis: Heparinized peripheral blood samples were taken from the patients, and the metaphase plaques obtained after cell culture were banded with GTG banding and examined under a microscope for karyotype analysis.

STR fragment analysis: Maternal contamination was detected via STR fragment analysis performed on the mother using *D5S818*, *D7S820*, *FGA*, *D21S11*, *D8S1179*, *D13S317*, *VWA*, *D18S51*, *THO-1*, *D3S1358* STR markers.

Rapid aneuploidy test: Again rapid aneuploidy panel in the FISH (13, 18, 21, X ve Y) was performed using *LS1 13*, *LS1 18*, *LS1 21*, *CEPX VE CEPY* probs on CVS material.

Next-Generation Sequencing (NGS)

Genomic DNA was extracted from the peripheral blood samples and CVS. NGS was performed by capture of the coding regions and splice sites of target genes via Illumina custom enrichment panel. Annotation of detected variants was carried out using Illumina BaseSpace Variant Interpreter, InterVar, Franklin, VarSome, ClinVar, OMIM, and Pubmed. dbNSFP (contains SIFT, PolyPhen-2, LRT, Mutation Taster) were used to predict the pathogenicity of variants. Rare variants were classified according to the American College of Medical Genetics and Genomics criteria (5).

Discussion

ZS was named Cerebrohepatorenal syndrome due to the prominent findings in the cases when it was first described by De Lange and Janssen in 1949 (6). It was first reported by Hans Zellweger, after whom the disease is named, that it is caused by a mutation in the *PEX1* gene, which consists of 24 exons located at 7q21.2. Later, other peroxisomal diseases such as infantile refsum disease, neonatal adrenolipodystrophy, rhizomelic chondromalacia punctata were identified. However, since peroxisomes are found in all tissues except mature erythrocytes, it is known that they affect almost all tissues, starting from the intrauterine period. ZS is a rare disease and usually causes mortality within the first year (7). Whole exome sequencing is important to exactly understand the roles and functions of genes associated with different diseases in the etiopathogenesis (8-13).

In such diseases, it is very important to perform family segregation analysis studies and provide genetic counseling in order to have healthy children. In the genetic analysis performed on our patient's mother and father, c.1804-2A>G class 2 OR transitional heterozygous novel mutation in *PEX1* gene intron in the mother, c.3693_3696delGTCA(p.Gln1231Hisfs)rs769836601 class 2 heterozygous mutation in exon 23 of the *PEX1* gene in the father were detected. Two ways were suggested to the family to have a healthy child: The first way is to have a child through in vitro fertilization, the second way is to conduct genetic examination of the baby with CVS at the appropriate week after normal pregnancy is achieved. Termination if the baby is sick, and continuation of the pregnancy if healthy.

Since the mother and father were young, it was decided to perform CVS after pregnancy was achieved normally. No pathology was detected in the mother's cranium examination (Figure 2). No malignancy or intraepithelial lesion was detected in the cervical or vaginal cytology performed on the mother. When the mother became pregnant in 2019, CVS sampling was performed at the 12th week of pregnancy. Karyotype analysis from CVS material was normal (Figure 3). No maternal contamination was detected in the STR fragment analysis performed using *D5S818*, *D7S820*, *FGA*, *D21S11*, *D8S1179*, *D13S317*, *VWA*, *D18S51*, *THO-1*, *D3S1358* STR markers from the CVS material. Again, a normal hybridization pattern was observed in the FISH (13, 18, 21, X ve Y) in rapid aneuploidy panel performed on CVS material. After the genetic examination was normal, the pregnancy continued and they had a healthy daughter. In 2021, the mother became pregnant again and in the amniocentesis, compound heterozygous c.1804-2A>G and c.3693_3696delGTCA/p.Gln1231Hisfs mutations were detected in the fetus, no maternal contamination was detected, and upon the presence of prefrontal thickness in the fetus, a decision was made to terminate by discussing with the family.

Conclusion

NGS technologies have an crucial place in the detection of rare disorders and appear as an important technology for better understanding the etiopathogenesis of different hereditary diseases and making an accurate diagnosis. Genetic counseling is of great importance to develop an effective treatment strategy for hereditary disorders and to reduce the risk of birth with abnormalities so that couples can have a healthy child.

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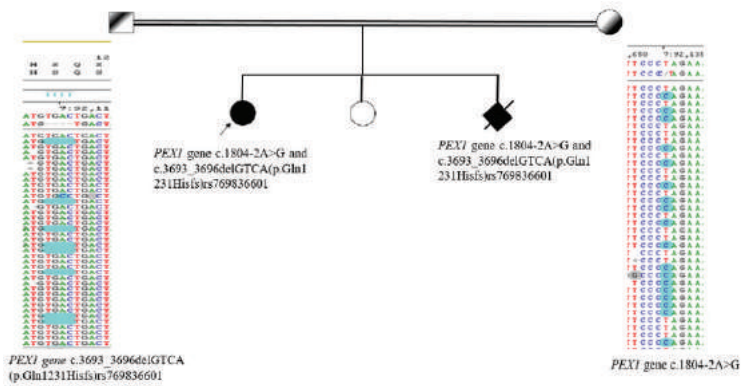


Figure 1. Family pedigree and detected variants

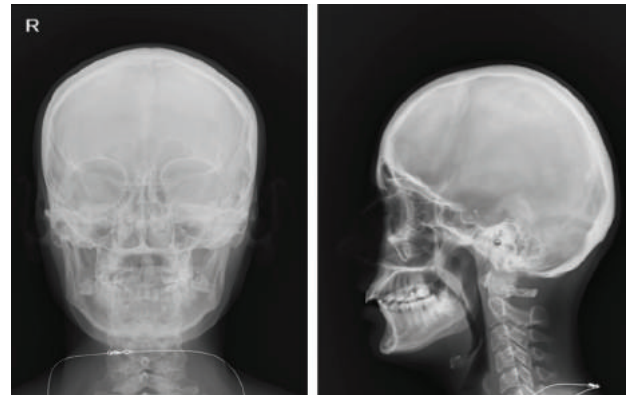


Figure 2. Image of mother's cranium



Figure 3. Karyotype analysis of fetal CVS material

A New Translocation in a Case of Recurrent Pregnancy Loss: t(2;7)(q31;p21)

Büşra Tan, İzem Olcay Şahin, Yusuf Özkul, Aslıhan Kiraz, Munis Dünder

Erciyes University Faculty of Medicine, Department of Medical Genetics, Kayseri, Türkiye

Abstract

Recurrent pregnancy loss (RPL) is when a woman has three or more miscarriages in a row. Chromosomal abnormalities are frequently seen in RPL cases. In this case report, we detected a balanced t(2;7)(q31;p21) translocation in the male partner of a couple who applied to Erciyes University, Department of Medical Genetics with the indication of RPL. Balanced translocation is a type of chromosomal anomaly in which two chromosomes swap parts, but the total amount of genetic material remains the same. Balanced translocations are generally harmless to the carrier, but may increase the risk of RPL and other pregnancy complications. The female partner of the couple in this case had a normal karyotype (46,XX), but the male partner had a balanced translocation between chromosomes 2 and 7 (46,XY,t(2;7)(q31;p21)). For this reason, the couple's children were also examined in terms of family segregation. The t(2;7)(q31;p21) translocation is new to the literature and has not previously been reported as a cause of RPL. In our study, by performing Gene Ontology Enrichment Analysis, we determined that morbid genes in the relevant translocation regions are effective in embryo development. In the light of all the analyses, we conclude that the unbalanced transfer of the 46,XY,t(2;7)(q31;p21) karyotype to the next generation negatively affects the survival of the embryo due to the effect of morbid genes.