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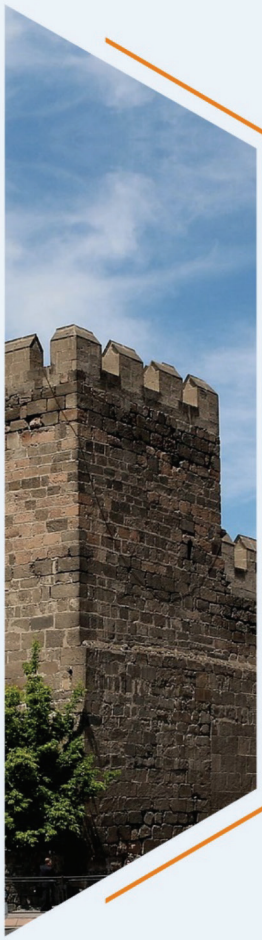
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5q35.2q35.3 Microduplication with Xp22.3 Microdeletion: A Rare 'Reverse' Sotos Syndrome with a New Chromosomal Rearrangement

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Speech Texts

SPEECH TEXTS

Who, When and Which Tests Should be Performed in Rare Childhood Hematological/Oncological Diseases?

Elif Yılmaz Güleç

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Abstract

In pediatrics, the majority of hematological and oncological disorders are rare. In most cases, manifestations usually accompany a rare genetic syndrome. When we classify these disorders in two main groups such as “hematological” and "oncological", hematological disorders include:

- Erythrocytic series diseases,
- Leukocyte diseases: non-erythrocyte myeloid series disorders and lymphoid series disorders,
- Platelet-related diseases,
- Primary immune-deficiencies,
- Coagulation pathway disorders.

Single gene analyses are preferred in phenotypes thought to be due to a single gene defect, while next generation sequencing panels are preferred in diseases with genetic heterogeneity, and methods such as whole exome sequencing and whole genome sequencing are preferred in cases with complex and syndromic phenotypes. Although all childhood cancers are rare, some are ultra-rare. The ultra-rare hematological malignancies in childhood are:

- Myelodysplastic neoplasms,
- Germline predisposition syndrome related hematological neoplasms: e.g. Noonan syndrome and juvenile myelomonocytic leukemia,
- Rare AML types: e.g. with *MECOM* rearrangement, *KAT6-CREBBP* fusion, *FLT3-ALM* fusion,
- Rare Lymphoid neoplasma: e.g. T-cell large granular lymphocytic leukemia.
- Ultra-rare solid tumors are classified as tumors with an incidence of <2/1,000,000 and include 14 malignancies: Nasopharyngeal carcinoma, adrenocortical tumors, pleuro-pulmonary blastoma, etc. Such rare tumors are usually predisposed by germline mutations of various genes: e.g. *DICER1* gene mutation and pleuro-pulmonary blastoma.

In addition to germline genetic tests, it is important to test somatic gene alterations and/or fusion genes and proteins, as well as SNVs, CNVs, rearrangements, methylations in tumor-associated genes. Appropriate tests are selected according to the tumor type.

Keywords: Rare pediatric cancers, rare pediatric hematological disorders, ultra-rare pediatric cancers, pediatric onco-genetics, pediatric hemato-genetics

Interpreting Genetic Results Accurately by Clinicians in Rare Childhood Hematologic and Oncologic Diseases

Özlem Sezer

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Abstract

In rare childhood hematologic and oncologic diseases, disease panels containing numerous genes are utilized for accurate diagnosis, risk assessment, prognosis prediction, and treatment strategy due to the diversity of genetic causes and unexpected genotype-phenotype relationships. The classification of variants identified in genetic tests is an ongoing process of data collection, and can change based on accumulated genetic data. Moreover, the compatibility of clinical findings with variants is influenced by numerous factors. Variants are interpreted using clinical findings, zygosity, minor allele frequency (<1%), population and patient data, segregation data, *de novo* data, functional data, and computational and prediction-based data. The most significant challenge in variant interpretation is the presence of “variants of uncertain significance” (VUS). When there is insufficient relevant data to determine whether a variant disrupts gene function, it is referred to as a VUS. VUS should not be used in clinical management or reproductive decision-making. Clinical decisions should be based on personal and family history. As genomic information advances and information for variant classification accumulates, a variant may be reclassified from one category to another. Only 10% of VUS can be reclassified to pathogenic/likely pathogenic upon accumulation of additional evidence. Segregation analysis often sheds light on VUS due to its familial specificity. *De novo* occurrence of the variant and consistent segregation in affected and unaffected siblings provide information about the variant’s pathogenicity. Advancements in genomic technologies and a more comprehensive understanding of the human genome will assist clinicians in accurately interpreting genetic results.

Keywords: Genetic, segregation, variant, VUS

SPEECH TEXTS

Gene Editing Therapies in Hemoglobinopathies

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Abstract

Hemoglobinopathies, such as transfusion-dependent β-thalassemia (TDT) and sickle cell disease (SCD), are prevalent monogenic disorders with significant global health impacts. Traditional treatments like transfusions, chelation therapy, and bone marrow transplantation have limitations and risks. Recent advancements in gene editing technologies, particularly CRISPR-Cas9, offer promising curative approaches. CRISPR-Cas9 technology uses a single guide RNA and the Cas9 protein to create precise genetic modifications, revolutionizing gene therapy. This abstract highlights cutting-edge developments in gene editing therapies for hemoglobinopathies, focusing on the mechanisms and clinical applications of treatments like Casgevy and Lyfgenia. Casgevy, the first CRISPR-based therapy to receive approval, targets the *BCL11A* gene, a transcription factor that represses γ-globin production postnatally. By disrupting *BCL11A*, Casgevy reactivates γ-globin expression, compensating for defective β-globin in TDT and SCD patients. Clinical trials show that a single dose of Casgevy significantly reduces severe vaso-occlusive crises and achieves transfusion independence in most patients. Lyfgenia, using a lentiviral vector, modifies hematopoietic stem cells to produce anti-sickling hemoglobin (HBs). It introduces a variant Hbs with an amino acid substitution that prevents sickle HBs polymerization. This anti-sickling variant, HbAT87Q, inhibits red blood cell sickling, reducing vaso-occlusive crises and hemolysis in SCD patients. Clinical trials demonstrate that Lyfgenia decreases painful crises frequency and improves overall HBs levels. These therapies represent a significant advancement in treating hemoglobinopathies, offering potential cures and improved quality of life. As gene editing technologies evolve, they promise to transform the therapeutic landscape for various genetic disorders.

Keywords: Hemoglobinopathies, transfusion-dependent β-thalassemia, sickle cell disease, CRISPR-Cas9

Current Treatment Approaches in Erythrocyte Membrane and Enzyme Defects

Sultan Aydın

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Abstract

Pyruvate kinase (PK) deficiency is the most common glycolytic pathway (Embden-Meyerhof pathway) enzyme defect that causes non-spherocytic hemolytic anemia. PK provides adenosine triphosphate (ATP) synthesis by catalyzing the conversion of phosphoenolpyruvate to pyruvate in the Embden-Meyerhof pathway. More than 600 families have been reported in the literature, and more than 300 mutations in the gene that causes PKLR have been identified. The clinic findings vary widely, such as mild, moderate, or severe anemia. Mitapivat or etavopivat treatment is among the current treatments for severe and transfusion-dependent PK deficiency. Mitapivate (AG-348) is a novel oral small molecule allosteric activator of the enzyme PK. Mitapivat regulates erythrocyte PK, increasing ATP production, decreasing 2,3-diphosphoglycerate levels, and increasing PK activity. It was found that anemia improved, and hemolytic anemia symptoms decreased in patients with PK deficiency. According to current literature, it was determined that the hemoglobin increase was higher in patients with homozygous missense mutations, and the PK protein level was higher in patients with missense mutations. Mitapivat or etopivat treatment is recommended, especially in patients with homozygous or heterozygous missense mutations. It was determined that PK deficiency patients without a missense mutation did not benefit from these treatments. In the open-label, single-arm ACTIVATE-T Phase 3 study conducted in 20 centers in Europe, North America, and Asia, patients over the age of 18 with no regular erythrocyte suspension (ES) transfusion and patients over the age of 18 with regular ES transfusion at least 6 times a year with PK deficiency were included in the ACTIVATE-T Phase 3 study. It was used for a period of 24 weeks by increasing the dose to 2x5 mg per day, 2x25 mg per day, and 2x50 mg per day. At the end of the study, A≥33% decrease in the number of ES transfusions and an improvement in hemolysis findings were detected. Side effects related to medication, such as increased liver dysfunction, headache, nausea, and vomiting, have been observed. Non-drug-related side effects such as joint swelling, hypertriglyceridemia, ovarian cysts, and renal colic have been reported. Etavopivate (FT-4202), another PK allosteric activator, was determined as the most effective dose of 400 mg once a day as a result of Phase 1 studies.

Keywords: Pyruvate kinase deficiency, mitapivate, etavopivate

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SPEECH TEXTS

Genetic Origins of Fanconi Anemia

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Abstract

Fanconi anemia (FA) is the most common inherited cause of aplastic anemia and represents a clinical spectrum characterized by susceptibility to hematologic malignancies and solid tumors. The majority of FA patients feature growth retardation, pigmentary abnormalities, and variable multisystem malformations including limb defects, genitourinary and gastrointestinal anomalies. FA is genetically heterogeneous, with mutations in 22 genes related to the interstrand cross-link repair pathway. Dysfunction in these genes results in cellular hypersensitivity to cross-linking agents, spontaneous chromosome breakage, genomic instability, and cell-cycle disturbance which underlie the clinical hallmark features of FA such as impaired growth, defective hematopoietic stem cell (HSC) proliferation, and predisposition to cancer. Currently, allogeneic HSC transplantation is the only curative treatment for FA-associated bone marrow failure. Recent successes in gene therapy studies, wherein autologous HSCs are genetically modified *ex vivo* to express wild-type FA genes prior to reinfusion into patients, offer promising alternatives for the future. In this talk, I will review the FA pathway genes according to functional roles in ICL repair, explore the clinical and cellular phenotypes associated with FA, and discuss genotype-phenotype correlations. In particular, I will spotlight a preclinical study examining whether caloric restriction might hold potential as a therapeutic strategy in FA (this study is ongoing as part of the TC-NER consortium brought together by the European Joint Programme, Rare Diseases 2020 Joint Transnational Call, and is funded by TUBITAK with project number 121N277).

Keywords: Fanconi anemia, therapy, gene therapy, caloric restriction

Fanconi Aplastic Anemia from the Perspective of a Pediatric Hematologist

Yeter Düzenli Kar, Sebahattin Gazioğlu

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Abstract

Fanconi anemia (FA) is a rare multisystem genetic disease, characterized by the triad of progresive bone marrow failure, physical abnormalities (including shortstature, microcephaly, developmental delay, café-au-lait skin lesions, hand, arm and other skeletal anomalies, kidney problems) and tendency to develop malignancy, primarily myeloid leukaemia and epithelial cancers. FA is caused by mutations in any of the 23 genes that are involved in the FA/BRCA pathway, named *FANC* genes. Bi-allelic mutations in FANCA are the most common mutations and are seen in 60-70% of patients. Demonstration of increased chromosome breakage, either spontaneously or with DEB or MMC, G2 phase arrest in flow cytometry, germline genetic tests are used to make a diagnosis. The current standard and curative treatment for Fanconi Aplastic Anemia patients is hematopoietic stem cell transplantation. Hematopoietic stem cell transplantation is a treatment associated to exposure to chemotherapy, immunological complications, plus opportunistic infections from prolonged immune incompetence or increased risk of morbidity. New gene therapy models include gene addition therapy, genome editing using CRISPR-Cas9 nuclease, and hematopoietic stem cell generation from induced pluripotent stem cells. Although gene therapy seems promising, it is not still in the routine use. Even if bone marrow failure disappears after hematopoietic stem cell transplantation, patients should be closely monitored throughout their lives for congenital anomalies and possible malignancies.

Keywords: Fanconi anamia, bone marrow failure, hematopoietic stem cell transplantation

SPEECH TEXTS

Genetics of Thalassemia in the Turkish Population

Aslıhan Kiraz

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Abstract

Hemoglobinopathies are the most common monogenic diseases in the world. Thalassemia is a hereditary disease caused by a production defect in the chains that form the protein (globulin) part of hemoglobin. There are 2 alpha and 2 beta chains in the protein structure of hemoglobin A. Damages in the production of these chains cause thalassemia. Defects in the alpha chain are called α-thalassemia, and defects in the beta chain are called β-thalassemia. It is estimated that more than 5% of the world's population is an α-thalassemia carrier and approximately 1.5% is a β-thalassemia carrier. In Turkish society, the frequency of α-thalassemia carriage varies from region to region and is generally around 0.25%, while the frequency of β-thalassemia carriage varies between 1.4-13% from region to region and is generally around 2.1%. Consanguineous marriages play a major role in the high frequency of thalassemia in these parts of the world. In genetic analysis, many variants with regional differences are observed. The 639 patients with a preliminary diagnosis of thalassemia who applied to the department of Medical Genetics at Erciyes University between 2012 and 2014 were examined. Sanger sequencing analysis was performed for *HBB* gene examination. A total of 2595 variants were detected. Fifty-seven percent of the detected variants were clinically relevant pathogenic/possibly pathogenic. The most common variant (44%) was the IVSI-110(G->A) variant, which is consistent with the literature. Multicenter genetic-based studies on thalassemias (especially α-thalassemia) need to be planned to obtain clearer information about the population genetics of hemoglobin A in Türkiye.

Keywords: Thalassemia, α-thalassemia, β-thalassemia, Turkish society, Türkiye

Erythrocyte Membrane and Enzyme Defects: A Pediatric Hematologist's Perspective

Serap Karaman

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Hemolytic anemias result from a shortened lifespan of erythrocytes due to either intrinsic erythrocyte abnormalities or external factors. These can include erythrocyte membrane disorders (membranopathies), erythrocyte enzyme disorders (enzymopathies), and erythrocyte hemoglobin (Hb) disorders (hemoglobin opathies).

I. Erythrocyte Membrane Disorders (MEMBRANOPATHIES)

The erythrocyte membrane consist of lipids, carbohydrates, and proteins. A loss of membrane proteins, whether hereditary or acquired, leads to lipid layer deterioration and a reduction in surface area. This disruption in the surface-volume ratio results in impaired membrane function.

Types of Erythrocyte Membrane Disorders:

A. Due to Loss or Dysfunction of Erythrocyte Membrane Proteins:

- Hereditary spherocytosis (HS),

- Hereditary elliptocytosis,

- Hereditary pyropoikilocytosis.

B. Due to Altered Cation Permeability of the Erythrocyte Membrane (Stomatocytosis Group Diseases):

- Hereditary stomatocytosis,

- Hereditary xerocytosis.

C. Due to Quantitative or Structural Disturbances of Erythrocyte Membrane Lipids:

- Erythrocyte membrane disorders characterized by acanthocytosis,

- Erythrocyte membrane disorders characterized by target cells.

Hereditary Spherocytosis (HS):

HS is the most common non-immune hemolytic anemia,

It is caused by vertical structural defects due to mutations in spectrin, ankyrin, and protein band 3, which are essential membrane proteins,

These defects cause the erythrocyte shape to change from a biconcave disc to a spherical form. As a result, erythrocytes are more readily trapped and destroyed in the spleen due to their reduced ability to deform and navigate capillaries.

Clinical Features:

- The diverse nature of membrane pathology results in a wide range of phenotypes and clinical presentations. Symptoms can appear at any time, reflecting the variability in membrane defects,

- Anemia, jaundice (jaundice in the first 24 hours of ND, prolonged jaundice) and splenomegaly are the most common findings.

SPEECH TEXTS

Laboratory Findings:

- Anemia,

- Spherocytic erythrocytes in peripheral blood smear,

- Normal or increased mean corpuscular volume, which may be due to folate deficiency or reticulocytosis,

- Typically, increased mean corpuscular Hb concentration (>35 g/dL) and increased red cell distribution width,

- Increased osmotic fragility and decreased resistance,

- Protein abnormalities in membrane electrophoresis,

- Indirect hyperbilirubinemia and increased lactate dehydrogenase LDH.

Osmotic Fragility (OF) Test:

- The OF test assesses the "resistance of erythrocytes to hypotonic solutions." Normal erythrocytes swell but do not hemolyze in 0.9%, 0.8%, 0.7%, 0.6%, and 0.5% NaCl solutions. Hemolysis typically begins at concentrations below 0.5% NaCl. In HS, hemolysis can occur at higher NaCl concentrations where normal erythrocytes remain intact. Thus, osmotic fragility is increased and resistance is decreased. In 30% of patients, the test may be normal. Sensitivity can be enhanced by incubating erythrocytes at 37°C for 2 hours before performing the test.

Diagnosis:

- Clinical findings,

- Presence of spherocytes in peripheral blood smear,

- Family history of hemolytic anemia,

- Increased osmotic fragility test results,

- neonates, anemia, reticulocytosis, and spherocytosis may be absent, and the reliability of the osmotic fragility test is reduced. An MCHC greater than 36 g/dL is indicative, with high specificity and sensitivity.

Complications of Hereditary Spherocytosis:

- Aplastic crisis (often due to parvovirus infection),

- Folate deficiency leading to megaloblastic crisis,

- Gallstones,

- Leg ulcers,

- Extramedullary hematopoiesis,

- Transfusion-related iron overload.

II. Erythrocyte Enzyme Disorders (Enzymopathies)

To maintain a normal erythrocyte lifespan:

1. The erythrocyte must be energetically efficient.

2. Hb and intracellular proteins must be protected from oxidative damage.

Erythrocytes lack mitochondria and other organelles, and they do not have the ability to proliferate, synthesize proteins, or perform oxidative phosphorylation. They rely on two primary pathways for energy production:

1. Anaerobic Glycolysis (Embden-Meyerhof Pathway): Provides approximately 90% of the energy. The key enzyme in this pathway is pyruvate kinase.

2. Pentose Phosphate Pathway: Contributes about 10% of the energy through the production of NADPH. The main enzyme here is glucose-6-phosphate dehydrogenase (G6PD).

Erythrocyte enzymes are crucial for glucose metabolism and nucleotide metabolism in the cytoplasm. These processes are essential for erythrocyte survival, functionality, and the removal of accumulated toxic metabolites (Figure 1).

The most common enzyme deficiencies are shown in Figure 2. The most common of these are deficiencies of G6PD, pyruvate kinase and pyrimidine 5' nucleotidase (P5N) enzymes.

Glucose-6-Phosphate Dehydrogenase (G6PD) Deficiency

Discovered in 1932, G6PD is known as the "enzyme that protects erythrocytes from oxidative damage." The deficiency was first described in the 1950s when American soldiers taking antimalarial drugs experienced acute hemolysis. While G6PD is present in all cells, erythrocytes are the most affected by this deficiency. In Turkey, the prevalence of G6PD deficiency is approximately 0.5%, with higher rates of 8.2% reported in the Çukurova region. The frequency of hyperbilirubinemia in newborns varies between 10.5% and 22.1%.

G6PD deficiency is most common in regions such as Africa, the Mediterranean, the Middle East, Southeast Asia, and India. It is more prevalent in individuals of African descent compared to those of European descent. The deficiency provides protection against Plasmodium falciparum malaria, particularly during childhood. The World Health Organization G6PD enzyme deficiency classification is shown in table 1. Certain G6PD variants are more prevalent in specific regions. The G6PD-A variant is commonly found in Africa; the Mediterranean variant is prevalent in southern Italy, Sardinia, and other Mediterranean areas; and the G6PD-Canton variant is frequently observed in southern China.

Pathophysiology of G6PD Enzyme Deficiency

G6PD is essential for glutathione metabolism. In the absence of G6PD, NADPH production is impaired, which in turn disrupts glutathione metabolism. Without NADPH, oxidized glutathione cannot be reduced back to its active form. As NADPH levels decrease, free oxygen radicals accumulate, and the antioxidant defense mechanism is compromised. The detoxification of hydrogen peroxide (H_2O_2) within erythrocytes is hindered. When exposed to oxidant substances, Hb becomes oxidized, forming Heinz bodies that precipitate and adhere to the cell membrane, compromising membrane stability. This disruption leads to hemolysis (Figure 3).

Clinical Presentation

- Jaundice, dark yellow-orange urine, pallor, and fatigue may be observed in the skin and mucous membranes,

- G6PD deficiency can manifest in four clinical forms: acute hemolytic anemia, neonatal jaundice, chronic hemolysis, and favism.

Acute Hemolytic Anemia

Hemolysis can be triggered by drugs, infections, and chemicals. During periods between hemolytic episodes, patients typically exhibit no clinical symptoms and may have normal Hb levels. Table 2 outlines the various triggers of hemolysis in G6PD deficiency. Patients diagnosed with G6PD deficiency should be provided with a comprehensive list of medications and foods to avoid to prevent hemolytic episodes.

Neonatal Jaundice

Hyperbilirubinemia is significantly more pronounced in neonates with G6PD deficiency, and approximately 20% of kernicterus cases are associated with this

condition. Jaundice typically appears on the 2nd or 3rd day of life and may become severe enough to require blood exchange. While anemia may not always be evident, oxidant exposure can exacerbate hemolysis.

Chronic Nonspherocytic Hemolytic Anemia

Reticulocytosis is commonly observed. Unlike in spherocytic anemia, spherocytes are absent from the peripheral smear, leading to the classification of this condition as chronic nonspherocytic hemolytic anemia. Hb levels usually range from 8 to 10 g/dL, and hemolysis in this case is primarily extravascular.

Favism

Favism involves the consumption of broad beans, which contain oxidants like divisin that increase reactive oxygen species. This condition is most prevalent in boys aged 1 to 5 years. Ingesting broad beans can lead to intravascular hemolysis and severe anemia within 5 to 24 hours (up to 48 hours) after consumption. Symptoms may include nausea, vomiting, abdominal pain, fever, and altered consciousness. Urine may appear dark due to Hburia.

Laboratory Findings:

- Anemia: Normocytic with a negative Direct Coombs test,

- Peripheral Blood Smear: Anisocytosis, poikilocytosis, bitten cells, and Heinz bodies may be present. Supravital staining with methylene blue or crystal violet reveals Heinz bodies precipitated in erythrocytes. The characteristic "bite cells" appear due to the removal of Heinz bodies by the spleen,

- Additional Tests: Increased LDH, reticulocytes, and indirect bilirubin; decreased haptoglobin levels,

- Urine Analysis: Hburia and red to black-colored dark urine,

- G6PD Levels: Decreased or normal.

Diagnosis of G6PD Deficiency:

- The diagnosis is established by demonstrating the absence of G6PD enzyme activity. Enzyme deficiency can be assessed qualitatively, using a fluorescence spot test to detect NADPH formation from NADP, or quantitatively, using spectrophotometric measurement. Results are expressed in units of enzyme activity per gram of Hb. It is important to repeat the test after 2-3 months, as results may be falsely negative during acute hemolysis periods. DNA analysis is crucial for a definitive diagnosis. PCR testing identifies specific mutations and is useful for family screening and prenatal diagnosis.

Pyruvate Kinase Deficiency:

- Pyruvate kinase deficiency is the most common congenital enzyme deficiency in the glycolytic pathway. It leads to the accumulation of 2,3-DPG, which inhibits Hb's oxygen binding. The clinical spectrum ranges from asymptomatic cases to severe, life-threatening anemia. In neonates, it can present as severe hyperbilirubinemia, anemia, and hydrops requiring blood exchange, whereas in adults, it may be first detected as compensated hemolysis.

Clinical Features:

- Common findings include extravascular hemolysis and iron accumulation. Symptoms may include fatigue, weakness, tachycardia, splenomegaly (80-85%), jaundice (40-70%), gallstones (30-45%), and cholecystitis. Less commonly, patients may experience aplastic crisis, bone deformities, extramedullary erythropoiesis, delayed puberty, hyperpigmentation, leg ulcers, and pulmonary hypertension.

Laboratory Findings:

- Anemia is typically macrocytic and normochromic, with Hb values usually ranging from 8-12 g/dL in older children. Reticulocytosis is often observed. Increased MCHC due to dehydration from ATP deficiency may occur, and spicule cells or spur cells can be seen in the peripheral smear.

Diagnosis:

- Diagnosis involves measuring increased 2,3-DPG levels (which can sometimes be normal), decreased pyruvate kinase activity (using spectrophotometric methods), and detecting mutations in the PKLR gene associated with primary deficiency. Mild hemolysis may occur in heterozygous carriers. Secondary deficiencies, such as those seen in HS or acute leukemia, can complicate diagnosis. Potential pitfalls include:

- Retrieval of pyruvate kinase levels post-transfusion,

- Elevated enzyme levels in reticulocytes due to reticulocytosis,

- Incomplete separation of leukocytes from erythrocytes (as pyruvate kinase activity in leukocytes is normal),

In suspicious cases, genetic examination is recommended.

Other Enzyme Deficiencies

Erythrocyte Nucleotide Metabolism Defects:

• Enzymes involved in nucleotide (purine and pyrimidine) metabolism are essential for removing toxic nucleotide precursors from erythrocytes. Key enzymes include P5N for pyrimidine metabolism, and adenylate kinase and adenosine deaminase for purine metabolism. Deficiencies in these enzymes can lead to hereditary nonspherocytic hemolytic anemia.

Pyrimidine 5'-Nucleotidase Deficiency (Pyr 5'-N):

- This is the most common nucleotide metabolism disorder and has two isoforms in erythrocytes: type 1 and type 2. Hemolytic anemia is primarily caused by type 1.

Pyrimidine 5'-Nucleotidase Deficiency:

- Deficiency leads to impaired RNA degradation and accumulation of basophilic punctuations. Patients may develop mild to moderate chronic anemia or may become transfusion-dependent. Gallstones, jaundice, and splenomegaly may also be present. Diagnosis is confirmed by decreased nucleotidase activity and increased pyrimidine nucleotides.

- Some of these enzymes are also found in muscle and brain tissues. Neurological deficits, mental retardation, and myopathy may occur. When these neurological symptoms are accompanied by hemolytic anemia, further evaluation for metabolic diseases is warranted.

Conclusion

Erythrocyte membrane and enzyme defects are types of hemolytic anemia resulting from intrinsic erythrocyte pathologies. Despite differing underlying mechanisms, these defects often present with similar clinical and laboratory features. Collaboration with genetics is essential for accurate diagnosis. Multidisciplinary meetings involving hematologists and geneticists can significantly reduce unnecessary tests and improve diagnostic accuracy.

SPEECH TEXTS

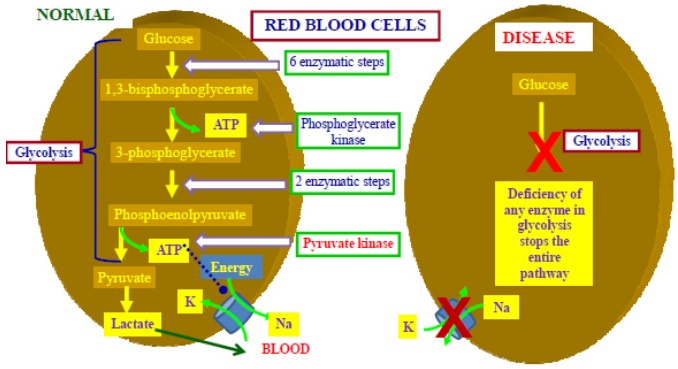


Figure 1: Glycolysis in normal erythrocytes and enzyme-deficient erythrocytes.
G6PD: Glucose-6-phosphate dehydrogenase

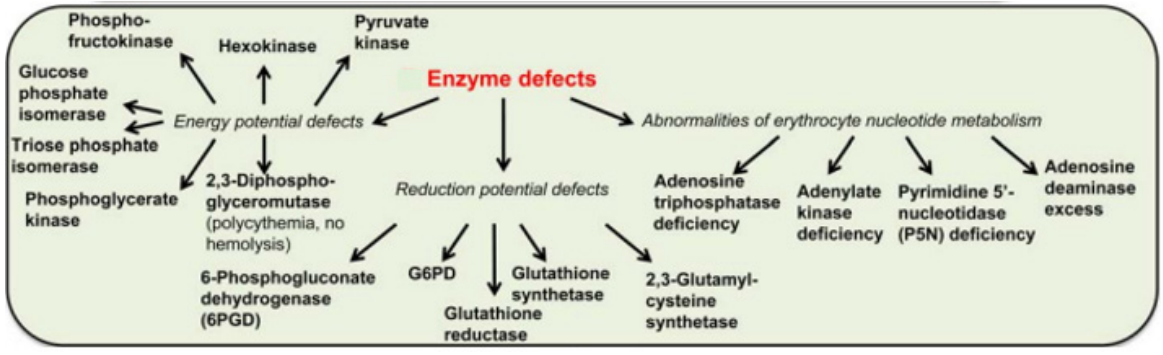


Figure 2. The most common enzyme deficiencies
G6PD: Glucose-6-phosphate dehydrogenase

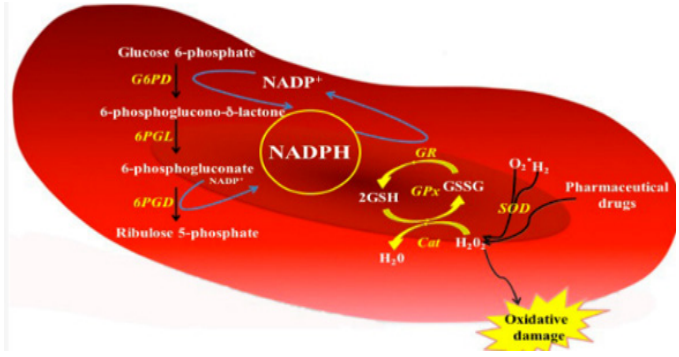


Figure 3. G6PD enzyme deficiency pathophysiology
G6PD: Glucose-6-phosphate dehydrogenase

SPEECH TEXTS

Table 1. World Health Organization (WHO) classification of G6PD variant

Class I variant	**Chronic hemolysis due to severe G6PD deficiency, e.g., G6PD deficiency Harilaou.**
Class II variant	**Intermittent hemolysis in spite of severe G6PD deficiency, e.g., G6PD Mediterranean.**
Class III variant	**Intermittent hemolysis associated usually with drugs/infections and moderate G6PD deficiency, e.g., G6PDA variant.**
Class IV variant	**No hemolysis, no G6PD deficiency, e.g., normal G6PD (B variant)**

G6PD: Glucose-6-phosphate dehydrogenase

Table 2. Triggers of hemolysis in G6PD deficiency

Drugs	Dapson, primacine and primethamine, chlorocine, metilen blue, nitrofrantoine, nalidixic acid, PAS, ciprofloxacin, sulfamethoxazole, sulfasalazine, chloramphenicol, rasbirucase, doxorubicin
Chemicals	Naphtalene, broad bean, benzene
Infections	Viral, bacterial,..

G6PD: Glucose-6-phosphate dehydrogenase

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Oral Presentations

ORAL PRESENTATIONS

[OP-01]

Combined Implementation of Local Outlier Factor Analysis and Extremely Randomized Trees Classifier on *MEFV* Gene Variants Display Outstanding Classification Performance

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Abstract

Introduction: More than half of the familial Mediterranean fever gene *(MEFV)* gene variants pathogenicity is unknown. The extremely randomized tree classifier (ETC), a type of ensemble learning, employs the outcomes of numerous unrelated decision trees arranged in a "forest" pattern to make a classification decision. ETC is applicable to the prediction of variants of uncertain significance variants of the *MEFV* gene.

Methods: We extracted more than 11,000 variants of the *MEFV* gene from the ensembl database. For most of the *in-silico* tools previously trained on the missense dataset, we selected 6,034 *MEFV* gene missense gene variants for our model. We excluded eight highly known *MEFV* gene pathogenic variants and 10 benign *MEFV* gene variants from the study. In the feature engineering step, we implemented local outlier factor analysis, and overall, 370 variants included our machine learning analysis. In the feature selection step, we determined the optimal number of *in-silico* tools for our model. After establishing our model on the training and test datasets, we deployed it into unknown variants.

Results: After hyperparameter tuning, our model obtained 100% accuracy on our training dataset and 98% accuracy on a 5-fold cross-validated dataset. After this step, we deployed our model on 8 *MEFV* gene pathogenic variants and 10 benign *MEFV* gene variants. Our model accurately classified all 18 (100%) known variants.

Conclusion: Many *in-silico* tools are trained on many genes. However, ClinGen recommended that gene-specific evaluation is necessary to improve the classification of *in-silico* tools. Re-evaluation of *in-silico* tools can improve the accuracy of *MEFV* gene variants.

[OP-02]

Two Different Cytogenetic Anomalies in a Case: Ring-(13) and Dup22q11

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Abstract

Introduction: Ring chromosome 13 [r(13)] is a rare chromosomal disorder characterized by missing genetic material from one or both ends of chromosome 13, resulting in the formation of a ring structure. Patients with r(13) have various phenotypic abnormalities that correspond to the amount of genetic material lost. Deletions of the distal locus 13q34 have been shown to be involved in symptoms such as growth and developmental retardation, microcephaly, facial dysmorphism, hand-feet anomalies and ambiguous genitalia. 22q11.2 duplication syndrome is an extremely variable disorder with a phenotype ranging from normal to learning disability and congenital defects. In this report, we described an r(13) patient who has distinct phenotypic traits and duplication of 22q11 besides deletion 13q34.

Methods: A 12-year-old female was referred to our genetics clinic for short stature, developmental delay, cardiac defect, and foot deformity. Karyotyping from peripheral blood culture were performed, following fluorescence *in situ* hybridization (FISH) and microarray analysis were planned.

Results: The karyotype imaging revealed 46, XX, r(13). FISH analysis was performed by using a 13qter specific subtelomeric probe (Cytocell, LPT13 QR/G), showing a one red signals in that region. Acro-p-arm FISH probe (Cytocell, LPENOR) had two signal. Microarray analysis (GenetiSureCyto8x60K) showed a deletion containing 17 *OMIM* genes (1.4 Mb) in the 13q34 and duplication containing 30 genes (2.4 Mb) in the 22q11.21q11.22. The parents' karyotypes revealed as normal and microarray analysis is ongoing for them.

Conclusion: To our knowledge, there is no previous report of a r(13) who has del13q34 and concomitant dup22q11. 21q11.22. The characterization of the structure and the additional genomic aberrations of the r(13) are valuable for future researchers to better understanding of the genotype-phenotype correlation.

Keywords: r(13), del13q34, dup22q11

ORAL PRESENTATIONS

[OP-03]

A Rare Syndrome from Physical Examination to Diagnosis: Short-Rib-Thoracic Dysplasia-3

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Introduction

Skeletal dysplasia is a heterogeneous group of genetic disorders affecting bones and cartilages. Short rib thoracic dysplasia-3 is a ciliopathy characterised by a narrow rib cage, short ribs, short tubular bones and a "trident" appearance of the acetabular roof. This condition is lethal in the perinatal period. The prevalence of DYNC2H1-associated short rib thoracic dysplasia-3 is currently unknown.

Case Report

Amniotic fluid was obtained from a 23-year-old gravida 2, para 0, abortus 1 mother due to the presence of skeletal dysplasia in the fetus. The chromosome analysis was normal. The case was born by C/S at 36+5 weeks, followed up in the intensive care unit due to respiratory distress and prematurity and died at the age of 1 day. The examination of the dead foetus revealed a narrow thorax, horizontal ribs, shortness and curvature of the long bones and brachydactyly. The radiological imaging revealed the presence of handlebar clavicle, trident acetabulum, and shortening of long bones. The parents were from the same village. In the next generation sequencing analysis, the c.7606C>T variant was identified as homozygous in the *DYNC2H1* gene. This variant was classified as a variant of unknown significance or possibly pathogenic according to the American College of Medical Genetics and Genomics criteria. The parents were included in the segregation analysis.

Discussion

Currently, there is no definitive treatment for short costal-thoracic dysplasia; therefore, the best strategy in the management of the disease is to determine the carrier status of the parents. Families in which the variant is identified have a chance of having a healthy child with preimplantation genetic diagnosis.

[OP-04]

Investigation of Innate Lymphoid Cells in Patients with Familial Mediterranean Fever and Spondyloarthritis

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Abstract

Introduction: Familial Mediterranean fever (FMF) is the most common monogenic autoinflammatory disease characterized by recurrent and self-limiting attacks of fever and serositis. FMF is the prototypical example of autoinflammatory diseases. Spontaneous activation of the innate immune system results in the release of IL-1B and typical clinical attacks occur. Musculoskeletal involvement of abdominal aortic aneurysm (AAA) includes acute arthritis, chronic arthritis, sacroiliitis, and spondyloarthropathy (SpA). In this study, changes in innate lymphoid cells (ILC) cells were examined to understand how AAA, an autoinflammatory disease, and SpA come together.

Methods: Ficoll-Hypaque density gradient was used to isolate peripheral blood mononuclear cells (PBMCs) from blood samples taken from healthy volunteers (n=6), FMF patients (n=17), SpA patients (n=12) and FMF patients with SpA (n=14). PBMCs were were counted with Tyrpan Blue staining, and stained with appropriate surface markers for ILCs examined on FACSAria III and all data was analyzed using FlowJo and Graphpad 9.

Results: In analyzes made from peripheral blood, it was observed that the frequency of the ILC2 subtype was significantly reduced in patients with SpA compared to patients with AAA. In patients with SpA + AAA, despite the increasing trend, statistical significance was not reached. It was observed that ILC3 cell frequencies and numbers tended to increase compared to the FMF group, although it did not reach statistical significance. Supported by ERÜ BAP unit with TDK-2022-11916 procect code.

Conclusion: Quantitative and phenotypic changes in ILCs in FMF patients with SpA may contribute to disease pathogenesis.

Keywords: Familial mediterranean fever, spondyloarthritis, innate lymphoid cells

ORAL PRESENTATIONS

[OP-05]

FBN2 Related Fibrillinopathy with New Phenotypic Findings in an Affected Family

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Abstract

Fibrilinopathies represent a group of diseases that microfibrils are disrupted by genetic variants in one of the genes encoding fibrillin molecules, large glycoproteins of the extracellular matrix. There are three isoforms of fibrillin molecules identified in mammals: fibrillin 1, fibrillin 2 (FBN2), and fibrillin 3. FBN2 is an important component of microfibers involved in the formation of elastic fibers in connective tissue throughout the human body. FBN2-associated fibrillinopathies have been associated with different connective tissue diseases such as congenital contractural arachnodactyl (CCA), macular degeneration, and myopathy. Congenital CCA is an autosomal dominant disorder caused by heterozygous disease-causing variants on the gene encoding FBN2. FBN2 is located on chromosome 5q23-q31, and it is the only gene known to be associated with CCA, which has similar features with Marfan syndrome. CCA is characterized by a marfanoid habitus (a long and slender build), dolichostenomelia, crumpled ear, arachnodactyly, flexion contractures of multiple joints, kyphoscoliosis. Some patients also have cardiovascular and ocular complications. Here we report 4 affected individuals in a family who were referred to us with findings such as marfanoid habitus, Chiari malformation, aortic dilatation, glaucoma, scoliosis and arachnodactyly. We detected the same heterozygous missense variant on FBN2 [NM_001999 c.680G>A p.(Gly227Glu)] in all affected individuals. We classified this variant as a likely pathogenic according to American College of Medical Genetics and Genomics Critierias. Our study broadens the phenotypic spectrum of CCA with findings that have not been reported before, including glaucoma and Chiari malformation. We emphasize the importance of understanding the phenotypic spectrum of the disease and genetic counseling.

Keywords: FBN2, fibrillinopathy, glaucoma, Chiari malformation

[OP-06]

Impact of Polymorphisms on Clinical Management

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Abstract

Genetics have been impacting healthcare and patient management for many years. The impact of genetics on healthcare is becoming more and more profound. Most of this management is based on the identification of pathogenic, likely pathogenic and even VUS variants. Precision Health Group (PHG), is the world's first healthcare and wellness provider that integrates genetics, functional and regenerative medicine, naturopathy, nutrition, fitness and healing under one roof to provide a unique "system of care" for patients and clients, with a stated mission of "from illness to wellness and beyond", PHG is set to change medicine and healthcare as we know it! This means going beyond the simple variant reporting, we go deeper to search the literature, investigating all variants and their clinical correlations, guided by and working with the patient's physician and the care team, we don't stop until we get the right answers to help the patients. Here we present to you with a case to demonstrate this concept of care, a 76 year old male presented to our care team asking for guidance to help him understand his mysterious post surgical bleeding following eye surgery. This case is a great example of how even a polymorphism may have profound impact on patient management.

ORAL PRESENTATIONS

[OP-07]

Hypoparathyroidism, Short Stature, Glaucoma, Epilepsy and Thalassemia: A Case with Blend Phenotype

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Introduction

Next generation sequencing technologies have the capacity to sequence the entire genome or many DNA fragments in small targeted regions. These technologies are an important tool for determining the etiology of diseases with genetic heterogeneity. Once the root cause of the disease is identified, more precise genetic counseling can be provided to patients and family members at risk can be rapidly screened. Especially in cases where various clinical findings coexist, more comprehensive gene panel tests can be preferred and diagnostic superiority can be achieved.

Case Report

A 10-year-old girl with congenital glaucoma, hypoparathyroidism, history of epilepsy starting at 2 months of age, profound anemia and short stature was referred to us from the pediatric endocrinology department. Physical examination revealed buphthalmos in the left eye, prominent ear and mild microcephaly. Clinical exome sequencing was performed to elucidate the etiology of the patient's current clinical findings. In the study, c.1336 C>T p.(Arg446*) non-sense homozygous pathogenic variant in *ABCG5* gene, c.868dup p.(Arg290Profs*37) frameshift homozygous pathogenic variant in *CYP1B1* gene and c.25_26del p. (Lys9Valfs*14) frameshift heterozygous pathogenic variant in *HBB* gene were detected.

Discussion

In this study, we emphasize that the presence of different clinical findings is not always associated with a single syndrome, that more than one gene alteration may cause clinical manifestations, and therefore, the superiority of studying broad panels instead of narrowly targeted panels in patients with complex phenotypes in making a diagnosis.

Keywords: Next generation sequencing, blended phenotype, sitosterolemia, congenital glaucoma

[OP-08]

Novel LRP5 Variant in a Patient with Multiple Fractures, Retinal Detachment and Epilepsy

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Introduction

Low-density lipoprotein receptor-related protein 5 (LRP5) is a 1,615 amino acid transmembrane receptor for the conserved Wnt-β-catenin signaling pathway, a pathway known to regulate bone metabolism in humans. Specific human polymorphisms in LRP5 have been hypothesized to affect bone density, in part by altering the anabolic response of bone to mechanical loading. Recessive loss-of-function mutations in LRP5 cause osteoporosis-pseudoglioma syndrome, a condition characterized by severe osteoporosis and occasional ocular abnormalities.

Case Report

A 17-year-old female patient was referred to us from pediatric endocrinology for osteogenesis imperfecta due to multiple fractures. She had a history of nearly 30 fractures. She was followed up with epilepsy but no active seizure was observed. There is bilateral visual loss due to retinal detachment. There was no pathology in prenatal follow-up and birth history. Physical examination revealed curved legs and inability to stand bilateral ptosis, large auricle, underdeveloped helix structure, bulbous nasal tip, faint filtrum, and scoliosis. Our patient's maternal and paternal grandparents are siblings. Eletroencephalogram shows the presence of epileptiform discharges. Vertebral radiography showed trocholumbar scoliosis.

Discussion

Clinical exome sequencing was performed. As a result of the analysis, we detected a homozygous c.2645T>A p.(Ile882Asn) variant in the exon 12 of the *LRP5* gene. When we examined the databases, we found that this variant has not been reported before. We hope that our case will contribute to the literature with this report.

Keywords: LRP5, osteoporosis-pseudoglioma syndrome, rare disease

ORAL PRESENTATIONS

[OP-09]

Comparative Analysis of Sickle Cell Trait Distribution Among Nigerian and Zimbabwean Students in Northern Cyprus

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Abstract

Sickle cell disease (SCD) is a genetic disorder caused by abnormalities in the *HBB* gene, which codes for the hemoglobin component. This research paper investigates the distribution of SCD among Nigerians and Zimbabweans in Northern Cyprus. The study found that in Nigerians with a sample size of 50 males (50%), and 50 females (50%), about 51% of individuals had normal hemoglobin, 47% had AS, and 1% had carrier individuals, with the observed values for A and SS being 0.75% and 0.25%, respectively, indicating a lower prevalence of the disease. In contrast, the study analyzed 108 Zimbabwean students studying in Northern Cyprus, with a sample size of 56 males (52%), and 52 females (48%). Polymerase chain reaction genotyping revealed that 27% had SCD features. The A allele frequency was 0.87%, while the S allele frequency was 0.12%. The genotype distributions were calculated using the gene- counting method, in addition, the Hardy-Weinberg equilibrium was observed in the *HBB 20A>T* gene. As a result, Nigerians have a higher prevalence of SCD compared to Zimbabweans. The allele frequencies also differ slightly between the two populations in Northern Cyprus.

Keywords: HBB, sickle cell trait, sickling, sickle cell anemia, sickle cell

[OP-10]

5q35.2q35.3 Microduplication with Xp22.3 Microdeletion: A Rare "Reverse" Sotos Syndrome with A New Chromosomal Rearrangement

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Abstract

In the genetic approach to short stature and developmental delay, microarray testing plays an important role. Microduplication of 5q35.2q35.3 region is associated with commonly including short stature, microcephaly, delayed bone age, and mild developmental delay. A 10-year-old female patient with short stature is referred to our clinic by pediatric endocrinology. The patient was found to have short limbs during prenatal follow-up. Her height was 112.5 cm (<3p), weight was 20.7 kg (<3p), head circumference was 48 cm (<3p). Her physical examination revealed disproportionate short stature (rhizomelic shortness in the upper limbs), microcephaly, low weight, upslanting palpebral fissure, periorbital fullness, hypotelorism, prominent broad nasal tip, low hanging columella, thin upper lip, cubitus valgus and pectus excavatum deformity. She had surgery due to strabismus. Karyotype analysis from the patient's peripheral blood was found to be 46,XX. In microarray analysis, 1.8 Mb heterozygous duplication was detected in the 5q35.2-35.3 chromosome region including *NSD1* gene and 1.1 Mb heterozygous deletion was detected in the Xp22.31 chromosome region. Although the phenotype of our index patient is similar to the phenotype of previously published patients, some dysmorphic findings observed in our patient have not been previously reported in the literature. Fewer than 50 cases have been reported in the literature. To the best of our knowledge, this is the first case report of 5q35.2q35.3 microduplication and Xp22.3 microdeletion together seen. We also present the first 5q35.2q35.3 microduplication syndrome from Türkiye. For these reasons, it is thought that this case report will contribute to the literature.

Keywords: 5q35.2q35.3 microduplication, reverse Sotos syndrome, NSD1, aCGH

ORAL PRESENTATIONS

[OP-11]

Case Presentation: Dravet Syndrome Patient with SCN1A Homozygous Mutation, Which Has Been Rarely Reported Worldwide

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Introduction

Mutations affecting the alpha subunit of the *SCN1A* gene can cause various clinical pictures including simple febrile seizures, febrile seizures plus, Dravet syndrome, generalized tonic-clonic seizures, intractable childhood epilepsy and developmental and epileptic encephalopathy. The varying severity of mutations appears to correlate with the clinical spectrum of epileptic disorders. The SCN1A related phenotypes typically have autosomal dominant inheritance, usually occurring *de novo*. In this case report, we report one of the few cases with a homozygous disease-causing variant in SCN1A.

Case Report

A 3-year-old male patient with consanguineous parents had his first seizure as a myoclonic seizure after routine vaccination in the infantile period. His seizures, which started febrile and continued non-febrile, were attenuated with multiple antiepileptic treatments but could not be completely controlled. The patient was referred with a prediagnosis of Dravet syndrome. Molecular analysis revealed a homozygous missense likely pathogenic variant in exon 29 of SCN1A (NM_001165963 : c.5053G>A : p.(Ala1685Thr).

Conclusion

We present a patient with a homozygous mutation of the *SCN1A* gene and a clinical presentation of Dravet syndrome. There are very few reported cases with biallelic pathogenic variants in SCN1A. Our report helps enlighten the clinical course of Dravet syndrome with recessive inheritance.

Keywords: Dravet syndrome, SCN1A, recessive inheritance

[OP-12]

Identification of Novel Variants in *HPRT1* and *OTOG* Genes

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Introduction

The *HPRT1* gene, located on the X-chromosome at Xq26.3, produces a crucial enzyme, hypoxanthine-guanine phosphoribosyltransferase, essential for purine synthesis. HPRT1 disorders, stemming from enzyme deficiency, present with clinical symptoms such as elevated uric acid levels, kidney stones, and neurological and/or behavioral issues. Three distinct phenotypes have been identified: Lesch-Nyhan disease, HPRT1-related neurologic dysfunction, and HPRT1-related hyperuricemia, ranging from severe to mild. Hearing loss is one of the most common deficiencies of the neural-sensory system and has a significant impact on the quality of life. The *OTOG* gene encodes otogelin protein, a non-collagenous component of the acellular gelatinous structures that cover the sensory epithelia of the inner ear. Mutations in OTOG are known to cause DFNB18B (OMIM 614945).

Case Report

In this study, we present a case of a 17-year-old male patient with elevated uric acid levels and bilateral sensorineural hearing loss. He was referred from the pediatric nephrology rheumatology service service for evaluation of hearing loss, gut, and learning difficulties. Whole exome sequencing revealed a novel missense variant, c.431A>C p.(Gln144Pro), of uncertain significance in the *HPRT1* gene, and a frameshift likely pathogenic novel variant, c.1366_1369del p.(Tyr456Alafs*33), in the *OTOG* gene. These variants detected in the patient were evaluated as a compound phenotype. The identification of novel variants contributes to the variant spectrum.

Discussion: The detected variants were evaluated as a compound phenotype, and the identification of novel variants contributes to the variant spectrum.

Keywords: HPRT1, Lesch-Nyhan, OTOG, hearing loss

ORAL PRESENTATIONS

[OP-13]

Case Report: A Derivative Chromosome Involving a Large Duplication in Distal Arm of Chromosome 15

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Abstract

Duplications in the distal arm of chromosome 15 reported in the literature so far have been associated with prenatal and postnatal overgrowth, mental retardation, and craniofacial malformations. The prevalence and severity of symptoms and physical findings may vary from case to case depending on the length and location of the duplication of chromosome 15q. Here we report a 21-month-old male patient with dysmorphic facial features, craniosynostosis, undescended testis, and afebrile seizures. The patient had intrauterine restriction of movement, and developmental delay due to the history of SGA, secundum autism spectrum disorder, and electroencephalography abnormality. The prominent dysmorphic features were scaphocephaly, epicanthal folds, low-set ears, wide nasal root, thin upper and lower lip, high palate, and abnormal teeth. We performed a series of genetic tests including conventional karyotyping, fluorescence in situ hybridization (FISH), and array comparative genomic hybridization (CGH). The karyotype result of the patient was 46,XY, der(15)(qter-->q23: :pter-->qter). FISH analysis using probes designed for 15qter and 15q11.2 revealed an additional signal from the 15qter probe proximal to the 15q11.2 signal. Array CGH result of the proband was Arr[GRCh37] 15q23q26.3(68487617_102465355)x3, revealing a 34 mb copy number gain. Previous reports of cases with 15q duplications including the terminal region presented with some overlapping clinical features such as hypotonia and craniosynostosis and carried smaller duplications. On the other hand, our case harbors a previously unreported larger duplication and an interesting chromosomal rearrangement. Our study reports a unique case of a derivative chromosome 15 broadening the clinical and genetic spectrum of 15q terminal duplications.

Keywords: Duplication 15q, array CGH, FISH, derivative chromosome

[OP-14]

ATP8A2 Homozygous Deletion: A Novel Presentation in Cerebellar Ataxia and Intellectual Impairment Syndrome

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Abstract

Cerebellar ataxia, impaired intellectual development, and dysequilibrium syndrome denote a genetically heterogeneous rare disorder characterized by congenital cerebellar ataxia and impaired intellectual development. *ATP8A2* is one of the responsible genes for the disorder. Furthermore, some patients with biallelic *ATP8A2* pathogenic variants have a slightly different clinical presentation with intellectual disability, hypotonia, hyperkinetic movements, and optic atrophy. In this instance, a 40-year-old mother, presenting with hypotonia, referred her 2-year-old daughter, who was born at 39 weeks weighing 2,700 grams. The physical examination unveiled low weight percentiles, low height percentiles, low head circumference percentiles, bilateral strabismus, overlapping 4th toes, Mongolian spots on the back, high-arched palate, and a tented mouth. Neurological examination indicated absent deep tendon reflexes, no pathological reflexes, a negative Babinski sign, negative clonus, and no fasciculations. Subsequent analysis of copy number variations from Next-Generation Sequencing data delineated a suspected homozygous deletion in exons 2, 3, 4, and 5 of the *ATP8A2* gene. Primers were tailored for the 2nd and 4th exons, and polymerase chain reaction (PCR) and gel electrophoresis were executed on both healthy controls and the patient. For the proband's sample, gel electrophoresis revealed no PCR product for either exon, contrary to the control samples with normal PCR products, confirming the suspected deletion. Consequently, our patient emerges as the first documented case in the literature with ATP8A2 homozygous deletion, thereby broadening the mutational spectrum of ATP8A2- linked disorders.

Keywords: Homozygous deletion, dystonia, hypotonia

ORAL PRESENTATIONS

[OP-15]

A Case of Beta Thalassaemia Major with Joubert Syndrome

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Abstract

Beta-thalassemia is an autosomal recessive disease that occurs as a result of a disorder in the β-globin chains synthesis.Hemoglobinopathies such as beta thalassaemia are highly prevalent along a belt stretching from the Middle East to the Far East, especially in Mediterranean countries such as Italy, Greece, Cyprus and Türkiye. The severity of disease expression is mainly related to the degree of alpha-globin chain excess that precipitates in red blood cell precursors, causing both mechanical and oxidative damage (ineffective erythropoiesis). Homozygous cases progress to severe anaemia requiring regular blood transfusions. Joubert syndrome is a rare autosomal recessive neurological disorder characterised by neurological findings such as hypotonia, abnormal respiratory pattern and eye movements, ataxia and psychomotor developmental delay. A hypoplastic cerebellar pedicle and complete or partial absence of the vermis are basic radiological findings and the cause of fourth ventricle deformity. These findings cause "molar tooth signs" on magnetic resonance. First described by the paediatric neurologist Marie Joubert in 1969, the prevalence of this condition is estimated to be 1/80,000-1/100,000 live births. Awareness of the characteristic clinical and radiological findings of Joubert syndrome will facilitate early diagnosis, appropriate counselling and rehabilitation. A case of Joubert syndrome associated with beta thalassaemia is presented in people with epilepsy and mental retardation.

Keywords: Thalassemia, joubert, mental retardation

[OP-16]

A Blended Phenotype of AChromatopsia and Conjenital Myotonia with Germline Novel ATF6 and Rare CLCN1 Variant

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Abstract

ATF6 is an endoplasmic reticulum stress-regulated transmembrane transcription factor that activates transcription of endoplasmic reticulum molecules. Studies have shown that mutations in ATF6 are one of the rare causes of achromatopsia. *CLCN1* is a muscle chloride channel and regulates striated muscle cell membrane excitability. Defects in *CLCN1* cause disturbances in electrical activity and myotonia. A 4-year-old male patient was admitted to our clinic with complaints of nystagmus, photophobia and myotonia. Physical examination revealed frontal bossing, flattened nasal root, hypertelorism, retrognathia, photophobia and peripheral nystagmus. Electromyelography revealed myotonic discharges in the muscles and the patient's findings were consistent with myotonia. Clinical exome sequencing was performed. Clinical exome sequencing was performed. As a result of the analysis, homozygous probable pathogenic variant in exon 15 of *ATF6* gene and homozygous probable pathogenic variant in exon 12 of *CLCN1* gene were detected. The variant in the *ATF6* gene was associated with the patient's nystagmus and photophobia clinic and the variant in the *CLCN1* gene was associated with the patient's myotonia findings and led to a blend phenotype. To the best of our knowledge, the variant in the *ATF6* gene was considered novel because it has not been reported before. We hope that this case will contribute to the literature because it has a blended phenotype and novel variant.

Keywords: ATF6, nystagmus, myotonia, photophobia

ORAL PRESENTATIONS

[OP-17]

Analysis of Methylation of Individuals with a History of COVID-19

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Abstract

Introduction: The coronavirus disease-2019 (COVID-19) pandemic has led to a global health situation due to severe acute respiratory syndrome-coronavirus-2 (SARS-CoV-2). This study provides clinical examination of methylations in COVID-19 patients infected with SARS-CoV-2.

Methods: Patients, survival and diseases, had chronic diseases, were divided into 4 groups, and N6-methyladenosine (m6A) pathway genes *METTL3*, *WTAP*, *FTO*, *ALKBH5* and *YTHDF2* gene expressions were measured by quantitative real-time polymerase chain reaction (RT-qPCR) method and global m6A methylation levels were evaluated with ELISA tests. Additionally, genes and intergroup details were evaluated.

Results: There are few studies in the literature on recorded *m6A* genes in patients infected with SARS-CoV-2 and showing chronic disease. There is a significant decrease in the *ALKBH5* gene between group 1a, b chronic, which contain mild protection at the level of gene expression. According to our findings, the *ALKBH5* gene stands out for its powers in growth in patients with mild COVID-19 and chronic disease. It is the common chronic pathogen DM and asthma virus that lives in group 1b. Especially in asthma patients, an improvement is achieved by the emergence of mRNA and protein of ALKBH5, and the growth is significantly reduced compared to the control group.

Conclusion: Findings show that with increasing disease changes, global m6A methylation increases and there are changes in *m6A* gene expressions. In conclusion, a complex relationship emerges between COVID-19 and m6A methylation and gene expressions. This study is an important step in supporting related mechanisms and disseminating therapy methods.

Keywords: COVID-19, SARS-CoV-2, M6A

[OP-18]

An Acute Myeloid Leukemia Case Transformed from Myelodysplastic Syndrome with Deletion of 6q and Monosomy 7 Cytogenetic Abnormality

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Abstract

Introduction: Myelodysplastic syndrome (MDS) is a clonal bone marrow neoplasm characterized by morphological dysplastic features in hematopoietic cells, peripheral cytopenias, ineffective hematopoiesis, recurring genetic abnormalities, and an increased risk of transformation to acute myeloid leukemia (AML). Detection of specific chromosomal abnormalities helps distinguish MDS from AML in some cases, assists in the classification of MDS, and is a major factor in determining prognostic risk groups and treatment decisions. Here, a 69-year-old female patient diagnosed with transformed acute myeloid leukemia from myelodysplastic syndrome is presented.

Methods: Bone marrow culture was studied for conventional karyotyping. Fluorescence in situ hybridization (FISH) was utilized to examine genomic abnormalities as outlined in the current guidelines for MDS, as well as to confirm the presence of 6q deletion and monosomy 7. Capillary electrophoresis-based fragment analysis method was used for FLT3-ITD mutation screening.

Results: The patient was referred to our clinic because of low platelet count level. In her bone marrow analyses revealed acute myeloid leukemia transferred from myelodysplastic syndrome diagnosis. Deletion of 6q and monosomy 7 was observed in her bone marrow. Analyzed cytogenetic findings were confirmed using the FISH method.

Conclusion: MDS is a highly heterogeneous disorder. The chromosomal abnormalities most commonly detected in MDS are generally also identified in AML cases. Deletions in the long arm of chromosome 6 (6q del) are a rare occurrence in MDS. To our knowledge, case reports regarding del (6q) in MDS are not commonly encountered, there are very few case reports worldwide.

Keywords: Acute myeloid leukemia, myelodysplastic syndrome, deletion 6q, monosomy 7, chromosomal abnormality

ORAL PRESENTATIONS

[OP-19]

In Vitro Investigation of the Role of Schizophrenia-Associated Potential miRNAs in the Regulation of *COMT* Gene

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Abstract

Introduction: There are potential miRNA-mRNA interactions associated with miRNAs and mRNAs related to schizophrenia. Therefore, our study aimed to biologically validate the association of miR-30a-5p, miR-30e-5p, and miR-34a-5p with the *Catechol-O-methyltransferase (COMT)* gene in the SH-SY5Y cell line for schizophrenia.

Methods: miR-30a-5p, miR-30e-5p and miR-34a-5p mimics were transfected into the SH-SY5Y cell line. Total RNA was isolated from transfected cells, followed by reverse transcription for miRNA and mRNA analysis. Changes in *COMT* gene expression levels were observed using RT-qPCR and western blotting. RNA immunoprecipitation was performed to determine RNA-protein interactions post miRNA mimic transfection.

Results: We observed that higher levels of miR-30a-5p and miR-34a-5p inhibited *COMT* gene expression at both mRNA and protein levels. Higher levels of miR-30e-5p led to an increase in *COMT* gene levels. Our data revealed an enrichment in *COMT* gene transcript post miR-30a-5p and miR-34a-5p transfection. To date, functional studies on the association of miR-30a-5p and miR-34a- 5p with the *COMT* gene have not been reported in the literature.

Conclusion: Following miR-30a-5p and miR-34a-5p mimic transfection, significant decreases in *COMT* gene expression levels were observed, while significant increases were noted post miR-30e-5p mimic transfection. RNA-IP data demonstrated an increase in *COMT* gene quantity post miR-30a-5p and miR-34a-5p mimic transfections, suggesting that these miRNAs directly target the *COMT* gene.

Keywords: Schizophrenia, miRNA, COMT

[OP-20]

Examining the Genotype of the ABO Blood Group System Using Next-Generation Sequencing

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Abstract

Introduction: The ABO blood group system, discovered by Landsteiner in 1901, stands as one of the most crucial blood group systems in transfusion medicine. The human *ABO* gene resides on chromosome 9 at locus 9q34.2, spanning 18 kilobases and consisting of 7 exons. The *ABO* gene encodes enzymes known as glycosyltransferases that produce A and B antigens. Within the ABO locus, three main alleles exist: A, B, and O. Following the sequencing and cloning of the *ABO* gene, variations in these alleles have also been identified, with the ABO blood group system holding particular significance in blood transfusion, forensic medicine, and disease relationships, routinely determined through serological methods. However, resolving certain issues like blood group incompatibility necessitates blood group genotyping.

Methods: We retrospectively analyzed 256 individuals who underwent next-gen sequencing of the *ABO* gene. Bioinformatics analysis of the *ABO* gene was performed using updated databases, *ABO* blood group allele types were determined and allele frequencies were calculated.

Results: In our study, all individuals evaluated demonstrated 100% concordance between serological and molecular blood group results. Among the 256 individuals, 98 (38.28%) were found to have the A blood group, 76 (29.69%) had the O blood group, 58 (22.66%) had the B blood group, and 24 (9.37%) had the AB blood group. We identified 22 alleles, including 4 alleles that are not currently present in the database, forming 56 genotype combinations.

Conclusion: This study is the first to evaluate the *ABO* genotype of the Turkish population using next-generation sequencing method.

Keywords: ABO, next-generation sequencing, allele frequency

ORAL PRESENTATIONS

[OP-21]

Prenatal Diagnosis of Fetal Ventriculomegaly Associated with a *De Novo* Partial Trisomy 13 and Terminal 1q Deletion

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Abstract

Introduction: Trisomy 13, also known as Patau syndrome, affects approximately 1 in 12,000 newborns and is characterized by midline facial defects, holoprosencephaly, and internal organ malformations, often resulting in high antenatal mortality. Early detection with first-trimester screening is possible. Previous studies suggested that partial duplications of 13q present with variable clinical features, with proximal segment duplications typically associated with more severe symptoms than distal ones. This case study aims to emphasize genotype-phenotype correlations in partial Trisomy 13.

Methods: Genomic DNA isolation performed directly from amnion tissue for quantitative fluorescence polymerase chain reaction (QF-PCR) analyses. Primary human amnion cell culture is studied for conventional karyotyping and SNP microarray. Metaphase fluorescence *in situ* hybridization (M-FISH) 13qter and 1qter used for confirmation of partial Trisomy 13. Conventional karyotyping is performed from peripheric blood leukocytes of parents to identify possible reciprocal translocations.

Results: QF-PCR analysis of chromosome 13 indicates grey zone for Trisomy 13. Conventional karyotyping of amnion cell culture identified additional chromosomal segment in terminal part of chromosome 1. Additionally, SNP-microarray identified 1q44 microdeletion and 13q31.3q34 duplication which explains derivative chromosome 1. M-FISH analysis showed three hybridization signals with 13qter locus. Parental karyotype analysis showed that this aberration occurred *de novo*.

Conclusion: Fetal ventriculomegaly and lemon sign were detected during ultrasound examination in a 29-year-old primigravida, at 22 weeks of pregnancy. First trimester screening tests indicated low risk pregnancy. Selective termination of pregnancy was performed upon parents' request. Compared with other studies, this case report will contribute knowledge about genotype-phenotype correlation in partial Trisomy 13.

[OP-22]

Investigation of Polycystic Kidney Disease by Molecular Methods

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Abstract

Introduction: Polycystic kidney disease (PKD) affects millions of people worldwide and is known as the most common cause of end- stage renal failure. In our study, we aimed to investigate mutations in *PKD1*, *PKD2* and *PKHD1* genes in patients with PKD using the next generation sequencing (NGS) method and to compare the phenotype-genotype relationship.

Methods: In the study, pedigrees of 31 patients were drawn, their inheritance types were determined, and clinical data and blood samples were collected. DNA isolated samples were analyzed on the NGS device after library preparation and target enrichment. Bioinformatics analysis was performed using databases. The phenotype-genotype relationship of the patients was evaluated.

Results: Variants detected were evaluated as: *PKD1* gene 3.53% pathogenic (P), 5.88% likely pathogenic (LP), 24.71% unknown clinical significance (VUS), 51.76% benign (B), 14.12% likely benign (LB); *PKD2* gene 20% P, 10% VUS, 50% B, 20% LB; *PKDH1* gene 1.79% LP, 30.36% VUS, 67.85% B. Ten variants detected in the *PKD1* gene and six variants detected in the *PKHD1* gene were evaluated as Novel. The variants detected in the genes, phenotype-genotype relationships were evaluated, were found to be compatible with the literature.

Conclusion: Our original results, obtained for the first time in Manisa province in the Aegean region, may contribute to future research focusing on diagnosis, prognosis and potential therapeutic interventions in PKD, and the variants considered as Novel will be included in the databases and may be a reference for researchers who will work on this subject.

Keywords: PKD1, PKD2, PKHD1

ORAL PRESENTATIONS

[OP-23]

The Importance of Further Examinations in Rare Cases

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Abstract

Growth is the most important indicator of a child's health. Short stature is a common clinical picture in children. However, in most children with short stature, a cause cannot be determined with routine screening. In this study, a case with a preliminary diagnosis of short stature and his family were examined. Chromosome, FISH and array-CGH analysis were performed on the peripheral blood sample taken from our case. After cell culture with peripheral blood, chromosome analysis, FISH analysis using SHOX Xp22.33 probe and array-CGH using Agilent CytoGenomics v5.3.0.14 program were performed. Chromosome and FISH analysis were performed on the entire family to examine familial segregation. In the patient's chromosome analysis, 46,X,der(X)(pter?)[50], *SHOX* gene deletion in the FISH analysis, and heterozygous deletion in the Xp22.33 in array-CGH analysis. In this regard, while the same deletion was detected in the mother and daughter whose family segregation was examined, it was not detected in the father and brother. In our case, chromosome analysis initially revealed a suspicion of a break in the X chromosome. After further examination, it was determined that there was a deletion in the Xp22.33 region and the same result was seen in all women of the family, but not in the men. This study shows that laboratory tests alone are not sufficient and that one should not jump to conclusions based on initial tests, and contributes to the literature by emphasizing the importance of conducting further tests by carefully examining clinical findings.

Keywords: Short stature, chromosome analysis, further examination

[OP-24]

A Rare Chromosome Anomaly, Isodicentric Chromosome 15: Case Report

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Abstract

It is known that eighty percent of marker chromosomes originate from acrocentric chromosomes. In particular, the 15th chromosome was found to be the chromosome that forms the most marker chromosomes, with a share of fifty percent. Isodicentric chromosome 15 [idic(15)], also known as marker chromosome 15 syndrome, is found at a rate of 1 in 30000. It is a rare chromosomal anomaly syndrome that causes epilepsy, psychomotor developmental delay, autism and hypotonia. In this report, we present a two-year-old girl with treatment-resistant tonic-clonic seizures, psychomotor developmental delay, autistic findings, and obesity. As a result of the patient's examination, karyotype analysis and Prader-Willi FISH (15q11-13) tests for chromosome 15 were requested. In the patient whose marker chromosome was detected in the karyotype analysis, four centromere signals belonging to the 15th chromosome were seen in the Prader-Willi FISH (15q11-13) analysis. The patient's family members were evaluated for idic(15). No chromosomal anomaly was detected in his mother, father, sister or brother. It was thought that the marker chromosome in the patient might be related to advanced maternal age (age of birth was forty-four years old). The 15th chromosome duplication was confirmed with the array CGH study. We present the clinical findings of idic(15) through this case, along with a literature review.

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Poster Presentations

[P-01]

Exploring and Expanding Secondary Findings Through Exome Sequencing in the Çanakkale/ Türkiye Population

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Introduction: Exome-sequencing (ES) methods enable accurate diagnosis in challenging cases, and uncover secondary findings (SFs) potentially linked to life-threatening or preventable diseases. The American College of Medical Genetics and Genomics (ACMG) publishes a list detailing which SFs should be reported and regulaly updates it. This study aims to compare results across different SF versions in patients and explore additional SFs to identify potential new recommendations for SF reporting.

Methods: We conducted a retrospective analysis of 724 patients who had previously undergone ES, utilizing the QIAGEN clinical insight interpret database to identify ACMG SFs. Furthermore, we investigated pathogenic/likely pathogenic variants in cancer and cardiovascular disease genes not listed in ACMG SFs, as well as genes associated with common diseases prevalent in our country (e.g., PKU, HBB, SMA, FMF, and G6PD deficiency).

Results: ACMG SF v3.2 variants were identified in 60 patients (8.2%), with no observed differences between ACMG v3.1 and v3.2. However, 51 patients (7%) had variants from ACMG SF v2.0. Additionally, our analysis revealed that 208 patients harbored non-ACMG SF variants. Among these, 67 variants in MEFV, 10 variants in CHEK2, 9 variants in G6PD are particularly noteworthy.

Conclusion: In this study, we focused on known SFs and identified additional variants that could be considered as new recommendations. While expanding the list of SFs can pose challenges during analyses and genetic counseling, a thoughtfully curated SF list has the potential to enhance patient care and improve clinical outcomes.

[P-02]

Multiple Congenital Anomalies-Hypotonia-Seizure Syndrome 1: A First Case from Türkiye

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Introduction

Mutations in the *PIGN* gene result in a condition characterized by severe developmental delay, hypotonia, and early-onset seizures, associated with multiple congenital anomalies, termed "Multiple Congenital Anomalies-Hypotonia-Seizures Syndrome 1." Its prevalence is estimated to be <1/1,000,000 (ORPHA: 280633). Genitourinary and gastrointestinal abnormalities can be observed. Here, we present a case of "multiple congenital anomalies-hypotonia-seizures syndrome 1".

Case Report

The patient, referred to us due to the manifestations of epilepsy and hypotonia, underwent an evaluation revealing hypotonia, a history of macrosomic birth, bitemporal narrowing, nystagmus, hypertelorism, bilateral hypoplastic distal fingers and toes. A contrast-enhanced brain magnetic resonance imaging examination indicated cerebral parenchymal and corpus callosum atrophy. Echocardiography showed a persistent foramen ovale. There was a first-degree cousin marriage between the parents. A similar clinical history of a deceased male sibling with comparable clinical features and a family history were present. The patient who applied to the Medical Genetics Clinic of Necmettin Erbakan University Faculty of Medicine for the investigation of the etiology of hypotonia were evaluated with their medical history, physical examination, and pedigree. Peripheral blood sample from the patient was examined using clinical exome sequencing. Variants were classified according to ACMG standards. CES analysis of the patient revealed a homozygous missense variant in the *PIGN* gene (ENST00000357637,c.996T>G,rs1060499763), classified as "likely pathogenic".

Discussion

The presentation of this case aims to guide clinicians and medical geneticists in considering rare clinical associations with the *PIGN* gene in patients presenting with hypotonia, macrosomia, and epilepsy, and to present the first Turkish case in the literature.

Keywords: *PIGN* gene, hypotonia, seizure

POSTER PRESENTATIONS

[P-03]

The Unusual Cause of a Common Association: Neuroocular Syndrome

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Introduction

Neuroocular syndrome (NOS) is an autosomal dominant syndrome that encompasses a wide range of systemic features. The type and severity of neuropsychiatric problems (developmental delay, intellectual disability, isolated speech delay, autism spectrum disorder) and eye anomalies (ptosis, coloboma, microphthalmia, retinal dysplasia, cataract, strabismus) exhibit marked variability. We present this rare NOS diagnosis found in a patient aiming to contribute to genotype-phenotype correlation studies.

Case Report

A 7-month-old male with a Noonan syndrome-like phenotype (ventricular septal defect, bilateral ptosis) was referred to the genetic diseases evaluation center. He was delivered by C/S at 31 weeks of gestation, with a weight of 1370 grams, from a 38-year-old G3P1 mother. No family history of similar conditions. The examination revealed various dysmorphic features (cryptorchidism, partial syndactyly on right foot, frontal bossing, ptosis, downslanting palpebral fissures, wide-set eyes, long philtrum, tented mouth, retrognathia) along with failure to thrive. Abdominal ultrasonography and brain MRI were normal. CES analysis (KAPA HyperCap Heredity Panel) uncovered a heterozygous nonsense variant (ENST00000418929.7:c.2545C>T/p.Gln849Ter) in exon 4 of the *PRR12* gene.

Discussion

In the ClinVar database, the majority of pathogenic variants identified in this gene consist of variants causing loss of function (54% frameshift, 23% nonsense). A stop-gain variant in a gene, where loss of function is the primary pathogenic mechanism, and its absence in population databases, along with clear clinical correlation, led to the likely pathogenic classification of this novel variant based on ACMG standards (PVS1, PM2). In the differential diagnosis of Noonan syndrome, considering NOS is recommended.

[P-04]

A Novel Variant in *CACNA1H* Gene in Childhood Absence Epilepsy

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Introduction

Absence seizures are characterized by a brief loss of consciousness and seen 4.6 per 100,00 in the general and 6 per 100,000 in children younger than 15 years. CACNA1H encodes a T-type calcium channel and associated with epilepsy susceptibility. With this case we aimed to enlighten a novel variants of *CACNA1H* gene.

Case Report

A 6-year-old patient with no additional disease history was referred to our outpatient clinic with a complain of loss of awareness, unresponsiveness and occasional incontinence. It was stated that symptoms were lasting about 10 seconds, were usually seen once a day. Generalized epileptiform anomaly was reported in electroencephalography. DNA isolation from patient's peripheral blood and clinical exome sequencing containing 3300 genes was performed for epilepsy, then analyzed on SEQ NGS analyze platform. Molecular analysis revealed two different heterozygous missense variants [c.5024G>A,p.Arg1675Gln (rs149367557) and c.4462G>T,p.Asp1488Tyr] in *CACNA1H* gene. Variants are classified as "Variant of Uncertain Significance" according to ACMG criteria. To identify the clinical importance, segregation analysis was also performed with Sanger sequencing. The former variant was found in patient's mother who has no related symptom and the latter variant was not detected in both parents. The variant was evaluated as *de novo* and thought to be responsible for complaints of the patient.

Discussion: Here we conclude that missense c.4462G>T:p.Asp1488Tyr variant which has never been encountered before may be responsible for childhood absence epilepsy. With this case report, we hope to make contribution to the literature.

Keywords: Absence seizure, epilepsy, *CACNA1H* gene

POSTER PRESENTATIONS

[P-05]

Pathogenic Variant in CBL Causing Juvenile Myelomonocytic Leukemia: A Case Report

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Introduction: Pathogenic variants altering RAS/MAPK pathway cause wide spectrum of diseases including developmental abnormalities, growth delay, various neoplasms and hematologic diseases. Juvenile myelomonocytic leukemia (JMML) is a rare and aggressive myelodysplastic disorder of early childhood. Somatic or germline pathogenic variants in 5 genes (*NF1*, *PTPN11*, *KRAS*, *NRAS*, *CBL*) affecting this pathway account for 90% of JMML cases. We report a patient diagnosed with Noonan syndrome-like disorder and CBL-mutated JMML (NS-JMML).

Methods: A 41-month-old female, the first child of a healthy unconsanguineous-couple, with growth and neurodevelopmental delay, absent speech, microcephaly and open-fontanel initially applied to hospital with abdominal distention and splenomegaly. After the detailed work-up, she was diagnosed with NS-JMML. Following consultation to our department, she was tested with next generation sequencing RASopathy panel from peripheral blood sample as well as cytogenetics and molecular tests from bone marrow biopsy.

Results: RASopathy panel revealed a c.1112A>C(pTyr371Ser) heterozygous likely pathogenic missense variant in *CBL(NM_005188.3)* gene with the allele frequency of 56% (reading depth: 375). Variants affecting Tyr371Ser has been reported several times in both isolated JMML and NS-JMML. Chromosomal analysis, monosomy 7 FISH and t(9;22) RT-PCR tests from bone marrow biopsy were all normal.

Conclusion: We present a heterozygous variant in the *CBL* gene detected in a female with NS-JMML. Considering that transplantation is not recommended in germline CBL mutations, it is valuable to detect these mutations for patients' follow-up and treatment. In addition, we aim to demonstrate that genetic counseling and monitoring for hematologic malignancies is crucial for Noonan syndrome-like phenotype.

Keywords: CBL, JMML, RASopathy, Noonan syndrome-like

[P-06]

A Rare Li-Fraumeni Syndrome Caused by a Homozygous TP53 Pathogenic Variant

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Introduction

Li-Fraumeni syndrome (LFS) inherited in an autosomal dominant manner, is a hereditary cancer predisposition syndrome, resulting from pathogenic variants in the *TP53* gene. TP53 is a tumor suppressor gene encoding the p53 transcription factor involved in the cell cycle regulation.

Case Report

A 3-year-old female patient was consulted with relapsed refractory choroid plexus carcinoma (CPC) and surrenal mass. An abdominal wall defect was detected prenatally at 24 weeks and the patient was operated twice for omphalocele. Cranial magnetic resonance imaging (MRI) revealed a mass almost completely filling the left cerebral hemisphere, causing midline shift and hydrocephalus. Pathological examination of the excised tumor tissue revealed a diagnosis of grade 3 CPC. Abdominal MRI was performed due to high androgen levels. A 38x23mm lesion was observed in the right adrenal gland. It was found compatible with adrenocortical carcinoma (ACC). Next generation sequencing revealed a homozygous c.375G>A (p.Thr125Thr) variant in the 4th exon of the TP53. This variant was evaluated as "Pathogenic Variant" according to the The American College of Medical Genetics and Genomics 2015 variant classification guide.

Conclusion

Although TP53 is the most frequently mutated gene in cancer patients, homozygous germline variants are very rare. In our case, two LFS-associated tumors (CPC and ACC) were seen at a very early age. CPC recurred multiple times and our patient died due to a severe clinical course. This prognosis indicates that the homozygous status, may have brought the age of tumor emergence earlier and aggravated the clinical course.

Keyword: TP53

POSTER PRESENTATIONS

[P-07]

Smith-Kingsmore Syndrome: A Rare Case of Intellectual Disability from Çanakkale

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Introduction

Smith-Kingsmore syndrome is a rare neurological disorder characterized by a macrocephaly, intellectual disability, seizures. It is caused by pathogenic mutations in the *MTOR* gene. We aimed to present a case of Smith-Kingsmore syndrome with a rare MTOR mutation.

Case Report

A 30-year-old male patient applied to the outpatient clinic with his family with the diagnosis of mental retardation. The patient additionally had newly diagnosed hypothyroidism and congenital macrocephaly. There was no other patient with intellectual disability in the family. We performed clinical exom analysis to the patient and detected heterozygous c.5395G>Ap. E1799K(NM_004958.4) variant in MTOR. *MTOR* gene is associated with autosomal dominant "Smith-Kingsmore syndrome" in Online Mendelian Inheritance in Man which shows compatibility with the case.

Conclusion

The *MTOR* gene affects the PI3k/Akt/mTor pathway and plays a role in the extracellular communication, growth and proliferation of cells. MTOR mutations that cause Smith-Kingsmore syndrome cause hyperactivation of this pathway, causing symptoms such as megaloencephaly, macrocephaly, intellectual disability and epilepsy. To date, 56 patients with a missense mutation in the *MTOR* gene that causes Smith-Kingsmore syndrome have been reported and 30 of these patients have the same mutation as our patient. Currently, there is no treatment for this disease and it has been reported that patients with gain-of-function mutations in the *MTOR* gene may benefit from MTOR inhibitors such as rapamycin. Therefore, functional studies are required for patients diagnosed with Smith-Kingsmore syndrome. Although there are not enough publications in the literature regarding the treatment of this disease, we think that the increase in the number of published cases will be beneficial in finding a treatment for the disease.

[P-08]

The Role of Array-CGH Analysis in the Diagnosis of Rare Diseases: A Single-Center Experience

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Abstract

Array-CGH analysis is an important tool in the diagnosis of rare diseases due to its ability to identify defined microdeletion-duplication syndromes, unique copy number variations in individuals, major chromosomal abnormalities, chromosomal numerical anomalies, single gene disorders, imprinting disorders; providing insight into mosaicism, and detecting loss of heterozigosity. Rare diseases were diagnosed in 47 out of the 695 patients we analyzed (6.7%). Copy number variations were detected in 10 patients, with a single gene (*SHANK3*, *CHRNA7*, *GLI3*, *HNF1B*, *NRXN1*, *ABCG2*, *NF1*, *HBA1*, *BHLHA9*) responsible for the clinical manifestations. Copy number variations were detected in rare regions among 14 patients, with sizes ranging from 145 KB to 7.7 MB, along with identified variations in microdeletion/duplication syndrome regions including 22q11.2 microdeletion syndrome in 4 patients, Prader-Willi/Angelman syndrome in 3 patients, 3q29 microduplication syndrome in 4 patients, and other syndromes found in 1 patient each such as Phelan McDermid syndrome, 1q21.1 duplication syndrome, 9q31.1q31.3 microdeletion syndrome, cat eye syndrome, 6q25 microdeletion syndrome, and 8q21.11 microdeletion syndrome. Additionally, major numerical and structural chromosomal anomalies, such as trisomy 9p, partial monosomy 11q, 6q25 microdeletion syndrome, Turner syndrome, and derivative Y chromosome, were detected in 7 of these patients, which also identified through chromosome analysis. In today's context where sequencing technologies have emerged as prominent tools for diagnosing rare diseases, the significance of array CGH technology remains crucial in the diagnosis of rare diseases due to its ability to diagnose genetic disorders arising from various mechanisms.

POSTER PRESENTATIONS

[P-09]

DeSanto-Shinawi Syndrome with *De Novo* WAC Variant in Çanakkale: A Case Report with Dysmorphic Face, Congenital Heart Disease, and Neonatal Convulsion

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Introduction: WAC-related intellectual disability, known as DeSanto-Shinawi syndrome (Online Mendelian Inheritance in Man #616708), is an autosomal dominant disorder caused by pathogenic variants in the WW domain-containing adapter with coiled-coil (*WAC)* gene, located on 10p12.1. This syndrome is characterized by global developmental delay, dysmorphic facial features, intellectual disability, and behavioral problems. We present the clinical findings and genetic analysis results of a newborn female patient with DeSanto-Shinawi syndrome.

Methods: A newborn female patient was referred to us due to congenital heart disease, hypotonia, respiratory distress, neonatal convulsion, and dysmorphic face (deep-seated eyes, bulbous nasal type, prominent ear). The patient was born via cesarean section at 34 weeks in a twin pregnancy, with her twin sibling showing no health issues. Transfontanellar ultrasound revealed multiple corticothalamic cysts. There was no consanguinity between the parents. The father, aged 28, exhibited a similar dysmorphic facial phenotype, mild ID and congenital heart disease.

Results: Since no pathogenic/likely pathogenic variant that could explain the clinic was detected in chromosome and microarray analyzes, we detected a heterozygous likely pathogenic *de novo* c.920-5delTTTAGinsTTAA variant in the *WAC* gene in the patient and his father in the clinical exom analysis.

Conclusion: DeSanto-Shinawi syndrome is an exceedingly rare genetic disorder, with only a limited number of cases reported in the medical literature. As far as we know, this is the first DeSanto-Shinawi syndrome reported in Türkiye. Our case contributes to the current literature by emphasizing the importance of advanced genetic testing methods such as CES in rare diseases.

Keywords: DeSanto-Shinawi Syndrome, *WAC* gene, CES

[P-10]

Dual Phenotype Patient with Congenital Hypothyroidism and Family History of Common Hearing Loss

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Introduction

Thyroid disorders and hearing loss may coexist in diseases such as Pendred syndrome, but they may also be present independently of each other. Numerous genetic aetiologies have been described for these two clinical conditions.

Case Report

A 2-year and 6-month-old male patient was consulted to us from pediatric endocrinology with a prediagnosis of Pendred syndrome because of congenital hypothyroidism and diffuse hearing loss in the family. There were no significant findings in prenatal follow- up and hypothyroidism was detected on heel prick screening. His parents were not related and he had a healthy sibling. In the family history, the mother had mild hearing loss. His father and some relatives on the paternal side had a history of hypothyroidism and hearing loss. The patient's neuromotor developmental milestones were normal. There were no significant dysmorphic findings on physical examination.

Discussion

The patient underwent clinical exome sequencing and the analysis revealed a heterozygous pathogenic variant c.167del in exon 2 of the *GJB2* gene. In the *TG* gene, c.7111C>T non-sense heterozygous probable pathogenic variant and intronic c.638+5G>A heterozygous probable pathogenic variant were detected in exon 41. Variants were detected in two different genes explaining the clinical status of the patient. We hope that this rare case will contribute to the literature.

Keywords: Hypothyroidism, hearing loss, dual phenotype

POSTER PRESENTATIONS

[P-11]

A Case with Kaufman Oculocerebrofacial Syndrome with an UBE3B Variant

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Abstract

Kaufman Oculocerebrofacial syndrome (KOS) or Blepharophimosis-Ptosis-Intellectual Disability Syndrome is an autosomal recessive developmental disorder characterized by reduced growth, microcephaly, ocular anomalies (microcornea, strabismus, myopia, and pale optic disc), distinctive facial features (narrow palpebral fissures, telecanthus, sparse and laterally broad eyebrows, preauricular tags, and micrognathia), mental retardation, and generalized hypotonia. Inactivating mutations in *UBE3B*, an *E3* ubiquitin ligase gene, are causative for KOS. At least 46 cases have been reported in the literature. Here, we report an infant with dysmorphic features, growth delay, cleft palate, laryngomalacia, respiratory distress, atrial septal defect, pectus carinatum, and talipes equinovarus. Dysmorphic facial features included low-set ears, dysplastic ears, bilateral preauricular skin tags, microphthalmia, blepharophimosis, hypertelorism, wide nasal bridge, anteverted nares, microretrognathia, and small mouth. Whole Exome Sequencing detected a homozygous splice acceptor variant in intron 1 of *UBE3B* (NM_130466.3 c.1623-1G>T). We classified the variant as likely pathogenic according to the ACMG criteria. KOS should be considered among the autosomal recessive causes of blepharophimosis-mental retardation syndromes, particularly in populations with a high rate of consanguineous marriages, even if there are dysmorphic facial features that are not typically associated with the phenotype. Our report contributes to the literature on this ultra-rare syndrome.

Keywords: Kaufman oculocerebrofacial syndrome, *UBE3B*, blepharophimosis, intellectual disability

[P-12]

Case Report: Patient with Blepharophimosis Ptosis Epicanthus Inversus Syndrome

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Abstract

Blepharophimosis, ptosis, and epicanthus inversus syndrome [(BPES), OMIM #110100] is a rare autosomal dominant disease of the eyelids and mid-face structures. There are two main types of BPES. Each type harbors the four classic clinical signs: PBE inversus, and telecanthus. Type 1 is associated with premature ovarian failure. Type 2 is characterized by the classic facial features alone. Here we present a family with multiple cases that prediagnosed with BPES due to their midface and eye features. Molecular analysis of the patients with whole exome sedquencing revealed *FOXL2* gene heterozygous frameshift likely pathogenic variant (c.11_12insGG p.(Ser4Argfs*147)) in exon 1. With respect to clinical and molecular data, patient's diagnosis is confirmed as BPES.

POSTER PRESENTATIONS

[P-13]

New Case with Thauvin-Robinet-Faivre Syndrome: Novel Variant and New Phenotypic Findings

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Abstract

Thauvin-Robinet-Faivre syndrome is an autosomal recessive disease characterized by intellectual disability, delayed psychomotor development, speech delay, macrocephaly, tall stature, large hands and feet, kidney anomalies, congenital heart diseases and dysmorphic facial findings. Biallelic pathogenic variants in the *Fibroblast Growth Factor, Acidic, Intracellular Binding Protein (FIBP)* gene are responsible for this phenotype. In this study, we present a case in which a loss of function variant was detected in this gene. The proband, a 4-year-9-month-old girl, was the fifth child of consanguineous parents. There was congenital heart disease, atrial septal defect (ASD), mitral valve prolapse (MVP) intellectual disability, speech retardation, delayed acquisition of motor developmental stages, abnormal electroencephalography, relative macrocephaly, typical dysmorphic facial findings, large hands and feet, camptodactyly in bilateral third toes, and clinodactyly in 4-5 toes. There was benign neutropenia and non-specific flare hyperintensities in the parenchymal area on cranial magnetic resonance imaging. In the whole exome sequencing performed on the patient, NM_004214.5 c.453dup p.(Arg152Alafs*16) frameshift homozygous novel probable pathogenic variant was detected in the *FIBP* gene. It was the ninth case reported in the literature and the fifth from Türkiye. The findings were consistent with the literature; the case had different ASD, clinodactyly and camptodactyly. This study contributes to the enrichment of the clinical findings and variant distribution of this very rare syndrome. More data are needed for phenotype-genotype correlation.

Keywords: FIBP, Thauvin-Robinet-Faivre syndrome, autosomal recessive

[P-14]

Partial Trisomy 4q Case

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Abstract

Partial trisomy of the long arm of chromosome 4 (distal duplication 4q; ORPHA:96096), is a rare chromosomal anomaly characterized by growth failure, intellectual disability, microcephaly, facial dysmorphism, finger anomalies, cryptorchidism, hearing loss, epilepsy, and heart/kidney malformations. Patients with partial trisomy 4q have highly variable phenotypes, therefore, it is very difficult to establish genotype-phenotype correlation in these cases. The number of cases reported to date is less than 100. Cases most often result from unbalanced inheritance of balanced parental chromosomal translocations. Here, we present an 8-day-old male infant, who was referred to our hospital with respiratory distress and suspicion of choanal atresia. He was born to a non-consanguineous parent, his mother had 9 pregnancies, 7 of which ended in abortion, and he had a healthy brother. In physical examination, he had a broad nasal root, bushy and wide eyebrow, low-set ear, prominent and overfolded ear helices, downslanting palpebral fissures, hypertrichosis, prognathia, high palate, preaxial polydactyly in the right hand, bilateral overlapping toes, sacral hypertrichosis, and bilateral undescended testicles. Based on the patient's findings, karyotype analysis was performed. Upon the detection of a derivative chromosome 15, karyotype analysis was performed from both parents. The mother was found to be a balanced translocation carrier t(4;15)(q21.3;qter), and our patient's karyotype was reported as 46,XY,der(15)t(4;15)(q21.3;qter)mat. To the best of our knowledge, this karyotype has not been published before in the literature. It is important to present such cases to better elucidate the variable phenotype and phenotype-genotype correlation of this disease.

POSTER PRESENTATIONS

[P-15]

Adult-Onset Atypical Absence Epilepsy Associated with a Novel MECP2 Variant: A Case Report

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Introduction

Variants in MECP2, located on Xq28 and encoding a methyl CpG binding protein, are well known to be associated with Rett syndrome in female patients. However, it is becoming increasingly clear that variants in the *MECP2* gene may have a wide range of consequences in terms of neurodevelopmental phenotypes beyond Rett syndrome. Here, we report the case of a female patient with adult-onset atypical absence epilepsy carrying a novel truncating variant in the *MECP2* gene.

Case report

A 27-year-old woman was admitted to the neurology department with epilepsy and intellectual disability. She had her first febrile convulsion at the age of 1.5 years and had no further seizures until the age of 25. The patient had mental retardation and was unable to continue her education after primary school due to poor school performance. At the age of 25, the patient commenced to experience treatment-resistant atypical absence seizures and tonic-clonic seizures. Following a comprehensive etiological workup, which yielded negative results, the patient underwent clinical exome sequencing. This revealed an 80 bp deletion in the *MECP2* gene, NM_001110792.2:c.1190_1269del, p.(Pro397Argfs*24).

Discussion

This case was not consistent with Rett syndrome in terms of clinical manifestations. MECP2 variants may result in a spectrum of neurodevelopmental phenotypes and may manifest as epilepsy in adulthood.

Keywords: MECP2, atypical absence epilepsy, adult-onset epilepsy

[P-16]

NGS as an Indispensable Tool in Cancer: An Overview of the Variants Detected in AML Patients

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Abstract

Introduction: Acute myeloid leukemia (AML) is a heterogeneous, aggressive form of cancer caused by clonal proliferation of malignant hematopoietic precursor cells in the bone marrow. Genetic alterations are common in AML, playing a crucial role in diagnosis, prognosis, and treatment. Recent advancements in next generation sequencing (NGS) have emphasized the crucial role of molecular genetics in disease categorization. In this study, we present the molecular findings of AML patients analyzed using the NGS method.

Methods: The findings of 40 AML patients who underwent NGS panel testing between January 2023 and April 2024 were evaluated retrospectively. NGS was performed on the samples using the QIAseq Myeloid Panel, which contains 32 genes. Variant calling and analysis were performed using the Qiagen Clinical Insight (QCI) interface. Reportable variants were classified according to the AMP/ASCO/CAP guidelines.

Results: Among the 40 patients included in the study, 22 (55%) were male and 18 (45%) were female with a median age of 53 years (11-94 years). Twenty four (60%) samples tested positive for one or more pathogenic or likely pathogenic variant(s). A total of 55 pathogenic/likely pathogenic variants were detected. Most of the variants were detected in *FLT3* (13%), *IDH2* (11%), *TET2* (11%), *NRAS* (9%), and *DNMT3A* (9%) genes. The most recurrent variant was detected on the *IDH2* gene {NM_002168.4:c.419G>A p.(Arg140Gln)}.

Conclusion: NGS panel testing is a fast and cost-effective method for genetic screening of myeloid malignancies. Our study illustrates the significant role of genetic profiling in diagnosis and prognosis of AML patients.

Keywords: Acute myeloid leukemia, hemato-oncology, molecular genetics, next generation sequencing

POSTER PRESENTATIONS

[P-17]

Two Siblings with Trisomy 9p and a Novel Finding

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Abstract

Trisomy 9p is one of the most common autosomal abnormality known to be the fourth frequency after full Trisomy 21, 13 and 18. Compatibility with survival can be explained that 9p is relatively gene poor region. In most cases, Trisomic 9p segment was inherited from reciprocal translocation carrier parent and a few of cases were due to *de novo* chromosomal aberrations. This syndrome has been featured by craniofacial dysmorphism, intellectual disability and developmental delay. We describe the case of a 14 years old girl who was referred to our clinic for evaluation of growth retardation and primary amenorhea. Clinical examination of patient revealed short stature and dysmorphic features such as bulbous nose tip, ocular hypertelorism, high arched eyebrows, strabismus and unilateral ptosis, low-set ears, short filtrum, downturned corners of mouth. Her height was 145 cm (<3p) and her secondary sex characters were prepubertal. Also according to evaluation of pediatric endocrinology clinic she diagnosed as central diabetes insipidus. It was noticed that her little sister has same facial dysmorhic features, mild intellectual disability and mild degre of growth retardation. The karyotype in proband and her sister was identified as 46,XX,der(12)t(9;12)(p24.3-p13.1) resulting from a maternal balanced reciprocal translocation t(9;12) (p24.3-p13.1). These two sibligns were diagnosed as Trisomy 9p syndrome. 9p duplication syndromes are particularly heterogenous because of breakpoint heterogenity. But until now, diabetes insipidus hasn't reported as a finding of Trisomy 9p. For detailed genotype-phenotype correlation, whole exome sequencing and copy number variation analysis will perform from our proband.

Keywords: Trisomy 9p, reciprocal translocation, diabetes insipidus

[P-18]

Ectodermal Dysplasia: A Single Medical Center Experience in Central Anatolia

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Abstract

Introduction: Ectodermal dysplasia is an uncommon genetic disorder known for its distinctive physical features. It is a hereditary condition that affects how the sweat glands, teeth, hair, and nails grow or function. Ectodermal dysplasia can also damage the skin, the inner ear, the development of fingers and toes, the retina or lens of the eye, the nerves, and other regions of the body, depending on the specific disease. We aimed to investigate the clinical characteristics of patients with ectodermal dysplasia in our center.

Methods: We conducted a retrospective analysis of 10 patients with ectodermal dysplasia diagnosed in our clinic from 2017 to 2024.

Results: This case series consisted of 6 males and 4 females aged 2 to 33, with a mean age of 10.8 years. The clinical features were as follows: hypodontia/oligodontia (100%), abnormal hair morphology (70%), abnormality of the skin (40%), onychodysplasia/dystrophic nails (30%), and sparse eyebrows (40%).

Conclusion: This study presents the clinical presentation and variant diversity of patients with ectodermal dysplasia as a single-center experience. In addition, the study introduces four novel variants to the literature.

Keywords: Ectodermal dysplasia, oligodontia, hypodontia, onychodysplasia

POSTER PRESENTATIONS

[P-19]

The Molecular Analysis of the Relationship Between the Pharmacogenetics of Slc19a1 and the Response to Methotrexate Therapy in Patients with Juvenile Idiopathic Arthritis: Preliminary Findings

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Introduction: Juvenile idiopathic arthritis (JIA) is a chronic rheumatic disease that affects children. Though its etiology and pathogenesis remain unclear, it is the most common childhood rheumatic disease. In Türkiye, the oligoarticular subtype is the most frequently observed form of JIA. Methotrexate (MTX) is commonly used for treatment of JIA. In cases of insufficient response, MTX is often combined with Biological disease-modifying anti-rheumatic drugs (bDMARDs). The SLC19A1 gene encodes a protein that plays a role in transporting folates into cells. Point mutations and downregulation of the SLC19A1 gene are key factors in antifolate resistance.

Methods: This study aims to investigate the relationships between the response to therapy and the pharmacogenetics of SLC19A1 in JIA patients who are treated with MTX or MTX+bDMARD. Genomic DNA was isolated from peripheral blood samples of 120 JIA patients. 230 bp SLC19A1 amplicon was amplified using conventional PCR with gene specific primers. RFLP analysis using Hha I restriction endonuclease enzyme was performed to genotype (GA, GG, AA) SLC19A1.

Results: The patients were divided into two groups based on the treatment they received: the MTX group (n=59) and MTX+bDMARD group (n=61). The analysis showed that hemoglobin level was a risk factor for the drugs used. However, this did not show significance within the drug groups ($p>0.05$). Additionally, no significant difference was observed among the three different genotypes in patients treated with MTX or MTX+bDMARD based on RFLP results.

Conclusion: It is planned to increase the number of patient volunteers treated with MTX or MTX+bDMARD and conduct re-analysis of the study.

Keywords: Juvenile idiopathic arthritis, pharmacogenetics, SLC19A1, Methotrexate

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02nd – 04th May 2024

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Full Texts

FULL TEXTS

[OP-25]

Genetic Analysis Results of Patients with a Prediagnosis of Monogenic Parkinson's Disease

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Introduction

The term Parkinsonism refers to a group of progressive neurodegenerative diseases characterized by bradykinesia, rigidity and tremor. The most common type seen in the clinic is Parkinson's disease (PD). PD is the second most common neurodegenerative disorder after Alzheimer's disease (1). For reasons that are not yet fully understood, the incidence and prevalence of the disease has increased rapidly in the last 20 years (2). The main pathophysiology of PD results from the complex interaction of abnormal alpha-synuclein accumulation, mitochondrial, lysosomal or vesicular transport dysfunction, synaptic transport problems and neuroinflammation. As a result of these mechanisms, loss of dopaminergic neurons occurs (3). Monogenic PD can be inherited as autosomal dominant, autosomal recessive or X-linked. Mutations in certain genes are responsible for about 5-10% of all cases (4). Mutations in some genes have been shown to cause mitochondrial dysfunction and are associated with familial PD. Mutations in the *PRKN*, *SNCA*, *DJ1*, *UCHL1*, *LRRK2*, *PINK1*, *VPS35*, *HTRA2* genes directly or indirectly lead to mitochondrial dysfunction (5). A better understanding of the genetic pathways that play a role in the neurodegenerative process is expected to provide benefits for diagnosis in the early prodromal period and contribute to the treatment process in the coming years. In this study, we aimed to examine the genetic etiology of cases evaluated with a prediagnosis of Monogenic PD retrospectively from the patients data and to evaluate the clinical correlates of the results.

Methods

Results of next generation sequencing (NGS) panel of 35 cases, results of MLPA panel of 9 cases, Sanger sequencing analysis of 1 patient and demographic and clinical characteristics of the patients who were clinically evaluated with the prediagnosis of monogenic PD were evaluated retrospectively. The cases' age, gender, clinical findings, head trauma history, environmental exposure, family history and past medical records were scanned in detail from their files. Family trees containing at least 3 generations have been drawn. Ethical approval for this study was received from the University of Health Sciences Türkiye, Gülhane Training and Research Hospital Clinical Research Ethics Committee (decision number: 2021-355, date: 21.10.2021).

Results

NGS panel was performed on 35 of 36 cases, MLPA panel was performed on 9 cases, and Sanger sequencing was performed on 1 case due to a known mutation in his family. Among the 36 patients included in the study, 13 different variants were detected in 12 (33.3%) patients. In 3 cases c.125G>C (p.Arg42Pro) homozygous pathogenic variant in *PRKN* gene and in 1 case promoter region, exon 1 and exon 3 deletions in *PRKN* gene were found. Heterozygous pathogenic variant in the *PRKN* gene was found in 1 case, and heterozygous class III (Clinically Uncertain-VUS) variant was found in the *ATP13A2, PRKN, VPS35, PINK1, PLA2G6* genes in 7 cases. Genotype-phenotype relationship of the patients were evaluated.

Conclusion

This study is one of the rare studies conducted in our country in which more than one molecular diagnostic methods were used for the prediagnosis of monogenic PD.

In our study, 13 different variants were detected in 12 cases, and pathogenic variants considered to be related to the disease were detected in 4 (11.1%) cases. This rate was found to be compatible with the literature (6). To date, rare variants in more than 20 genes conforming to autosomal dominant and autosomal recessive inheritance models have been reported to be associated with PD. LRRK2 mutations are the most common. It has been associated with 4% of familial forms and 1% of sporadic cases. The fact that no variant was detected in the *LRRK2* gene in our study can be explained by the small number of our cases. We believe that our study will contribute to the literature by examining the detected variants and their clinical effects. Results of our study would contribute to provide genetic counseling to other family members at risk.

Keywords: Parkinson's disease, next generation sequencing, MLPA

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FULL TEXTS

[OP-26]

Role of Retinoic Acid and Brain-Derived Neurotrophic Factor in Cholinergic Neuron Differentiation of Human Neuroblastoma SH-SY5Y Cells

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Introduction

Alzheimer's disease (AD) is a complex neurodegenerative disease. *In vitro* experimental models are of great importance in elucidating the molecular mechanisms and therapeutic approaches of diseases. Although human neuroblastoma SH-SY5Y cells are frequently used in this field, there is a need to differentiate the cells to reflect their neuronal properties. In our study, we aimed to compare differentiation protocols using retinoic acid (RA) and brain-derived neurotrophic factor (BDNF) to determine the experimental protocol that best represents the cholinergic neuron model for use in *in vitro* models of AD.

Methods

SH-SY5Y cells were subjected to differentiation protocols with all-trans RA and BDNF for 5 days and 7 days. Morphological changes in differentiated SH-SY5Y cells during the treatment period were imaged and neurite lengths were analyzed in Neuron J (image J) program. It was evaluated by changes in gene expression levels of neuronal and cholinergic markers microtubule-associated proteins (MAP2), neuronal nuclear protein (NeuN), choline acetyltransferase and AChE using real-time reverse transcriptase-polymerase chain reaction.

Results

Our results showed that there were significant morphologic changes in the cells in the 5 day RA treatment and 5-day RA +2-day BDNF supplementation groups compared to the control. Neurite lengths were the highest in the RA + BDNF group ($p<0.05$). *MAP2*, *NeuN* and *AcHE* gene expressions were increased in RA + BDNF group compared to the control ($p<0.001$, $p<0.001$, $p<0.05$).

Conclusion

Our study demonstrated that SH-SY5Y cells differentiate into cholinergic cells at morphological and molecular levels. According to our results, we can suggest that RA+BDNF treatment protocol for 7 days can be used in *in vitro* AD models in addition to the protocols with different contents and durations in the literature.

Keywords: Alzheimer's disease, SH-SY5Y, retinoic acid, BDNF, neuronal differentiation

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FULL TEXTS

[OP-27]

The Significance of Gene Dosage Analysis in Patients Undergoing Microarray Testing

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Introduction

An eight-month-old male patient was referred to the clinical genetics department with a history of postpartum neonatal intensive care unit hospitalization for 4.5 months. Born via cesarean section at 36 weeks and 5 days of corrected gestational age, the patient weighed 2780 grams at birth. The mother, aged 35, had her first pregnancy with no consanguinity between the parents. The patient experienced cyanosis after delivery and required tracheostomy and intubation due to postpartum respiratory distress. At four months old, the frontal fontanelle measured 2x2 cm. During hospitalization, the patient experienced electrolyte abnormalities, including diarrhea and hyponatremia, leading to suspicion of congenital secretory chloride diarrhea related to the *SLC26A3* gene. Clinical examination revealed several physical abnormalities, including a concave nasal bridge, suspected midface hypoplasia, short neck, brachytelephalangy, flattened nasal base, reduced nasal tip protrusion with short columella, crescent-shaped nostrils, and postnatal short stature. The patient also had a history of recurrent respiratory infections. Neurological examination indicated global developmental delay, with cognitive, and social adaptation functions severely impaired. Echocardiography revealed tricuspid regurgitation alongside a patent foramen ovale. Imaging studies revealed preserved cervical lordosis, significant spinal canal narrowing at the C1-2 level, and vertebral body calcifications on computed tomograph scan of cervical vertebrae. Cranial magnetic resonance imaging revealed dilated lateral ventricles, diffuse brain atrophy, widened periventricular spaces, diffuse thinning of the corpus callosum secondary to hydrocephalus, and normal brainstem and basal ganglia. No evidence of acute ischemia or mass lesions was observed. Abdominal ultrasound showed normal findings for the liver, spleen, gallbladder, kidneys, and bladder, with no evidence of intra-abdominal abscess or significant pathology. Emergency abdominal ultrasound ruled out significant pathology or free fluid. Pedigree anamnesis was clear except for the presence of an uncle from the mother's side with nasal bone hypoplasia. At the time of consultation, the patient's length was 60 cm, corresponding to the 0.1st percentile for preterm 8-month-old male infants according to World Health Organization growth standards. The weight was 6900 grams, placing the patient at approximately the 14th percentile for preterm 8-month-old male infants.

Methods

The comprehensive evaluation of the patient's condition underscores the complexity of the case, with multiple systemic abnormalities and developmental delays. Further genetic testing and clinical management strategies are warranted to address the patient's needs effectively. Karyotyping analysis using G banding technique at 500-550Kb resolution and clinical exome sequencing (CES) utilizing the Roche CES kit followed by sequencing using the DNBSEQ-G400™ sequencing platform (MGI Tech Co., Ltd.) revealed no diagnosis. The patient was then indicated for single nucleotide polymorphisms (SNP)-microarray analysis using Illumina beadchip array technology Infinium high-throughput screening platform using Gene Set Analysis-Booster kit (>700K SNP).

The primary analysis of microarray data revealed no compatible copy number variations (CNVs) with the clinical features or any variants of uncertain significance, likely pathogenic, or pathogenic CNVs using Decipher and ClinGen Database. Advanced analysis was conducted using the GenomeStudio v2 software, focusing on gene dosages.

At our center, two different approaches for gene dosage analysis are employed: inward and outward approaches. In the inward approach, a hotspot gene list is selected, and each gene is individually evaluated for possible coverage by CNV areas. Accordingly, *Hotspot* genes were chosen based on the GeneCards database, and since the phenotypical features resembled skeletal dysplasia, keywords of "chondrodysplasia" and "dysplasia" were chosen for database searching. The results were sorted by relevance score from the GeneCards database, and the most relevant hotspots were listed within the first 80% of the score intervals to prevent missed genes (generally 40% interval seems to sufficient). For example, if the most relevant score is 130, scores from 130 to 26 (highest score x0.20) are included. Then the created hotspot analaysis was performed using the GenomeStudio software's genome chromosome browser tab utilizing gene search menu, where CNV bookmarks facilitating analysis by showing related bookmarks colors for each copy number.

In the outward approach, detected CNV area coordinates were exported to the Decipher database, and each gene's data was evaluated separately. The choice between these approaches depends on the patient's status; for example, the inward approach may be preferred when facing multiple CNVs, while the outward approach may be chosen based on the analyzer's preference or when fewer CNVs are detected.

Results

In our patient, a deletion was detected at the Xp22.33 (1095534-2986771) locus, which is not compatible with any reported significant CNV according to both Decipher and University of California Santa Cruz databases. However, as an outward approach, when the coordinates were exported to the Decipher database, 26 genes were revealed under the coordinate coverage. Analysis of these 26 genes showed that only ARSL and CSF2RA exhibited haploinsufficiency clinical evidence by ClinGen database, with associated pLI and loss-of-function observed/expected upper-bound fraction scores of 0.59 and 0.49 for ARSL and 0 and 1.12 for CSF2RA. *ARSL* gene deletion is directly associated with CDPX1, while CSF2R deletions and loss-of-function mutations affecting both copies of CSF2RA are associated with hereditary pulmonary alveolar proteinosis (PAP) which is a very rare lung disorder characterized by the accumulation of surfactant-derived lipoproteins within pulmonary alveoli, presenting in early childhood, which often leads to severe respiratory distress or failure.

Since, Inward analysis only included the *ARSL* gene but not CSF2RA result were sam efor *ARSL* but not CSF2RA. The reason is the difference between specificity and sensitivity of two approaches.

Since, *ARSL* gene is located outside of the protease-activated receptor-1 (PAR-1) region and the *CSF2RA* gene is located inside the PAR-1 region, the haploinsufficiency score of these two genes is expected to be different. The PAP disease can only be seen in 0 copy number or whole function loss for CSF2RA, equivalent to autosomal

recessive haploinsufficiency. In contrast, ARSL can be sensitive to one copy deletion, as it has only one copy of the gene in normal circumstances. Thus, a deletion of one copy can bring about phenotypic features, and this deletion can be evaluated as an X-linked disease. Accordingly since we have one copy of the detected area, only non-PAR-1 regions may have problematic expression level depends on their sensitiveness to haploinsufficiency.

Accordingly, patient was diagnosed with CDPX1. CDPX1 is caused by genetic changes involving the *ARSL* gene. This gene provides instructions for making an enzyme called arylsulfatase L. The function of this enzyme is unknown, although it appears to be important for normal skeletal development and is thought to participate in a chemical pathway involving vitamin K. Evidence suggests that vitamin K normally plays a role in bone growth and maintenance of bone density. Between 60 and 75 percent of males with the characteristic features of CDPX1 have a mutation in the *ARSL* gene. These mutations reduce or eliminate the function of arylsulfatase L. Another 25 percent of affected males have a small deletion of genetic material from the region of the X chromosome that contains the *ARSL* gene. These individuals are missing the entire gene, so their cells produce no functional arylsulfatase L. Researchers are working to determine how a shortage of arylsulfatase L disrupts the development of bones and cartilage and leads to the characteristic features of X-linked chondrodysplasia punctata 1 (CDPX1). CDPX1 is characterized by chondrodysplasia punctata (stippled epiphyses), brachytelephalangy (shortening of the distal phalanges), and nasomaxillary hypoplasia. Although most affected males have minimal morbidity and skeletal findings that improve by adulthood, some have significant medical problems including respiratory involvement, cervical spine stenosis and instability, mixed conductive and sensorineural hearing loss, and intellectual disability.

CDPX1 is inherited in an X-linked manner. If the mother of a proband has the *ARSL* pathogenic variant identified in the proband, the chance of transmitting it in each pregnancy is 50%. Males who inherit the pathogenic variant will be affected; females who inherit the pathogenic variant will be carriers and thus far have not been affected. Males with CDPX1 pass the pathogenic variant to all of their daughters and none of their sons. Carrier testing for at-risk relatives and prenatal testing for at-risk pregnancies are possible if the ARSL pathogenic variant has been identified in the family.

According to the GeneReviews® guidelines diagnosis can be made in 88% by *ARSL* gene sequance analysis and 12% percent by gene-targeted deletion/duplication analysis. Our investigation revealed a contiguous deletion within the proximal region of the X chromosome, encompassing the haploinsufficient *ARSL* gene, leading to the diagnosis of CDPX1 in the patient. This discovery highlights the critical role of gene dosage analysis in microarray testing, particularly in cases with suspected chromosomal rearrangements and CNV issues, where conventional karyotyping may lack the necessary resolution.

Conclusion

Of particular interest is the observation that the detected deletion is located between two ALU repeats, which are short interspersed nuclear elements known for their repetitive nature in the human genome. The presence of these flanking sites suggests a potential mechanism for the recurrence of CNV in this region. ALU repeats are prone to mediating non-allelic homologous recombination, which can lead to CNV formation through unequal crossing over between repetitive sequences during meiosis. Such events may result in the deletion or duplication of genetic material, contributing to the development of genetic disorders. To further elucidate the inheritance pattern and assess the risk of recurrence, segregation analysis from the patient's mother and uncle (who also presents with nasal hypoplasia) should be considered for inherited CNV research. Investigating the presence of the deletion in these family members can provide valuable insights into the genetic basis of the condition and inform genetic counseling. Additionally, examining the flanking ALU repeats and their potential role in mediating CNV recurrence may shed light on the underlying molecular mechanisms driving the disorder. Moreover, the identification of a potential genetic predisposition in family members with similar phenotypic features underscores the importance of thorough clinical and genetic evaluations in such cases. Early detection and intervention can help in implementing tailored management strategies and providing appropriate support for affected individuals and their families.

FULL TEXTS

[OP-28]

Investigation of *de novo* Mutations in Clinical Exome Sequence Trio Samples

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Introduction

Of particular interest is the exploration of *de novo* mutations (DNMs), which arise spontaneously in parental germ cells and are inherited by the next generation, shaping genetic diversity and influencing disease susceptibility. Recent studies using next generation sequencing have revealed a range of DNM rates in human germ cells for single nucleotide variations (SNVs), ranging from 1.0 to 1.8×10^{-8}. Various recent studies have attempted to explore the reasons behind this phenomenon, yielding different interpretations. *In vitro* studies have shown that DNA polymerase ε and δ involved in DNA replication can perform unique base pairings during replication at rates ranging from 10^{-4} to 10^{-5}. Additionally, replication timing influences the occurrence of these errors. Regions with late replication in the cell cycle exhibit higher mutation rates compared to early replicating regions. This is due to a decrease in dNTP and protein pools contributing to replication during late replication periods. Furthermore, recent whole genome sequencing studies have highlighted the clustering of mutations in specific regions of the genome, indicating the formation of mutation hotspots in these areas. Currently, there is no consensus on whether DNMs coincide with these hotspots. Mutations can be found in regions ranging from 10 to 100 kb within mutation clusters. Additionally, the analysis indicates that the rate of transition mutations is higher than that of transversion mutations, often associated with base changes in CpG islands abundant throughout the genome. These regions are particularly prone to errors during replication due to their repetitive nature and sensitivity to methylation during replication. The prevalence of transversion mutations in regions harboring mutation clusters is remarkable. It has also been suggested that this may stem from dysfunctional replication forks and errors in DNA repair processes. In pioneering trio studies, it is suggested that the majority of DNMs identified in the germline originate from the father, and the mutation rate is associated with the father's age. Furthermore, it has been reported that approximately 80% of identified DNMs originate from the father, and this is associated with the father's age. Limited studies have been conducted to determine the relationship between maternal age and DNMs. Some studies have shown a mild association between maternal age and an increase in DNMs, while others have not supported these findings. However, the limited number of studies on this topic complicates interpretation. Although there are few reports on the impact of the mother on DNM formation, the dominant use of proteins and enzymes from the mother's cytoplasm during early embryonic development, especially during initial cell divisions, suggests that this maternal factor should be considered in the formation of replication errors at these early stages. Therefore, further research is needed to clarify the possible factors and origins of DNM formation.

Methods

In this study, we conducted a comprehensive analysis of DNMs in 69 families undergoing trio clinical exome sequencing (CES) analysis at the Department of Medical Genetics, Selçuk University Faculty of Medicine, between January 2017 and December 2023. Our objectives were to investigate the relationship between parental age at conception and DNMs, characterize the frequency and distribution of DNMs, and explore their potential molecular mechanisms.

We observed a weak correlation between parental age and the number of DNMs, with maternal and paternal ages showing no significant predictive power when combined. Despite recent studies suggesting a paternal age effect, our findings emphasize the need for further research to clarify the impact of maternal age on DNM formation. Our analysis identified 407 *de novo* variants in CES samples, with the majority classified as variants of uncertain significance (VUS). The coexistence of benign (B) and likely benign (LB) mutations with VUS suggests that DNMs may give rise to newly identified variants with no clearly defined significant pathogenic importance according to previous clinical association studies. However, this should not diminish the importance of these mutations in clinical evaluations. Even if categorized at the lowest level of importance, such mutations can contribute to diversifying the clinical presentations or susceptibilities of probands. Therefore, reporting and archiving these mutations should be considered for tracking evidence levels and potential revisions in the near future. Additionally, these mutations may harbor digenic or polygenic profiles and may require further investigation. Indeed, determining the pathogenicity of a variant requires comprehensive evaluation of statistical and functional evidence from the literature, variant databases, and population studies, but these are still insufficient today. While large-scale population studies provide valuable data, the occurrence of variants in these databases should not be solely accepted as benign. Public variant databases may contain outdated or conflicting data, emphasizing the importance of primarily consulting the literature for variant classification. For Mendelian disorders, considering factors such as disease inheritance model, prevalence, and penetrance is crucial when assessing variant frequencies in the general population. Therefore, high allele frequency variants in the general population do not exclude pathogenicity. Although gene databases are valuable resources, they should not be the sole criterion for determining pathogenicity, especially when it comes to DNMs. Accurately assessing the clinical importance of a variant requires comprehensive evaluation of statistical and functional evidence.

Results

In this study, the most common type of mutation observed is 3'UTR variants, constituting 40.54% of mutations. In the 5'UTR region, this rate is 8.84%. Variants in this regions can affect post-transcriptional regulation, mRNA stability, and translational efficiency. Disruptions in UTRs can contribute to irregular gene expression and susceptibility to disease progression or sensitivity. Following 3'UTR variants, missense variants, accounting for 19.4% of identified mutations, occur. These variants can alter protein structure and function, leading to various phenotypic outcomes, and are commonly involved in Mendelian and complex diseases, making them important in disease etiology and therapeutic approaches. The frequency of synonymous variants is 13.5%. Although they do not alter the amino acid sequence, synonymous variants play a regulatory role in gene expression and protein function, affecting mRNA stability, splicing efficiency, and translation kinetics. Splicing site variants, representing 7.86% of identified mutations, represent disruptions in splicing consensus sequences. These variants can lead to abnormal splicing patterns and the production of dysfunctional protein isoforms, playing roles in various diseases, emphasizing their importance in understanding disease mechanisms and developing targeted therapies. Variants causing frameshift mutations have a frequency of 3.19%. These mutations often lead to the formation of early stop codons and subsequently short, non-functional protein products. Therefore, frameshift variants are associated with severe forms of the disease.

FULL TEXTS

Stop loss and gain variants are observed at a frequency of 0.74% each. These variants affect the translation start site, resulting in complete loss of protein length. Therefore, these variants have significantly destructive effects on the respective gene, resulting in increased disease phenotype. DNMs occurring in protein-coding genes are categorized into three classes in the literature, associated with the effects mentioned above: 1) likely gene-disrupting SNVs (stop codon, frameshift, splice donor, and acceptor), 2) missense, and 3) synonymous mutations. The impact of these mutations has been extensively studied in various types of diseases, such as neurodevelopmental disorders (NDDs); LGD and missense mutations are more frequently encountered in patients with NDDs. On the other hand, synonymous mutations, which play a role in regulating gene expression, are associated not only with NDDs but also more broadly with neuropsychiatric disorders.

Genes located on the same chromosome may sometimes share regulatory elements or participate in similar biological pathways due to their proximity. In this study, we observed the frequency of DNMs in genes located on chromosomes 10 and 3, which could potentially represent shared regulation or functional relationships. Chromosome 10 hosts a cluster of genes including vinculin (VCL), which is located at cytoband 10q22.2 and plays a role in cell-cell adhesion and cell-matrix interactions. MTPAP, located at cytoband 10p12.31, is vital for the polyadenylation of mitochondrial RNA transcripts. PTEN, found at cytoband 10q23.31, functions as a tumor suppressor gene regulating cell growth and survival. ANKRD1, located at cytoband 10q23.33, plays a role in muscle function and cardiovascular development. CACNB2, located at cytoband 10p12.33, is involved in calcium channel regulation. Chromosome 3 also harbors a pair of genes including activin A receptor type 2B (ACVR2B) and FYVE and Coiled-Coil Domain Autophagy Adaptor 1 (FYCO1). ACVR2B functions as a receptor for activin and influences various cellular processes, while FYCO1 regulates autophagosome traffic. Interestingly, out of the 31 DNM mutations identified in genes on chromosome 10, 28 (90%) are located in UTR regions (23 in 3'UTR and 5 in 5'UTR), and three are in splicing-related regions. Of these, 25 are insertion/deletion and the remainder are SNVs. The clustered genes on chromosome 3 all contain 3'UTR mutations and 11 mutations related to insertion/deletion mechanisms. These two groups of chromosomes collectively contain 42 DNMs, the majority of which (85.7%) are VUS. Notably, exceptions include MTPAP and CACNB2, which exhibit a high density of B and LB variants. Interestingly, all LB and B variants originate from regions outside of the 3'UTR for these two genes. These findings underscore the importance of 3'UTR regions in determining pathogenicity and the relationship with DNM mechanisms, warranting further investigation. However, without considering the classification of 3'UTR and 5'UTR regions, 203 out of 407 are associated with CNV (<50 bp) and 204 are associated with the SNV mechanism. These findings contradict some findings in other studies indicating the predominance of SNVs (1 bp). However, the limitations of these studies and statistical biases should be considered. Molecular events underlying nucleotide changes in DNA sequences are also defined as transitions and transversions; transitions occur more frequently than transversions and lead to a higher transition/transversion ratio across the genome. Transitions are often attributed to variability in CpG dinucleotides. Methylation of cytosine in CpG dinucleotides produces 5-methylcytosine, which is chemically unstable and prone to deamination, leading to G:T mismatches. CpG dinucleotides exhibit significantly higher mutation susceptibility compared to other dinucleotides. Interestingly, the mutation susceptibility of CpG dinucleotides varies across genomic regions. Contrary to expectation, CpG-rich regions exhibit a lower mutation rate compared to the rest of the genome. This difference is attributed to factors such as lower methylation levels, selective pressures associated with gene regulation, or physical prevention of spontaneous deamination by stronger DNA strand binding. Understanding mutational signatures associated with specific mutational processes is crucial for identifying the underlying mechanisms leading to genetic variations. Mutational signatures characterized by different mutation patterns have been identified in somatic cells, and correlations have been observed between these signatures and DNMs. Mutational signatures representing a significant portion of germline DNMs, signatures 1 and 5, are associated with high rates of C->T transitions in CpG dinucleotides and A->G transitions, respectively. Although the exact mechanisms underlying these signatures are unclear, they likely involve processes such as deamination of methylated cytosine and spontaneous deamination of adenine. The presence of these mutational signatures has potential implications for genetic variations in both somatic and germ cells, necessitating further investigation of these mechanistic foundations. Consequently, our study confirms the predominance of transition mutations over transversion mutations (75.9%: 24.1% of total SNV). Among transitional SNVs G->A and C>T were the highest as expected (G->A: 26.33%, C->T: 23.96%, T->C: 13.17%, A->G: 12.43%, C->A: 5.62%, C->G: 4.59%, G->T: 4.14%, G->C: 3.40%, T->G: 2.37%, A->T: 1.63%, A->C: 1.33%, T->A: 1.04%). G->A and C->T mutations are common genetic alterations observed in cancer genomes, often influenced by environmental factors. G->A mutations can arise from exposure to UV radiation and certain chemical mutagens, such as polycyclic aromatic hydrocarbons (PAHs). UV radiation induces the formation of pyrimidine dimers, particularly thymine dimers, which can lead to G->A mutations in skin cells, contributing to skin cancer development. PAHs, found in tobacco smoke and grilled food, can undergo metabolic activation to form reactive intermediates that bind to DNA, inducing G->A mutations, especially in tumor suppressor genes like TP53. On the other hand, C->T mutations can result from spontaneous deamination of cytosine, accelerated by environmental factors like heat or acidic conditions. Nitrous acid, a chemical mutagen found in air pollution and food preservation, can also directly deaminate cytosine to uracil, contributing to C->T mutations. PAHs can further exacerbate C->T mutations by forming bulky DNA adducts that interfere with DNA replication and repair processes. Additionally, PAHs can inhibit DNA repair enzymes, leading to the accumulation of DNA damage and subsequent mutations, including C->T transitions. CpG island analysis for our patients was also conducted using the UCSC online database, but no correlation was found. Conversely, some mutations, such as those associated with HPS4, DSPP, and PTEN, were found in regions with GC content above 50%. This may contribute to higher mutation in these regions, but the same is not true for other genes with the highest number of DNMs like VCL, which did not exhibit high GC content in the mutation region.

Tandem repeats (TRs) were also examined using the UCSC online database platform. Interestingly, some genes with a high number of DNMs (DNM as CNV<50 bp) were exactly located at TR sites. According to literature, germline mutations in STR repeats are well-documented, with mutation rates estimated for each STR based on data from paternity testing laboratories. These repeats, characterized by repetitive sequences adjacent to each other, are prone to DNA replication errors, slippage, and other mutagenic processes. Consequently, they serve as hotspots for the formation of copy number variations (CNVs) and other genomic rearrangements. Our study's identification of variants within TR sites of specific genes, such as CACNB2, ACVR2B, PTEN, HPS4, ANKRD1, and DSPP, aligns with previous knowledge indicating that TRs are potential sites for CNV (<50 bp) formation. These findings confirm the relationship between TRs and CNVs, as supported by literature reports of variant or off-ladder alleles at Combined DNA Index System STR loci, contributing to allele dropout and small insertion/deletion polymorphisms in the surrounding regions. The mechanisms underlying CNV formation, such as non-allelic homologous recombination and non-recurrent events, as described in the literature, shed light on the structural changes observed in the study. Recurrent CNVs are typically associated with low copy repeats (LCRs), suggesting homologous recombination between repeated sequences. In contrast, non-recurrent CNVs occur at sites of limited homology and may involve complex chromosomal structural changes. However, the known breakpoints in the form of LCRs cannot be the cause of our CNV mutations, as we are discussing DNMs. Therefore, if the hypothesis of short TRs (STRs) is applicable here, the cause of mutation could be the genome instability at the STR flanking sites rather than the LCR breakpoints that typically cause bigger structural changes. Overall, the study's findings, combined with existing literature, highlight the significance of TRs in CNV formation and genomic instability, providing valuable insights into the mutational landscape and mechanisms underlying structural changes in the human genome.

FULL TEXTS

Conclusion

A total of 242 genes were found to have DNM variants. Pathway analysis for these genes was conducted using the Reactome online platform. The obtained analysis was transferred to a CSV file, filtered for those with p value <0.05, and sorted according to the filters defined in the pathway analysis. The signaling transduction pathway (R-HSA-162582) was identified as the most common pathway among the defined genes. Signal transduction is a critical cellular process where external signals cause changes in cell behavior. Transmembrane receptors including receptor tyrosine kinases (RTKs) and transforming growth factor-beta (TGF-beta) receptors perceive these signals and initiate downstream cascades affecting cellular functions such as proliferation and survival. While RTKs activate pathways involving RAF/MAP kinases and AKT, TGF-beta receptors phosphorylate SMAD proteins, regulating gene expression. WNT receptors initially classified as G-protein coupled receptors use beta-catenin to regulate gene transcription. Integrins activated by extracellular matrix components affect cell adhesion and shape through cytosolic kinases. Rho GTPases respond to signals by altering cytoskeleton organization, affecting cell polarity and connections. These mechanisms allow cells to dynamically respond to their environment. Our analysis indicates that our genes primarily contribute to Hedgehog and TGF-beta family members in this pathway. Research suggests that Hedgehog signaling activates a mammalian heterochronic gene regulatory network that controls differentiation timing among cell lineages of different origins. However, it is important to confirm this assumptions with further studies.

FULL TEXTS

[OP-29]

Ovarioleukodystrophy: A Novel Variant in *EIF2B4* Gene

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Introduction

Childhood ataxia with central nervous system hypomyelination/vanishing white matter, also known as leukoencephalopathy with vanishing white matter, is characterized by ataxia, spasticity, variable optic atrophy, and sometimes associated ovarian insufficiency. Genetic diagnosis can be made by identifying pathogenic variants in one of the five genes encoding the five subunits of eukaryotic translation initiation factor 2B (EIF2B1-5). The disease is classified according to the time of onset as prenatal/congenital form, subacute infantile form (<1 year), early childhood onset form (1-4 years), late childhood/juvenile onset form (4-18 years) and adult onset form (>18 years). Disease progression is correlated with the age of onset. Juvenile and adult forms are usually associated with primary or secondary ovarian insufficiency, a syndrome called "ovarioleukodystrophy"; however, ovarian insufficiency can occur in any form, regardless of the age of onset. In this case, we aimed to present variant unknown/uncertain significance (VUS), a VUS detected in the *EIF2B4* gene in the clinical exome sequencing (CES) analysis performed with the next-generation sequencing method after the evaluation and preliminary analysis of a 20-year-old female patient who was referred to us for evaluation in terms of leukodystrophies from the neurology department. The DNA material isolated from the patient's peripheral blood sample was processed using the Roche HyperCap DS CES kit for CES analysis of the targeted regions of 4.133 genes, and the raw data were analyzed using the online Genomize SEQ analysis version 16.7.6, and Ensembl annotation. Each variant was analyzed using databases such as ClinVar, OMIM, HPO, GnomAD, dbSNP, scientific publications, clinical correlation and inheritance pattern. Mutation terminology was used as recommended by the Human Genome Variation Society.

Case Report

A 20-year-old female patient who was followed up in the neurology department due to recurrent headaches and an appearance compatible with leukodystrophy on cranial magnetic resonance imaging (MRI) and who was regularly receiving lidocaine treatment for nerve blockade at intervals was referred to us in terms of leukodystrophies. In the evaluation of the patient, in addition to recurrent headaches, mild ptosis in the left eye and mild impairment in speech fluency were observed, and other neurological examinations were normal. There was no history of seizures. The patient did not have any other diseases or complaints. The results of metabolic and hormonal tests were normal. On cranial MRI, prominent, patchy, butterfly shaped hyperintense signal alterations with a tendency to merge were observed in the periventricular deep white matter areas, particularly in the periatrial region, with preservation of the subcortical U fibers. Echocardiographic imaging revealed a mild mitral regurgitation. *PSAP* single-gene sequence analysis was previously performed with a prediagnosis of metachromatic leukodystrophy, and the results were normal. Both parents were consanguineous and were the first cousins, and no significant familial history was identified in the pedigree. Ptosis was observed in both the male and female siblings. We planned a CES analysis for leukodystrophies in the patient and detected a novel homozygous variant, c.638 C>G (p.Thr213Ser) in the EIF2B4. This variant is known as VUS in the current literature and has not been previously reported in Clinvar. A literature review associated this gene with late-onset premature ovarian failure, and the patient was referred to the gynecology department in this regard. Gynecologic ultrasonography did not show any abnormality, and the anti-Müllerian hormone level in peripheral blood was found to be low (0.7 ng/mL and the patient was presented with the option of oocyte cryopreservation. The segregation analysis of the parents revealed that both parents were heterozygous for this variant.

Discussion

This case highlights the complex interplay between genetic findings and clinical practice in the diagnosis and management of leukodystrophies intertwined with reproductive health problems such as ovarian failure. The identification of a novel EIF2B4 variant in this investigational case of leukodystrophies has drawn attention to several critical points. Leukodystrophies exhibit diverse genetic and phenotypic characteristics. *EIF2B* genes, which are integral to the regulation of cellular stress response, contribute to white matter integrity. Variability within the same family, such as different neurological symptoms between siblings, illustrates the challenges in predicting clinical outcomes from genetic data alone. The early identification of genetic mutations can significantly impact the management and therapeutic strategies for leukodystrophies. For this patient, identification of the EIF2B4 variant led to immediate consultation with gynecologists to address potential reproductive issues, demonstrating the practical implications of genetic insights. The effective management of leukodystrophies requires collaboration across multiple specialties, such as neurology, genetics, gynecology, and radiology, to provide holistic patient care. Reporting of VUS may be necessary if it is clinically relevant, especially in populations with high inbreeding rates. The psychological impact of such information requires comprehensive genetic counseling to help understand these complex scenarios. Further research is essential to clarify the effects of EIF2B4 mutations and to develop targeted interventions. Advances in gene therapy may ultimately offer therapeutic prospects in these progressive conditions. This case highlights the need for a comprehensive clinical evaluation and the benefits of incorporating genetic testing into diagnostic protocols, especially in populations such as Türkiye, where consanguineous marriages are common. The identified EIF2B4 variant provides new insights into the genetic basis of leukodystrophies, enriching existing literature and improving our understanding of these complex disorders.

Keywords: Ovarioleukodystrophy, EIF2B4, VUS, leukodystrophies

FULL TEXTS

[OP-30]

SCN1A-Related Epilepsy Phenotypes with the Same Mutation in A Family

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Introduction

The *SCN1A* (MIM#182389) gene, which encodes the voltage-gated Na+ channel type 1 (Nav1.1) α-subunit, is located on chromosome 2q24.3 (1). *SCN1A* mutations result in epilepsy syndromes ranging from self-limiting epilepsies to developmental and epileptic encephalopathies (2). Additionally, *SCN1A* has been shown to play a role in other diseases, such as hemiplegic migraine and autism spectrum disorders. The most well-known epilepsy phenotype associated with *SCN1A* is Dravet syndrome (DS), an epileptic encephalopathy leading to developmental delay with multiple seizure types, including myoclonic, absence, and atonic seizures (2). The prevalence of DS is estimated at 1.2-6.5 per 100,000 (3). While 90-95% of DS cases result from *de novo* mutations, 5% of cases inherit a mutation from a mildly affected or asymptomatic parent (4).

Here we present a patient diagnosed with generalized epilepsy and whose child was followed up with DS. The same mutation was detected in both of them.

Case Report

A 35-year-old female patient was admitted to our neurology outpatient clinic due to epileptic seizures for 3 years. The patient's seizures consisted of dizziness, nausea, and loss of consciousness that developed after behavioral pauses. The patient reported that drowsiness and amnesia in the post-ictal period could last up to 2 days. The patient had no medical history of chronic disease. Her parents were non-consanguineous, and she and her husband were also non-consanguineous. She had two children, and both of her children died at an early age. The patient's first child was a girl who was followed up in the neonatal intensive care unit for one month due to complications associated with premature birth. She had developmental delays and cognitive retardation since early childhood and was diagnosed with epileptic encephalopathy at the age of 3, then died at the age of 9 due to status epilepticus. The second child, a boy, started having generalized tonic-clonic and myoclonic seizures at the age of 9, accompanied by cognitive impairment. He was diagnosed with DS and died at the age of 13 due to status epilepticus. His genetic test for the *SCN1A* gene revealed a variant of unknown significance (VUS) alteration, c.1889G>A, chr2-166900333 C>T, p. Arg630Gln, NM_001165963.4 rs145670933. Sanger sequencing was performed on both parents and the c.1889G>A variant was detected in the mother, who we describe as our patient.

The patient's mother was evaluated additionally for an epilepsy gene panel using a next-generation sequencing technique. Additionally, NM_006920.6 c.-537G>A missense variant was detected in the 5'UTR of the *SCN1A* gene, which was also classified as VUS. Unfortunately, we could not check this variant because their children were not alive.

The patient's systemic and neurological examination was normal. Blood tests were within normal limits. The patient's cranial magnetic resonance imaging was not an epileptogenic lesion. Due to the patient's long post-ictal period, a cerebrospinal fluid examination was also performed and was found to be normal.

Valproate treatment was started for the patient, and a 4:1 ketogenic diet was added to the treatment.

Discussion

SCN1A mutations are responsible for over 80% of Dravet syndrome (5). Only 5% of DS cases inherit the mutation from a mildly affected or asymptomatic parent (4). In a cohort study conducted in the Turkish population, *SCN1A* variants were evaluated in patients with Generalized epilepsy with febrile seizure plus and DS, and a total of 17 variants were detected in 18 index cases; 7 of these have been reported as new variants. The variants have been detected *de novo* in all DS cases (6). Our case presents as a mildly affected parent of a child with Dravet disease. The c.1889G>A variant was shown on the *SCN1A* gene in both the patient's child and the patient. The variant we detected is among the possible benign variants in the Clinvar database. However, the patient was diagnosed with generalized epilepsy, the patient's child had DS, and the same missense variant was present in both of them. Therefore, the variant needs to be investigated further to be considered benign.

The phenotypes caused by *SCN1A* pathogenic variants are highly variable, ranging from severely affected DS patients to much milder cases of genetic epilepsy febrile seizure plus (7). *SCN1A* mutations have been shown in several studies to be characterized by a wide range of different phenotypes within families, even when they have the same mutation (8-10). Goldberg-Stern et al. (10) studied a large Ashkenazi Jewish family with a novel *SCN1A* mutation. They reported a family with phenotypes including genetic epilepsy without febrile seizures, a wide spectrum of genetic epilepsy with febrile seizures, and DS. They suggested that even in families with familial DS, the same mutation may be found in unaffected carriers (10). In another study, a clinically heterogeneous family with a novel mutation of the *SCN1A* gene was reported. Different clinical phenotypes were identified, including generalized epilepsy with febrile seizures plus, DS, and partial epilepsy with febrile seizures plus (9). Our patient and her deceased child described in this study confirm the findings of the existing literature.

We recommend tracking these variants in patients with a preliminary diagnosis of DS, as they are currently classified as VUS. We also must keep in mind that the co-existence of these variants may be related to patients' seizures.

FULL TEXTS

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FULL TEXTS

[OP-31]

A Rare Case of Muscular Dystrophy: Walker-Warburg Syndrome

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Introduction

Walker-warburg syndrome (WWS) is a rare syndrome associated with muscle, brain and eye abnormalities (1,2). It is one of the most severe types of congenital muscular dystrophy, with most infants dying before the age of three years. *FKTN*, *FKRP*, *LARGE1*, *POMT1*, *POMT2*, *CRPPA* are some of the genes related to WWS (3-8). In this study, we aimed to present the clinical and genetic features of the syndrome by presenting a case of WWS.

Methods

Our patient was a 1.5 years old female patient who was consulted to our clinic from paediatric intensive care unit. The patient whose parents were distant relatives was the third child of the family.

Our patient was hospitalised in the intensive care unit due to sepsis after urinary tract infection and was also followed up with hydrocephalus and a venriculoperitoneal shunt was inserted in the neonatal period. Height: 80 cm (25-50 p), weight: 13.5 kg (90-97 p), head circumference: 52 cm (>97 p). The patient had several seizures and was being fed with percutaneous endoscopic gastrostomy because of feeding and swallowing disorders. The patient was planned to have a tracheostomy and was also receiving nasal oxygen support because of respiratory difficulties.

Results

Bilateral anophthalmia, swallowing dysfunction, seizure history, hypotonia were present. Creatinine kinase (CK) value was measured as 5,225 U/L, ferritin value as 1,601 ng/mL. Echocardiography revealed wide persistent left svc, secundum asd (2 pieces) Partial Abnormal Pulmonary Venous Return was suspected. In the whole abdomen ultrasonography, the vagina appeared dilated and fluid was observed in it, the vaginal dimensions were measured 9x10 mm in width and imperforate himen or haematocolpos prediagnoses were considered. Computed tomography (CT) cardiac-angiography showed situs solitus appearance and the superior vena cava was located on the left.

The left vena cava opened into the right atrium via the superior coronary sinus. Brain CT findings showed severely dilated 3rd and lateral ventricles and 4th ventricle and dysmorphic appearance in the brain stem, cerebellar hemisphere and vermis.

In brain diffusion magnetic resonance examination; Dilatation in the 3rd, 4th and Lateral Ventricles, Hydrocephalus, Ventriculomegaly, Thinning Secondary to Compression in Both Cerebral Hemispheres, Appearances Compatible with Cortical Dysplasia in the Form of Diffuse Cobblestone in the Parenchyma, Diffuse Thinning in the Corpus Callosum, Dilatation and Deformation in the 4th Ventricle in the Posterior fossa in the Intratentorial Sections, Hypoplasia and Dysmorphic Appearance in the Cerebellar Hemispheres and Vermis, Abnormal Foliation in Both Cerebellar Parenchyma. Dilatation and Deformation in the Ventricle, Hypoplasia and Dysmorphic Appearance in the Cerebellar Hemispheres and Vermis, Abnormal Foliation in Both Cerebellar Parenchyma. Lens cannot be selected in both bulbs in the microophthalmic view in both bulbus oculi. Thick septal structure crossing the posterior camera from anterior to posterior in both orbitas, suggesting a preliminary diagnosis of primary persistent hyperplastic vitreous. When the orbital, cerebral and cerebellar findings were evaluated together, congenital muscular dystrophies with cortical malformations presenting in a cobblestone shape came to mind and WWS was considered as the primary diagnosis.

The karyotype analysis of the patient revealed 46,XX. Microarray analysis was planned for the diagnosis. The microarray analysis of our patient revealed homozygous deletion at 7p21.2 (16,212,542-16,287,021)x0. This region contained the *ISPD* (*CRPPA*) gene related to the etiology of the disease.

The incidence of WWS is estimated to be 1/100,000 live births (9). Patients usually have mental retardation, developmental delay, hypotonia, seizures and microphthalmia. WWS is caused by defective glycosylation of α-dystroglycan, which is important for muscles and neuronal migration (10-12). Laboratory investigations usually show high levels of CK. In our case, macrocephaly, frontal bossing, retrognathia, microphthalmia, cataract, optic nerve hypoplasia, hypotonia, cerebellar and brainstem hypoplasia were present.

WWS is one of the most severe forms of congenital muscular dystrophies (13). Bi-allelic loss of function mutations in the *ISPD* gene located on chromosome 7p21 may be the second most common cause of WWS (13). It is a syndrome that should be considered in the differential diagnosis especially in infants with muscle weakness, eye and brain findings. In patients diagnosed with ISPD mutations, most of the individuals were diagnosed in the postnatal period rather than the prenatal period (14-18).

Conclusion

In this syndrome, which is extremely rare and fatal, it is very important to diagnose the disease, to provide genetic counselling to families in terms of prognosis and to evaluate patients in terms of prenatal diagnosis.

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FULL TEXTS

[OP-32]

Evaluation of Nucleolin, Nucleophosmin and Upstream Binding Transcription Factor Gene Expressions in Patients Diagnosed with Lung Cancer

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Introduction

Lung cancer is one of the most common cancers that cause death worldwide (1). Nucleolar organizing regions (NORs) are located on the short arms of human acrocentric chromosomal (chromosomes 13, 14, 15, 21, and 22) regions that contain ribosomal genes with essential roles for protein synthesis and organization of the nucleolus. The NORs related proteins are transcriptionally active or transcribed rDNA sites of non-histon type acidic proteins. Because these proteins have the affinity of silver and selectively bind silver ions, they are named Argyrophilic nucleolar organizing region associated protein (AgNOR) and may be used as crucial biomarkers to obtain knowledge about the activity of the nucleus and therefore the active capacity of the cell (proliferation, metabolic activity, etc.) (2-4). Nucleolin (NCL), Nucleophosmin (NPM1) and Upstream Binding Transcription Factor (UBTF) are major AgNOR proteins. The expression Levels of *NCL*, *NPM1* and *UBTF* were studied in human hair loss (5) and wound healing (6). The *NCL* and *NPM1* serum levels was studied in Non-small cell lung (7). But to our knowledge no studies about the evaluation together expression levels of *NCL*, *NPM1* and *UBTF* in cases with lung cancer. Therefore we performed the current study.

Methods

Totaly 80 patients (20 control, 30 pre-treatment patient group and post-treatment patient group) were included in the current studies. The study was approved by Düzce University Local Ethics Committee.

RNA Isolation and cDNA Synthesis

Then RNA was isolated from the Peripheral blood samples of patients via QIAamp RNA Blood Mini Kit (Catalog no. 52304) according to the manufacturer's instructions. Using the QuantiTect Reverse Transcription cDNA Synthesis Kit (Catalog No: 205310), cDNA was obtained from isolated RNA.

Relative Gene Expressions of *NCL*, *NPM1* and *UBTF* Gene by Real-Time qPCR

The expression levels of *NCL, NPM, UBTF* and the reference gene (*ACTB*) were detected via the QuantiNova™ SYBR® Green PCR for each cDNA sample of the patients. The QuantiNova SYBR Green PCR Kit (Catolog No: 208052) was used for the PCR. *ACTB* transcript was used as a reference for quantitation of mRNA expressions and normalized according to the control group. Calculation of fold change had been calculated via processing ΔΔCt values as $2^{-\Delta\Delta Ct}$.

The data were analyzed via the Statistical Package for Social Sciences (IBM Corp., Armonk, NY, USA) for Windows 23.0. The Shapiro-Wilk test was used for the detection of data distribution. Because the data were not normally distributed ($p<0.05$), non-parametric tests were used for statistical analysis. The $p<0.05$ was accepted as statistically significant.

Results

Totaly 50 patients were included in the current studies. The study groups were included control, pre-treatment patient groups and post-treatment patient groups. When the cancer subgroups to be considered, 14 (46.7%) patients with adenocarcinoma, 15 (50%) patients with squamous cell carcinoma and 1 (3.3%) patients with large cell carcinoma.

The differences among the groups (control, pre-treatment and post-treatment patient groups) were statistically significant for both *UBTF* and *NPM1* expression levels. Statistically significant differences were detected between pre-treatment and post-treatment patient groups for WBC, neutrophil, leucocyte, hemoglobin, MWC, RDW and PLT ($p>0.05$). Also the differences between pre-treatment and post-treatment patient groups for the expression levels of the *NCL*, *NPM1* and *UBTF* were significant ($p<0.05$).

There are various studies about the AgNOR in different diseases such as discrimination of benign from malign thyroid tissues (2-4,8,9), Xeroderma Pigmentosum Group E (10), testicular torsion (11), different doses of acute and chronic CO poisoning in brain (12,13), coronary artery diseases (14-16), clinical exacerbation of chronic obstructive pulmonary disease (17), comparison of FNAB and paraffin embedded tissue sections (18), renal ischemia/reperfusion (I/R) injury (19), hair root cells of humans (20,21), buccal epithelial cells of healthy individuals (22), Down syndrome (23,24), diagnostic marker for detection of the most reliable dose of rhamnetin (25), curcumin (26), and capsaicin (27), for detection of tissues damage caused by CO intoxication in the literature (28-32), oncocytology (33,34).

NCL, *NPM1* and *UBTF* are major AgNOR proteins. *NCL* and *NPM1* are found mostly in the nucleolus but also they are seen in the nucleoplasm and cytoplasm (35,36). *NPM1* has role as a suppressor and promotor of carcinogenesis (37). upregulating proliferation, transformation and invasion of cancer cells (38). As a pro-oncogenic protein, *NCL* promotes proliferation and blocks apoptosis (39). Altered *NCL* and *NPM1* expression has been found in many diseases including cancer (40,41). *UBTF* plays a critical role in ribosomal RNA transcription, chromatin remodeling and pre-rRNA processing (42). According to the our results, statistically significant differences were detected between pre-treatment and post-treatment patient groups for WBC, neutrophil, leucocyte, hemoglobin, MWC, RDW and PLT ($p>0.05$). Additionally, the differences beween pre-treatment and post-treatment patient groups fort he expression levels of the *NCL*, *NPM1* and *UBTF* were significant ($p<0.05$).

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Conclusion

Determining the expression levels of NCL, NPM1 and UBTF may provide information on the discrimination of benign and malignant lesions, prognosis of the disease and treatment strategy.

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FULL TEXTS

[OP-33]

Identification of Genes Associated with Autism Spectrum Disorder with Bioinformatics Tools

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Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by limited interests, repetitive behaviors, and difficulties in social interaction and communication. Recent data has shown that ASD affects 1 in 36 children (1). Although ASD is believed to arise from a combination of environmental, biological, and genetic factors, the causal mechanisms of ASD have not been fully elucidated. Early intensive intervention programs initiated after diagnosis are among the limited intervention methods available for ASD treatment. Despite the recognized importance of early diagnosis, a meta-analysis published in 2021 indicated that the global average age of ASD diagnosis is 60.48 months (2). Current data underscores the importance of developing more objective screening and diagnostic tools for early detection and intervention of ASD. However, there is currently no biological marker or genetic signature available for ASD screening and early diagnosis. In our study, aiming to address this need, we sought to identify genes that illuminate the etiopathogenesis of ASD and have the potential to serve as biomarkers. For this purpose, we utilized the dataset from a study with the accession number GSE42133, which investigates the relationship between gene expression obtained from blood samples in male children with ASD and total brain volume.

Methods

The GSE42133 microarray dataset was downloaded from the Gene Expression Omnibus database. The study with the code GSE42133 explored the relationship between gene expression obtained from blood samples and total brain volume in 91 male children with ASD and 56 controls. Co-expression module analysis and module activity analysis were performed using the microarray data from autism and control groups in this dataset with WebCEMITool (3). The microarray dataset had been previously processed and transformed using $\log_2$ for analysis. The parameters used in the co-expression module analysis were as follows: Beta value= 6, correlation method= pearson, Dissimilarity threshold value= 0.8, phi= 0.855, r2= 0.906, gene filter= yes, merge similar modules= yes, minimum gene in module= 20.

Results

Thirteen modules were identified in the co-expression module analysis. In the module activity analysis, it was observed that module-3 (M3) exhibited significant positive activity in the ASD group, while module-5 (M5) showed significant negative activity. The hub genes in the positive active module were *CTDSPL*, *GP9*, *TUBB1*, *PDE5A*, and *SH3BGRL2*, whereas the hub genes in the negative active module were *CPM*, *CCDC198*, *MIGA1*, *LRRN4CL*, and *TNFSF15*.

In our study, we aimed to elucidate the etiopathogenesis of ASD and identify hub genes that may serve as biomarkers. Co-expression module analysis was applied through WebCEMITool using the microarray dataset of the *GSE42133* to create an associated gene network. We found that hub genes within modules M3 and M5 were associated with ASD. Module M3, which exhibited the highest significant positive activity with ASD, contained hub genes *CTDSPL*, *GP9*, *TUBB1*, *PDE5A*, and *SH3BGRL2*, while module M5, which exhibited the highest significant negative activity, contained hub genes *CPM*, *CCDC198*, *MIGA1*, *LRRN4CL*, and *TNFSF15*. Review of the literature suggests that among the hub genes identified in our study, *TUBB1*, *PDE5A*, and *MIGA1* genes may be associated with ASD and neurodevelopmental disorders.

While there is no direct evidence linking the *TUBB1* gene, a member of the tubulin gene family, to autism, there is limited evidence associating it with another neurodevelopmental disorder, intellectual disability. A single case report in the literature has linked a mutation in the *TUBB1* gene with intellectual disability in conjunction with a DCX mutation causing pachygyria. Although the DCX mutation was reported as a major pathogen for pachygyria, the TUBB1 variant was only observed in patients with severe intellectual disability (4). Due to the limited number of reported cases, the pathogenicity of TUBB1 remains uncertain (5).

Phosphodiesterase 5A (PDE5A) is abundant in cerebellar Purkinje neurons. Based on the association of cerebellar Purkinje cells with the pathogenesis of autism, it has been suggested that the application of a PDE5A inhibitor in mice resulted in reduced social deficits, and cyclic guanosine monophosphate-specific PDE5A inhibitors could potentially serve as therapeutic targets for correcting impaired social behavior (6).

The *MIGA1* gene encodes mitoguardin 1 protein, which is located on the outer membrane of mitochondria and regulates mitochondrial fusion. Mitoguardin has been reported to play a significant role in maintaining neuronal homeostasis (7). Additionally, the literature highlights the potential role of mitochondrial dysfunction in the etiology of autism. Various studies have investigated mitochondrial dysfunction in relation to autism, including mitochondrial metabolism, DNA deletions and variations, mitochondrial morphology, and nuclear gene expression (8). Our study draws attention to the relationship between ASD and the *MIGA1* gene, in addition to existing data. Future studies elucidating the relationship between mitochondrial dysfunction and autism may lead to the development of predictive biomarkers and treatments targeting specific metabolic abnormalities.

Conclusion

This study suggests that *TUBB1*, *PDE5A*, and *MIGA1* genes may be potential biomarkers for ASD and have the potential to illuminate the pathogenesis of ASD. Further studies investigating the relationship between ASD and these genes may pave the way for early diagnosis, early intervention, and the development of new treatment options for ASD.

Keywords: Autism, co-expression modular analysis, MIGA1

FULL TEXTS

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FULL TEXTS

[P-19]

Diagnostic Importance of MLPA: The Case of Tuberous Sclerosis

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Introduction

Tuberous sclerosis complex (TSC) is a highly variable disease that can involve multiple organs. Clinical manifestations of TSC include seizures, autism spectrum and cognitive impairment, along with various solid tumors. TSC is inherited in an autosomal dominant manner and studies have shown that the genes involved in its pathogenesis are *TSC1* and *TSC2*. Genetic diagnosis of the disease can be made using a pathogenic variant in the *TSC1-TSC2* gene in approximately 97% of cases, whereas copy number variation has been detected in approximately 3% of patients. In this case, a 17-month-old Syrian male patient referred to our clinic with a prediagnosis of TSC was initially diagnosed using Cauda Equina syndrome (CES) analysis followed by Multiplex ligation-dependent probe amplification (MLPA) analysis.

Methods

We performed CES analysis of the DNA material isolated from the patient's peripheral blood sample using the Roche HyperCap DS CES kit and the targeted regions of 4133 genes, and the analysis was performed with online Genomize SEQ analysis version 16.7.6 and Ensembl annotation. For MLPA analysis, DNA samples isolated from the patient's peripheral blood were analyzed using the SALSA MLPA Probemix P046 TSC2 kit and the Coffalyser program.

Case Report

A 17-month-old Syrian boy was referred to our clinic with a prediagnosis of tuberous sclerosis and suspected giant cell astrocytoma. It was observed that he had seizures at 5 months postnatally, epileptiform anomaly was observed on electroencephalogram and nodules were observed in both lateral ventricle frontal horns on brain magnetic resonance imaging. In addition, cardiac echocardiography revealed a rhabdomyoma in the left ventricle and urinary system ultrasonography revealed an angiomyolipoma in the right kidney. Physical examination revealed no dermatologic findings except hypomelanotic macules. The vision and audition were normal. The parents were distant relatives and he had a 3.5-year-old healthy sister. CES and *TSC2* MLPA analyses were planned for prediagnosis of TSC.

Results

No clinically compatible variants were detected in the CES analysis. *TSC2* MLPA analysis revealed a heterozygous deletion in exons 11-16 of TSC2. In the segregation analysis, no deletion/duplication was observed in the parents. No significant history was found in pedigree analysis.

Conclusion

Genetic diagnosis of patients with TSC is often performed using sequence analysis. However, in less than 3% of patients, the diagnosis can be made using MLPA. In this study, we aimed to demonstrate the importance of MLPA in patients who could not be diagnosed using sequence analysis.

Keywords: Tuberous sclerosis, *TSC1*, *TSC2*, MLPA

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FULL TEXTS

[P-20]

An Autosomal Recessive Cerebellar Ataxia: A Case Report

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Introduction

Although the most common causes of hereditary ataxia are nucleotide repeat disorders, there are other mechanisms too. One group is caused by sequence changes which some are autosomal recessively inherited. Ataxia with oculomotor apraxia is one of the examples. There are several types of it, the most common of which are types 1, 2, and 4 (1). Autosomal recessive Spinocerebellar ataxia with axonal neuropathy-2 is a neurodegenerative disorder characterized by juvenile onset of progressive cerebellar ataxia which is often the first symptom, axonal sensorimotor peripheral neuropathy, and increased serum alpha-fetoprotein (AFP). Oculomotor apraxia is a common but inconsistent finding, found in about 50% of patients (2). It is estimated to occur in 1 in 900,000 individuals worldwide.

It results from variants in *SETX* gene, encoding senataxin protein, a DNA/RNA helicase localized in nucleus which is implicated in DNA break repair (3).

We report a young male with progressive ataxia phenotype with a positive family history.

Case Report

A 21-year-old male patient who had been suffering from progressive speech disorder and walking difficulty for 4 years was referred to our clinic. He did not have any history of an illness, medicine or substance use. His parents were consanguineous. He had a cousin with similar complaints who was also from a consangineus couple. In the neurological examination, an ataxic gait, romberg sign, dysmetria, dysdiadochokinesia, dysarthria in speech and difficulty in swallowing were observed. His vision, hearing and intelligence were normal. He was clinically diagnosed with Spinocerebellar ataxia. A brain magnetic resonance imaging (MRI) was ordered.

Brain MRI revealed cerebellar atrophy and spinal MRI was normal.

In the next step, a clinical exome analysis done and showed a homozygous single base insertion (c.7147dupG p.Asp2383fs) in *SETX* gene that resulted in a frameshift in the code. The variant was not defined in the Clinvar database and not found in GnomAD exomes and genomes. As loss of function predicted, it was evaluated as likely pathogenic according to PVS1 and PM2 ACMG rules. There was no other clinically relevant variant and the parents were tested heterozygous for it. He is diagnosed with Spinocerebellar ataxia, autosomal recessive, with axonal neuropathy 2 (MIM 606002) SCAN2.

The cousin with the similar phenotype was later tested homozygous for the variant.

Discussion

SCAN2 is mostly an adolescent onset autosomal recessive disorder, that manifests as progressive cerebellar ataxia associated with peripheral neuropathy, cerebellar atrophy, occasional oculomotor apraxia, pyramidal signs, head tremor, dystonia, strabismus, chorea. In the case we presented peripheral neuropathy and oculomotor apraxia were not prominent.

Diagnosis of SCAN2 is based on clinical features, progressive evolution to motor handicap, absence of extra-neurologic findings and family history. Laboratory findings show an elevated AFP serum level. Electromyography findings may reveal axonal sensory-motor neuropathy. Oculographic recordings may demonstrate OMA. Cerebral magnetic resonance imagery displays cerebellar atrophy. Diagnosis is confirmed by molecular analysis.

No specific treatment exists and management is mainly supportive. Most patients will become wheelchair bound at a mean age of 29.9 years.

A family history should be taken with attention to relatives with manifestations of hereditary ataxia. Consanguinity may suggest autosomal recessive inheritance. Findings in the family may assist in narrowing the scope of relevant hereditary ataxias and a prompt molecular diagnosis.

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FULL TEXTS

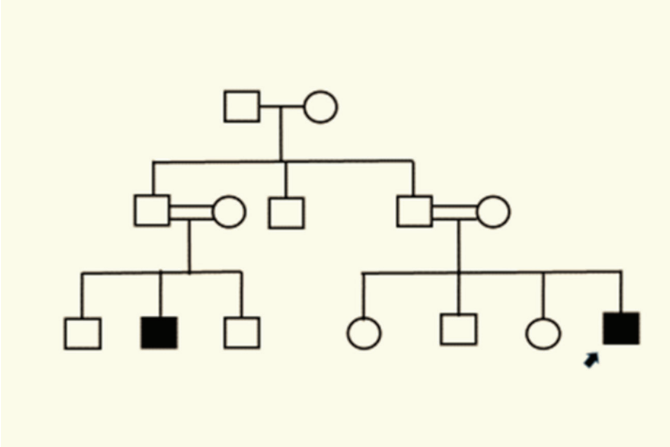


Figure 1. Affected members of the family are seen on the pedigree

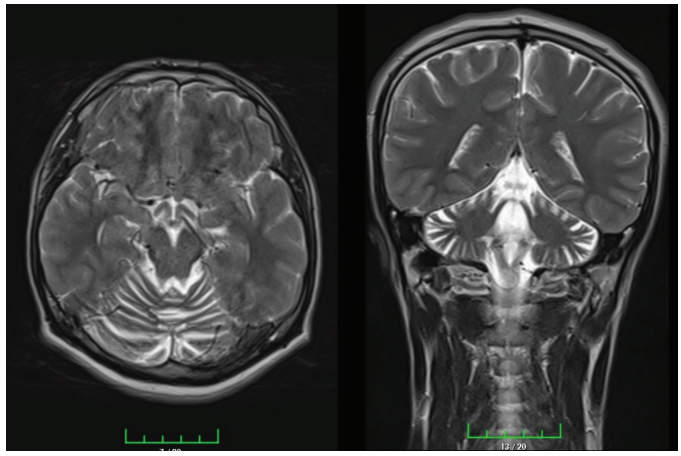


Figure 2. Magnetic resonance imaging of the brain shows widening of the cerebellar folia due to cerebellum cortex atrophy

FULL TEXTS

[PA-21]

A Case Diagnosed Familial Hypercholesterolemia with A Pathogenic c.1678A>T (p.Ile560Phe) rs1131692213 Variant in *LDLR* Gene

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Introduction

Familial hypercholesterolemia (FH) is a common autosomal dominant forms of genetic disorder related with elevated low-density lipoprotein (LDL) and cholesterol. The pathogenic variations in the *LDLR*, *Apo-B100* and *PCSK9* genes roles in the etiopathogenesis of the disease (1). The development of next generation sequencing (NGS) technologies has contributed significantly to the diagnosis of different diseases at an earlier stage and to a better understanding of their etiopathogenesis. (2-8). In the current study, we aimed to contribute to the literature to present a case with a pathogenic c.1678A>T (p.Ile560Phe) rs1131692213 variant in *LDLR* gene.

Case Report

A 31-year-old male patient was applied to the hospital with complaints of headache. The albumin, alkaline phosphatase, alanine aminotransferase, amylase, aspartate aminotransferase, total bilirubin), phosphorus, gamma glutamyl transferase, fasting blood sugar, Ca, Cl, creatinin, lipase, Mg, K, Na, urea, bun, uric acid, high-density lipoprotein, triglyceride, vitamin B12, UIBC, TIBC, phosphorus, thyroglobulin, anti TPO ab*, sedimentation, TSH, Free T4), Free T3, folate, hemoglobin, white blood cell, platelet, lymphocyte, monocyte and neutrophil values of the case were normal range. Our case has high LDL (131.6 mg/dL), cholesterol (233.60 mg/d) levels and low 25-hydroxyvitamin D (20.9) levels. So the *PCSK9* gene and *LDLR* gene were sequenced with NGS. While *PCSK9* gene sequence analysis was normal, pathogenic heterozygous class 1 c.1678A>T (p.Ile560Phe) rs1131692213 variant was detected in Exon 11 of *LDLR* gene, and a diagnosis of FH was made due to the high LDL (131.6 mg/dL) and cholesterol (233.60 mg/d) levels. The entire exome dataset including Genome Aggregation Database, conservation status, predictions of pathogenicity based on the American College of Medical Genetics and Genomics recommendations were given in Table 1.

Discussion

Because the limeted number of studies on variation of *LDLR*, *Apo-B100* and *PCSK9* genes roled in the etiology of the disease, the very heterogeneous most common variation with different distribution in Türkiye are not known (9). In the studies conducted around the world showed that the *LDLR* gene mutation is common. Additionally the pathogenic variation related with the FH were detected on the *Apo-B100* gene and *PCSK9* genes (1-10).

In our case with high levels of LDL (131.6 mg/dL) and cholesterol (233.60 mg/d). While *PCSK9* gene sequence analysis was normal, pathogenic heterozygous class 1 c.1678A>T (p.Ile560Phe) rs1131692213 variant was detected in exon 11 of *LDLR* gene. We think that it will be important to screen the relevant genes with NGS and conduct population-based studies in order to identify the frequency and type of variation in genes related to FH (*LDLR*, *Apo-B100* and *PCSK9* genes) in our country.

Keywords: Familial hypercholesterolemia, *LDLR* gene, *PCSK9* gene, *Apo-B100* gene, NGS

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FULL TEXTS

Table 1. The entire exome dataset including gnomAD, conservation status, predictions of pathogenicity based on ACMG recommendations

Gene	LDLR
Variation ID	NM_430782
dbSNP	rs1131692213
Transcript ID	NM_000527.5
Variant	c.1678A>T (p.Ile560Phe)
Variant location	Exon 11
Variant type	Missense
MutationTaster	Uncertain
PROVEAN	Uncertain
MutPred	Pathogenic
SIFT	Pathogenic
FATHMM-MKL	Pathogenic
gnomAD (exomes)	f=0.000000685
ClinVar	Likely pathogenic, pathogenic
Conservation	Conserved
Conservation score	9,206
ACMG classification	Pathogenic
ACMG pathogenicity criteria	PM1, PM2, PP4 (Moderate), PS4 (Moderate)

ACMG: American College of Medical Genetics and Genomics, gnomAD: Genome Aggregation Database