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Developmental and Epileptic Encephalopathy due to Biallelic Pathogenic Variants in *PIGM*

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ABSTRACT

Objective: *PIGM* encodes a critical enzyme in the glycosylphosphatidylinositol (GPI)-anchor biosynthesis pathway. While promoter-region mutations in *PIGM* have been associated with a relatively mild phenotype characterized by portal vein thrombosis and absence seizures, recent evidence suggests that coding-region mutations result in a more severe multisystemic disorder.

Methods: Whole-exome sequencing reanalysis was performed in a patient with early-onset developmental and epileptic encephalopathy, and subsequent matchmaking identified another patient with a similar phenotype. The pathogenicity of the variant was evaluated through multiple functional assays. To further delineate the genotypic and phenotypic spectrum of *PIGM*, we reviewed all patients reported to date with *PIGM* variants.

Results: We describe two unrelated patients, both carrying the same homozygous missense variant in *PIGM* (c.1001A > C, p.Gln334Pro), presenting with early-onset developmental and epileptic encephalopathy, profound neurodevelopmental impairment, multi-organ involvement, and distinctive neuroimaging findings including hypomyelination. Both patients died in infancy due to super-refractory status epilepticus. Treatment with sodium phenylbutyrate was attempted in one patient without clinical benefit. Flow cytometry revealed partial GPI-anchor deficiency. Comparative analysis with previously reported cases highlights a potential genotype–phenotype correlation between coding region variants and disease severity.

Interpretation: Our findings establish *PIGM* as a causative gene of early-onset developmental and epileptic encephalopathy and expand the clinical and radiological spectrum of *PIGM* deficiency to include hypomyelination and prenatal onset. This study underscores the importance of including *PIGM* in the differential diagnosis of developmental and epileptic encephalopathy and leukoencephalopathies and provides further insight into the molecular mechanisms underlying phenotypic variability of GPI-anchor disorders.

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1 | Introduction

Glycosylphosphatidylinositol-anchored proteins (GPI-APs) are a diverse group of membrane proteins tethered to the cell surface via glycosylphosphatidylinositol (GPI) anchors. This posttranslational modification, essential for proper protein localization and function, is mediated by a complex biosynthetic pathway involving over 30 genes, including the PIG and PGAP families [1, 2]. GPI-APs play critical roles in embryonic development, neurogenesis, signal transduction, and immune response. Pathogenic biallelic variants in some of these genes encoding components of the GPI-anchor biosynthesis pathway result in glycosylphosphatidylinositol biosynthesis defects (GPIBDs), a group of rare disorders characterized by early-onset, often severe, neurodevelopmental and systemic manifestations, including global developmental delay, epilepsy, and multisystemic involvement.

Among these genes, *PIGM* (MIM 610273) encodes the enzyme responsible for the initial mannosylation step of the GPI-anchor, a crucial event in the synthesis of the trimannosyl core of the GPI membrane anchor [3]. Until recently, most pathogenic variants in *PIGM* were restricted to a hypomorphic promoter mutation associated with a distinct clinical syndrome encompassing portal and cerebral vein thrombosis, refractory absence seizures, and macrocephaly [4, 5]. However, a recent report identified the first patients harboring biallelic coding region variants in *PIGM*, revealing a broader and more severe phenotypic spectrum, including profound developmental delay, drug-resistant epilepsy, and variable multisystem involvement [6–8].

In this article, we expand the phenotypic and genotypic spectrum of *PIGM*-related disorders by describing two unrelated patients carrying the same biallelic pathogenic variant in the *PIGM* coding region. Both individuals present with severe developmental and epileptic encephalopathy accompanied by neurological and systemic features. Our findings support the emerging evidence of a genotype–phenotype correlation in *PIGM*-associated GPIBDs and highlight the need for clinical awareness of this underdiagnosed condition.

2 | Materials and Methods

2.1 | Genetic Studies and Variant Assessment

Genomic DNA was extracted from whole blood after informed consent for DNA analysis was provided by the family. Whole exome sequencing (Kit xGen Exome Panel v2.0, IDT; NovaSeq6000, Illumina), arrayCGH (ogt 020045, CytoSure Constitutional v3 8×60K), karyotype (standard cytogenetic protocols, using G-banding staining, 30 metaphases were evaluated) and mitochondrial DNA sequencing (NEBNext Ultra II DNA library prep kit, Illumina) was done and a subsequent WES reanalysis at Vall d'Hebron Genetics Department. Variant segregation was performed by Sanger sequencing. Variant interpretation has been done according to ACMG guidelines [9].

2.2 | Patient Recruitment

We sought genotypic and phenotypic information from further patients with probable pathogenic *PIGM* variants (following *in silico* criteria), identified via Varsome [10], and an individual with a similar phenotype carrying the same homozygous variant was identified (patient 2). We reviewed clinical information related to neurodevelopment, multiple neurological manifestations, dysmorphology, and systemic features, neuroimaging, and any other tests. Written informed consent for genetic testing and publication was obtained from patients' legal guardians.

2.3 | Flow Cytometry

2.3.1 | Sample Collection and Preparation

Peripheral blood samples were collected in EDTA tubes and processed within 24 h to ensure optimal antigen stability. In parallel, a peripheral blood sample from a healthy donor was processed under the same conditions to serve as an internal control.

2.3.2 | Antibody Staining

Whole blood samples were directly stained with fluorochrome-conjugated monoclonal antibodies targeting GPI-anchored proteins. The antibody panel included CD55-FITC (Clone: JS11KSC2.3), CD24-PE (Clone: ALB9), and CD16-PC7 (Clone: 3G8) (all from Beckman Coulter, Brea, CA, USA). A volume of 100 µL of whole blood was incubated with the antibody mixture for 15 min at room temperature in the dark. After incubation, red blood cells were lysed using a commercial lysis buffer (Beckman Coulter, Brea, CA, USA) according to the manufacturer's protocol. The samples were then washed with phosphate-buffered saline (PBS) and resuspended in a staining buffer and analyzed using a DxFlex flow cytometer (Beckman Coulter, Brea, CA, USA). Acquisition was performed on at least 50,000 events, gating granulocyte subsets based on forward scatter (FSC) and side scatter (SSC) parameters. Mean fluorescence intensity (MFI) values for each marker (CD55, CD24, and CD16) were quantified using Kaluza Analysis Software (Beckman Coulter, Brea, CA, USA).

2.4 | Functional Analysis

The HEK293 *PIGM* knockout cell line was generated by the Cas9/CRISPR system. Cells were transiently transfected with vectors carrying different promoter driven wild type or mutant *PIGM*: a strong aSR promoter (pME EB-*PIGM*-FLAG-GST) or a weak TATA-box promoter (pTA-*PIGM*) using Lipofectamin 2000 (Thermo Fisher Scientific). Restoration of FLAER, CD59, and DAF expression levels was assessed 2 days later. Cells were stained with FLAER (fluorescent-labeled inactive toxin aerolysin), mouse anti-DAF (IA10), and CD59 (5H8) antibody, followed by a PE-conjugated anti-mouse IgG antibody and analyzed with flow-cytometer (MACSQuant Analyzer; Miltenyi

Biotec, Bergisch Gladbach, Germany) using Flowjo software (Tommy Digital, Tokyo, Japan). Immunoblotting was also performed, after 2 days of transfection, in order to assess the *PIGM* levels in cells using an anti-GST antibody. *PIGM*-band intensities were quantified with Image Gauge software and were normalized for band intensities of endogenous GAPDH and for transfection efficiencies determined by luciferase activities in the transfected cells.

2.5 | Structure Protein Model

The structure protein model used was the AF-Q9H3S5-F1-v4 predicted by AlphaFold Protein Structure Database [11, 12]. Predicted structure features from Uniprot database [13] were used to localize the different variants reported in *PIGM* patients.

3 | Results

3.1 | Patient 1

The proband is an 11-month-old boy with severe developmental and epileptic encephalopathy.

He was the first child of healthy, consanguineous parents (first cousins), conceived by in vitro fertilization. Transient polyhydramnios was detected at 31 weeks of gestation, with no other concerns during pregnancy. He was born at term via cesarean section due to arrested labor and fetal tachycardia. At birth, he showed signs of mild hypoxic-ischemic encephalopathy (HIE) and a first MRI showed mild diffuse white matter changes.

The patient showed very early signs of a severe developmental encephalopathy and a multisystemic disease, with clinical features nonattributable to the perinatal insult. Seizures began at 3 months of age, presenting as focal tonic and/or clonic seizures and cyanotic events. Neurological evaluation revealed progressive microcephaly, profound global developmental delay, including absent head control, minimal social interaction, and no purposeful visual or motor responses. A hyperkinetic movement disorder was present, alongside irritability and disturbed sleep-wake cycles, partially managed with levomepromazine. He also suffered severe sensorineural hearing loss and visual impairment, with absent fixation and abnormal ocular tracking. Additional findings included hepatomegaly and a cutaneous pigmentation disorder.

Epilepsy remained refractory with intractable daily seizures despite multiple antiseizure medications (including valproate, levetiracetam, lacosamide, oxcarbazepine, rufinamide, pyridoxine, perampanel, topiramate, brivaracetam, and ketogenic diet), often requiring daily rescue midazolam. Starting at 8 months, he began to suffer from recurrent suprarrefractory *status epilepticus*, for which he needed to be admitted to the PICU on different occasions. He received treatment with continuous ketamine infusion and high-dose steroids, with transient improvement of his status.

The initial EEG showed multifocal and bilateral epileptiform discharges with diffuse background slowing. From 8 months of age onward, all EEGs demonstrated bilateral overlapping focal epileptic seizures, alternating between hemispheres, occupying more than 50% of the EEG recording and consistent with *status epilepticus* (Figure 1).

A brain MRI at 11 months old showed diffuse supratentorial hypomyelination, prominent frontotemporal subarachnoid spaces, signs of left-sided hippocampal sclerosis, and bilateral involvement of the central pontine tegmental tract (Figure 2). MRS was normal.

An extensive metabolic workup was performed, with normal results. After a thorough genetic analysis, a *PIGM* pathogenic variant was detected (see below at “Genetic data” subheading) and a therapeutic trial with phenylbutyrate was carried out, which did not bring any positive effects on epileptic seizures in the advanced stage of the patient’s disease.

Considering the disease’s progression, severity, poor prognosis, and the absence of therapeutic options, a multidisciplinary pediatric palliative care approach was initiated in agreement with the family. After progressive neurological deterioration and refractory *status epilepticus*, palliative sedation was done, and the patient passed away at the age of 11 months.

3.2 | Patient 2

The second proband was a 4-year-old male with early-onset, severe developmental and epileptic encephalopathy.

He was the first live-born child of consanguineous parents (coefficient of inbreeding $f = 1/16$), following a previous pregnancy terminated due to a polymalformative syndrome that was not genetically investigated but included lissencephaly, intrauterine growth restriction, facial dysmorphism with bilateral cleft lip and palate, and pyelocaliceal dilatation.

The pregnancy was uneventful, although decreased fetal movements were noted compared to a sibling. He was delivered at term via vaginal delivery and presented with initial transient cyanosis that did not require resuscitation. Early clinical signs indicated severe encephalopathy. Psychomotor development was profoundly delayed, with head control achievement at 2 years of age, independent sitting at 3 years, and no walking acquirement. Language and comprehension were absent, and abnormal visual behavior and lack of social interaction were also noted.

Epileptic seizures began at 3 months of age as generalized clonic seizures, with initial good response to diazepam and phenobarbital. He later developed recurrent focal and generalized seizures, prompting multiple therapeutic changes, including sodium valproate, clonazepam, and levetiracetam, without achieving adequate seizure control. The clinical course progressed to drug-resistant epilepsy, characterized by epileptic spasms, myoclonic seizures, and recurrent episodes of Todd’s paresis.

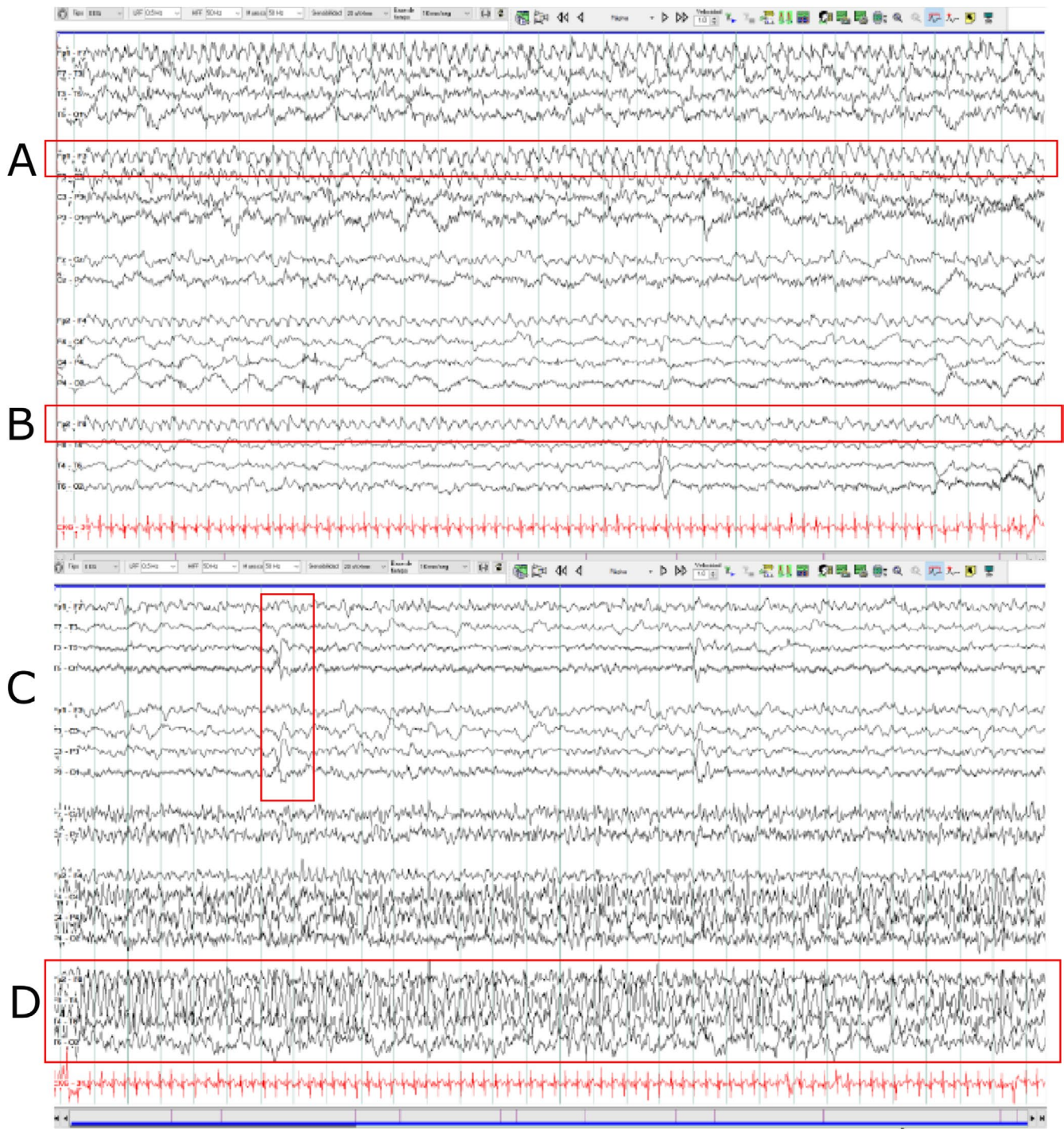


FIGURE 1 | EEG at 8 months demonstrates multifocal and bilateral epileptiform discharges with diffuse background slowing. The upper panel illustrates two independent focal electrographic seizures with overlapping onsets: A left frontal focal electrographic seizure (A) and a right parieto-central focal electrographic seizure (B). Right temporal focal interictal epileptiform discharges are also observed. The lower panel shows left temporo-parietal focal interictal epileptiform discharges (C) together with the progression of a right hemispheric seizure (D). Overall, bilateral overlapping focal epileptic seizures alternate between hemispheres, occupying more than 50% of the EEG recording and are consistent with *status epilepticus*. This electrographic pattern and the associated clinical status were persistent during the last 3 months of life.

Neurological examination revealed progressive microcephaly (-3 SD), facial dysmorphism (left divergent strabismus, horizontal nystagmus), hepatomegaly, poor social interaction, absent visual tracking, severe axial hypotonia, and spastic tetraparesis with joint contractures of the limbs.

Serial EEGs showed severely abnormal activity, including multifocal epileptiform discharges and hypersarrhythmia. Brain MRI at 10 months of age revealed progressive cortical and subcortical atrophy, more prominent in the left hemisphere, with subcortical white matter hyperintensities on FLAIR and

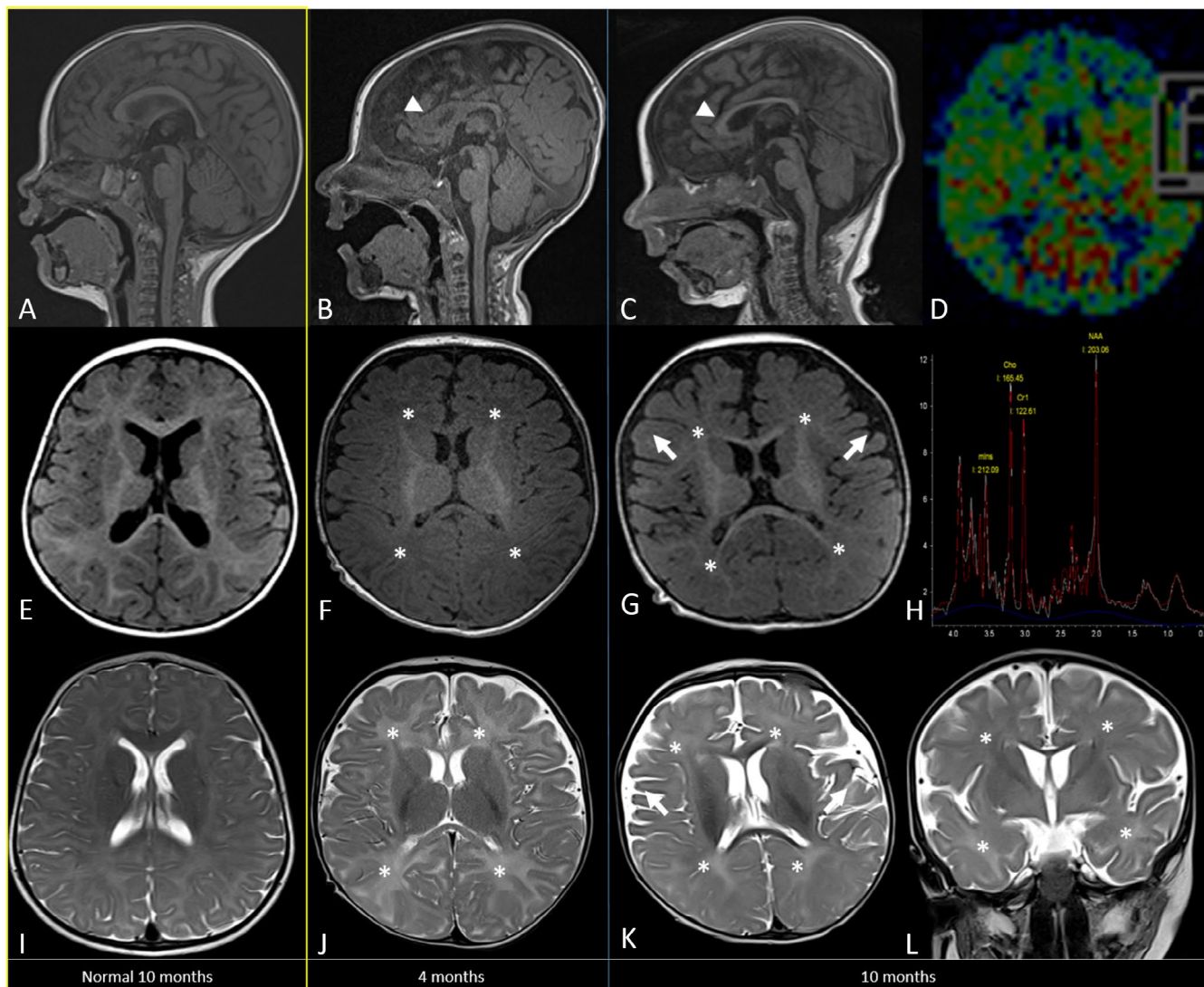


FIGURE 2 | Sequential MRI assessment of myelination in Patient 1 at 4 and 10 months of age, compared with age-matched normative myelination atlases. (A) Midline sagittal T1-weighted image from a 10-month-old normal myelination atlas. (B) Midline sagittal T1-weighted image from the patient at 4 months. (C) Midline sagittal T1-weighted image from the patient at 10 months. (D) Arterial Spin Labeling (ASL) perfusion map from the patient at 10 months. (E) Axial T1-weighted image at the level of the internal capsule from the atlas. (F) Axial T1-weighted image at the level of the internal capsule from the patient at 4 months. (G) Axial T1-weighted image at the same level from the patient at 10 months. (H) Short echo-time MR spectroscopy map from the patient at 10 months. (I) Axial T2-weighted image at the level of the internal capsule from the atlas. (J) Axial T2-weighted image at the same level from the patient at 4 months. (K) Axial T2-weighted image at the same level from the patient at 10 months. (L) Coronal T2-weighted image at the level of the frontal horns and third ventricle from the patient at 10 months. Arrowheads in (B) and (C) highlight delayed myelination of the corpus callosum, particularly involving the genu. Asterisks in (F), (G), (J), (K), and (L) denote hypomyelination of the internal capsule. Additionally, images (G) and (K) show volume loss and prominent subarachnoid spaces in the frontotemporal regions. Both the ASL map (D) and MR spectroscopy (H) demonstrate normal findings.

T2-weighted sequences. Magnetic resonance spectroscopy was normal. Fundoscopy showed optic atrophy. Abdominal ultrasound demonstrated homogeneous hepatomegaly with patent portal and suprahepatic veins. Metabolic investigations were unremarkable.

The clinical picture was dominated by profound developmental impairment, medically refractory epilepsy, and multisystemic involvement. The disease followed a progressive course, culminating in the patient's death at 4 years of age.

3.2.1 | Genetic Data

Initial genetic testing of patient 1, including Whole Exome Sequencing, arrayCGH, karyotyping, and mitochondrial DNA sequencing, did not reveal conclusive pathogenic variants at the first analysis. However, a subsequent reanalysis identified a homozygous missense variant in the *PIGM* gene (NM_145167.3, c.1001A > C, p.Gln334Pro). This variant was validated by Sanger sequencing, and segregation analysis demonstrated that both parents are carriers. The variant has an extremely low allele

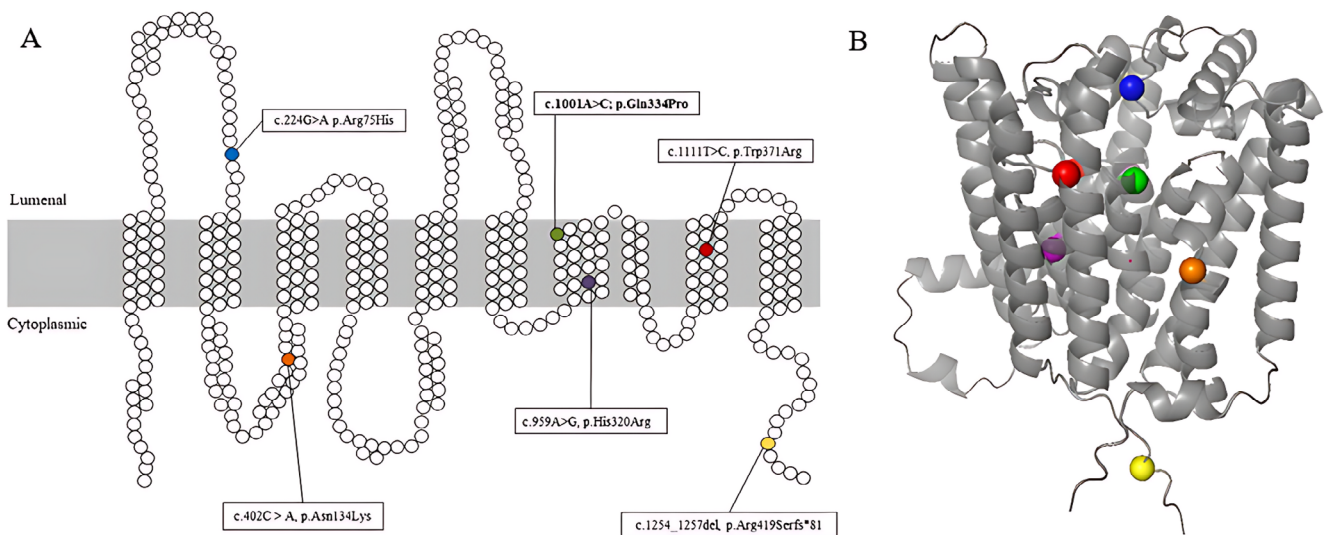


FIGURE 3 | *PIGM* protein structure representation. A: Structural domains and localization within the endoplasmic reticulum membrane. B: Predicted 3D conformation of *PIGM* (AF-Q9H3S5-F1-v4). Variants are color-coded as follows: Blue, c.224G>A (p.Arg75His), homozygous in patients 8–10; yellow, c.1254_1257del (p.Arg419Serfs*81), found in compound heterozygosity with red, c.1111T>C (p.Trp371Arg), in patient 12, and with orange, c.402C>A (p.Asn134Lys), in patient 13; violet, c.959A>G (p.His320Arg), homozygous in patient 11; green, c.1001A>C (p.Gln334Pro), homozygous in patients 14 and 15 (present study).

frequency of approximately 0.0000037 (3 alleles out of 1,614,054) in the gnomAD v4.1.0 database, with no homozygous individuals reported in the control population. In silico pathogenicity prediction tools suggest a potentially damaging effect, with scores from PolyPhen [14] (1.00), CADD [15] (29.4), and REVEL [16] (0.65). However, the variant is currently classified as a variant of uncertain significance (VOUS) in the ClinVar database (ID: VCV001055901.8).

For patient 2, standard karyotype analysis revealed a normal 46, XY result. Genetic analysis by whole-exome sequencing identified the same homozygous missense variant in *PIGM*. Sanger sequencing confirmed that both parents were heterozygous carriers.

According to the ACMG guidelines, considering the information stated above, together with the effect observed in functional studies detailed below and the finding of two different patients harboring the same variant and with a similar phenotype that goes in line with the one already reported, variant c.1001A>C, p.Gln334Pro can be considered Likely Pathogenic (PS3_moderate, PS4_supporting, PM2, PM3, PP3).

Regarding protein structure, mapping of the six reported coding variants onto the *PIGM* protein 3D structure (AF-Q9H3S5-F1-v4) reveals that the missense variant c.224G>A p.Arg75His is located in the luminal domain and does not appear to form any specific structural feature. The variant c.402C>A, p.Asn134Lys, observed in compound heterozygosity with another variant, is positioned in the cytoplasmic domain and appears to connect two consecutive α -helices. The frameshift variant c.1254_1257del (p.Arg419Serfs*81) also localizes to the cytoplasmic domain, affecting the C-terminal region of the protein and resulting in an extended protein compared to the wild-type. Finally, the c.1111T>C; p.Trp371Arg variant, which is identified in compound heterozygosity with p.Arg419Serfs*81, as well as the homozygous variants c.959A>G; p.His320Arg and

c.1001A>C; p.Gln334Pro, are situated within the transmembrane helices (Figure 3).

3.2.2 | Flow Cytometry

Flow cytometry analysis of granulocytes from patient 1 revealed a partial deficiency of GPI-anchored proteins. The MFI values showed a marked decrease for CD24 and CD16 in the patient sample, indicating impaired GPI anchoring of these proteins. In contrast, CD55 expression was preserved, with fluorescence intensity levels similar to those observed in the control sample. The reduction in fluorescence intensity for CD24 and CD16 is evident in the histograms, where a distinct leftward shift is observed in the patient sample (Figure 4). This pattern suggests a selective deficiency rather than a global impairment of GPI-anchor biosynthesis, leading to variable surface expression of GPI-APs. The differential impact on specific proteins likely reflects distinct sensitivities to reduced GPI anchor availability or structural quality. Proteins such as CD24 and CD16 appear more dependent on optimal GPI anchor synthesis, whereas others like CD55 may be more stably expressed despite partial biosynthetic defects. These findings are consistent with a partial defect in GPI biosynthesis, such as that described in *PIGM* deficiency, and highlight the importance of assessing multiple GPI-APs when evaluating suspected GPI biosynthesis disorders.

3.2.3 | Functional Analysis

Transfection of HEK293 *PIGM* KO cells with strong promoter driven *PIGM*, *PIGM* variant protein expressed normally and restored the GPI-AP expression to the same level as wild type *PIGM*. However, when cells were transfected with weak promoter driven *PIGM*, the *PIGM* mutant failed to restore GPI-AP

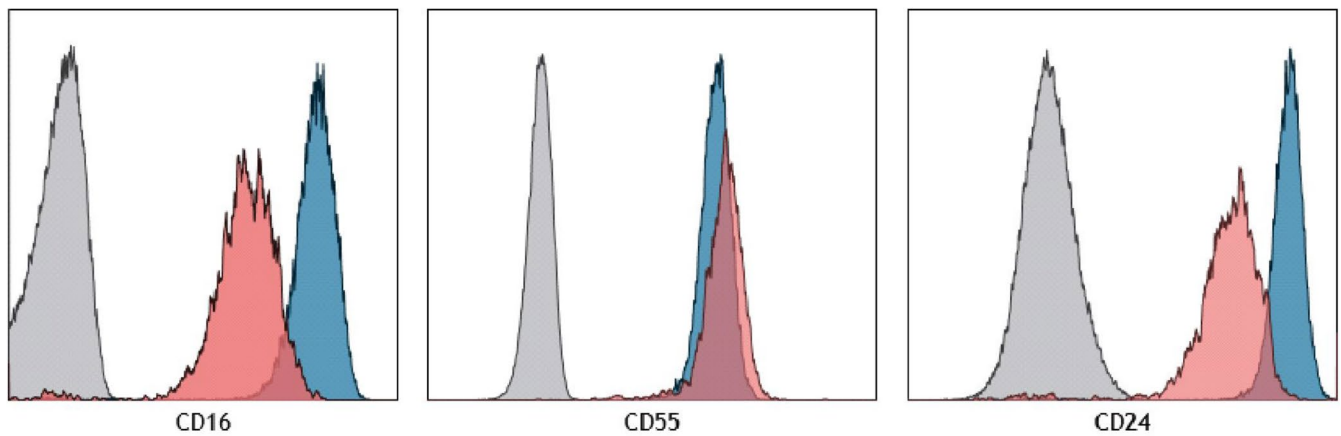


FIGURE 4 | Flow cytometry histograms of GPI-anchored proteins (CD16, CD55, and CD24) in granulocytes. Representative flow cytometry histograms comparing the fluorescence intensity of CD16, CD55, and CD24 between a healthy control and the patient sample. The gray histogram represents unstained cells (negative control), the blue histogram corresponds to the healthy control, and the red histogram represents the patient sample. A leftward shift in fluorescence intensity is observed for CD16 and CD24, indicating a reduced expression of these markers in the patient. In contrast, CD55 expression remains preserved, with no significant difference between the patient and the control. These findings suggest a partial GPI-anchor deficiency, selectively affecting CD16 and CD24 while maintaining CD55 expression.

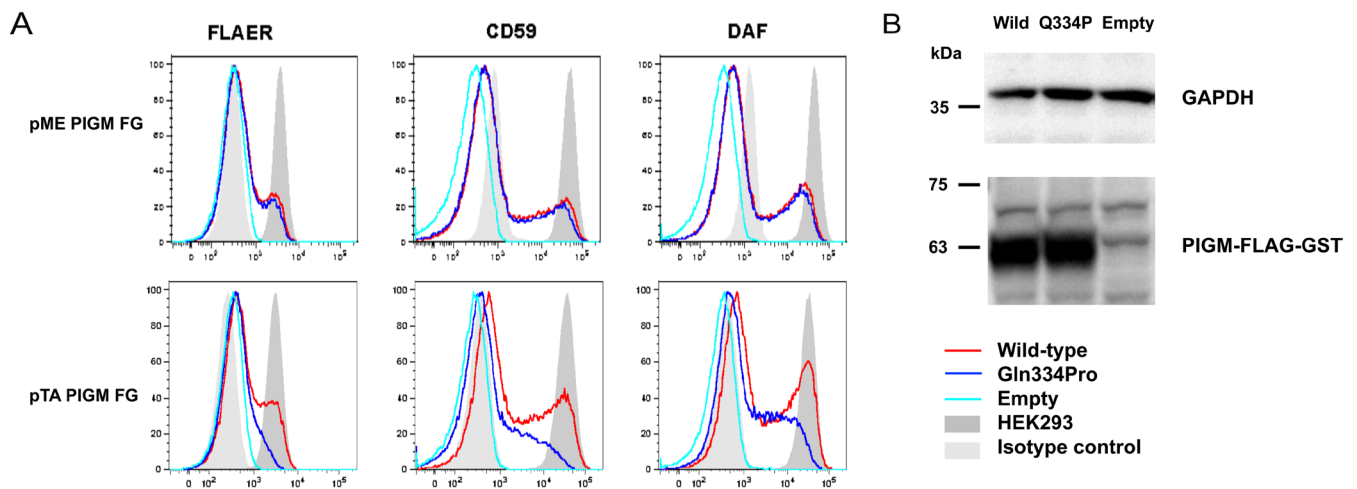


FIGURE 5 | Functional analysis of *PIGM* in HEK293 knockout cells generated by CRISPR/Cas9. (A) Flow cytometry analysis of DAF and CD59 cell-surface expression following transient transfection with constructs under promoters of different strengths. Expression of the p.Gln334Pro *PIGM* variant restored DAF and CD59 surface levels under the strong promoter (pME, upper panels). In contrast, the weak promoter (pTA) failed to support restoration, suggesting reduced activity of the altered *PIGM* (lower panels). (B) Immunoblot of cell lysates from cells transfected with the strong promoter (pME) showed, after 2 days, similar expression levels of wild-type and mutant *PIGM*. GAPDH was used to normalize protein levels.

expression to the same levels as the wild type *PIGM*, suggesting impaired activity of *PIGM* p.Gln334Pro (Figure 5).

4 | Discussion

In this work, we describe two new unrelated patients harboring the same homozygous variant in *PIGM* (c.1001A>C; p.Gln334Pro) presenting with early-onset severe developmental and epileptic encephalopathy and multisystemic disease with notable hypomyelination in the MRI.

To our knowledge, this is the fourth report of patients with autosomal recessive *PIGM* deficiency associated with coding region genetic variants. *PIGM* disease was initially described by Almeida et al. in 2006, reporting three patients from two

different families presenting with portal vein thrombosis and refractory absence seizures, attributed to biallelic variants in the gene promoter (c.-270C>G) [4]. The same group described an impressive clinical and biochemical improvement of one of these patients after initiating or while following treatment with sodium phenylbutyrate [17]. Some years later, Podeshkovskaya et al. expanded the cohort identifying four new patients with a similar phenotype along with thrombotic cerebral events [5]. It was not until 2024 that pathogenic variants within the coding region of *PIGM* were reported as causative of the disease [7]. From then on, six patients with different biallelic variants have been published (patients summary in Tables 1 and S1).

Two distinct types of biallelic pathogenic variants have been identified in the *PIGM* gene: promoter mutations (c.-270C>G) and mutations affecting the coding region. These two

TABLE 1 | Summary of clinical and genetic data from patients reported to date with *PIGM* genetic defects.

Patient ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Publication	Almeida et al. 2006				Pode-Shakked et al. 2019				Heimer et al. 2025				Nkolle et al. 2025		Current report
Patient group					Promotor variant				Coding variant						
Genetic variant	-270C>G	-270C>G	-270C>G	-270C>G	-270C>G	-270C>G	-270C>G	c.224G>A p.Arg75His	c.224G>A p.Arg75His	c.224G>A p.Arg75His	c.959A>G, p.His320Arg	c.1254_1257del, p.Arg49Serfs*81 + *81 + c.1111T>C, p.Trp371Arg	c.1254_1257del, p.Arg49Serfs*81 + c.402C>A, p.Asn134Lys	c.1001A>C; p.Gln34Pro	c.1001A>C; p.Gln34Pro
Epidemiology	Family	A	B		C		D		E		F	G	H	I	J
Gender		F	M	M	F	F	M	M	F	M	m	F	F	M	M
Age at last follow-up or Death	14years	4years (Death to sepsis)	NA	7years (death to complications of cirrhosis)	NA	NA	4years	20years	18years	13years	9 weeks (death; disease progression)	5weeks (death to sepsis)	7years	11 months (Death, disease progression)	4years (Death; disease progression)
Clinical manifestations	Head circumference	NA/NR	NA/NR	Macrocephaly	Macrocephaly	Progressive macrocephaly	Macrocephaly	Normal	Normal	Normal	Macrocephaly	NA/NR	NA/NR	Progressive microcephaly (HC -42.5cm) (-3DS)(11 m)	Progressive microcephaly (-3DS at 4years)
Seizure types	Absence sz (4years), Tonic-clonic sz (5 years), Non-Convulsive Status Epilepticus (9years)	Absence sz, atonic sz (3 years)	Absence sz	Absence sz	Atypical absence sz	No seizures	No seizures (4years)	Febril sz beginning at 1year, absence sz from early childhood and later GTC sz	Febril sz beginning at 1year, myoclonic absence sz early childhood and later GTC sz (2 years)	Febril sz beginning at 1year, myoclonic absence sz early childhood and later GTC sz	No seizures	Myoclonic seizures	Episodes of absence, head drop, atonic and generalized tonic-clonic seizures	Refractory epilepsy with multiple seizure types: variable onset of focal motor sz, tonic sz, autonomic sz, tendency to prolonged seizures with ultra-refractory myoclonic seizures until death	Refractory epilepsy with multiple seizure types: variable onset of focal motor sz, tonic sz, autonomic sz, tendency to generalized sz, epileptic spasms, with ultra-refractory myoclonic seizures until death
Seizure onset	4years	3years	5years	3.5 years	3-4years	No seizures	No seizures	1year	1year	1year	NA/NR	4days	7 months	3 months	3 months
Developmental status	Progressive deterioration	No	No	NA/NR	Mild-moderate GDD	Mild-moderate GDD	NA/NR	ID and learning disabilities - Special Education	Borderline-mild ID	Moderate ID	Not valuable	Not valuable	Moderate GDD/ID	Profound	Profound
Other neurological manifestations	Coma (5y) following varicella bleed and hepatic encephalopathy; Progressive encephalopathy and epilepsy. Improvement after sodium phenylbutyrate.	NA/NR	NA/NR	NA/NR	Increased peripheral tone, brisk tendon reflexes, cerebellar dysfunction	Initially normal, posterior sequelae of ACM stroke at 16 months	Hemiplegia and aphasia due to right ACM stroke	NA/NR	NA/NR	NA/NR	NA/NR	NA/NR	Not reported	Hypotonic tetraparesis, hyperkinetic movement disorder with chorea	Severe axial hypotonia and spastic tetraparesis with joint contractures of the limbs.

(Continues)

TABLE 1 | (Continued)

Patient ID		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Additional tests	Brain MRI	Normal, MRA and MRV normal	Normal, MRA and MRV normal	Normal, MRA and MRV normal	Prominent cerebellar atrophy, multiple diffuse cerebral infarcts and an old left cerebellar hemorrhage. Normal MRA	Mild cerebral and cerebellar atrophy, an old right parietal infarct and an old right frontal hemorrhage. Normal MRA	7 months—Chronic venous thrombosis in the left Sylvian fissure area. 16 months—Acute large cerebral infarct of right MCA. Normal MRA	Mild cerebral atrophy and a few small frontal lesions with hypertensive signal on FLAIR suggestive of micro-ischemic events	NA/NR	NA/NR	NA/NR	1 week. Severe multicystic encephalomalacia, cerebellar hypoplasia, atrophy of the basal ganglia and thalami, thin brainstem, severe ventriculomegaly, and bilateral multifocal hemorrhages. 8 weeks—worsening multicystic encephalomalacia and severe cerebral atrophy	10 days—Multiple subdural hematoma (bilateral occipital, unilateral frontal and falx cerebri), enlarged frontal and temporal pericerebral spaces and, enlarged sylvian valley	4 years—Brain atrophy	Neonatal and 3 months: mild bilateral parietal and periventricular subcortical white matter hyperintensity. 10 months: delayed diffuse hypomyelination predominantly supratentorial, with prominence of subarachnoid spaces in both frontotemporal convexities.	Cortical and subcortical atrophy (yo)/Left hemispheric cerebral atrophy, sub-cortical white matter hyperintensity on FLAIR and T2 with normal MRS
	EEG	Frequent multifocal and generalized epileptiform discharges and a massive photoconvulsive response, consistent with nonconvulsive status epilepticus	Normal	Normal	NA/NR	Periods of high amplitude generalized background delta activity and irregular 2.5–3 Hz general spike wave bursts ranging from 2 to 8 s	Decreased amplitudes on the right that subsequently improved, but without generalized discharges or the high amplitude delta activity suggestive of encephalopathy	Normal	Progressive background slowing	Interictal epileptiform activity with posterior dominance and ictal 3 per sec generalized spike wave activity	Generalized epileptiform discharges with posterior dominance.	Asymmetric burst suppression pattern suggestive of a diffuse encephalopathy	Diffuse slow waves	Mild-moderate active generalized epileptiform discharges and slow cerebral activity for age	Normal at birth. Progressive worsening of EEG pattern with increasing multifocal epileptiform activity and progressive slowing of background activity. Persistent status epilepticus during the last 3 months	Progressive worsening of EEG pattern with increasing multifocal epileptiform activity and progressive slowing of background activity. Persistent status epilepticus during the last 3 months
Treatments	Flux cytometry	Granulocytes—CD24 severely reduced. Erythrocytes, granulocytes and platelets—CD59 partially reduced	Granulocytes: CD24 severely reduced. Fibroblasts—CD59 reduced	Not performed	Granulocytes—CD14, CD24, CD59 decreased. No clear or sustained changes after initiation of sodium phenylbutyrate. Fibroblasts—CD59/CD87 severely reduced	Granulocytes—CD14, CD24, CD59 globally decreased. Transient improvement after sodium phenylbutyrate	Granulocytes, monocytes and erythrocytes—Normal expression of CD59, CD24, CD14 and FLAER	Granulocytes, monocytes and erythrocytes—Normal expression of CD59, CD24, CD14 and FLAER	Granulocytes, monocytes and erythrocytes—Normal expression of CD59, CD24, CD14 and FLAER	Drug resistant epilepsy (not specified de drugs)	Drug resistant epilepsy (not specified de drugs)	Not seizures	Not performed	Low level of CD16 and moderate decrease for total GF anchor (FLAER method) and CD55. CD59 profile was normal.	Granulocytes—low level of CD16, 24 and normal level of CD55	Not performed
	Antiseizure medication	VPA, LTG, LEV, TPM, CLB	NA/NR	None	Drug resistant epilepsy (not specified de drugs)	Drug resistant epilepsy (not specified de drugs)	No seizures	No seizures	Drug resistant epilepsy (not specified de drugs)	Drug resistant epilepsy (not specified de drugs)	Drug resistant epilepsy (not specified de drugs)	Not seizures	Phenytoin and DZP	KD, LEV, VPA, TPM, NZP, B6	VPA, LEV, B6, CLB, DZP, PB, LEV, CZP, PER, RFN (transient improvement), OXC, LCM, DXM, ketamine, KD (3:1), MDZ, propofol	VPA, LEV, B6, CLB, DZP, PB, LEV, CZP, PER, RFN (transient improvement), OXC, LCM, DXM, ketamine, KD (3:1), MDZ, propofol

Abbreviations: ASD, autism spectrum disorder; B6, piridoxine; CLB, clobazam; CZP, clonazepam; DXM, dexametazone; DZP, diazepam; F, female; GDD, global developmental delay; ID, intellectual disability; KD, ketogenic diet; LEV, levetiracetam; LTG, lamotrigine; M, male; NA/NR, No available/No reported; NZP, nitrazepam; OXC, oxcarbazepine; PB, phenobarbital; RFN, rufinamide; Sz, seizures; TPM, topiramate; VPA, Valproate.

categories are associated with markedly different clinical phenotypes. Individuals carrying promoter mutations typically present with a relatively restricted phenotype characterized by portal vein thrombosis, refractory absence seizures, and, in some cases, cerebral thrombotic events [4, 5]. In contrast, patients with coding region mutations exhibit a more severe multisystemic disorder, including early-onset epilepsy, global developmental delay, and multi-organ involvement. To date, this dual mutational mechanism, which correlates with distinct phenotypic spectra, has only been reported in one other gene within the glycosylphosphatidylinositol (GPI) anchor biosynthesis pathway, *PIGY* [18, 19]. In these cases, children with homozygous coding mutations suffered from a more severe and multisystemic disease resulting in early death, whereas the phenotype of the homozygous promoter mutation was limited to global developmental delay and microcephaly [18]. Individuals with pathogenic variants affecting the coding regions of *PIGM* present with a clinical phenotype that closely resembles other inherited disorders of GPI-anchor biosynthesis [19, 20]. Core features include early-onset epilepsy, intellectual disability, visual impairment, and characteristic craniofacial dysmorphisms. Additionally, these patients may present with varying degrees of multi-organ involvement, reflecting the systemic nature of GPI deficiencies. In contrast, portal vein thrombosis, a hallmark of individuals who are homozygous for the c.-270C>G *PIGM* promoter variant, is an atypical manifestation among GPI-related disorders and has rarely been reported outside this specific genetic context.

Polyhydramnios during pregnancy, as observed in one of our patients (Patient 1), appears to be a common prenatal finding among individuals with *PIGM* mutations (Table S1), along with other antenatal abnormalities. Perinatal complications such as difficulties during the transition to extrauterine life, observed in both of our patients, and preterm birth are also frequently reported. Notably, these findings have only been described in patients harboring mutations in the coding region of *PIGM*, and not in those with promoter mutations. This pattern suggests a prenatal onset of disease and a more severe phenotype, more closely resembling the one seen in other GPIopathies.

Our two patients harboring the c.1001A>C variant exhibited a severe and early-onset neurological and multisystemic disease, similar to other disorders of GPI-anchor biosynthesis. Neurological features included refractory epilepsy beginning in early infancy and profound global neurodevelopmental delay, with minimal or absent psychomotor acquisition. Clinically, they presented with hypotonic tetraparesis, and one of them exhibited a hyperkinetic movement disorder consistent with chorea. Additionally, both showed hepatomegaly without signs of portal hypertension. Distinctive features observed in at least one of the patients included a non-ichthyosis skin pigmentation disorder, visual impairment, severe congenital sensorineural hearing loss, and progressive dysphagia. At the time of molecular diagnosis (10 months of age), Patient 1 was treated with sodium phenylbutyrate; however, the treatment was ineffective. Unfortunately, both patients died prematurely due to uncontrollable epileptic encephalopathy associated with disease progression.

A striking feature in our two patients was the presence of developmental and epileptic encephalopathy, a phenotype that

has not been well documented or clearly specified in previously reported *PIGM* cases. Both patients presented with very early-onset epilepsy at 3 months of age, characterized by a tendency toward super-refractory focal *status epilepticus*, and showed no sustained response to any antiepileptic medication. They experienced multiple types of daily seizures, particularly toward the end of the disease course. Similar DEE phenotypes have already been described in other genes involved in the GPI-anchor biosynthesis pathway, including *PIGA* [21], *PIGK* [22], *PIGO* [23], *PIGP* [24] and *PIGQ* [25]. These findings underscore the importance of considering *PIGM* as a causative gene for early-onset DEE.

4.1 | Neuroimaging and Hypomyelination in *PIGM* Patients

Neuroimaging in patients with *PIGM* mutations shows a wide range of findings. While initial cases with promoter variants had normal brain MRIs [4], later reports described progressive supratentorial and infratentorial atrophy along with diffuse thrombotic-ischemic lesions [5]. This suggests a broader vascular-neurodegenerative spectrum than previously recognized. Imaging data from patients with coding-region variants are limited, but cerebral atrophy appears to be a consistent feature, as seen in Patient 2. More severe abnormalities have also been reported, including multicystic encephalomalacia [7] and subdural hematomas [8]. Notably, Patient 1 exhibited hypomyelination, a feature not previously associated with *PIGM* deficiency, though described in some GPI-anchor biosynthesis disorders (GPIopathies). Collectively, these observations suggest that the neuroradiological spectrum of *PIGM*-related disease is wider and potentially more aggressive than previously recognized.

The mechanism responsible for the observed hypomyelination is not fully delineated. We hypothesize that it could be consistent with the concept of secondary hypomyelination proposed by Wolf et al. [26]. According to this model, normal axon–glia signaling is required for the onset and progression of myelination; early-onset neuronal dysfunction can disturb this interplay and secondarily hamper myelin formation. Similar cascades have been invoked for *SPTAN1*, *HSPD1* and *UFM1* encephalopathies. Hypomyelination has been documented in some patients with *PIGA*-related encephalopathy [27], underscoring that GPI-anchor biosynthesis defects can affect white-matter development. Although hypomyelination is not universal across GPIopathies, its occurrence in both *PIGA* and *PIGM* deficiencies suggests shared downstream mechanisms, which might be related to impaired neuronal signaling or altered complement regulation that warrant deeper exploration.

Recognition of hypomyelination within the neuroradiological repertoire of *PIGM* deficiency can be relevant in clinical practice. First, clinicians should remain alert to early white-matter changes when evaluating children with unexplained developmental epileptic encephalopathies. Second, longitudinal MRI follow-up becomes essential for monitoring disease trajectory and assessing potential therapeutic interventions. Finally, these findings strengthen the rationale for considering the sequencing of *PIGM* and other GPI-anchor genes when hypomyelination co-occurs with severe developmental and epileptic encephalopathy.

Also, progressive microcephaly (-3 SD), likely secondary to cerebral atrophy, was observed in both of our cases, an atypical finding not previously described in *PIGM*-related disorders. Conversely, four patients reported in the literature (three patients with the promoter variant c.-270 [5]) and one with the c.959A>G; p.(His320Arg) variant [7] presented with static or progressive macrocephaly despite concurrent cerebral atrophy, acquired thrombotic cerebral complications or multicystic encephalomalacia.

Similarly to what has been observed in previously reported patients with *PIG*-pathies, flow cytometry analysis in granulocytes from our Patient 1 demonstrated reduced expression of CD16 and CD24, with normal levels of CD55. These findings suggest a partial GPI-anchor deficiency. Most published patients with biallelic mutations in *PIGM*, except for the three individuals with a milder phenotype carrying the c.224G>A (p.Arg75His) variant, have shown abnormal flow cytometry results (Table 1). Drawing more definitive conclusions is challenging due to the small number of cases, the use of different tissues (granulocytes, fibroblasts), and the variability in markers analyzed, such as CD16, CD24, CD55, and CD59. Moreover, these findings overlap with those observed in other *PIG*-pathies [2, 28]. Although not a specific marker, flow cytometry abnormalities may serve as a useful biomarker in the diagnostic evaluation of *PIG*-pathies.

Finally, regarding therapeutic approaches, Patient 1 was treated with sodium phenylbutyrate following the protocol published by Almeida et al. (2007); however, the treatment was ineffective. It is important to note that the therapeutic trial was initiated during a terminal phase of the disease, when the patient was in a state of super-refractory status epilepticus, and he passed away a few days later due to advanced disease progression. To date, no other patients with coding region mutations have been reported to undergo a therapeutic trial with phenylbutyrate. The rationale for using sodium phenylbutyrate lies in its role as a histone deacetylase (HDAC) inhibitor intended to enhance *PIGM* transcription. In cases involving promoter region mutations, disruption of the SP1 binding site compromises transcriptional activation, resulting in decreased gene expression due to reduced histone acetylation. HDAC inhibition in these patients can increase histone acetylation and partially restore *PIGM* expression. HDAC inhibition in these patients can increase histone acetylation and partially restore *PIGM* expression [17]. However, this therapeutic mechanism is not applicable in cases with coding region mutations, where the pathogenic effect stems from altered protein structure rather than transcriptional dysregulation. Although early treatment has shown encouraging outcomes in initial reports of individuals with promoter mutations, according to this hypothesis, earlier administration of sodium phenylbutyrate would likely not have improved the clinical course in our patient with a coding region mutation.

4.2 | Phenotype–Genotype Correlation

In this study, we present a comparative analysis of patients carrying different *PIGM* mutations, aiming to elucidate potential genotype–phenotype correlations. Our findings suggest that both the location of the variants within the protein domains

and their predicted structural consequences may underlie the observed variability in clinical severity.

Three patients from the same family harboring the homozygous p.(Arg75His) missense variant exhibited a comparatively milder phenotype [6]. This variant is situated within the luminal region of the protein and does not map onto any defined secondary structural element in the current 3D models. It is plausible that while this substitution affects protein function, it does not critically disrupt the folding or membrane anchoring of the protein, thus allowing partial residual activity.

In contrast, five patients carrying other variants demonstrated a more severe clinical presentation. Among these, two patients were homozygous for missense variants predicted to impact transmembrane helices (p.His320Arg and p.Gln334Pro). The disruption of these helical domains likely exerts a substantial effect on the global conformation of the protein, potentially impairing its correct insertion or stability within the membrane. This hypothesis is further supported by the identification of compound heterozygous variants in two patients: in one case, the combination of a truncating frameshift mutation (p.Arg419Serfs*81) with a missense variant in a transmembrane helix (p.Trp371Arg); in the other, a missense variant located in the cytoplasmic domain (p.Asn134Lys).

Interestingly, although p.(Asn134Lys) has been annotated in the literature as residing within the cytoplasmic domain, 3D structural data indicate that it contributes to linking two contiguous alpha helices, suggesting that this substitution could destabilize local folding or impair interactions essential for *PIGM* transferase activity. On the other hand, the frameshift variant p.(Arg419Serfs*81) is predicted to elongate the protein by introducing an aberrant C-terminal extension. This modification could interfere with the proper termination of translation or alter the folding landscape of the protein, further compromising function.

Taken together, these observations support the notion that mutations potentially affecting transmembrane helices formation or introducing frameshift elongations are associated with more severe phenotypes, likely due to their pronounced impact on protein conformation and membrane localization. Conversely, missense variants located outside defined structural elements, such as p.(Arg75His), may partially preserve protein function, resulting in milder clinical manifestations. This genotype–phenotype correlation underscores the importance of considering both the positional context and the structural consequences of *PIGM* mutations when interpreting pathogenicity and predicting disease severity.

In conclusion, our report expands the phenotypic and genotypic spectrum of *PIGM*-related disease by describing two unrelated patients harboring a homozygous coding-region variant (c.1001A>C; p.Gln334Pro) associated with early-onset developmental and epileptic encephalopathy, multisystem involvement, and hypomyelination. These findings establish DEE as a prominent and previously underrecognized clinical hallmark of *PIGM* deficiency due to coding-region mutations, aligning it with other severe GPI-anchor biosynthesis disorders. Furthermore, the presence of hypomyelination suggests that *PIGM* should

be considered in the differential diagnosis of genetic leukoencephalopathies with early-onset epilepsy. Structural analysis of pathogenic variants supports a genotype–phenotype correlation, whereby mutations affecting transmembrane domains or introducing frameshift elongations are associated with more severe disease. Finally, our data emphasize the need for early genetic diagnosis, comprehensive neuroimaging follow-up, and further exploration of targeted therapies in this devastating disorder.

Author Contributions

Júlia Sala-Coromina, Anna Marcé-Grau, Barbara Masotto, and Alfons Macaya contributed to the conception and design of the study. Júlia Sala-Coromina, Anna Marcé-Grau, Barbara Masotto, RB, and Yasmina Elaribi designed the analysis. Júlia Sala-Coromina, Marta Codina, Lamia BenJemaa, Yasmina Elaribi, Mónica Martínez-Gallo, Angel Sanchez-Montañez, Irene Valenzuela, and Yoshiko Murakami performed the analysis. Anna Marcé-Grau and Alfons Macaya supervised the study. Júlia Sala-Coromina and Anna Marcé-Grau drafted the manuscript. All authors read, revised, and approved the manuscript.

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The authors have nothing to report.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** Additional clinical and genetic features from patients reported to date with *PIGM* genetic defects. F: Female. M: Male. NA/NR: No Available/No Reported. Sz: seizures. ASD: Autism Spectrum Disorder. Y: Years. Mo: Months.