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RESEARCH ARTICLE
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IDENTIFICATION AND BIOINFORMATICS ANALYSIS OF TWO NOVEL VARIANTS IN THE *SEMA4A* AND *SCP2* GENES IN A PATIENT WITH EARLY-ONSET VISUAL IMPAIRMENT AND LEUKODYSTROPHY.

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Abstract
Optic Nerve Atrophy (ONA) is the destruction of the nerve fibers of the optic nerve, reducing the number of retinal ganglion cells that generate nerve impulses and transmit them from the eye to the brain. In this case, the information arriving in the brain is distorted, which is accompanied by deterioration of vision and can lead to irreversible blindness.
We conducted a genetic analysis of a six-year-old male patient with vision problems, cognitive impairment and leukodystrophy. DNA samples were collected from the patient and his mother and father. Whole-genome sequencing (WES) and PCR-Sanger sequencing were used to identify genetic variants. Two novel mutation variants were detected in the patient: a frameshift mutation in the SEMA4A gene (NM_001193300.2):c.2272_2273insT and a substitution in the SCP2 gene (NM_002979.5):c.712A>T. These mutations were confirmed in the parents and were not detected in the control group, which consists of 400 healthy people from the same ethnic group. In addition, such variants were absent from other published genetic databases, including 1000 Genomes, ExAc, gnomAD, and Iranome. Extensive bioinformatics analysis, the absence of these mutations in healthy human controls, and the early onset of symptoms confirm the pathogenicity of these variants.

Keywords: SEMA4A gene, SCP2 gene, Whole Exome Sequencing, (NM_001193300.2):c.2272-2273insT, (NM_002979.5):c.712A>T.

INTRODUCTION

• Optic atrophy (ONA) is the thinning and destruction of optic nerve fibers and a decrease in the number of retinal ganglion cells that generate nerve impulses and transmit them from the eye to the brain [1]. The following genetic factors (diseases) may contribute to optic atrophy: mitochondrial disorder (Leber's optic atrophy), OPA1-related optic atrophy , Inherited optic atrophy (recessive) (mutations in several genes including OPA3, TMEM126A, and RTN4IP1), Wolfram syndrome (mutations in the WFS1 gene), and leukoencephalopathy with dystonia and motor neuropathy (mutations in the SCP2 gene) [2, 3]. The incidence and prevalence of ONA can vary depending on

the specific underlying causes of the disease and the population studied [4]. It is important to note that ONA is not an independent disease, but rather a consequence of other pathologic processes that may lead to optic nerve degeneration. Given the wide range of underlying causes of the disease, it is difficult to provide accurate data on the incidence and prevalence rates of ONA in general [5, 6].

One disorder in which ONA is observed is leukoencephalopathy with dystonia and motor neuropathy (L-DMN), also known childhood ataxia with hypomyelination of the central nervous system . The disease is an extremely rare genetic and difficult to diagnose condition, and as such, accurate data on the incidence and prevalence of L-DMN in the general population is limited. However, the disease is known to occur more frequently in childhood. L-DMN is characterized by a range of neurological manifestations, and symptoms and clinical presentation may vary from person to person. However, some common features of L-DMN include dystonia, motor neuropathy, ataxia, cognitive impairment, seizures, spasticity, and optic atrophy [7, 8].

In this report, we describe two new pathogenic mutations in a patient with ONA and L-DMN. The variants are in a homozygous state: (NM_001193300.2):c.2272_2273insT, located at exon 16 of the SEMA4A gene, and (NM_002979.5):c.712A>T, located at exon 9 of the SCP2 gene. The first variant is a frameshift mutation resulting in the replacement of threonine with isoleucine at position 758 and an early stop codon. The second variant results in the replacement of serine at position 238 with cysteine. Testing 400 healthy people from the same ethnicity did not show the presence of these variants among the healthy individuals.

MATERIAL & METHOD

A 6-year-old boy from the Iranian –Turkish- Azeri population was referred for genetic analysis due to autosomal recessive early-onset severe visual impairment, seizures, and leukodystrophy. Whole-exome sequencing (WES) analysis was performed to determine

the causative gene(s). The patient had a history of bilateral optic atrophy, but no retinal degeneration at this age. Leukodystrophy was observed in the patient without neuropathy or dystonia, although it is more likely that neuropathy and dystonia may develop later in life. The patient was born to parents who were in an incestuous marriage. From the patient's parents, after consent was obtained, their blood samples and the patient's blood sample were collected using EDTA tubes. Next, a DNA sample was isolated and received WES according to previously discussed methods (9). The obtained variants, including single nucleotide variants (SNVs) and insertions or deletions (indels), were analyzed using the Wannovar web tool (10). Variant filtering pipelines based on ACMG and Sherloc guidelines were employed to identify disease-causing variants. Only rare variants (MAF > 0.05) in dbSNP, 1000G, ExAc, Iranome and gnomAD were included for subsequent analysis. Exonic variants and those impacting protein structure/function were prioritized for mutation assessment, while deep intronic, upstream, downstream, and highly similar variants were excluded.

To validate the detected genetic mutations in the patient and his unaffected parents, Sanger sequencing was conducted utilizing an ABI 3500 genetic analyzer (Applied Biosystems, Foster City, CA, United States). Primers were specifically crafted targeting the relevant genomic regions, and PCR, followed by Sanger sequencing, was executed on all DNA specimens.

RESULTS

Patient was a member of a family with consanguineous marriages from the Iranian-Azeri-Turkish ethnic group. He was the only sick child of his parents and there were no other people in the family with the same medical condition. The subject was diagnosed early-onset severe visual impairment accompanied by Leukoencephalopathy with dystonia and motor neuropathy (L-DMN).

Whole-exome sequencing (WES) analysis identified two novel variants in a homozygous state in two different genes: *SEMA4A* and *SCP2*. In the *SEMA4A* gene, an insertion of T at position c.2272 (NM_001193300.2) in exon 16 was detected (Figure 1). In the *SCP2* gene, a transition of A to T at position c.712 (NM_002979.5) in exon 9 was found (Figure 2). The variant in the *SCP2* gene resulted

in the alteration of the amino acid serine at position 238 with cysteine. This substitution led to a structural change in the encoded protein (Figure 3).

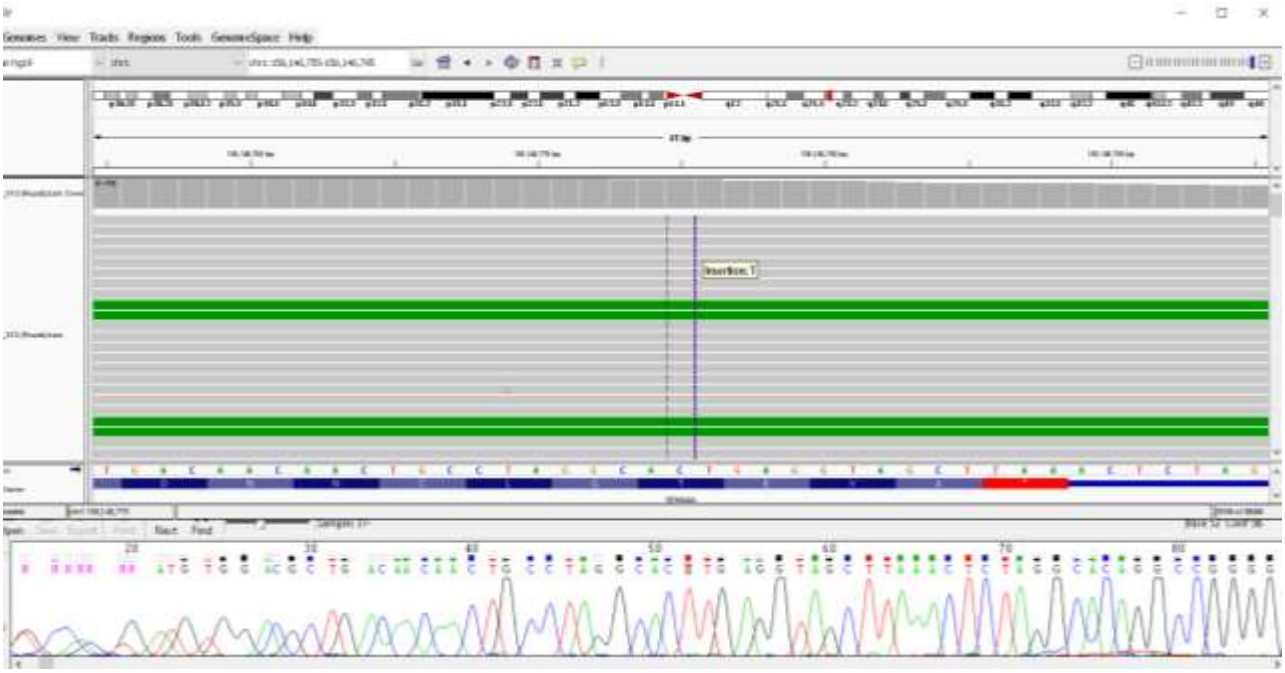

Figure 1: WES results in the proband (upper graph) shows the homozygous mutation c.2272_2273insT, located at exon 16 of the *SEMA4A* gene. Sanger sequencing result in the patient (lower graph) confirms the finding of WES results.

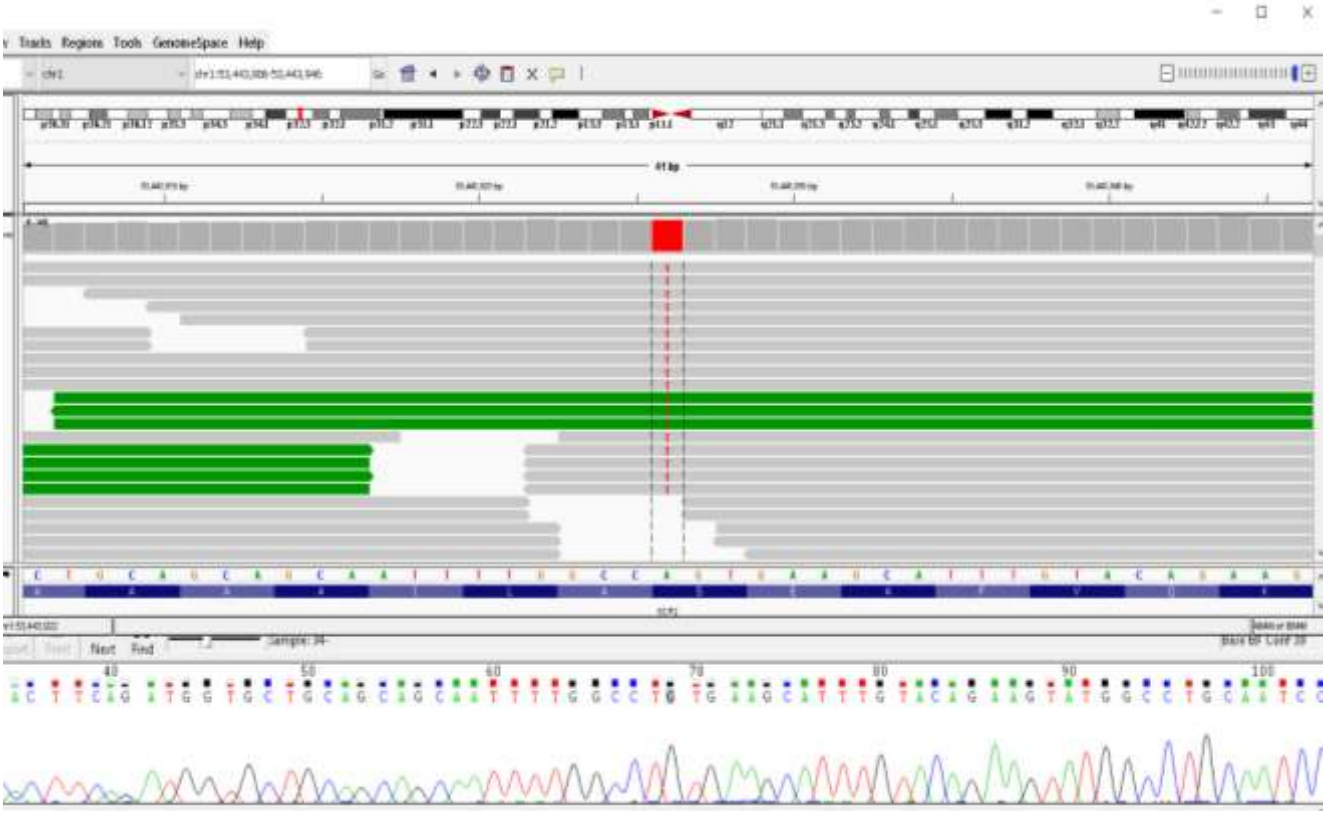

Figure 2: WES results in the proband (upper graph) shows the homozygous mutation c.712A>T, located at exon 9 of the SCP2 gene. Sanger sequencing result in the patient (lower graph) confirms the finding of WES results.

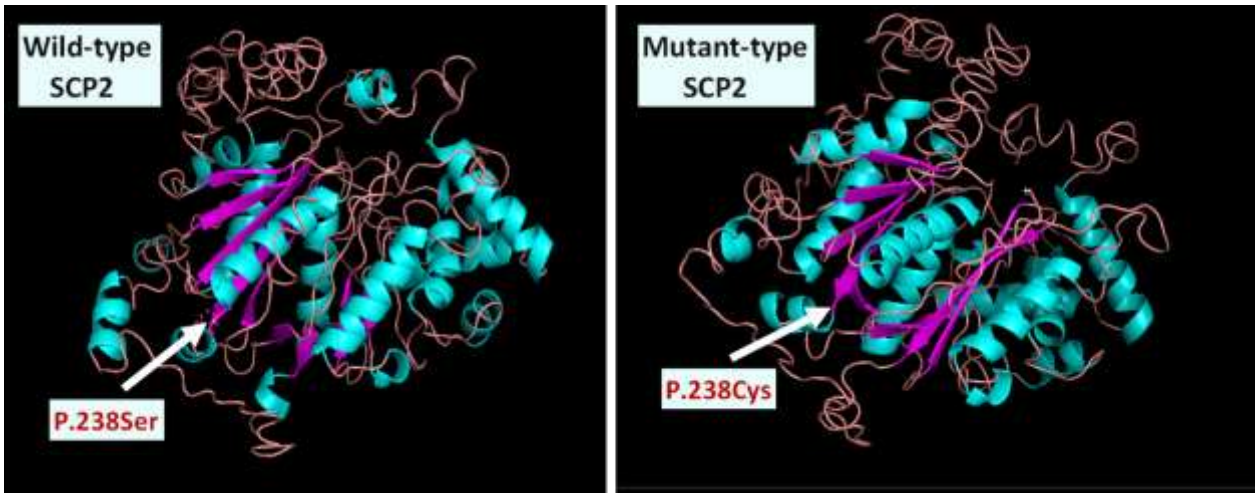

Figure3: The variant in the SCP2 gene resulted in the replacement of the amino acid serine at position 238 with cysteine. We also analyses the 3D structure of wild-type and mutant-type proteins using I-TASSER web tools. Sarine is a polar amino acid, while cysteine is a nonpolar amino acid.

Converting a polar amino acid to a nonpolar one can have consequences such as changes in protein's three-dimensional structure, alterations in solubility, and modifications in molecular interactions.

The second variant, located in the SEMA4A gene, caused an early stop codon at position p.758, resulting in the deletion of three terminal amino acids (Glu-Val-Ala) in the encoded protein. These amino acids are present in the cytoplasm of the protein , specifically in the topological domain (Figure 4). This domain spans amino acids 705 to 761, comprising 57 amino acids in total.

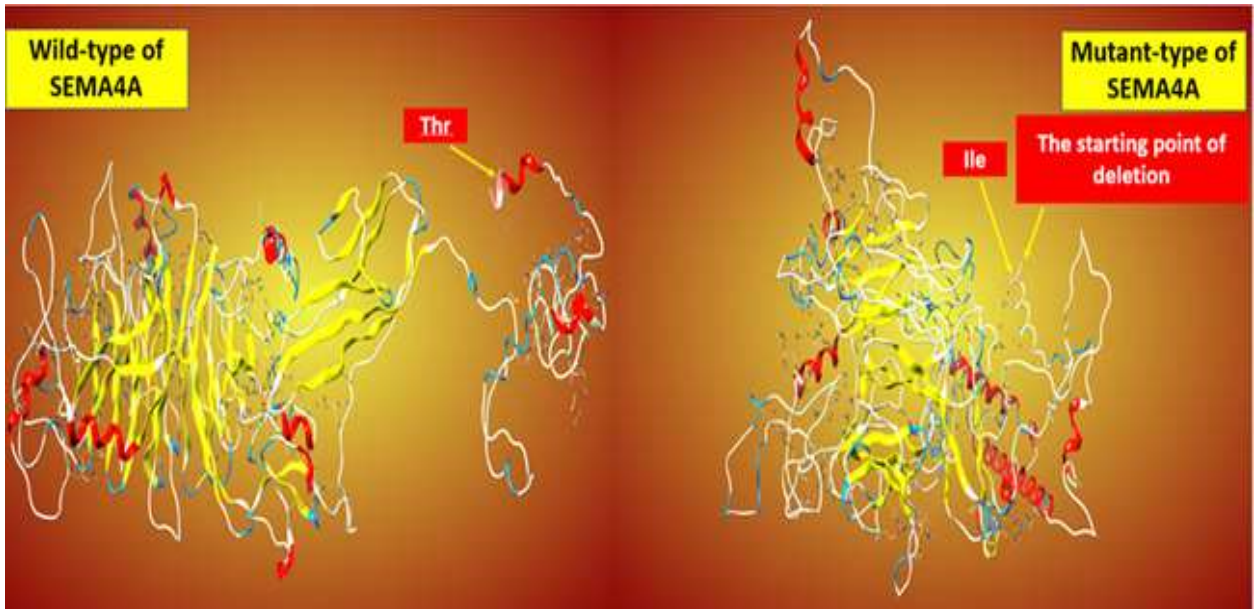

Figure 4: This variant resulted in the replacement of threonine with isoleucine at position 758, followed by an early stop codon, resulting in the deletion of three terminal amino acids (Glu-Val-Ala) in the encoded protein. These amino acids are located in the cytoplasmic region of the protein, specifically in the topological domain.

WES-identified pathogenic variants were confirmed in both parents, who were found to be heterozygous carriers. (Figures 1 & 2). Protein analysis using the I-Tasser web-based software was performed To determine how the variants affect the secondary structure of the mutant proteins. (Figures 3 & 4).

To determine the frequency of these variants in the population, 400 healthy individuals from the Iranian Azeri Turkish ethnic group were screened. None of the individuals in the cohort carried these variants. Furthermore, these variants were not replicated in any of the available databases, including GenomAD and the 1000 Genomes Project, despite the fact that these databases cover the relevant loci. These findings provide additional evidence supporting the pathogenicity of the identified variants.

DISCUSSION

Here we're talking about two new mutations in the *SEMA4A* gene (NM_0011933 00.2:c.2272_2273insT) and the *SCP2* gene (NM_002979.5:c.712A>T) with autosomal recessive segregation patterns in a case originating from the Azeri -Turkish, Iranian ethnicity . This patient had early-onset leukoencephalopathy with dystonia and motor neuropathy (L-DMN) accompanied by severe bilateral optic nerve atrophy (OPA). Clinical examination of the patient did not reveal any signs of retinitis pigmentosa (RP) until the age of six, and there was no development of dystonia or neuropathy by that age.

The first novel variant resulted in the replacement of threonine with isoleucine at position 758, followed by an early stop codon. The patient's parents were carriers of this variant. A population study conducted on a cohort of 400 persons from the same ethnic group did not have this variant. among healthy individuals.

The *SEMA4A* gene encodes a protein consisting of 760 amino acids and belongs to the Semaphorin family of transmembrane proteins (11, 12). SEMA4A is a protein that helps T cells and APCs communicate with each other in the immune system. (13, 14). It is highly expressed in both the sclera and retina (5).

Animal studies have demonstrated that knockout animals for this gene exhibit severe retinal degeneration in mice (4). Abdi et al. (1) screened 190 unrelated patients with various eye disorders, including RP, Leber congenital amaurosis (LCA) and cone-rod dystrophy, for the *SEMA4A* gene and identified 2 pathogenic mutations: p.F350C and p.D345H. They also identified another variant, p.R713Q, and claimed that this variant segregated with a dominant inheritance pattern. However, further studies performed on knock-in mice (3) did not confirm its pathogenicity.

Only two other studies have reported pathogenic variants in the *SEMA4A* gene: p.Arg658Trp and p.Asn135Asn (in heterozygous states) (5,6) in patients with high myopia and/or RP.

The second novel variant identified in our study, leaded to the replacement of serine with cysteine at position 238. Two independently controlled promoters within the SCP2 gene give rise to different proteins: SCPx, a peroxisomal thiolase involved in branched-chain fatty acid breakdown, and SCP2, likely an intracellular lipid transport protein. [15].

Mutations in the *SCP2* gene can have various consequences depending on the specific mutation and its impact on the protein structure and function. Some mutations can lead to decreased function of the SCP2 protein, disrupting normal lipid transport within cells. This can affect lipid metabolism and cellular processes, leading to abnormalities in the absorption, transport, and storage of lipids in the body. In addition, mutations that alter the structure of SCP2 protein may impair its function, namely, its ability to interact with other molecules, disrupt its normal folding, stability, or localization in cells [16].

SCP2 mutations can also cause metabolic disorders characterized by abnormalities in lipid metabolism. Peroxisomal disorders or lipid storage diseases can lead to the accumulation of fatty acids or cholesterol in tissues. In addition, SCP2 mutations may have implications for the central nervous system. Disorders of lipid metabolism can affect the development and maintenance of the central nervous system, potentially leading to neurological abnormalities, cognitive impairment, or developmental delay [17].

It is important to note that the specific effects of SCP2 gene mutations can vary depending on the nature and location of the mutation, as well as individual genetic and environmental conditions. To

fully understand the impact of specific SCP2 gene mutations on SCP2 gene function and associated phenotypes, comprehensive clinical evaluation and further observations are required.

Observing developmental delays, cognitive impairments, and leukodystrophy in our case could be attributed to the novel variant (NM_002979.5:c.712A>T) observed in a homozygous state in the SCP2 gene. The other phenotype, ONA, observed in the patient, could be due to the variant observed in the SEMA4A gene (NM_001193300.2:c.2272_2273insT). However, there is a possibility that this clinical manifestation (ONA) is also due to the mutation that occurred in the *SCP2* gene, and the phenotypic consequence of the *SEMA4A* gene mutation (such as RP) may emerge in the later stages of life.

Mutations were absent in the genomes of 400 healthy individuals of the ethnic group. In addition, none of these variants were found in any of the available databases, including the 1000 Genomes and GenomAD Project. All the provided information strongly suggests the potential pathogenicity of the identified variants. However, to confirm these results, further validation would be required through functional studies using knock-in animal models specific to each variant.

CONCLUSION

The reported variant (NM_002979.5:c.712A>T) in The SCP2 gene is absent from population data. ,and bioinformatics analyses indicate its deleterious nature. Furthermore, the presence of early-onset leukodystrophy and cognitive deficiency in the case further strengthens the evidence of the variant's pathogenicity. To confirm the pathogenicity of the second variant (NM_001193300.2: c.2272_2273insT) observed in our case, it is necessary to monitor the patient's clinical manifestations, such as retinitis pigmentosa (RP), in later stages of life.

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