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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 ($p<0.05$).

FULL TEXTS

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

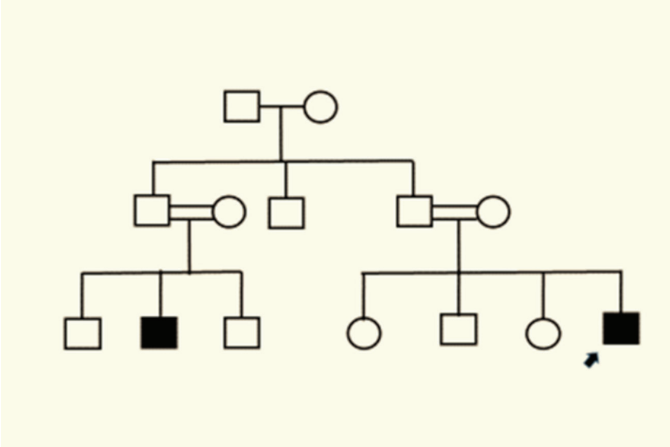


Figure 1. Affected members of the family are seen on the pedigree

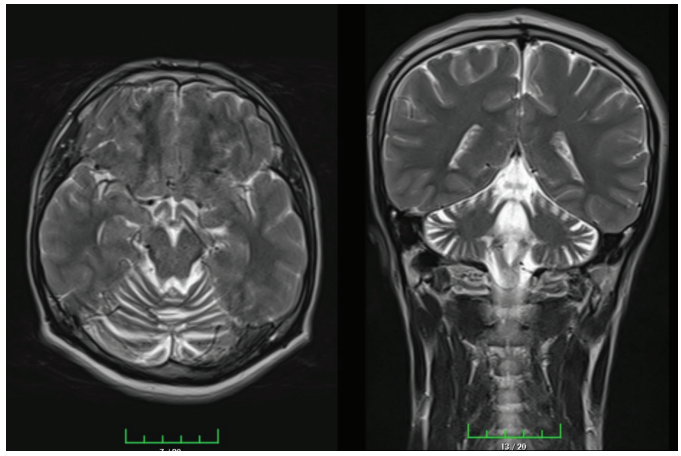


Figure 2. Magnetic resonance imaging of the brain shows widening of the cerebellar folia due to cerebellum cortex atrophy

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