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Novel mutation in PLP1 gene in an individual with Pelizaeus-Merzbacher disease (PMD) using whole exome sequencing

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A B S T R A C T

Background: Pelizaeus-Merzbacher disease (PMD) is a rare X-linked genetic disorder affecting the central nervous system. This disease is associated with abnormalities of the white matter of the brain and spinal cord. It is a subgroup of leukodystrophies caused by abnormalities in one or more components (mainly fat or protein) that contribute to the formation of the white matter (myelin sheath) of the brain. Point mutations account for the remaining 20% of cases and are associated with highly variable phenotypes. These cases can range from mild to severe congenital clinical forms. Case Presentation: A family with one affected person studied in Baghdad, Iraq. The causative gene identified by exome sequencing. Exome sequencing results showed that a c.772A > C; p. Met258Leu mutation exists in the affected son of this family. To confirm the presence of the pathogenic PLP1 mutation, we performed direct Sanger sequencing on the patient and his mother. He was a hemizygous for this mutation while his mother was heterozygous. Conclusion: Our findings broaden the range of pathogenic mutations in PLP1 associated with the PMD disease, which is essential for the disease's genetic diagnosis and screening. This finding supports earlier studies indicating a connection between mutations in the PLP1 gene and PMD. Our research can assist in offering proper genetic counseling to the families impacted and helps enhance our understanding of how the PLP1 gene functions in PMD.

Introduction

Pelizaeus-Merzbacher disease, or PMD, is a disorder that causes loss of myelin in the central nervous system and is classified among the hypomyelination leukodystrophies (Koeppen and Robitaille 2002). The prevalence of PMD in the United States is estimated at 1 in 200,000 to 500,000 men. Although the clinical onset and characteristics of PMD are extremely variable across genotypes, the majority of individuals experience cognitive impairment, motor symptoms such as hypotonia that progresses to spastic paraplegia, ataxia, and dystonia, as well as nystagmus (Elitt and Tesar 2024). Respiratory problems caused by the patient's motor impairments can lead to death as early as infancy. The most severe form of PMD is congenital PMD, which results in death by the second decade of life, while classical PMD is relatively milder, resulting in death between the third and seventh decades of life (Osório and Goldman 2018; Hobson and Garbern 2012).

PLP1 gene on the X chromosome encodes the most abundant myelin protein (PLP). Point mutations in

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the PLP1 gene occur at low frequency in the population and usually result in Pelizaeus-Merzbacher disease (PMD) or spastic paralysis 2 (SPG2) in males. At the same time, female carriers are usually asymptomatic (Alghamdi et al. 2025). Numerous mutations in the proteolipid protein 1 (PLP1) gene, lead to symptoms ranging from severe demyelination in infancy to delayed demyelination and axonal degeneration in adulthood, which are linked to demyelinating diseases, such as Pelizaeus-Merzbacher disease (PMD) and hereditary spastic paralysis (SPG)(Alghamdi et al. 2025).

The introduction of next-generation sequencing (NGS) in clinical settings, notably targeted sequencing panels and whole-exome sequencing (WES), has improved the diagnostic yield of inherited neurological illnesses that have a high degree of genetic diversity and a low mutational burden (Gonzaga-Jauregui et al. 2015; van De Warrenburg et al. 2016; Vanderver et al. 2016). The analysis of NGS data continues to be difficult, particularly when choosing and interpreting variants, which is crucial for solitary exomes or in cases where family cosegregation/linkage investigations cannot be conducted (Schlüter et al. 2022). After filtering by frequency below 1% and deleteriousness, WES genotypes produce between 500 and 1,000 variants per individual. As a result, setting up a priority system based on the patient's phenotype (Köhler et al. 2017; Boudellioua et al. 2019) or gene interaction networks (Yang, Robinson, and Wang 2015; Cornish, David, and Sternberg 2018) may be helpful in speeding up the selection of potential variants.

To find the gene responsible for PMD in an Iraqi family, we conducted whole-exome sequencing (WES), given the unmet diagnostic need among PMD patients and the possibility of next-generation sequencing (NGS) to provide insight into these problems.In this study, a family with a child with a movement disorder in Baghdad, Iraq, was studied.

Case Presentation

Clinical parameters, including age of onset, previous history, family history, clinical symptoms, and laboratory signs, were extracted from the patient's medical records. After collecting this information and biochemical tests, the family completed a consent form. The affected individual in the pedigree is a 9-year-old Iraqi boy who has a healthy sister and brother. He had a history of two strokes with seizures at the ages of 9 and 11 months. After that, he developed a motor disorder, and motor weakness was observed. The affected boy was a late walker in childhood and currently has poor vision. The marriage of the affected boy's parents is consanguineous, and his parents and his siblings appear to be healthy and have no specific symptoms.

We only conducted WES on the proband. First, 10 cc of blood was collected from all family members in falcon tubes containing 0.5% EDTA anticoagulant for DNA extraction and molecular testing. The collected samples were stored in a -20 freezer until the experiment. In this study, the Blood DNA Extraction Mini kit (Dynabio, Takapozist, Iran, and Tehran) was used for DNA extraction, and the quality and quantity of extracted DNA were evaluated. The electrophoresis technique was used for qualitative analysis, and the Nanodrop device was used for quantitative analysis. As stated by the manufacturer's procedures, the Illumina HiSeq 4000 instrument (San Diego, CA, USA) was used to sequence the patient's DNA regions that had been captured using the SureSelect Human All Exome Kit V6 (Agilent Technologies Inc., Santa Clara, CA, USA). The typical read depth exceeded 100 ×, with 98.0% of the intended genomic sequence reaching a depth of 20 × or higher. The method of performing exome sequencing is as follows: first, genomic DNA is randomly fragmented, and then adapters are attached to the resulting library. The library is then enriched for exonic sequences, and in the next step, biotinylated DNA fragments are hybridized, followed by sequencing using massively parallel sequencing, and finally, the resulting data is analyzed using relevant software (Sherman et al. 2021). Prioritization of candidate genes is performed in several steps. In the first step, variants with low allelic frequency (less than 1%) were filtered in the 1000 Genomes databases https://www.internationalgenome.org/ and the ExAC database. In the second step, those genes or positions are checked in reliable databases such as OMIM (Online Mendelian Inheritance in Man). Then, the symptoms of that gene are examined with the symptoms of PMD, and unrelated genes are eliminated at this stage. In the third stage, based on the type of variant, we predict the effect of the

desired variant on the protein product of the gene and the phenotype using In Silico programs such as Polyphen2 and Mutation Taster.

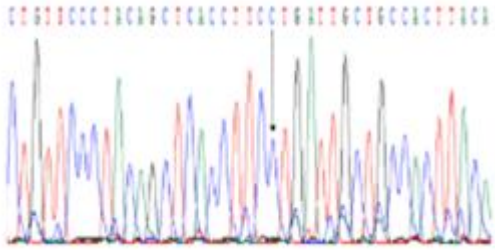
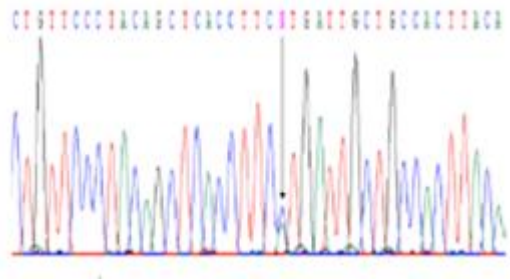


Fig. 1- Result of PLP1 gene sequencing in a patient carrying a hemizygous mutation.



Fi. 2- Result of PLP1 gene sequencing in the patient's mother who is heterozygous for the identified mutation.

A new missense mutation was found in the PLP1 gene (c.772A > C; p.Met258Leu), suggesting a link to PMD. This mutation suggests a change in the way codons are translated, resulting in the replacement of methionine with leucine in the amino acid sequence. This mutation is classified as likely pathogenic according to American College of Medical Genetics and Genomics (ACMG) guidelines. Software tools in bioinformatics, including Mutation Taster suggested that this mutation could be deleterious and lead to disease. To confirm the presence of the pathogenic PLP1 mutation, we performed direct Sanger sequencing on the patient and his mother. We created a specific pair of primers (the forward primer: CAGAGATGTCTCAGGGACTGC and the reverse primer: GCATTTTCCATTCAGGGACTGTG) to amplify the mutated area in the genome using the PCR method. To perform PCR, 8 µL of Master Mix was added to 1 µL of each of the Forward and Reverse primers, 3 µL of extracted DNA, and 12 µL of sterile distilled water. PCR was then performed by the following protocol: five minutes at 95°C, followed by 30 cycles at 95°C for 1 min, 62°C for 45 seconds, and 72°C for 1 min, and finally, for the final extension, 10 minutes at 72°C. Following the amplification of the PLP1 sequences, we conducted direct sequencing of the PCR products using an automated genetic analyzer (Applied Biosystems 3130xl; USA), with the results displayed in Fig. 1 and 2. We used the NCBI website (http://www.ncbi.nlm.nih.gov/blast) to blast the sequences and compare them against normal sequences. Interestingly, this finding has not been reported in the other patients.

Discussion

In this research, we discuss a family that has a birth-related movement issue. Whole exome sequencing (WES) helped us reach a clear diagnosis by detecting a new missense mutation in the PLP1 gene, which indicates Pelizaeus-Merzbacher disease (PMD). Due to the wide range of clinical severity in PMD and the absence of genotype-phenotype correlation in many cases, it is essential to have an accurate molecular diagnosis of PMD because it is not possible to forecast the phenotype of a patient with a given genotype based only on clinical features (Duan et al. 2022).

The PLP1 gene, which is located on chromosome Xq22. 2, is the cause of PMD. This gene, which encodes the myelin protein proteolipid protein 1 (PLP1), is made up of seven exons. Toxic gain-of-function mutations and loss-of-function mutations in PLP1 are thought to be the underlying pathophysiologic mechanisms of PMD. In animal models of PMD or human cell lines, a variety of downstream pathophysiologic processes have been demonstrated, including misfolding of PLP/DM20, aberrant endoplasmic reticulum stress responses, alterations in the iron metabolism and susceptibility of oligodendrocytes, and/or disruption of the regular oligomerization and myelin compaction of PLP/DM20 (Ruskamo et al. 2022). The severity of the condition in PMD is thought to be caused by the degree of resultant oligodendrocyte dysfunction or cell death from these several processes, with the most pathological, harmful gain-of-function mutations causing congenital PMD. BoespflugTanguy and coworkers suggested a five-grade classification of clinical phenotypes of PMD based on the patient's best motor attainment (forms 0 to 4); the most severe condition is form 0 (congenital PMD), while milder cases (classic PMD) are classified as

forms 2 and 3. Patients with congenital illnesses experience a range of symptoms, including low motor and cognitive abilities and an early onset of the disease. The majority of these patients also exhibit neurological symptoms like dystonia and seizures. The patients in the classic group gain more skills but exhibit signs of hypotonia. Other motor and cognitive impairments appear as the condition worsens, even though nystagmus lessens (Duan et al. 2022).

The most common genetic defect in this group of patients is a duplication in PLP1, which results in overexpression of PLP1 but with a normal structure. Point mutations and small rearrangements of PLP1 account for approximately 15–20% of all PMD-causing alleles and can occur throughout the gene. Another group has null mutations in PLP1. These patients, which are also rare, both develop clinical symptoms later and experience a milder phenotype of PMD and usually live to be 40–60 years of age compared with those with point mutations or duplications (Sarmadi et al. 2020; Cambi et al. 1996). In general, various mutations correlate to different phenotypes. Since duplications of PLP1 often result in form 1 and form 2, the phenotype of point mutations in PLP1 can range from the most severe (form 0) to the mildest (form 4). The phenotype of deletion and nonsense/frameshift mutations, which make up a null mutation, is always mild to severe, falling between forms 3 and 4 (Duan et al. 2022).

In this study, we identified a point mutation (c. 772A>C; p. Met 258 Leu) in a 9-year-old boy with PMD in an Iraqi family. This is the first report of c.772A>C in PMD. The identified mutation was X-linked. This mutation is novel in this disease. Although the sample size in this family was small, the mutations matched well with affected or carrier members in the pedigree. We believe that the identified mutation could be responsible for PMD for the following reasons. This mutation results in reduced protein stability due to the substitution of an evolutionarily conserved amino acid. In general, such mutations are thought to result in the retention of the mutant PLP/DM20 protein in the ER. The main pathogenesis of PMD is protein misfolding and retention, which causes oligodendrocyte apoptosis (Sherman et al. 2021). Ultimately, the amino acid changes caused by the mutation interfere with the regular folding and build-up of PLP1 in the ER, which blocks the movement of PLP1 from the ER to the Golgi complex. The buildup of these improperly folded proteins increases ER stress-induced folding, which must then be removed to preserve cellular homeostasis (Duan et al. 2022).

As mentioned above, patients who are congenitally affected by PMD have poor motor or cognitive skills. They have a young age of onset and often have dystonia, seizures, and other neurological symptoms. The patient in this study also has these symptoms. As mentioned, he showed symptoms of the disease as a newborn, had two strokes with seizures at 9 and 11 months of age, and was also late to walk in infancy. Given that the mutation identified in this affected boy is a point mutation, and the complications of point mutations, including complete loss of function or gain of function, can be a factor in the underlying pathophysiological mechanisms in PMD, the identified mutation is completely consistent and related to the patient's phenotype.

In this respect, Takashi Shiihara and colleagues described a 5-year-old boy who had been diagnosed with a less severe form of PMD that was brought about by a novel mutation in exon 3 of the PLP1 gene, namely c. 300delC (p. I100IfsX13). Although he had spasticity in his lower limbs, he was able to walk with assistance at the age of 19 months after having experienced growth retardation from the age of a few months. Brain MRI at 12 months of age revealed hypomyelination and motor nerve conduction tests revealed a drop in velocity and amplitude. This patient was discovered to have a single nucleotide deletion that causes a frameshift and early termination of PLP1, which is the cause of the illness (Shiihara et al. 2015). Furthermore, in 2018, Jaber Lyahyai and his team conducted a complete exome sequencing (WES) analysis on several members of a big family. This family was diagnosed with Pelizaeus-Merzbacher disease due to the discovery of a new pathogenic missense mutation in the PLP1 gene, c. 251C > A (p. Ala84Asp) (Lyahyai et al. 2018). The results of the present study are consistent with these two studies in some ways and different in others. Among the similar results is that in all of these studies, the individuals with PMD were born with a congenital form, had problems with the lower limbs, or could walk with delay and assistance. However, there is a very clear difference between the study and previous studies, and that is that the individual studied had poor vision. This symptom

distinguishes the patient in the present study from other affected patients in similar studies and can be attributed to the specific mutation identified in this individual (c. 772A>C; p. Met 258 Leu) in the PLP1 gene. In other words, one of the unique phenotypes caused by the identified mutation is a disorder in the visual system, which is classified as a nervous system disorder.

The affected boy in this study has two unaffected siblings. This observation in the family pedigree is fully consistent with the X-linked inheritance of this mutation. As mentioned, the boy's mother was heterozygous for the identified mutation. Thus, the mother's mutated allele was transmitted to the affected boy and the mother's normal allele to his unaffected siblings. However, due to existing limitations, including the small size of the studied family and the lack of functional validation of the software, databases, and analyses used in this study, further and complementary studies are needed in this field to prove this hypothesis.

Conclusions

We have effectively used whole exome sequencing (WES) in an Iraqi family to examine mutations in genes linked to PMD. During this process, we discovered a new substitution mutation in the PLP1 gene. This finding supports earlier studies indicating a connection between mutations in the PLP1 gene and PMD. Our research can assist in offering proper genetic counseling to the families impacted and help enhance our understanding of how the PLP1 gene functions in PMD.

Declarations

Ethics approval and consent to participate

All procedures performed in this study were by the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or compare ethical strands.

Consent for publication

Written informed consent was obtained from the family for this publication.

Competing interest

The authors declare that they have no conflict of interest.

References

Alghamdi, Malak, Essa Alharbi, Salman Aljarallah, Ghaida Alghamdi, Reham M Balahmar, Nisserin Jado, Hebattalah Hamed, Dima Jamjoom, Fahad A Bashiri, and Naif AM Almontashiri. 2025. 'A copy number variant overlapping the 3′UTR of PLP1 causes spastic paraplegia', *Journal of Human Genetics*: 1-6.

Boudellioua, Imane, Maxat Kulmanov, Paul N Schofield, Georgios V Gkoutos, and Robert Hoehndorf. 2019. 'DeepPVP: phenotype-based prioritization of causative variants using deep learning', *BMC bioinformatics*, 20: 1-8.

Cambi, F, X-M Tang, P Cordray, PR Fain, LD Keppen, and DF Barker. 1996. 'Refined genetic mapping and proteolipid protein mutation analysis in X-linked pure hereditary spastic paraplegia', *Neurology*, 46: 1112-17.

Cornish, Alex J, Alessia David, and Michael JE Sternberg. 2018. 'PhenoRank: reducing study bias in gene prioritization through simulation', *Bioinformatics*, 34: 2087-95.

Duan, Ruoyu, Haoran Ji, Huifang Yan, Junyu Wang, Yu Zhang, Qian Zhang, Dongxiao Li, Binbin Cao, Qiang Gu, and Ye Wu. 2022. 'Genotype–phenotype correlation and natural history analyses in a Chinese cohort with pelizaeus–merzbacher disease', *Orphanet Journal of Rare Diseases*, 17: 137.

Elitt, Matthew S, and Paul J Tesar. 2024. 'Pelizaeus–Merzbacher disease: on the cusp of myelin medicine', *Trends in Molecular Medicine*.

Gonzaga-Jauregui, Claudia, Tamar Harel, Tomasz Gambin, Maria Kousi, Laurie B Griffin, Ludmila Francescatto, Burcak Ozes, Ender Karaca, Shalini N Jhangiani, and Matthew N Bainbridge. 2015. 'Exome sequence analysis suggests that genetic burden contributes to phenotypic variability and complex neuropathy', *Cell reports*, 12: 1169-83.

Hobson, Grace M, and James Y Garbern. 2012. "Pelizaeus-Merzbacher disease, Pelizaeus-Merzbacher-like disease 1, and related hypomyelinating disorders." In *Seminars in neurology*, 062-67. Thieme Medical Publishers.

Koeppen, Arnulf H, and Yves Robitaille. 2002. 'Pelizaeus-merzbacher disease', *Journal of Neuropathology & Experimental Neurology*, 61: 747-59.

Köhler, Sebastian, Nicole A Vasilevsky, Mark Engelstad, Erin Foster, Julie McMurry, Ségolène Aymé, Gareth Baynam, Susan M Bello, Cornelius F Boerkoel, and Kym M Boycott. 2017. 'The human phenotype ontology in 2017', *Nucleic acids research*, 45: D865-D76.

Lyahyai, Jaber, Bouchra Ouled Amar Bencheikh, Siham C Elalaoui, Maria Mansouri, Lamia Boualla, Alexandre DIonne-Laporte, Dan Spiegelman, Patrick A Dion, Patrick Cossette, and Guy A Rouleau. 2018. 'Exome sequencing reveals a novel PLP1 mutation in a Moroccan family with connatal Pelizaeus-Merzbacher disease: a case report', *BMC pediatrics*, 18: 1-4.

Osório, M Joana, and Steven A Goldman. 2018. 'Neurogenetics of Pelizaeus–Merzbacher disease', *Handbook of clinical neurology*, 148: 701-22.

Sarmadi, Akram, Aliasgar Mohammadi, Fatemeh Tabatabaei, Zahra Nouri, Morteza Hashemzadeh Chaleshtori, and Mohammad Amin Tabatabaiefar. 2020. 'Molecular genetic study in a cohort of Iranian families suspected to maturity-onset diabetes of the young, reveals a recurrent mutation and a high-risk variant in the CEL gene', *Advanced biomedical research*, 9: 25.

Schlüter, Agatha, Agustí Rodríguez-Palmero, Edgard Verdura, Valentina Vélez-Santamaría, Montserrat Ruiz, Stéphane Fourcade, Laura Planas-Serra, Juan José Martínez, Cristina Guilera, and Marisa Girós. 2022. 'Diagnosis of genetic white matter disorders by singleton whole-exome and genome sequencing using interactome-driven prioritization', *Neurology*, 98: e912-e23.

Sherman, Larry S, Weiping Su, Amanda L Johnson, Samuel M Peterson, Cassandra Cullin, Tiffany Lavinder, Betsy Ferguson, and Anne D Lewis. 2021. 'A novel non-human primate model of Pelizaeus-Merzbacher disease', *Neurobiology of disease*, 158: 105465.

Shiihara, Takashi, Mio Watanabe, Kengo Moriyama, Mitsugu Uematsu, and Kiyoko Sameshima. 2015. 'A novel PLP1 frameshift mutation causing a milder form of Pelizaeus–Merzbacher disease', *Brain and Development*, 37: 455-58.

van De Warrenburg, Bart P, Meyke I Schouten, Susanne T de Bot, Sascha Vermeer, Rowdy Meijer, Maartje Pennings, Christian Gilissen, Michèl AAP Willemsen, Hans Scheffer, and Erik-Jan Kamsteeg. 2016. 'Clinical exome sequencing for cerebellar ataxia and spastic paraplegia uncovers novel gene–disease associations and unanticipated rare disorders', *European Journal of Human Genetics*, 24: 1460-66.

Vanderver, Adeline, Cas Simons, Guy Helman, Joanna Crawford, Nicole I Wolf, Genevieve Bernard, Amy Pizzino, Johanna L Schmidt, Asako Takanohashi, and David Miller. 2016. 'Whole exome sequencing in patients with white matter abnormalities', *Annals of neurology*, 79: 1031-37.

Yang, Hui, Peter N Robinson, and Kai Wang. 2015. 'Phenolyzer: phenotype-based prioritization of candidate genes for human diseases', *Nature methods*, 12: 841-43.