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Integrative Genomic and Genotype-Phenotype Analysis of a Homozygous PIGQ Frameshift Variant in MCAHS4

Yousef Al-Hajaya

Department of Biological Sciences, Faculty of Science, Mutah University, Karak 61710, Jordan.
*Corresponding Author E-mail: Yousef.hajaya@mutah.edu.jo

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Multiple congenital anomalies-hypotonia-seizures syndrome type 4 (MCAHS4, OMIM: 618548) is a rare autosomal recessive neurological disorder caused by biallelic causal variants in PIGQ gene (OMIM 605754). Whole genome sequencing was performed in a 4-year-old male presenting with clinical features consistent with MCAHS4. A homozygous frameshift variant in PIGQ gene (c.1673del; p. Gly558fs*25) was identified and subsequently confirmed through parental segregation analysis. This variant is located in the final exon and is not predicted to efficiently undergo nonsense-mediated mRNA decay, potentially resulting in the production of a truncated protein with impaired enzymatic function. However, it remains classified as a variant of uncertain significance according to ACMG/AMP guidelines due to the absence of functional validation. Additional carrier status and pharmacogenomic findings were identified but are not directly related to the proband's presenting clinical phenotype. Collectively, these results provide additional interpretative evidence consistent with PIGQ-related disorder and underscores the importance of integrative genome-wide analysis and cautions variant interpretation in rare neurodevelopmental disorders.

Keywords: Carrier Status; Developmental Delay; Frameshift Variants; Pharmacogenomics Association; PIGQ; Whole Genome Sequencing.

Glycosylphosphatidylinositol (GPI) is a complex lipid anchor for many cell surface proteins in eukaryotes and most likely in some Archaea.[1] GPI-anchored proteins (GPI-APs) are integral proteins found on the outer surface of cell membranes.[2] Approximately 150 different human proteins are post-translationally modified by GPI at the carboxyl terminus.[2,3] These proteins have various functions such as adhesion molecules, cell surface antigens, hydrolytic enzymes, complement regulatory proteins, receptors, and prions, playing critical roles in processes like cell signaling, neurogenesis, embryogenesis, and immunological response.[4,5] Currently, over 31 genes have been identified as contributors to the GPI-anchor biosynthesis pathway that is divided into transamidase, remodeling, and synthesis groups. 25 of them have been implicated in specific human diseases. [6-8]

Phosphatidylinositol glycan anchor biosynthesis Class Q (PIGQ) is a protein coding gene. PIGQ is a key co-enzyme in the initial step of glycosylphosphatidylinositol (GPI)-anchor formation. [9-12] The PIGQ gene (OMIM 605754) encodes a N-acetylglucosaminyl transferase core component that is a critical part of the complex that catalyzes transfer of N-acetylglucosamine from UDP- N-acetylglucosamine to phosphatidylinositol (PI). [9,11,13]

Multiple congenital anomalies-hypotonia-seizures syndrome type 4 (MCAHS4, OMIM: # 618548) is an autosomal recessive neurodevelopmental disorder caused by biallelic causal variants in *PIGQ* gene, located on chromosome 16p13.[11] MCAHS4 typically presents with severe global developmental delay, generalized hypotonia, visual defects, craniofacial dysmorphism, renal anomalies, early-onset therapy-resistant epileptic encephalopathy, and frequently premature death. At the cellular level, the mutation in the *PIGQ* gene leads to a defect in the early steps of Glycosylphosphatidylinositol GPI synthesis, resulting in reduction in the surface expression of GPI-anchored proteins.[14,15]

In the present study, comprehensive genetic characterization of a 4-year-old male presenting with clinical features consistent with MCAHS4 was conducted using whole genome sequencing (WGS). The analysis included primary findings, carrier status testing, and pharmacogenomic profiling. Parental genetic testing was performed to confirm the segregation pattern and validate the proband's homozygous status. Integration of primary diagnostic findings, carrier status and pharmacogenomic insights provided a detailed genotype-phenotype evaluation. Collectively, this work aims to enhance genotype-phenotype correlations and emphasize the broader clinical utility of genome-wide sequencing in rare neurodevelopmental disorders.

MATERIALS AND METHODS

Clinical evaluation

The child, a 4-year-old male, was evaluated by a specialized physician for recurrent seizures and developmental delay. Detailed history, brain imaging, neurological examination, and family pedigree were obtained.

Genetic testing

Genetic analysis for the affected child was conducted using myLifeGenome™ test (Arcensus GmbH, Rostock, Germany), which applies a whole genome sequencing-based diagnostic test. This comprehensive assay identifies genetic variants directly relevant to the patient's clinical presentation. This test also enables the identification of incidental findings, carrier status, and pharmacogenomic association. Findings are interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[16-18] To confirm inheritance, both parents were tested for the identified variant by targeted Sanger sequencing (Revvity Omics, Pittsburgh, USA).

Written informed consent was obtained from the parents prior to testing. Whole genome sequencing was selected to expand the diagnostic yield by identifying coding and non-coding variants. Additionally, it is selected over whole-exome sequencing to enable the detection of structural variants and pharmacogenomic markers. Segregation analysis by targeted Sanger sequencing was performed to reduce false-positive results and confirm variant inheritance.

Carrier Status and Pharmacogenomic Associations

A dried blood spot specimen was used to carry out the carrier status and pharmacogenomic associations. To detect pathogenic or likely pathogenic variants that do not affect the proband but can increase the risk of having affected children (heterozygous variants in a gene associated with a recessive or X-linked disorder). Pharmacogenomics testing was conducted to identify different variants associated with drug use and dose, and the results were generated based on CPIC and PharmCAT.

RESULTS

Primary findings

The child was born as a late preterm infant, delivered at 9 months of gestation through a normal vaginal delivery, with oligohydramnios (deficiency of amniotic fluid) and subsequent admission for three days in the NICU. Child's clinical features include developmental delay, marked motor and speech impairment. Seizure episodes were diagnosed by postictal fatigue and cyanosis, accompanied by recurrent infections. Brain MRI scans demonstrated abnormal findings, including shrinkage of part of the cerebellar vermis (middle part) and diffuse cerebellar atrophy. Regarding family history, parents are consanguineous. There is no family history of neurological disorders. The child has one healthy brother. The only cancer in the family is a paternal aunt who had a history of endometrial carcinoma.

Child genetic findings reveal that the child has a homozygous uncertain variant in the PIGQ gene, which could be associated with autosomal recessive multiple congenital anomalies-hypotonia-seizures syndrome Type 4. The child is also a carrier for three unrelated conditions. PIGQ c.1673del; p. Gly558fs*25, a specific change in *PIGQ* gene, is a frameshift deletion in exon 11 of the *PIGQ* gene. The single nucleotide deletion at coding position 1673 causes a shift in the reading frame starting at codon glycine 558, generating a premature termination codon 25 amino acids downstream (p. Gly558fs*25). This variant is not predicted to undergo nonsense-mediated mRNA decay (NMD) because it lies within the last exon and may therefore result in a truncated protein, leading to complete loss of function. This variant is present in the local database and in gnomAD (allele frequency:0.00050, 0.0000159, respectively.) It is also listed in ClinVar as a variant of uncertain significance (VUS), accession ID: 2434846.

Segregation testing was performed, confirming that the proband is homozygous for the PIGQ c.1673 del; p. Gly558fs*25 variant, whereas both parents are heterozygous carriers. This is consistent with autosomal recessive inheritance. The child's clinical features are compatible with the reported spectrum of MCAHS4. Currently, this variant is classified as a VUS according to ACMG guidelines. This is due to insufficient functional evidence and limited independent case-level evidence. While PVS1 may be considered for predicted loss-of-function, its strength is reduced given the position in the final exon and uncertain likelihood of NMD.

Although the identified PIGQ c.1673del; p. Gly558fs*25 variant is classified as a VUS, several lines of supportive evidence suggest

Table 1. Segregation analysis

Individual	Gene	Variant	Zygosity	Classification
Proband	PIGQ	c.1673del; p. Gly558fs*25	Homozygous	Variant of Uncertain Significance (VUS)
Mother	PIGQ	c.1673del	Heterozygous	VUS (carrier)
Father	PIGQ	c.1673del	Heterozygous	VUS (carrier)

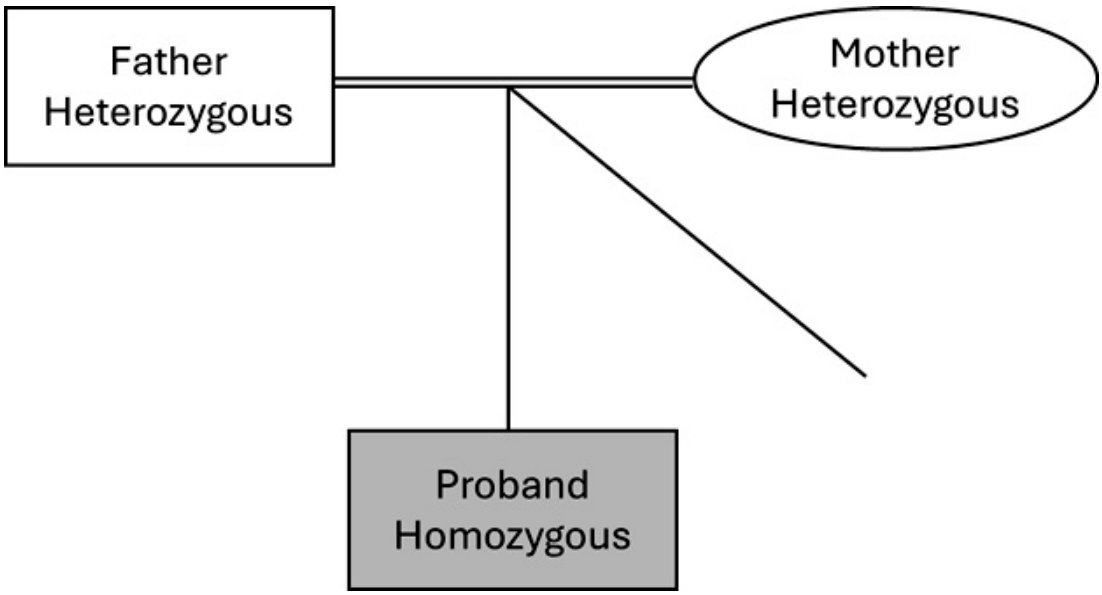

Fig. 1. Family pedigree demonstrating autosomal recessive inheritance. The proband is homozygous for the PIGQ c.1673del variant, while both parents are heterozygous carriers

Table 2. Carrier Status findings

Gene	Variant	Zygosity	Related disease (OMIM)	Mode of Inheritance (MOI)	Clinical assessment	Clinical significance (ACMG criteria)
ABCG8	c.965-1G>C	Heterozygous	Sitosterolemia Type 1	Autosomal recessive	Carrier	Pathogenic PVS1, PS4, PM2
HPS6	c.1819C>T	Heterozygous	Hermansky-Pudlak Syndrome Type 6	Autosomal recessive	Carrier	Likely pathogenic PVS1, PS4, PM2
SORD	c.757del	Heterozygous	Neuronopathy, Distal Hereditary Motor, Type 8	Autosomal recessive	Carrier	Pathogenic PVS1, PS3, PS4

Table 3. Comparison of the present case with previously reported PIGQ-related MCAHS4

Reference	Variant	Zygosity	Developmental Delay	Hypotonia	Seizures	Brain Abnormalities
Present	c.1673del (p.Gly558fs*25)	Homozygous	Severe	Yes	Yes	Cerebellar atrophy
Kušíková et al.[8]	Novel variants	Biallelic	Severe	Yes	Yes	Brain malformations
Johnstone et al.[12]	Multiple variants	Biallelic	Severe	Yes	Yes	Cerebellar anomalies
Starr et al.[15]	Various	Homozygous Compound Heterozygous	Severe	Yes	Yes	Structural CNS abnormalities

biological plausibility, including homozygosity in an affected individual from a consanguineous family, heterozygous carrier status in both unaffected parents, a predicted truncating effect on the PIGQ protein and strong phenotypic overlap with previously reported MCAHS4 cases. Conversely, in the absence of functional validation, a definitive causal relationship cannot be established.

Pedigree diagram (family tree, Fig.1) shows the parents as heterozygous carriers and the child as homozygous affected. The child inherited both copies from his parents, resulting in homozygosity.

Carrier Status Findings

To determine the child's risk for transmitting inherited genetic conditions to offspring, carrier status testing was performed. Carriers are typically healthy and/or asymptomatic. Though if an individual is found to be a carrier of genetic conditions, biological relatives of the individual are at risk of harboring the same mutation. Accordingly, it is recommended that patients inform their family members of the potential genetic risk and the availability of molecular carrier screening.

If a patient is identified as a carrier of a specific condition, their reproductive partner should undergo genetic testing to receive informed counseling on reproductive outcomes. If both partners carry the same genetic conditions, specialized genetic counseling should be offered to discuss reproductive options, family planning and preventive strategies.

Carrier status analysis indicated that the proband is a heterozygote carrier of three genetic conditions (Table 2, see supplement data for more details). The proband is heterozygous for three likely pathogenic or pathogenic variants linked to recessive disorders: ABCG8 gene (c.965-1G>C; sitosterolemia type 1), HPS6 gene (c.1819C>T; Hermansky-Pudlak syndrome type 6), and SORD gene (c.757del; distal hereditary motor neuronopathy type 8). Nevertheless, these results do not affect the patient's clinical status but do have reproductive consequences, as carriers can transmit pathogenic alleles to their offspring. The parental carrier status analysis was not performed because these variants are unrelated to the proband's phenotype. However, identification of carrier status has crucial implications for future reproductive planning and risk assessment, especially in consanguineous families, where shared pathogenic alleles may increase the risk of recurrence.

Pharmacogenomic Analysis

Pharmacogenomic (PGx) analysis demonstrates the association between drugs and genetic variants based on their drug metabolizing status as defined by Clinical Pharmacogenetics Implementation Consortium (CPIC) using Pharmacogenomics Clinical Annotation Tool (PharmCAT). Therefore, genetic variants linked to drug use and dosing were identified. Various additional gene-drug interactions with implications for clinical prescribing were also identified, spanning immunological, cardiovascular, and neurological therapies. Such variants influence the metabolism of different commonly prescribed drugs, including immunosuppressants, cardiovascular drugs, psychiatric medications, and anticonvulsants. For some drugs, actionable recommendations from CPIC guidelines were provided, such as dose adjustments or avoidance. The full pharmacogenomic associations, including drugs, PGx phenotypes, genes/genotypes, and CPIC recommendations, are provided in the supplementary data. These findings underscore the clinical utility of pharmacogenomic screening in tailoring drug therapy, improving efficacy, and minimizing toxicity. While the results of pharmacogenomic analysis are not directly linked to the proband's current therapeutic treatments, these findings may provide anticipatory guidance for long-term clinical management.

DISCUSSION

This work reports a homozygous PIGQ frameshift variant (c.1673del; p. Gly558fs*25) in a 4-years-old male exhibiting clinical features of MCAHS4. Targeted parental testing confirmed that both unaffected parents are heterozygous carriers. This result provides supportive evidence consistent with previously described PIGQ-related phenotypes.[7,8] The family presented in this study represents one of the few documented segregation-confirmed cases. The proband's homozygosity and consanguinity are consistent with autosomal recessive inheritance.

Clinically, the proband presented with

profound developmental delay, cerebellar atrophy and recurrent seizures. These clinical features are consistent with previous studies of PIGQ-related MCAHS4, in which patients often present early infantile epileptic encephalopathy, structural abnormalities of the central nervous system (CNS) and hypotonia.[12] To contextualize the present results with the previous studies, a comparison with previously reported PIGQ-related MCAHS4 cases is summarized in Table 3.[8,12,15] The concordance between the proband's clinical presentation and previously reported cases strengthens the genotype-phenotype correlation and supports biological plausibility, although causality cannot be definitively established.

Prior to molecular diagnosis, the differential diagnosis remained broad and included other genetic epileptic encephalopathies, congenital disorders of glycosylation, and syndromes associated with structural brain malformations.

The presence of parental consanguinity significantly increased the likelihood of detecting a homozygous autosomal recessive variant, thereby supporting the use of genome-wide sequencing in this clinical setting.

The detection of a homozygous variant in the last exon is particularly significant, as it is unlikely to undergo nonsense-mediated mRNA decay, potentially resulting in a truncated yet stable protein with impaired enzymatic function. Such a mechanism may contribute to the severe clinical phenotype observed in this case and highlights the importance of functional studies to further clarify the pathogenic consequences of this variant.[12,15]

According to ACMG guidelines, this variant is classified as a VUS due to the absence of functional studies and limited population-level evidence. However, segregation analysis and the phenotype, in line with previously reported cases, strongly support the clinical relevance of this variant. [16] These results presented in this study highlight the importance of systematic variant reporting in regions with high consanguinity, such as the Middle East, where limited representation in global genomic databases may contribute to persistent variant uncertainty. Expanding regional genomic datasets is necessary to improve the accuracy of variant interpretation and to reduce health disparities in the molecular diagnosis of rare diseases.

In addition to the primary findings, this study also examined carrier status and pharmacogenomic associations. The proband was found to be a heterozygous carrier for three genetic conditions: sitosterolemia type 1 (ABCG8), Hermansky–Pudlak syndrome type 6 (HPS6), and distal hereditary motor neuronopathy type 8 (SORD). While these likely pathogenic and pathogenic variants are unrelated to the proband's main clinical features, they raise significant reproductive considerations. In populations where the parents are consanguineous, the risk of autosomal recessive conditions is increased; carrier screening and counseling are recommended. Broader application of carrier testing may facilitate reproductive planning and reduce the prevalence of rare genetic disorders in such contexts. [7]

To provide additional clinically relevant insights, including gene-drug interactions that may affect drug metabolism and treatment response, pharmacogenomic testing was conducted. For example, variants affecting the metabolism of antidepressants, anticonvulsants and cardiovascular drugs were identified, some of which are associated with specific dose modification or contraindications based on CPIC guidelines. The integration of pharmacogenomic testing with patient-specific clinical and genetic information may enhance drug therapy. When prescribing a drug, knowledge of a patient's genotype can guide the determination of appropriate dosage, selection of therapeutic strategy and/or the evaluation of the likelihood of benefit or toxicity leading to improve overall patient management. [8] To our knowledge, the current study represents one of the first studies from Jordan integrating rare genetic disorder diagnostics with pharmacogenomic analysis, demonstrating the feasibility and additional clinical benefit of implementing precision medicine strategies in regional healthcare systems.

Functional impact of the PIGQ c.1673del variant and rationale for VUS classification

PIGQ encodes a vital component of the N-acetylglucosaminyl transferase complex responsible for the first step catalyzing the biosynthesis of the GPI-anchor.[9-11] The c.1673del variant introduces a frameshift resulting in a premature termination codon 25 amino acids downstream (p.Gly558fs*25) in the final exon of the gene. Because this variant lies within the

last exon, it is not predicted to efficient undergo nonsense-mediated mRNA decay, which may in turn produce a truncated protein with impaired enzymatic function.[19] The mutation in the *PIGQ* gene leads to a defect in the early steps of Glycosylphosphatidylinositol GPI synthesis, resulting in reduction in the surface expression of GPI-anchored proteins, a mechanism implicated in MCAHS disorders. [14,15]

Although the supportive evidence suggests biological plausibility, the variant remains classified as a VUS according to ACMG/AMP guidelines, primarily due to insufficient functional or clinical studies demonstrating reduced GPI-anchored protein expression and limited number of reported cases carrying this specific variant.

Additional evidence, such as enzymatic assays, independent identification of the variant in unrelated affected individuals, or functional assays examining GPI-anchored proteins, would likely support the likelihood of reclassifying a VUS.

Study limitations

The study is limited by the lack of clinical follow-up and by the absence of functional validation assays, including quantification of GPI-anchored protein expression. Additionally, this study is based on a single affected individual, which limits the generalizability of the results. Future studies, including independent replication and long-term clinical follow-up, are required to explore the biochemical and pathogenic effects associated with truncating mutations in the final exon of *PIGQ*, thereby clarifying the pathogenic role of the identified variant.

CONCLUSION

This study extends the molecular and clinical understanding of PIGQ-related MCAHS4 and illustrates the importance of integrating primary genetic testing with carrier detection and pharmacogenomic testing. The combination of these findings enables a more comprehensive genetic assessment which is not only diagnostic but also predictive and therapeutic. Future investigations should prioritize functional validation of the variant, systematic follow-up of affected patients, and broader application of combined genetic and pharmacogenomic testing in clinical practice. Such efforts will improve variant interpretation (i.e., the accuracy of variant classification), optimize patient care, and enhance clinical management of rare genetic disorders.

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Conflict of Interest

The author(s) do not have any conflict of interest

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

Ethical approval for this study was obtained from the Research Ethics Committee, Mutah University, Jordan (Approval No. 20/143366).

Informed consent statement

Written informed consent for publication of anonymized clinical and genetic data was obtained from the proband's parents.

Clinical Trial Registration

This study does not involve any clinical trials.

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Not applicable.

Author Contributions

The sole author was responsible for the conceptualization, methodology, data collection, analysis, writing, and final approval of the manuscript.

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