International Journal of Medical Laboratory 2025;12(1): - .

International Journal of Medical Laboratory
Shahid Sadoughi University of Medical Sciences

Case Report

A Novel *PEX3* Gene Mutation in a Patient with Zellweger Syndrome

Seyed Ali Madani Manshadi [1] MS.c., Mojtaba Movahedinia [2] M.D., Seyed Mehdi Kalantar [3] Ph.D., Negin Yazdian [1] MS.c., Maliheh Hashemi Nejad [4] Ph.D., Mahya Ebrahimi Nasab [1] MS.c., Ehsan Zare Mehrjardi [5,6*] Ph.D.

[1] Yazd Science and Technology Park, Yazd, Iran
[2] Children Growth Disorder Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3] Research and Clinical Center for Infertility, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[4] Hasti Medical Genetic Laboratory, Yazd, Iran
[5] Department of Biology, Ashk.C., Islamic Azad University, Ashkezar, Iran
[6] Medical Biotechnology Research Center, Ashk.C., Islamic Azad University, Ashkezar, Iran

Article history
Received: 23 Aug 2025
Accepted: 6 Dec 2025
Available online: 23 Dec 2025

Keywords
Bioinformatics analyses
NGS
PEX gene
Zellweger syndrome

A B S T R A C T

Introduction: Zellweger syndrome (ZS) is a severe peroxisome biogenesis disorder characterized by a deficiency in peroxisomal function due to mutations in various *PEX* genes.

Case Report: This report describes a 15-year-old male patient diagnosed with ZS, who was found to carry a homozygous variant in the *PEX3* gene, specifically p.Glu266Lys. Genetic analysis was performed using next-generation sequencing to identify the mutation, which was subsequently confirmed by Sanger sequencing. The clinical presentation of the patient included developmental delay, hypotonia, and characteristic imaging findings associated with ZS. Bioinformatics analyses were conducted to assess the functional impact of the p.Glu266Lys mutation.

Results: Predictions from various tools indicated that the variant is likely deleterious: SIFT predicted it to be deleterious, PolyPhen-2 classified it as probably damaging, and MutationTaster indicated that it is disease-causing. Structural analyses revealed altered hydrogen bonding and electrostatic interactions that may impair binding with PEX19, a crucial partner for peroxisome biogenesis. Stability predictions showed that the mutation decreases protein stability ($\Delta\Delta G = +2.5$ kcal/mol), suggesting a destabilizing effect on the PEX3 protein.

Conclusion: This case highlights the significance of *PEX3* mutations in the pathogenesis of Zellweger syndrome and underscores the utility of next-generation sequencing combined with Sanger sequencing in uncovering genetic variants that contribute to this disorder. Further investigation into the functional consequences of the p.Glu266Lys variant may provide insights into potential therapeutic strategies and enhance our understanding of the molecular mechanisms underlying peroxisome biogenesis disorders.

***Corresponding Author:** Department of Biology, Ashk.C., Islamic Azad University, Ashkezar, Iran, and Medical Biotechnology Research Center, Ashk.C., Islamic Azad University, Ashkezar, Iran. **Tel:** +9832725951, **Email:** Ehsan_zare98@yahoo.com

Introduction

Zellweger syndrome (ZS) is one of the most severe forms of peroxisome biogenesis disorders, which are characterized by defects in peroxisome assembly and function due to mutations in genes known as *PEX* genes [1, 2]. These disorders lead to an accumulation of very-long-chain fatty acids and other metabolites that are normally oxidized within peroxisomes, resulting in significant clinical manifestations affecting multiple organ systems, particularly the liver, brain, and kidneys [3, 4]. The clinical presentation of ZS includes developmental delays, hypotonia, seizures, liver dysfunction, and characteristic craniofacial dysmorphisms. The prognosis for patients with ZS is poor, with many individuals not surviving beyond infancy or early childhood [5]. Genetic analysis plays a crucial role in confirming diagnoses and understanding the underlying mechanisms of this disorder [6].

Case Presentation

The patient is a 15-year-old male born to first-cousin parents who presented for evaluation due to developmental delays noted since infancy. He was born at term via normal vaginal delivery to consanguineous parents. His birth weight was within normal limits; however, he exhibited significant hypotonia from birth (Fig. 1).

Clinical findings

Upon examination at our institution, the following clinical features were noted:

•**Developmental delay**: The patient has not achieved major developmental milestones appropriate for his age. He is non-verbal and exhibits limited social interaction.

•**Hypotonia**: Marked hypotonia has persisted since infancy; he requires assistance for mobility.

•**Feeding difficulties**: The patient has experienced chronic feeding difficulties leading to recurrent aspiration pneumonia.

•**Craniofacial features**: Distinctive facial features include a high forehead, wide-set eyes (hypertelorism), and a flat nasal bridge.

Neurological examination: Neurological assessment revealed poor muscle tone and reflexes consistent with central nervous system involvement.

Genetic analysis

Given the clinical suspicion of ZS based on physical examination and imaging findings, genetic testing was initiated. Blood samples were collected for DNA extraction, followed by next-generation sequencing (NGS) targeting known *PEX* genes.

Whole exome sequencing was conducted on the patient's genomic DNA. DNA was extracted using Qiagen. The quantity and quality of the extracted DNA were assessed using a NanoDrop spectrophotometer (Epoch, Biotek, USA) and 1.5% agarose gel electrophoresis. The DNA was then sent for Whole exome sequencing to enrich all coding regions of the proband by a HiSeq6000 instrument. Whole exome libraries were prepared using the Agilent SureSelect Human All Exon V7 kit sequencing was performed on an Illumina NovaSeq 6000 to generate 150 bp

paired-end reads. Raw sequencing data were assessed for quality using FastQC (version 0.11.6). Adapter sequences and low-quality reads were removed using Cutadapt (version 1.18). Sequence reads were aligned to the UCSC hg38 reference genome using BWA (version 0.7.17). Variants, including single-nucleotide polymorphisms and insertion/deletion events (indels), were called using SAMTools (version 1.10). Initial variant calls for nonsynonymous single-nucleotide variants were made, followed by further filtering to isolate high-confidence variants for downstream analysis.

Variant annotation and filtering

Variants were annotated using the ANNOVAR tool (version 2020), which provides detailed information on their functional impact, frequency in population databases (e.g., gnomAD, 1000 Genomes), and predicted pathogenicity based on various algorithms (e.g., SIFT, PolyPhen-2, MutationTaster). We focused on non-synonymous variants, splice-site alterations, and frameshift indels, as they are more likely to have a functional impact on protein function.

For initial filtering, we excluded common variants with a minor allele frequency greater than 5% in the general population, using gnomAD and the 1000 Genomes database. Additionally, we focused on variants located within coding regions or essential regulatory regions of genes known to be involved in disease mechanisms.

To narrow down potential disease-causing variants, we conducted a gene-based filtering approach, where only variants in genes associated with known phenotypic manifestations similar to the patient's presentation were kept. The final list of candidate variants was manually reviewed by cross-referencing with available literature and databases, including ClinVar, OMIM, and the Human Gene Mutation Database.

Identification of mutation

NGS analysis revealed a homozygous missense variant in the *PEX3* gene at position c.797G>A, leading to an amino acid substitution p.Glu266Lys. This variant was confirmed through Sanger sequencing, which validated its presence in both alleles.

Bioinformatics analysis

To evaluate the potential impact of the identified p.Glu266Lys mutation on protein function, several bioinformatics tools were utilized:

1. SIFT: This tool predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids [7].

2. PolyPhen-2: This predictor assesses possible impacts of an amino acid substitution on the structure and function of a protein using structural information [8].

3. MutationTaster: This tool evaluates if a genetic alteration is disease-causing based on various parameters, including conservation across species and splice site predictions [9].

Structural analysis

To further investigate how the p.Glu266Lys mutation might affect PEX3 functionality, we conducted a comprehensive analysis of protein stability. This analysis included evaluations of hydrogen bonding, electrostatic interactions,

and stability predictions using algorithms such as I-Mutant 3.0 [10]. By assessing these factors, we aimed to elucidate the potential impact of the p.Glu266Lys mutation on the structural integrity and functional capacity of the PEX3 protein.

Results

A 15-year-old male patient diagnosed with ZS was found to carry a homozygous variant in the *PEX3* gene, specifically p.Glu266Lys. Genetic analysis was performed using NGS, which identified the mutation and was subsequently confirmed by Sanger sequencing (Fig. 2). The clinical presentation of the patient included developmental delay, hypotonia, seizures, liver dysfunction, and characteristic craniofacial dysmorphisms consistent with ZS. Imaging studies revealed significant abnormalities in the brain and liver, corroborating the diagnosis. Bioinformatics analyses were conducted to assess the functional impact of the p.Glu266Lys mutation. Predictions from various tools indicated that the variant is likely deleterious: SIFT predicted it to be deleterious (score < 0.05), PolyPhen-2 classified it as probably damaging (score > 0.85), and MutationTaster indicated that it is disease-causing. Structural analyses demonstrated altered hydrogen bonding and electrostatic interactions that may impair binding with PEX19, a crucial partner for peroxisome biogenesis. Stability predictions showed that the mutation decreases protein stability, with a $\Delta\Delta G$ value of +2.5 kcal/mol, suggesting a destabilizing effect on the PEX3 protein.

These findings underscore the significance of *PEX3* mutations in the pathogenesis of ZS and highlight the utility of NGS combined with Sanger sequencing in identifying genetic variants that contribute to this disorder. Further investigation into the functional consequences of the p.Glu266Lys variant may provide insights into potential therapeutic strategies and enhance our understanding of the molecular mechanisms underlying peroxisome biogenesis disorders.

Discussion

ZS is a complex disorder that exemplifies the intricate relationship between genetics, biochemistry, and clinical manifestations [11]. The identification of a homozygous mutation in the *PEX3* gene (p.Glu266Lys) in our patient underscores the critical role of genetic analysis in diagnosing and understanding this condition. The following discussion will elaborate on the implications of this case, the pathophysiological mechanisms involved, and potential future directions for research and therapy.

The clinical features exhibited by our patient are consistent with those typically observed in individuals with ZS. The severity of symptoms often correlates with the specific *PEX* gene affected and the nature of the mutation [12]. PEX3 is crucial for peroxisome biogenesis, particularly in the import of peroxisomal matrix proteins and the assembly of peroxisomal membranes. Mutations in this gene can lead to severe disruptions in peroxisome function, resulting in a cascade of metabolic disturbances [13].

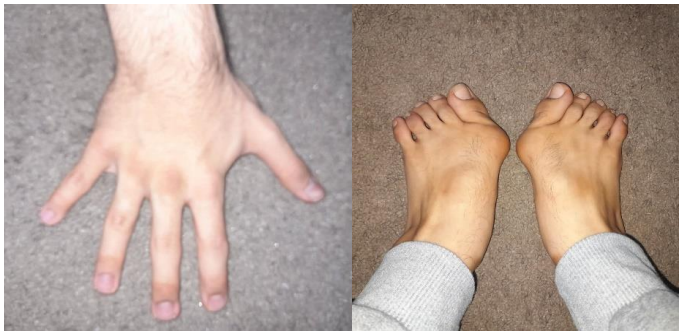

Fig. 1. Clinical photographs of patient's appearance at the time of evaluation

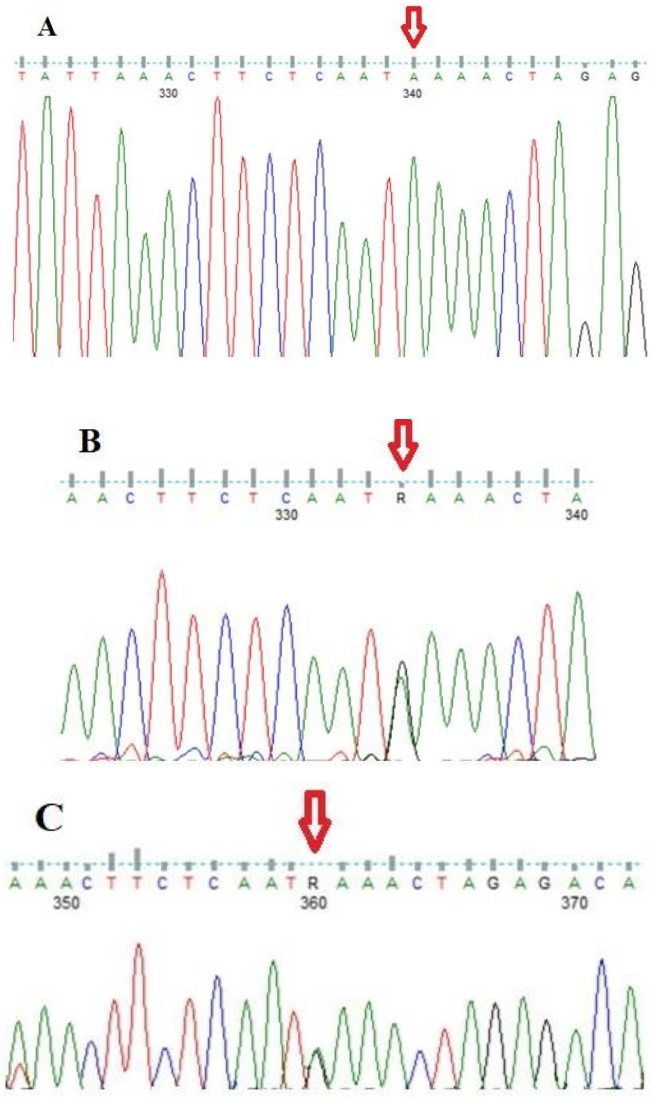

Fig. 2. Sequencing results, the site of the variant was marked by arrows. (A. proband, B. Mother, C. Father)

The developmental delays and neurological impairments observed in our patient are indicative of the profound impact that impaired peroxisomal function can have on brain development. Peroxisomes are involved in lipid metabolism, including the synthesis of plasmalogens, which are essential for myelin formation. Disruptions in myelination can lead to significant cognitive and motor deficits [14]. The presence of cerebral atrophy and polymicrogyria on neuroimaging further supports the notion that peroxisomal dysfunction adversely affects neurodevelopment [15].

Liver involvement is a hallmark feature of ZS, often presenting as hepatomegaly and elevated liver enzymes [16]. In our patient, chronic feeding difficulties and recurrent aspiration pneumonia may be partially attributed to hepatic dysfunction, which can complicate nutritional intake and overall health. The accumulation of very-long-chain fatty acids due to defective peroxisomal β-oxidation contributes to liver injury, leading to hepatic steatosis and dysfunction [17].

Although renal anomalies were not prominently featured in our patient's presentation, it is essential to note that they are frequently reported in ZS cases. Renal cysts or structural abnormalities may arise due to disrupted lipid metabolism affecting renal development. This highlights the multi-systemic nature of ZS and the need for comprehensive evaluations across various organ systems [18].

The identification of a specific mutation within the *PEX3* gene provides valuable insights into the genetic landscape of ZS. Previous studies have identified numerous mutations across various *PEX* genes, each contributing to distinct phenotypic presentations within the spectrum of peroxisome biogenesis disorders [19-21]. Understanding these genotype-phenotype correlations is crucial for predicting clinical outcomes and guiding management strategies. The p.Glu266Lys mutation specifically alters an amino acid that is likely involved in critical interactions necessary for PEX3's function. The predicted destabilization of the protein structure due to this mutation may hinder its ability to interact effectively with other proteins involved in peroxisome assembly, such as PEX19. This disruption can lead to a failure in importing essential matrix proteins into nascent peroxisomes, exacerbating the clinical manifestations observed in our patient.

The bioinformatics analyses conducted on the p.Glu266Lys variant provide a deeper understanding of its potential functional consequences. By employing tools such as SIFT, PolyPhen-2, and MutationTaster, we were able to predict that this mutation is likely deleterious to protein function. Furthermore, structural analyses indicated that alterations in hydrogen bonding and electrostatic interactions could significantly impair PEX3's ability to interact with its partners. The stability predictions suggest that this mutation may lead to a less stable protein conformation ($\Delta\Delta G = +2.5$ kcal/mol), which could result in reduced availability of functional PEX3 protein within cells. This finding emphasizes

the importance of structural integrity for protein function and highlights how even single amino acid changes can have profound effects on cellular processes.

Conclusion

In summary, this case report highlights a novel homozygous mutation (p.Glu266Lys) in the *PEX3* gene associated with ZS in a 15-year-old male patient. The integration of clinical findings with advanced genetic analysis underscores the multifaceted nature of this disorder and emphasizes the importance of genetic testing in confirming diagnoses and guiding management strategies. As research continues to evolve, understanding these genetic variants will be crucial for developing targeted interventions aimed at improving outcomes for individuals affected by peroxisome biogenesis disorders like ZS.

Ethical Considerations

Ethical approval for this study was obtained from the Ethics Committee of Shahid Sadouqhi University of Medical Sciences under the approved number: IR.SSU.MEDICINE.REC.1404.094.

Funding Statement

The responsible author covered the experiment and other associated costs.

Conflicts of Interests

The authors declare no potential conflict of interest concerning the research, authorship, and publication of this report.

Acknowledments

This manuscript benefited from AI-assisted refinement and formatting support, including language polishing and abbreviation standardization.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

Authors' Contributions

SA.M: Data curation and analyzing data, M.M: Data curation and investigation, SM.K: project administration, N.Y: Data curation, M.H: Data curation and investigation, M.E:, Data curation, investigation, E.Z.M: writing the manuscript, analyzing data, and interpreting analyses.

References

[1]. Fazi C, Lodi L, Magi L, Canessa C, Giovannini M, Pelosi C, et al. Case report: Zellweger syndrome and humoral immunodeficiency: The relevance of newborn screening for primary immunodeficiency. Frontiers in Pediatrics 2022; 10: 852943.

[2]. Alayoubi AM, Ijaz A, Wali A, Hashmi JA, Alharbi A, Basit S. Zellweger syndrome; identification of mutations in PEX19 and PEX26 gene in Saudi families. Annals of Medicine 2025; 57(1): 2447400.

[3]. Braverman NE, Raymond GV, Rizzo WB, Moser AB, Wilkinson ME, Stone EM, et al. Peroxisome biogenesis disorders in the Zellweger spectrum: An overview of current diagnosis, clinical manifestations, and treatment guidelines. Molecular Genetics and Metabolism 2016; 117(3): 313-21.

[4]. Celik M, Ipek MS, Ozgun N, Akdeniz O, Tuzun H, Bulbul A. Clinical diagnosis, biochemical findings, genetics and incidence of Zellweger syndrome. Iranian Journal of Pediatrics 2018; 28(1). [In press]

[5]. Bose M, Yergeau C, D'souza Y, Cuthbertson DD, Lopez MJ, Smolen AK, et al. Characterization of severity in Zellweger spectrum disorder by clinical findings: A scoping review, meta-analysis and medical chart review. Cells 2022; 11(12): 1891.

[6]. Enns GM, Ammous Z, Himes RW, Nogueira J, Palle S, Sullivan M, et al. Diagnostic challenges and disease management in patients with a mild Zellweger spectrum disorder phenotype. Molecular Genetics and Metabolism 2021; 134(3): 217-22.

[7]. Sim NL, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Research 2012; 40(W1): 452-57.

[8]. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Current Protocols in Human Genetics 2013; 76(1): 1-7.

[9]. Schwarz JM, Rödelsperger C, Schuelke M, Seelow D. MutationTaster evaluates disease-causing potential of sequence alterations. Nature methods. 2010; 7(8): 575-76.
[10]. Marabotti A, Scafuri B, Facchiano A. Predicting the stability of mutant proteins by computational approaches: an overview. Briefings in Bioinformatics 2021; 22(3): 74.
[11]. Yalçınkaya B, Sağlam KA, Terali K, Tekin E, Taslak H, Türkyılmaz A. Biallelic deletion of PEX26 exon 4 in a boy with phenotypic features of both Zellweger syndrome and infantile refsum disease. Molecular Syndromology 2024; 15(5): 380-88.
[12]. Su L, Peng MZ, Chen XD, Wu S, Liu L. Severe Zellweger spectrum disorder due to a novel missense variant in the PEX13 gene: A case report and the literature review. Molecular Genetics & Genomic Medicine 2024; 12(1): 2315.
[13]. Wanders RJ, Baes M, Ribeiro D, Ferdinandusse S, Waterham HR. The physiological functions of human peroxisomes. Physiological Reviews 2023; 103(1): 957-1024.
[14]. Deb R, Joshi N, Nagotu S. Peroxisomes of the brain: distribution, functions, and associated diseases. Neurotoxicity Research 2021; 39(3): 986-1006.
[15]. Dutton G, Bax M. Visual impairment in children due to damage to the brain: John Wiley & Sons; 2010.
[16]. Thompson RJ, Portmann BC, Roberts EA. Genetic and metabolic liver disease. MacSween's Pathology of the Liver E-Book; 2011: p. 157.
[17]. Lu Q, Zong W, Zhang M, Chen Z, Yang Z. The overlooked transformation mechanisms of VLCFAs: peroxisomal β-oxidation. Agriculture 2022; 12(7): 947.
[18]. Gazeu A, Collardeau-Frachon S. Practical approach to congenital anomalies of the kidneys: Focus on anomalies with insufficient or abnormal nephron development: Renal dysplasia, renal hypoplasia, and renal tubular dysgenesis. Pediatric and Developmental Pathology 2024; 27(5): 459-93.
[19]. Waterham HR, Ebberink MS. Genetics and molecular basis of human peroxisome biogenesis disorders. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 2012; 1822(9): 1430-441.
[20]. Braverman NE, D'Agostino MD, MacLean GE. Peroxisome biogenesis disorders: Biological, clinical and pathophysiological perspectives. Developmental Disabilities Research Reviews 2013; 17(3): 187-96.
[21]. Walter C, Gootjes J, Mooijer PA, Portsteffen H, Klein C, Waterham HR, et al. Disorders of peroxisome biogenesis due to mutations in PEX1: Phenotypes and PEX1 protein levels. The American Journal of Human Genetics 2001; 69(1): 35-48.