

Expanding the Genotypic and Phenotypic Spectrum of PGAP1 Deficiency: Clinical and Functional Insights from 15 Patients

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Abstract

Glycosylphosphatidylinositol-anchored proteins (GPI-APs) play essential roles in neuronal development, synaptic organisation, and signal transduction. Defects in GPI-anchor biosynthesis and remodelling cause rare multisystem neurodevelopmental disorders, among which post-GPI attachment to proteins 1 (PGAP1) deficiency is particularly uncommon. PGAP1 encodes an inositol deacylase that removes an acyl chain from the inositol ring of the GPI anchor, an early remodelling step required for proper trafficking and membrane localisation of GPI-APs. Loss of PGAP1 function leads to structurally abnormal GPI-APs and impaired cellular processes. Fewer than 40 affected individuals have been described, limiting understanding of the clinical spectrum.

We systematically characterised 15 individuals with PGAP1 deficiency from 11 unrelated families across 11 countries using standardised phenotyping, molecular analysis, and functional assays. Eight previously unreported pathogenic variants were identified across six exons, including splice-site, truncating, and missense variants, with exon 2 containing the largest cluster. Individuals ranged from 8 months to 23 years. Intellectual disability and global motor delay were the most common features (93.3%), and only 40.0% achieved independent ambulation. Seizures occurred in 66.7% of cases. Scoliosis was observed in 40.0%, and peripheral neuropathy in 33.3%, the latter representing an under-recognised aspect of the disorder. Functional studies showed resistance to phosphatidylinositol-specific phospholipase C cleavage of GPI-APs, confirming impaired GPI-anchor remodelling.

These findings expand the genotypic and phenotypic landscape of PGAP1 deficiency, highlight neuropathy as part of the disease spectrum, and emphasise the need for genetic testing and functional validation for accurate diagnosis and management.

Introduction

Glycosylphosphatidylinositol-anchored proteins (GPI-APs) are essential for numerous cellular functions, particularly within the central nervous system (CNS), where they influence neuronal activity and synaptic organization. These proteins are tethered to the cell membrane via GPI anchors; complex glycolipids synthesized in the endoplasmic reticulum. (1) Beyond the CNS, GPI-APs also play key roles in physiological processes such as reproduction, immune response and metabolism underscoring their broader systemic importance. (2)–(3)

Mutations in genes responsible for GPI anchor biosynthesis are frequently implicated in CNS disorders. The phosphatidylinositol glycan (PIG) and post-GPI attachment to proteins (PGAP) gene families are central to this biosynthetic pathway. Variants in genes such as *PIGV*, *PIGO*, *PGAP2*, *PIGW*, and *PGAP3* have been linked to hypophosphatasia with mental retardation syndrome (HPMRS), (4-8) emphasizing the role of GPI-APs in neurodevelopment. Disruptions in this pathway have also been associated with psychiatric conditions including schizophrenia, bipolar disorder, depression, and panic disorder, likely due to their impact on neuronal connectivity and synaptic function. (9-11)

Among these genes, *post-GPI attachment to proteins 1* (*PGAP1*) encodes a critical inositol deacylase involved in GPI anchor remodelling. This enzyme removes an acyl chain from the inositol ring, a necessary step for the correct structural conformation, membrane localization, and function of GPI-APs (Figure 1).(12-15) Mutations in *PGAP1* (OMIM *611655) lead to defective GPI-APs that are improperly anchored to the cell surface, resulting in a range of neurodevelopmental abnormalities (“Neurodevelopmental disorder with dysmorphic features, spasticity, and brain abnormalities”, OMIM #615802). Individuals with *PGAP1* mutations commonly exhibit intellectual disability, seizures, and delayed motor development. (16-22) These phenotypes highlight the essential role of *PGAP1* in maintaining neuronal function and synaptic integrity.

Diagnosing *PGAP1*-related disorders in clinical settings is often challenging due to their heterogeneous and non-specific presentation and can be confused with other disorders due to overlapping symptoms, especially within the scope of Congenital Disorders of Glycosylation (CDG). Affected individuals typically present in early infancy with developmental delays, varying levels of intellectual disability, and neurological features such as hypotonia and seizures. Clinical examination may suggest CNS involvement, but findings are often non-localizing. Neuroimaging, particularly magnetic resonance imaging (MRI), may reveal nonspecific anomalies such as delayed myelination or corpus callosum thinning, indicative of diffuse neurodevelopmental disturbance.(18)

Genetic testing, particularly whole exome sequencing (WES), plays a pivotal role in establishing a definitive diagnosis. Identifying pathogenic *PGAP1* variants confirms the diagnosis and delineates the molecular aetiology. Advances in high-throughput sequencing have facilitated the detection of GPI-pathway gene mutations, including *PGAP1*, in individuals with unexplained neurological syndromes. Nevertheless, *PGAP1* variants remain exceedingly rare, with only seven studies, as of the time of writing, reporting a total of 11 affected individuals. Most reported variants are unique to individual families, with recurrence observed primarily in siblings.

This study aims to enhance our understanding of *PGAP1*-related disorders by reporting novel pathogenic variants and systematically correlating these with detailed clinical phenotypes. By analysing 15 individuals from eleven unrelated families diagnosed through combined clinical, radiological, and molecular assessment, we expand the phenotypic and genotypic spectrum of *PGAP1* mutations. Our findings aim to support improved diagnostic precision and inform future management strategies for affected individuals, grounded in a deeper comprehension of the pathology's natural history.

Case Description

2.1 Subjects

This study characterizes a cohort of individuals with *PGAP1*-related disorders from multiple familial lines across eleven countries, highlighting the diverse clinical and genetic manifestations associated with the condition. The cohort comprised 15 individuals, aged 8 months to 23 years, diagnosed based on clinical

presentation, physical examination, laboratory findings, and confirmed genetic analysis. The participants, representing both sexes and various ethnic backgrounds, exhibited a spectrum of symptoms consistent with GPI-anchor deficiencies, with intellectual disability and motor developmental delay being the most common features.

In all cases, parents reported no prior family history of similar disorders, suggesting that *PGAP1* mutations occurred de novo or were inherited in an autosomal recessive manner. Detailed family histories and medical records were reviewed to identify any recurring phenotypes or related conditions, and pedigrees were constructed to illustrate familial relationships and inheritance patterns.

Each patient's clinical records were reviewed and findings on clinical examination including sensory and motor function, muscle tone, and reflexes were recorded. The results of neuroimaging and EEG recordings (where available) were also reviewed. Diagnostic neuroimaging, specifically brain MRI, was performed in several cases and frequently revealed structural abnormalities such as thinning of the corpus callosum. Electroencephalography (EEG) were used to look for epileptiform activity.

Results of developmental assessments were reviewed to quantify the degree of intellectual disability and characterize developmental delay patterns. These evaluations provided critical insights into the functional impairments associated with *PGAP1* mutations. Together with the clinical and genetic data from each individual, they contribute to a more comprehensive understanding of the phenotypic spectrum and natural history of this ultra-rare disorder.

Methods

Clinical

To assess the global burden and aiming to find genotype–phenotype correlations associated with *PGAP1* mutations, 17 new individuals were identified through international research collaborations, of whom 15 were included in this study. A dedicated registry was established, and data was collected on confirmed pathogenic or likely pathogenic variants in the *PGAP1* gene, provided by contributing clinicians, geneticists and families. Molecular data included variant type and copy number information.

Parents/legal guardians provided informed consent, and participating clinicians or patient families completed a standardized phenotyping questionnaire. This included information on facial dysmorphism, microcephaly, intellectual disability, speech development, motor milestones (e.g., independent walking), hypotonia, neuropathies, feeding and respiratory issues, stereotypic movements, ophthalmologic findings, seizures, EEG abnormalities, neuroimaging, and other relevant clinical features.

Genetic and Sequencing Analyses

Genetic diagnoses were established using clinical exome sequencing and, where available, RNA sequencing. Exome sequencing was performed at accredited diagnostic sequencing facilities following standard capture-based library preparation and next-generation sequencing protocols, as previously

described(23). Variant calling and annotation were conducted using validated clinical bioinformatics pipelines at the respective laboratories.

For selected individuals, transcriptome analysis was performed using RNA sequencing from patient-derived samples to assess the impact of PGAP1 variants on transcript structure and expression, following established protocols(24). RNA-seq data were analysed using standard alignment, quantification, and splice-aware methods at the contributing sequencing facilities. Identified variants were subsequently integrated with clinical data and *in silico* predictions for downstream interpretation.

Molecular Studies

Functional assessments of *PGAP1* activity were performed. These analyses followed previously established methodologies.¹⁶ The functional status of GPI-anchored proteins (GPI-APs) was evaluated by assessing cellular sensitivity to bacterial phosphatidylinositol-specific phospholipase C (PI-PLC), which normally cleaves the protein portion of GPI-APs (e.g., CD55, CD59, CD73, or uPAR) from the cell surface.

In *PGAP1*-deficient cells, this cleavage is impaired, resulting in resistance to PI-PLC treatment, a key diagnostic indicator due to the lack of specific PGAP1 antibodies. To quantify PGAP1 activity, fibroblasts were incubated with 50 µL of 1 U/mL PI-PLC (P6466 Invitrogen) in Opti-MEM I solution (Invitrogen) at 37°C for 1 to 1.5 hours. Post-treatment, cells were stained for GPI-AP markers (CD55, CD59, CD73) and analysed by flow cytometry. This enabled quantitative comparison of GPI-AP surface expression in patient versus control cells following PI-PLC treatment, providing a robust assessment of PGAP1 functionality.

PGAP1 deficient CHO cells (C10)(25) were transfected with the strong promoter SRα (pME) driven wild-type or mutant *PGAP1* and five days later, cells were treated with PIPLC and stained for GPI-AP markers (CD55, CD59, uPAR) and analysed by flow cytometry.

In silico Variant-Level Analysis

In addition to clinical characterization and registry data collection, molecular analyses were performed using *in silico* prediction tools and functional annotation software to assess the pathogenicity of each PGAP1 variant. Annotated genomic coordinates were aligned to transcript NM_024989.3. All reported variants were evaluated using Alamut Visual Plus v1.12 (SOPHiA GENETICS), which integrates multiple pathogenicity predictors (CADD, REVEL, MutationTaster, PolyPhen-2, SIFT), conservation metrics (phyloP), splice-site algorithms (MaxEnt, NNSPLICE, SSF), and ClinVar classification.

Variants were categorized into missense, nonsense, splice-site, or frameshift changes. For splice-site variants, disruptions to canonical donor/acceptor motifs and predicted changes in splice efficiency were examined. Frameshift and nonsense variants were assessed for potential to induce premature termination codons (PTCs) upstream of the nonsense-mediated decay (NMD) threshold. Variants were classified per ACMG guidelines and supported with transcript-level predictions when available. This

approach enabled the mechanistic interpretation of loss-of-function consequences for the majority of detected *PGAP1* variants.

Results

Genetic Analysis of *PGAP1* Variants

Sequencing of the *PGAP1* gene in 15 individuals revealed that 9/15 (60.0%) carried homozygous pathogenic variants, while 6/15 (37.5%) had compound heterozygous mutations. Most variants were private to a single family; however, the truncating combinations c.2199del p.(Leu735*) and c.2357_2358insTA p.(Ser787*) were each observed in two unrelated families (D-II and J-II). Siblings within the same family shared zygosity, consistent with autosomal recessive inheritance. In total, 11 unrelated families were represented in this cohort.

Among the detected variants, six were splice site mutations (see Supplementary 1 - Family A), two were missense and four were a frameshift or truncating variant. Four patients carried compound heterozygous mutations. Only one variant was shared between unrelated families (c.2199del p.(Leu735*) and c.2357_2358insTA p.(Ser787*)), underscoring the allelic heterogeneity of *PGAP1*-related disorders. Pedigree analyses (see Supplementary Figure 2 - Families A, B and C) demonstrated autosomal recessive inheritance patterns. Two families reported to be consanguineous.

Clinical Features and Summary

The clinical characteristics of all 15 newly identified individuals are summarized in Table 1, which also incorporates data from 11 previously published cases to support comparative analysis. The ages of the individuals at evaluation ranged from 8 months to 23 years, with nine females and six males included in the cohort.

This expanded case series integrates genotypic and phenotypic data from 15 new patients with *PGAP1* mutations and previously reported cases, bringing the total number of recorded cases to 27. Although the small sample size limits statistical power, this section discusses the observed phenotypic range, highlighting features that appear recurrent, rare, or previously unreported.

Physical abnormalities

Physical abnormalities, notably facial dysmorphisms, were observed in 11/15 (73.3%) of the newly reported *PGAP1* deficient patients under evaluation. These included distinctive features, such as arched eyebrow, synophrys (joining of the eyebrows), hypertelorism (widely spaced eyes), and prognathism (protruding jaw), prominent forehead, pronounced nose with characteristics such as a bulbous nasal tip and a high nasal bridge. Club foot was additionally noted in one *PGAP1*-related disorder patient, with a single patient also reporting shortened phalanges and clinodactyly of their fifth digit.

Beyond facial dysmorphisms, microcephaly was reported in 6/15 (42.9%) of the assessed *PGAP1*-related disorder patients, indicating incomplete brain development. For the first time, scoliosis was noted in 6/15 (40.0%), strabismus (and history of strabismus) in 5/15 (33.3%) and nystagmus in one of the *PGAP1*-deficient patients. It should be noted that scoliosis was often not assessed, likely as it has not been previously associated with *PGAP1* deficiencies (however, it has been associated with other GPI anchor protein disorders, such as *PIGT* disorder(26)).

Cognitive Function

Intellectual disability was diagnosed in 14/15 (93.3%) individuals and represents a core clinical feature of *PGAP1* deficiency. Intellectual disability is typically defined as an IQ < 70, with impairments in reasoning, problem-solving, learning, and adaptive functioning(27). Speech impairments were also reported in 13/15 (86.6%), ranging from delayed to absent verbal communication.

Motor Development and Function

Motor developmental delays were prominent symptoms observed in 14/15 (93.3%) of the patient cohort. In addition to the delays in motor development, the overall motor function was significantly compromised across the *PGAP1*-deficiency cohort. Self-feeding was documented in only 4 of 15 individuals (26.7 %), suggesting that most either lacked this ability or it was not reported. Of those four, three were able to eat semi-solid and solid foods, while one self-fed only with milk bottles. Difficulty biting hard foods was reported in 4/15 (26.7%) participants.

Furthermore, only 5/15 (33.3%) of the patients were able to walk independently, and 3/15 (20.0%) required some form of external support. This highlights the extensive impact of *PGAP1* gene mutations on basic motor functions and independence in daily activities.

Neuromuscular Findings

PGAP1 deficiency is known to cause spastic paraplegia type 47 (SPG47), which is classified as a neurodegenerative and neuromuscular disorder due to its prominent motor involvement. In our cohort, hypotonia and peripheral neuropathy were observed in 10/15 (66.7%) and 5/15 (33.3%) individuals, respectively. The presence of hypotonia and delayed gross motor milestones is consistent with previously reported cases of SPG47.

The finding of neuropathy in several patients represents a potentially novel observation within *PGAP1*-related disease, although similar features have been described in other GPI-anchor biosynthesis disorders. Where available, nerve conduction studies are being reviewed to further characterize this finding and confirm whether it reflects a true peripheral neuropathy or secondary motor impairment.

Sleep apnea was documented in one individual and may also reflect neuromuscular dysfunction within the spectrum of this disorder.

Neurological Manifestations

Seizures were observed in 10/15 (66.7%) individuals, consistent with the high prevalence of epilepsy previously reported in *PGAP1* deficiency. EEG data were available for seven patients, with abnormal findings in five (5/7). The EEG abnormalities were described as non-specific background slowing and multifocal epileptiform discharges, although detailed reports were not available for all cases.

Stereotypic movements, including repetitive hand or body movements, were noted in 8/15 (53.3%) patients. While these movements are not considered neuromuscular in origin, they are frequently reported among individuals with neurodevelopmental disorders and may reflect cortico-striatal circuit dysfunction.

Together, these findings highlight the broad neurological spectrum associated with *PGAP1* deficiency, encompassing epileptic activity, motor stereotypies, and global developmental impairment.

Brain Anatomy Assessment (MRI)

MRI findings were available for seven individuals. Among them, 3/7 (42.8%) exhibited abnormalities such as a thin or partially absent corpus callosum, translating to 13.3% (2/15) across the entire cohort. These findings are consistent with global neurodevelopmental impairment and defective interhemispheric connectivity.

In silico Characterization

In our cohort of fourteen individuals, we identified a diverse set of *PGAP1* pathogenic variants (Figure 2), spanning splice-site, frameshift/truncating, in-frame deletion, and missense classes, observed in both homozygous and compound-heterozygous configurations. Six individuals carried canonical splice-site variants: c.1221-3A>G (A-II:1), c.2286+5G>A (B-II:1-3) and c.2286+5G>T (I-II). Splice prediction (MaxEnt/NNSPLICE/SSF) indicates substantial weakening or loss of canonical donor/acceptor recognition (see Supplementary Table 1). Experimental confirmation of splicing disruption caused by the c.1221-3A>G variant is shown in Supplementary Figure 1, using blood- and fibroblast-derived mRNA from the proband in Family A (published as case A034-*PGAP1* in Bournazos et al 2022). In addition, one homozygous junctional deletion, c.1081_1089+3del (G-II), spans the exon-intron boundary and is predicted to abolish donor recognition across algorithms; the submitted protein annotation for this allele is p.(Val360_Asn363del), consistent with exon skipping/in-frame loss.

We also identified three individuals with homozygous frameshift or truncating variants: c.289del (p.Ser97Valfs30) (F-II), c.2024delT (p.Leu681Argfs4) (H-II), and C.1367A>G (p.Glu456Gly) (E-II). Each of these introduced premature stop codons well upstream of the nonsense-mediated decay (NMD) threshold, supporting a mechanism of transcript degradation. The variant, c.1367A>G affects a highly conserved residue and is predicted to be damaging by PolyPhen (0.999), SIFT, and MutationTaster.

Compound heterozygosity was observed in four families. Two siblings (C-II:1 and C-II:2) carried splice-donor variants c.1089+1G>A and c.1089+5delG, both affecting the intron-9 donor and consistent with exon skipping. Consistent with loss of function, bulk RNA-seq from Family C showed reduced PGAP1 transcript abundance in both siblings, with junction reads dominated by canonical splice sites at the coverage displayed (Supplementary Figure 4). Two unrelated individuals (D-II and J-II) harboured the recurrent truncating combination c.2199del (p.Leu735*) and c.2357_2358insTA (p.Ser787*/p.Arg786Serfs*35), which introduces premature termination codons. Additionally, one individual from Family K (K-II) carried a truncating–missense combination in trans: c.2357_2358insTA (p.Ser787*) and c.2241T>G (p.Cys747Trp).

Collectively, these variants span multiple pathogenic mechanisms, including splice disruption, loss-of-function through premature truncation, and potential structural compromise of PGAP1 enzymatic activity. The intolerance of *PGAP1* to loss-of-function alleles (gnomAD LOEUF 0.42; pLI ~0) further supports the pathogenic nature of the identified variants. Validation in tested individuals revealed resistance to PI-PLC cleavage of GPI-anchored markers, confirming disrupted GPI-anchor remodelling and providing cellular evidence for PGAP1 deficiency.

A detailed breakdown of each variant’s predicted impact, conservation, and functional annotation is provided in Supplementary Table 1.

In terms of reported pathogenicity, as of 31 Oct 2025, only a subset of the variants observed in our cohort have ClinVar entries. The splice-donor variant c.1089+1G>A is catalogued as Likely pathogenic (VCV000986862; single-submitter; criteria provided). The intronic c.2286+5G>A is listed in ClinVar as a variant of uncertain significance in the context of *PGAP1*-related neurodevelopmental disorder, per ClinVar Miner’s index of ClinVar submissions. The truncating insertion c.2357_2358insTA appears among pathogenic PGAP1 variants in ClinVar-derived listings (reported as p.Arg786fs), indicating at least one pathogenic ClinVar submission for this allele. For the remaining cohort alleles: c.1221–3A>G, c.2286+5G>T, c.1089+5delG, c.2199del, c.1367A>G (p.Glu456Gly), c.289del (p.Ser97Valfs*30), c.1081_1089+3del (p.Val360_Asn363del) and c.2024delT (p.Leu681Argfs*4), we found no dedicated ClinVar records at the time of review; where ClinVar is silent or uncertain, our classification rests on RNA/functional data (e.g., exon-23 skipping confirmed for c.2286+5G>T), predicted loss-of-function, and segregation within families, consistent with prior PGAP1 literature.

PGAP1 functional analysis

In the current report we show functional analysis of PGAP1 activity of two patients. As previously described, bacterial phosphatidylinositol-specific phospholipase C (PI-PLC) typically cleaves GPI-anchored proteins such as CD59, CD55, CD73, or uPAR from the cell surface. In *PGAP1*-deficient cells, this cleavage is impaired due to abnormal GPI structure, rendering cells resistant to PI-PLC.

In our assays, fibroblasts from affected individual were treated with PI-PLC, followed by flow cytometry using antibodies against CD59, CD55, CD73. In the tested patients, cell surface levels of GPI-APs

remained high post-treatment, resembling untreated control cells, whereas healthy control cells exhibited marked reduction in GPI-AP expression after PI-PLC treatment (Supplementary Figure 3, Family A). These results confirm impaired PGAP1 function in the affected individual. As fibroblasts from the Family E patient were not available, a functional assay was performed using PGAP1-deficient CHO cells. Cells transfected with Glu456Gly-mutant PGAP1 cDNA remained resistant to PI-PLC treatment, whereas cells transfected with wild-type PGAP1 were sensitive, indicating that the mutant PGAP1 protein exhibits severely reduced enzymatic activity (Supplementary Figure 3, Family E).

Discussion

This study significantly expands the clinical and molecular understanding of *PGAP1*-related disorders by characterizing 15 new individuals carrying twelve unique pathogenic variants, including both novel and previously unreported combinations of splice-site, frameshift, and missense changes(16-18, 20-22). The phenotypic features observed in this cohort, such as global developmental delay, intellectual disability, epilepsy, and facial dysmorphism, are consistent with previously reported presentations of *PGAP1*-related disease. Notably, peripheral neuropathy was identified in several individuals, representing a potentially novel feature of *PGAP1* deficiency. While neuropathy has not been reported in prior *PGAP1* cases, it has been observed in other GPI-anchor biosynthesis disorders (e.g., *PIGL*, *PIGO*, *PIGT*, *PIGA*, *PIGB*), suggesting that peripheral nerve involvement may occur within this broader disease spectrum(28-31). These observations broaden the clinical spectrum of *PGAP1* deficiency.

Our findings also support prior reports of intra-familial variability in clinical expression, likely influenced by additional genetic or epigenetic modifiers¹⁵. Neuroimaging in several patients revealed corpus callosum thinning and reduced white matter volume, consistent with earlier studies and reinforcing the role of *PGAP1* in CNS myelination and interhemispheric connectivity(16, 17, 32). Additionally, homozygous *PGAP1* variants have been implicated in hereditary spastic paraplegia and non-syndromic intellectual disability(20, 33), suggesting a broader phenotypic continuum than previously recognized. However, the pathogenic relevance of some reported alleles remains to be validated.

From a molecular standpoint, the variants identified in our cohort overwhelmingly support a loss-of-function mechanism. The most informative were the homozygous or compound-heterozygous splice-disrupting alleles. For c.1221-3A>G (acceptor -3) and c.2286+5G>A / c.2286+5G>T (donor +5), *in silico* predictions indicate marked weakening of splice-site strength, and exon 23 skipping was experimentally confirmed for c.2286+5G>T. The compound-heterozygous pair c.1089+1G>A and c.1089+5delG targets the same donor region in intron 9 and is expected to ablate proper splicing. The homozygous c.1081_1089+3del, spanning an exon-intron boundary, was predicted to eliminate canonical donor recognition by all tested algorithms, further implicating aberrant splicing.

Loss-of-function coding variants included homozygous c.289del (p.Ser97Valfs*30) and c.2024delT (p.Leu681Argfs*4), as well as the compound-heterozygous truncating combination c.2199del with c.2357_2358insTA (identified in two unrelated individuals, D-II and J-II); each introduces a premature

termination codon well upstream of the final exon–exon junction (c.2580), consistent with nonsense-mediated decay. The additional compound-heterozygous genotype in Family K combined truncating c.2357_2358insTA with missense c.2241T>G (p.Cys747Trp). The sole homozygous missense variant in the cohort was c.1367A>G (p.Tyr456Cys), alters a highly conserved tyrosine and was uniformly predicted to be damaging by PolyPhen-2, SIFT, MutationTaster, and phyloP.

Functional validation of the variants was achieved through flow cytometry in all available patient-derived samples. Cells displayed resistance to PI-PLC-mediated cleavage of GPI-anchored proteins (e.g., CD55, CD59), confirming defective inositol deacylation and disruption of GPI-anchor remodeling, hallmarks of PGAP1 deficiency¹⁵. Studies on B lymphoblastoid and CHO cells expressing PGAP1 variants have previously demonstrated complete loss of enzymatic activity¹⁵, consistent with our findings.

No recurrent positional mutational hotspots were apparent. Variants were largely private; however, we observed a recurrent truncating combination (c.2199del + c.2357_2358insTA) in two unrelated individuals, and the insertion c.2357_2358insTA occurred in three individuals from three families (two in trans with c.2199del, one with c.2241T>G), underscoring allelic heterogeneity with occasional recurrence. The predominance of private variants likely reflects the ultra-rare nature of PGAP1-related disease and/or broad vulnerability of *PGAP1* to deleterious missense and truncating changes. This is consistent with the gene's high evolutionary conservation ($\approx$ 98% identity across primates and strong conservation in vertebrates), underscoring its essential biological function.

From a therapeutic perspective, *PGAP1*-related disorders represent strong candidates for gene replacement strategies, given their autosomal recessive inheritance and primary LoF etiology. The compact size of PGAP1 cDNA is compatible with AAV-mediated delivery, and the disease's cellular signature (PI-PLC resistance) offers a quantifiable biomarker for preclinical optimization. In addition to gene therapy, pharmacological chaperones, ER stress modulators, or small molecules enhancing GPI-anchor trafficking may provide adjunctive or standalone treatments, particularly in cases involving hypomorphic missense variants.

In summary, although the marked phenotypic variability among reported cases precluded definitive genotype–phenotype correlations, this study advances the clinical and mechanistic understanding of *PGAP1* deficiency by integrating detailed phenotypic analysis with computational and functional validation. These findings help lay the groundwork for better diagnostics and targeted therapies for this rare neurodevelopmental disorder.

Declarations

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethical Approval

This study was approved by the Sydney Children's Hospitals Network Human Research Ethics Committee (SCHN HREC), Sydney, Australia (protocol 2024/ETH02025).

Author Contributions

FH, OTWO, WG and LL conceived and designed the study. FH and OTWO collated collaborator datasets, FH carried out the in silico variant analysis and wrote the manuscript. Molecular and functional experiments were performed by OTWO, AB, IV, DH, TK and YM. Clinical data and patient management details were contributed by ZA, RW, FS, JS, HS, IV, JA, TL, ALK, RB and TBH. JM assisted FH with manuscript revisions. WG and LL provided supervision. All authors critically reviewed the manuscript, contributed to subsequent revisions and approved the final version.

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Table

Table 1 is available in the Supplementary Files section.

Figures

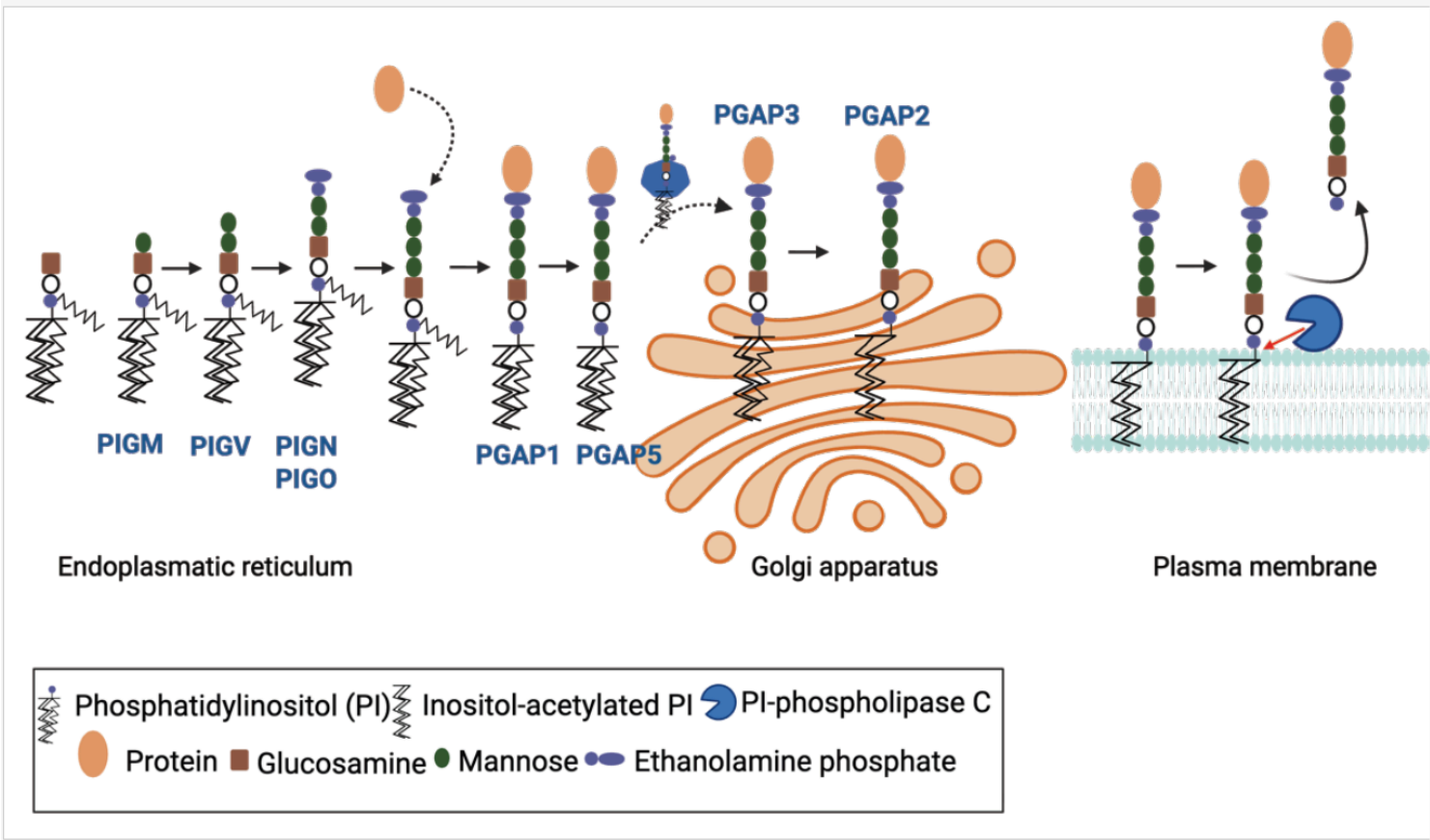


Figure 1

Overview of GPI anchor biosynthesis and remodeling of GPI-APs. GPI anchor synthesis is initiated in the endoplasmic reticulum (ER) through the stepwise assembly of the GPI precursor, followed by attachment of the protein via the GPI transamidase complex. After protein attachment, PGAP1 catalyzes deacylation of the inositol-linked acyl chain in the ER, representing the first remodeling step required for proper GPI maturation. In the absence of PGAP1 activity, inositol-acylated GPI-AP intermediates accumulate, impairing subsequent remodeling and intracellular trafficking. During passage through the Golgi apparatus, PGAP5 removes the ethanolamine phosphate side chain from the second mannose,

and PGAP3 catalyzes deacylation of the unsaturated fatty acid at the sn-2 position of the phosphatidylinositol moiety. This is followed by PGAP2-mediated reacylation, generating a mature, stable GPI anchor competent for efficient plasma membrane expression. Fully remodeled GPI-APs are transported to the cell surface, where they may be released by phosphatidylinositol-specific phospholipase C (PI-PLC) cleavage under experimental conditions.

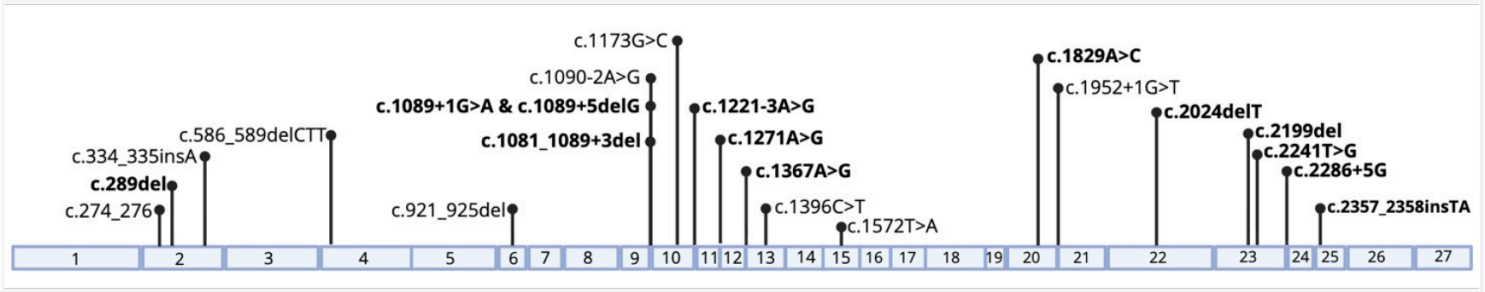


Figure 2

Schematic distribution of PGAP1 variants across the 27 coding exons of transcript NM_024989.3. This diagram maps both novel (bolded) and previously reported PGAP1 variants along the coding exons of the canonical transcript NM_024989.3. Variants include homozygous and compound heterozygous splice site mutations, frameshift deletions/insertions, nonsense, and missense changes. The figure illustrates the broad distribution of pathogenic variants across the 27 exons, without evidence of mutational clustering, consistent with the gene’s intolerance to loss-of-function mutations across its entire coding region.

Supplementary Files

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- [SupplementaryMaterialPGAP1.docx](#)
- [Table1.xlsx](#)