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ORIGINAL ARTICLE

Uncovering the genetic basis of hyperphosphatasia with impaired intellectual development syndrome type 2: identification of a novel biallelic nonsense mutation in *PIGO* gene

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ABSTRACT

Background: Glycosylphosphatidylinositol (GPI) is a glycolipid containing phosphatidylinositol related to the protein surfaces by covalent attachment. Inherited GPI deficiencies have various phenotypic chrematistics, which range from intellectual disability to dysmorphic features, epilepsy, and other severe anomalies.

Methods: Molecular diagnosis was performed using whole exome sequencing (WES) followed by Sanger sequencing.

Results: WES revealed a novel homozygous nonsense variant (c.250C>T; *p*.Gln84Ter) in the exon 2 of the phosphatidylinositol glycan anchor biosynthesis class Ogene that might explain the disease phenotype in the patient.

Conclusion: This study will help in proper genetic counselling of the family and help in genotype-phenotype correlation in the future.

Keywords: GPI, WES, missense variant, PIGO, ID, novel variant.

Introduction

Glycosylphosphatidylinositol (GPI) is a glycolipid containing phosphatidylinositol that covalently attaches proteins to the plasma membrane (cell surface). In forming GPI-anchored proteins (GPI-APs), almost 26-30 genes are involved (1,2). The GPI-APs group include different receptors, enzymes having hydrolytic nature, adhesion molecules, immune system-associated proteins, and complement regulatory proteins (1,2). Disease-causing variants have been identified in various components of the GPI-anchored-synthesis pathway, thus causing diverse phenotypes referred to as congenital disorders of glycosylation (1). Inherited GPI deficiencies include features such as epilepsy, ID, dysmorphic facial features, and multiple organ anomalies depending on the gene involved and the position of the identified variant. Pathogenic sequence variants in different genes have been reported in the GPI biosynthesis, such as the phosphatidylinositol glycan anchor biosynthesis class O (*PIGO*), *PIGV*, *PIGW*, *PGAP2*, *PGAP3*, and the *PIGY* reported to cause hyperphosphatasia with mental retardation syndrome (HPMRS; MIM # 614749; Table 1) also known as

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Table 1. *Genes associated with HPMRS and their clinical comparison.*

OMIM number	# 239300	# 616809	# 614749	# 614207	# 616025	# 615716
Disorder name	Hyperphosphatasia with impaired intellectual development syndrome 1; HPMRS1	Hyperphosphatasia with impaired intellectual development syndrome 6; HPMRS6	Hyperphosphatasia with impaired intellectual development syndrome 2; HPMRS2	Hyperphosphatasia with impaired intellectual development syndrome 3; HPMRS3	GPI biosynthesis defect 11; GPIBD11	Hyperphosphatasia with impaired intellectual development syndrome 4; HPMRS4
Gene	*PIGV* - 610274	*PIGY* - 610662	*PIGO* - 614730	*PGAP2* - 615187	*PIGW* - 610275	*PGAP3* - 611801
Inheritance (6/6)	- AR	- AR	- AR	- AR	- AR	- AR
Growth (3/6)	N.A.	- Poor growth	- Poor growth	N.A.	N.A.	- Poor growth
	N.A.	- Microcephaly	- Microcephaly	- Microcephaly	N.A.	- Microcephaly - Large anterior fontanel
	Face - Midface hypoplasia - Prognathism	*Face* - Bitemporal narrowing	N.A.	N.A.	N.A.	N.A.
	Hearing impairment	Thickened helices	Hearing impairment	Hearing impairment	N.A.	- Hearing loss
Head & neck (6/6)	*Eyes* - Hypertelorism - Upslanting palpebral fissures - Long palpebral fissures - Arched eyebrows	*Eyes* - Congenital cataracts - Cerebral visual impairment - Deep-set eyes - Long palpebral fissures - Strabismus	*Eyes* - Hypertelorism - Long palpebral fissures - Upslanting palpebral fissures	N.A	N.A	*Eyes* - Hypertelorism - Upslanting palpebral fissures - Epicanthal folds
	Nose - Broad nasal bridge - Broad nasal tip - Short nose	*Nose* - Depressed nasal bridge - Upturned nares - Bulbous nasal tip	*Nose* - Short nose - Broad nasal bridge - Broad nasal tip	*Nose* - Broad nasal bridge - Broad nasal tip - Short nose	*Nose* - Broad nasal bridge	*Nose* - Broad nasal bridge - Broad nasal tip
	Mouth - Cleft palate (rare) - Short philtrum - Tented mouth	*Mouth* - High-arched palate - Wide mouth	*Mouth* - Tented mouth - Cleft palate	*Mouth* - Cleft palate - Tented upper lip	*Mouth* - Tented upper lip - Large tongue	*Mouth* - Cleft palate - Bruxism -Abnormal dentition
Cardiovascular (3/6)	*Heart* - Cardiac defects - Ventral septal defect (rare)	N.A.	*Heart* - Heart defects - Atrial septal defect	N.A.	N.A.	*Heart* - Congenital heart defects (in 1 family)

Continued

OMIM number	# 239300	# 616809	# 614749	# 614207	# 616025	# 615716
Skeletal (4/6)	- Plagiocephaly	- Joint contractures - Osteopenia - Hip dysplasia - Proximal limb shortening	- Plagiocephaly - Coronal synostosis	N.A.	N.A.	N.A.
	- Hypoplastic toes - Bilateral adducted forefoot (rare)	N.A	Brachytelephalangy - Broad halluces	N.A	N.A	- Pes equinovarus
Neurologic (6/6)	*Central Nervous System* - Hypotonia - Seizures - Mental retardation, severe - Athetoid and dystonic hand movements - Moderate cortical atrophy - Delayed myelinization - Speech delay - No speech development	*Central Nervous System* - Delayed psychomotor development - Delayed speech - Developmental regression - Seizures, intractable - Truncal hypotonia	*Central Nervous System* - Delayed psychomotor development, moderate to severe - Delayed speech and language development - Hypotonia - Seizures - Enlarged ventricles	*Central Nervous System* - Delayed psychomotor development - Mental retardation, severe - Intellectual disability, mild - Hypotonia - Poor or absent speech - Seizures - Disordered sleep pattern - Cerebral atrophy	*Central Nervous System* - Delayed psychomotor development - Intellectual disability - Seizures, variable types - Poor or absent speech - Abnormal EEG	*Central Nervous System* - Delayed psychomotor development, severe - Inability to walk - Lack of speech development - Seizures, generalized - Seizures, myoclonic - Involuntary movements - Hypoplastic corpus callosum - Hypoplastic cerebellum with absent vermis - Cerebellar vermis hypoplasia
Laboratory abnormalities (6/6)	- Elevated alkaline phosphatase (varies from 1.3-20 times the age-adjusted upper limit of normal) - Hyperphosphatasia	- Increased serum creatine kinase - Increased alkaline phosphatase - Decreased expression of GPI-APs on fibroblasts	- Increased serum alkaline phosphatase - Hyperphosphatasia	- Increased serum alkaline phosphatase - Hyperphosphatemia	- Increased serum alkaline phosphatase - Decreased expression of GPI -anchored membrane proteins	- Increased serum alkaline phosphatase

Mabry syndrome (2-5). Herein, we report a proband (the first case from the Pakistani population) with epileptic encephalopathy caused by a novel disease-causing variant in the *PIGO* gene.

Subjects and Methods

For the present study, a family with an autosomal recessive (AR) inheritance pattern was recruited from the Khyber Pakhtunkhwa province of Pakistan (Figure 1A). The patient was evaluated by taking a medical history and performing biochemical tests at a local government hospital. Consent in written form was obtained from the participants for the genetic analysis in compliance with the Helsinki Declaration. The University of Education, Lahore, Pakistan's Institutional Review Board approved the current study. Blood samples were collected and processed further for DNA extraction and quantification using standard methods (6). WES was performed using DNA from the proband (IV-1). WES and variants filtering steps were performed as described earlier (7). Standard-screening principles were used to search for different functional variants associated with the patient phenotype (8). The genes already reported in the Online Mendelian Inheritance in Man and literature (PUBMED) were given priority. Prioritized disease-causing variants were Sanger sequenced for segregation analysis (9,10). The pathogenic nature of the identified variant was calculated using different tools. The Exome Aggregation Consortium (ExAC) and genomAD were searched to see if the variant was reported in the general population. Amino acid conservation was determined using NCBI-HomoloGene.

Protein modelling

The structure sequence of PIGO full length was retrieved from the Protein Data Bank. The protein modelling was executed according to the previously outlined methodology (11,12). Figures were made using the Py-Molecule molecular viewer (https://pymol.org/) (Figure 2A and B) (13).

Results

Clinical description

The proband (boy: IV-1) was the first child of a healthy Pakistani Pashto-speaking family. Pregnancy was unremarkable, and he was born with vaginal delivery having an average birth weight. Shortly after birth, features such as feeding difficulties, severe axial hypotonia, and muscular dystrophy were observed. He could not recognize his parents and did not establish eye contact presenting the features of global developmental delay (GDD). The proband also showed frequent seizures and drooling. He is on several antiepileptic drugs like phenobarbitone and topiramate. Recurrent episodes with pneumonia were observed in the second year of life, which led to respiratory insufficiency, and a gastrostomy tube was used to fulfil the feeding difficulties. Serum alkaline phosphatase was unremarkable; however, slightly in the upper ranges, i.e., 235, 247, 263, and 257 U/l (Normal range: 96-297 U/l). His younger brother is healthy with no epileptic or any other complications.

Molecular investigation

WES was performed as described earlier (14). Screening and filtering different homozygous and compound heterozygous variants manifested a novel homozygous stop gain variant (c.250C>T; *p*.Gln84Ter) in the exon 2 of *PIGO* (NM_032634.4) located on chromosome 9p13.3-9p13.3. The variant was also screened in ExAC, genomAD, and 145 control exomes (Figure 1B), and the variant was not observed in the homozygous state using both databases. The Gln84 amino acid was also conserved across different species (Figure 1C).

Protein modelling

3D modelling of the mutated PIGO and wild-type PIGO was performed (15). Their structural comparison showed that the mutated PIGO protein would result in a more minor, non-functional protein that loses its main domains. Thus, the mutated PIGO will not perform a proper function.

Discussion

In this study, we report an affected child having GDD, severe epileptic seizures, ID, and little elevation of ALP. We performed WES and identified a biallelic stop gain variant (c.250C>T; p.Gln84Ter) residing in the transmembrane domain of the protein (Figure 1B) of the PIGO, thus expanding the clinical and variant spectrum of *PIGO*-related pathogenesis. To date, disease-causing variants in the *PIGO* gene have only been reported in a few studies, including seven females and two males from six families (1-5,16). If the mRNA molecule coding for a protein avoids degradation through the nonsense-mediated decay pathway, the resulting truncated protein may exhibit a distinct structural formation that deviates from the full-length version of the protein, which can lead to improper functioning and potential cellular dysfunction.

Kuki et al. (17) and Nakamura et al. (3) reported patients that possessed missense variants in the alkaline phosphatase domain (core domain). They showed more progressive and severe phenotypes than the affected individuals reported by Krawitz et al. (2) and in the present study. The mild neurodevelopmental feature in our patient can be associated with the location of the variant identified in the transmembrane domain. Thus, the position of a variant in the protein might play a role in the diverse phenotypic presentation. Similarly, Nakamura et al. (3) reported that severe phenotypes might be associated with the location of variants identified in the specific PIGO domain, such as the core domain. These observations might lead toward genotype-phenotype correlation associated with *PIGO*-pathogenesis. However, more substantial evidence and functional analysis are required to elucidate phenotype–genotype correlations and to prove such a hypothesis. Neurological dysfunction in the affected individual

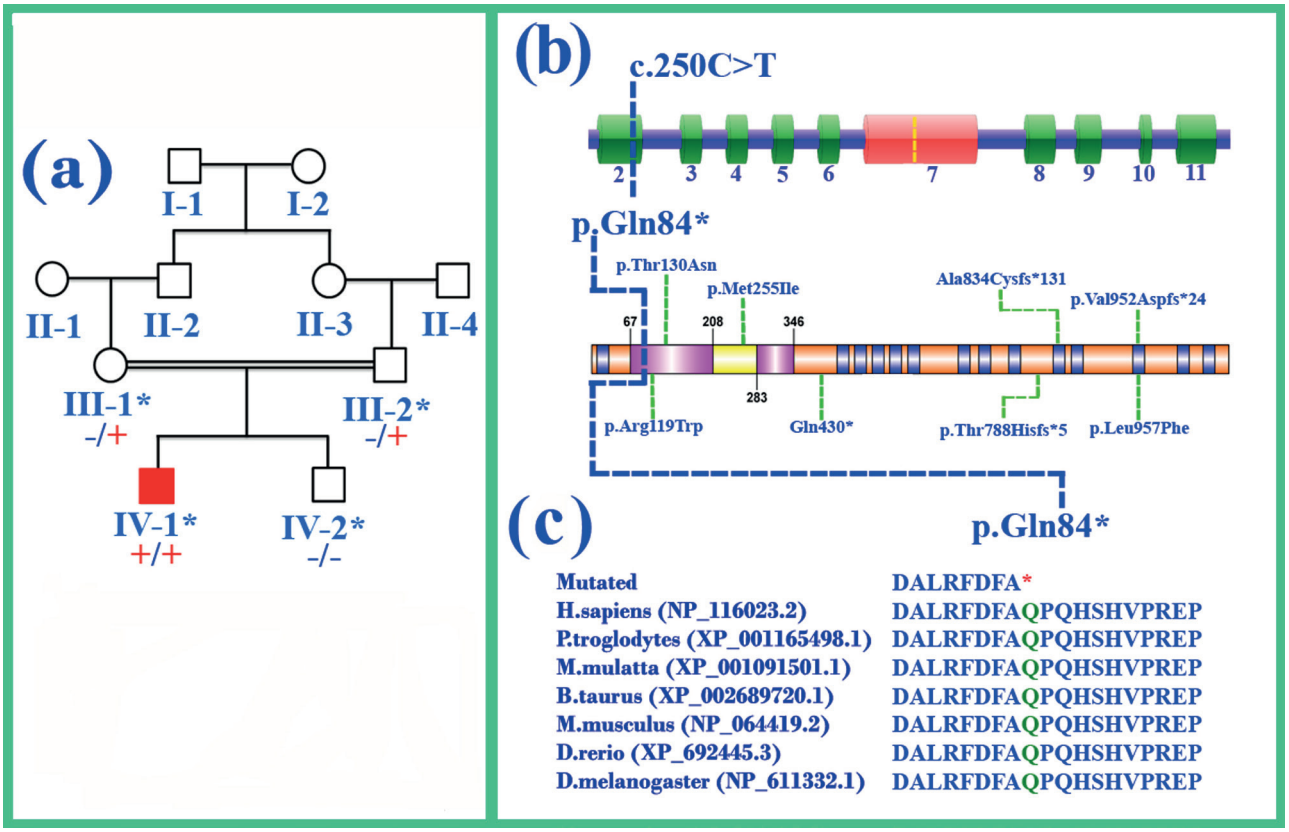

Figure 1. *(A) Pedigree of the present family along with genotype. (B) Surface expression of CD16 antigen. (C) Exons and domains of the PIGO and location of the identified variant. (D) Conservation of Gln84 across different species.*

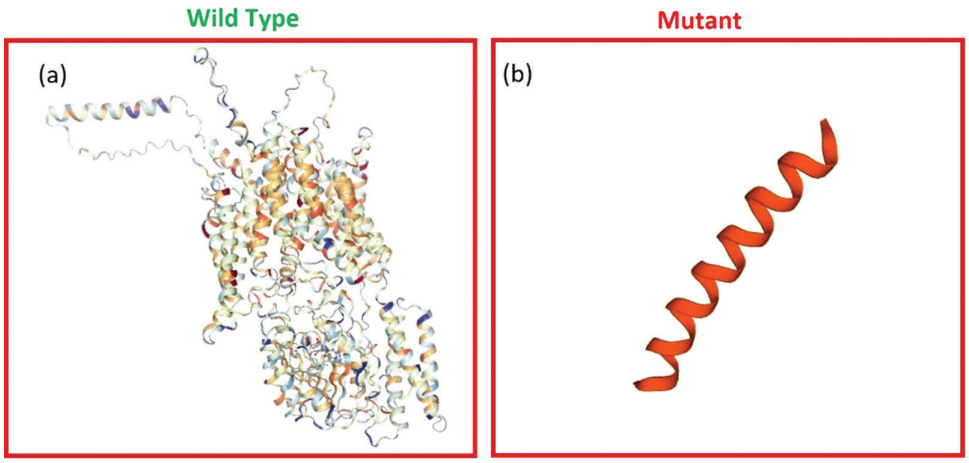

Figure 2. *Comparison of Wild Type and Mutant Protein Structures. (A) Representation of the wild-type protein structure. (B) Representation of the mutant protein structure. The structural differences between the wild type and mutant proteins include loss of 3/4th amino acid residues.*

reported here and in the patients said previously (4,17) is complex and thus cannot be related to alkaline phosphatase impairment.

Recent research and technological advancement have compelled scientists to think outside the box and develop a better understanding of neurodevelopmental disorders and their etiological bases (18-20) Given the complex nature of such disorders, any theoretical model designed to explain the disease pathogenesis will depend on advanced functional studies involving novel disease-gene identification, cohort studies, and available studies using animal models (21-24). Identification of such variants will help build a database that might lead to future therapeutic interventions and help conduct clinical trials (25,26). We revealed that homozygous loss-of-function variants in *PIGO* cause hyperphosphatemia with impaired intellectual development syndrome-2. Furthermore, novel variant identification for rare genetic disorders and making a database will help add such variants to the newborn screening program. In addition, preimplantation genetic testing for aneuploidies, noninvasive prenatal testing, and PGT-M can be employed for parents wishing to have future pregnancies (27-30). Identification of

additional families and functional studies are required to understand the cellular role of PIGO associated with neurodegeneration.

Conclusion

In conclusion, we have detected a novel homozygous variant in the *PIGO* gene in an affected individual having mild epileptic encephalopathy, along with slightly increased serum alkaline phosphatase levels and decreased CD16 expression but normal CD59 and CD24 expression. Furthermore, we suggest a genotype-phenotype correlation concerning the association between the location of the identified variant in the transmembrane domain and milder clinical phenotypes.

List of Abbreviations

ExAC The Exome Aggregation Consortium
PIGO Phosphatidylinositol glycan anchor biosynthesis class O

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None.

Declaration of conflicting interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Consent for publication

The consent for publication for this case was obtained from the parents.

Ethical approval

Ethical approval was granted by the Institutional Research Board of the University of Education, Lahore, Pakistan (UE/S&T/2019/222).

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