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Biallelic variants in *PIGN* cause Fryns syndrome, multiple congenital anomalies-hypotonia-seizures syndrome, and neurologic phenotypes: A genotype–phenotype correlation study

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Conflict of Interest

The authors declare no conflict of interest.

Ethics Declaration

Patients 2, 3-I, 3-II, 4, 5-I, and 6 were participants of the Deciphering Developmental Disorders study that has UK Research Ethics Committee (REC) approval (10/H0305/83 granted by the Cambridge South REC and GEN/284/12 granted by the Republic of Ireland REC). Patients 1, 5-II, and 7 to 19 were sequenced in clinical diagnostic laboratories. Informed consent was obtained from the legal guardian before genetic sequencing, and *PIGN* variants were identified in routine clinical analysis. Subsequently for all patients, written informed consent for publication of detailed clinical information and *PIGN* variant data was obtained from the legal guardians by the case-acquiring clinician.

Additional Information

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Abstract

Purpose: Biallelic *PIGN* variants have been described in Fryns syndrome, multiple congenital anomalies-hypotonia-seizure syndrome (MCAHS), and neurologic phenotypes. The full spectrum of clinical manifestations in relation to the genotypes is yet to be reported.

Methods: Genotype and phenotype data were collated and analyzed for 61 biallelic *PIGN* cases: 21 new and 40 previously published cases. Functional analysis was performed for 2 recurrent variants (c.2679C>G p.Ser893Arg and c.932T>G p.Leu311Trp).

Results: Biallelic-truncating variants were detected in 16 patients—10 with Fryns syndrome, 1 with MCAHS1, 2 with Fryns syndrome/MCAHS1, and 3 with neurologic phenotype. There was an increased risk of prenatal or neonatal death within this group (6 deaths were in utero or within 2 months of life; 6 pregnancies were terminated). Incidence of polyhydramnios, congenital anomalies (eg, diaphragmatic hernia), and dysmorphism was significantly increased. Biallelic missense or mixed genotype were reported in the remaining 45 cases—32 showed a neurologic phenotype and 12 had MCAHS1. No cases of diaphragmatic hernia or abdominal wall defects were seen in this group except patient 1 in which we found the missense variant p.Ser893Arg to result in functionally null alleles, suggesting the possibility of an undescribed functionally important region in the final exon. For all genotypes, there was complete penetrance for developmental delay and near-complete penetrance for seizures and hypotonia in patients surviving the neonatal period.

Conclusion: We have expanded the described spectrum of phenotypes and natural history associated with biallelic *PIGN* variants. Our study shows that biallelic-truncating variants usually result in the more severe Fryns syndrome phenotype, but neurologic problems, such as developmental delay, seizures, and hypotonia, present across all genotypes. Functional analysis should be considered when the genotypes do not correlate with the predicted phenotype because there may be other functionally important regions in *PIGN* that are yet to be discovered.

Keywords

Epilepsy; Fryns syndrome; GPI deficiency; MCAHS1; *PIGN*

Introduction

Glycosylphosphatidylinositol-anchored proteins (GPI-APs) are a family of >150 proteins in mammalian cells that are attached to the extracellular leaflet of the plasma membrane using GPI-anchors.[1] GPI-APs fulfill a diverse range of functions, eg, as enzymes, receptors, adhesions molecules, protease inhibitors, and complement regulators.[2]

A broad spectrum of clinical manifestations has been described in association with biallelic variants in genes that encode proteins involved in the biosynthesis, transfer, and modification of GPI-anchors.[1] The phenotypic expression can include congenital anomalies, intellectual disability, epilepsy, and characteristic facial features, alongside reduced in vivo expression of GPI-APs at the cell surface.[3–6] This clinical variety has been proposed to be partially related

to the position of the defect in this pathway.[1] Collectively these genetic conditions are known as inherited GPI deficiencies (IGDs) and are part of the broader group of conditions known as congenital disorders of glycosylation. IGDs are recognized as a rare cause of developmental disorders, with rare biallelic variants in genes involved in GPI-anchor biosynthesis found in 0.15% of probands with developmental delay among 4125 patient–parent trios in the Deciphering Developmental Disorders (DDD) study.[7]

The *PIGN* gene encodes a protein expressed in the endoplasmic reticulum that is involved in the addition of phosphoethanolamine to the first mannose in the conserved core of the GPI precursor molecule.[8] Homozygous or compound heterozygous variants in *PIGN* have been described in patients with Fryns syndrome (OMIM 229850)[9–11] and multiple congenital anomalies-hypotonia-seizures syndrome 1 (MCAHS1; OMIM 614080).[4,5,12–14] Fryns syndrome is the most common syndrome associated with congenital diaphragmatic hernia (CDH),[15] accounting for 1.3% to 10% of all cases.[16] It is a clinical rather than molecular diagnosis and has been observed in association with variants in several genes and chromosomal aberrations.[17] The clinical presentation can also include polyhydramnios, characteristic facial features, orofacial clefting, distal digital hypoplasia, pulmonary hypoplasia, and other anomalies affecting the eyes, kidneys, brain, genitalia, cardiovascular, and gastrointestinal systems. It is fatal in the antenatal or early neonatal period.[15,18] MCAHS1 is characterized by multiple congenital anomalies and dysmorphic features. Affected individuals can survive the neonatal period and have severe neurologic impairment with hypotonia, chorea, and seizures.[5] A third purely neurologic phenotype was described by Thiffault et al[6] in 2017 in a 2-year-old patient with biallelic variants in *PIGN* who presented with hypotonia, global developmental delay, and focal epilepsy but no significant dysmorphic features or congenital anomalies.

To date, 40 individuals have been reported with biallelic variants in *PIGN*.[3–6,9–14,19–23] Some of these reports have suggested a possible correlation between the effects of variants on residual PIGN function and clinical severity.[6,9,13] In this article, we report a further 21 patients and compare their clinical and molecular features with those of all previously reported cases, identifying many novel variants and gaining previously unreported insights into genotype–phenotype correlations.

Materials and Methods

Identification of patients

Patients 2, 3-I, 3-II, 4, 5-I, 5-II, and 6 were ascertained through the DDD-study[24–26] and included 2 sibling pairs (3-I and 3-II and 5-I and 5-II). In total, 14 cases were contributed from collaborators in epilepsy and genetic centers in Europe, which had been identified by clinical molecular testing. Patients 1, 2, 3-I, 3-II, 4, 5-I, 5-II, 13, and 15 are previously unpublished. Case 6[7] and cases 7 to 12, 14, and 16 to 19[27] are published cases for which we have gathered and present additional new clinical details.

Written informed consent for the publication of clinical information was obtained for all the patients recruited. A geneticist or neurologist examined each patient.

To identify additional cases with *PIGN* variants, a literature review of published case reports was carried out. We searched in PubMed using the keywords “PIGN,” “Fryns syndrome,” “Multiple congenital anomalies hypotonia seizures syndrome,” and “MCAHS.” Care was taken to avoid double-counting of patients who may be reported in >1 publication by comparing demographics, clinical details, and variants reported. Only scientific publications in English were considered.

Molecular testing and variant analysis

Trio exome sequencing in the DDD patients was carried out using the standard DDD pipelines published previously.[28] Targeted sequencing of the familial variant was carried out through Sanger sequencing in 1 case (Patient 5-II). Next generation sequencing panel testing for GPI-anchor genes was used to reach a diagnosis in 1 family because of the clinically suspected diagnosis of Fryns syndrome. For all 15 cases for which variant ascertainment details were available, biparental inheritance had been confirmed (Table 1). Variant ascertainment and biparental inheritance details for 6 cases were not available.

In silico predictions of variant effect by Polymorphism Phenotyping (PolyPhen) v2,[29] Sorting Intolerant from Tolerant (SIFT) v2,[30] Combined Annotation Dependent Depletion (CADD),[31] and maximum entropy modeling (MaxEnt)[32] were retrieved using the Ensembl Variant Effect Predictor. SpliceAI[33] predictions (https://spliceailookup.broadinstitute.org/#) and variant frequencies (Genome Aggregation Database v2.1.1 exomes) were also retrieved. Variants are annotated according to GRCh37 assembly and reference sequence NM_176787.5.

Functional analysis

As described previously,[7] *PIGN*-null HEK293 cells were generated and transfected with human wild-type or variant *PIGN* complementary DNA (cDNA) under a strong promoter-driven expression vector. Restoration of the cell surface expression of CD59, fluorescein-labeled proaerolysin (FLAER), and DAF was evaluated using flow cytometry. Levels of expressed wild-type and mutant PIGN were analyzed using western blotting.

Molecular characterization of splice site variants

RNA analysis was performed in the mother of patient 2 (heterozygote for c.548_549+6del) and in patient 5-I (compound heterozygous for p.Leu311Trp and c.2180+1G>T) and his mother (heterozygote for p.Leu311Trp). RNA was extracted from lymphocytes using the PAXgene Blood RNA Kit (PreAnalytiX) according to the manufacturer’s instructions. Reverse transcription was performed using random primers to create a cDNA library of RNA transcripts. The region of interest was amplified using polymerase chain reaction using primers within exons 5 and 10 for the mother of patient 2 and within exons 21 and 25 for patient 5-I and his mother. The polymerase chain reaction products were then purified and Sanger sequenced to identify the nature of the altered splicing.

Results

Molecular results

Among the 21 cases presented, there were 1 homozygous and 20 compound heterozygous *PIGN* genotypes, comprising 24 different variants (13 missense, 6 predicted splice-altering, 2 frameshifting indels, 2 premature stop codons, and 1 inframe deletion). Functional analyses were conducted for the 2 most prevalent variants in our 21 presented cases with p.Leu311Trp (found in 10 patients) and p.Ser893Arg (3 patients), and RNA analyses were conducted for the 2 most prevalent splice variants c.548_549+6 del (2 patients) and c.2180+1G>T (found in siblings 5-1 and 5-II). The in silico predictions and population frequencies for the variants identified are presented in Supplemental Table 1. The location and predicted effect of the variants identified in our patients and from literature review are presented in Figure 1.

Functional analysis published previously by our group showed on western blot that p.Leu311Trp does not significantly affect protein expression, but *PIGN*-null HEK293 cells transfected with p.Leu311Trp variant cDNA showed reduced restoration of CD59 expression compared with human wild type, indicating that the variant is hypomorphic.[7] Novel functional analysis of p.Ser893Arg showed on western blot that this variant was highly expressed, yet, failed to restore expression of CD59, DAF, or FLAER in *PIGN*-null HEK293 cells transfected with p.Ser893Arg variant cDNA, indicating that the variant has null activity (Figure 2).

RNA analysis in the mother of patient 2 (heterozygote for c.548_549+6del) detected 2 shorter transcripts in addition to a transcript of normal length. The normal length transcript appeared to be produced exclusively from the wild-type allele. Exon 7 was missing from one of the abnormal transcripts r.[443_549del] and is predicted to lead to a frameshift and premature termination of translation. In the other shorter transcript, 85 nucleotides from the beginning of exon 8 were missing in addition to exon 7 because of the use of an alternative splice site within exon 8 (r.[443_634del]). This transcript is predicted to lead to an inframe deletion of 64 amino acids (p.Ala149_Gly212) from within the phosphodiesterase domain of the protein.

RNA analysis in patient 5-I (compound heterozygous for p.Leu311Trp and c.2180+1G>T) detected the presence of a shorter transcript that was absent from his mother (heterozygote for the p.Leu311Trp), which was consistent with paternal inheritance of the splice variant. Sequencing showed that the splice variant created an alternative splice donor site at the end of exon 23, resulting in exon 23 ending 1 basepair early (r.[2180del]). This led to 2 additional RNA species: RNA terminating in exon 24 (p.Tyr728Metfs*7) and RNA with exon 24 skipped and terminating in exon 25 (r.[2180_2283del]:p.Gly727Alafs*9).

Clinical results

Of the 61 cases analyzed, 11 fit the existing definition of Fryns syndrome[15,18] (2 died in utero, 4 pregnancies were terminated, 5 died as neonates [1–39 days]), 13 fit the original description of MCAHS1[5] (9 living [4 months to 20 years], 4 deceased [1–17 months]), and 35 cases have predominantly neurologic manifestations with variable mild dysmorphic

features without visceral congenital anomalies (30 living [16 months to 30 years], 5 deceased [3 months to 15 years]). Two published cases lacked sufficient clinical information to be categorized.

Phenotype and genotype information for our 21 presented cases is presented in Table 1. Table 2 shows the prevalence of clinical findings among all cases and among groups of genotypes: biallelic missense, biallelic truncating (defined as intragenic deletions, premature stop codons, frameshifting indels, and splice variants), and mixed genotypes (consisting of 1 missense and 1 truncating variant). Also presented are the relative risks (RR) for each clinical finding in the biallelic-truncating genotype group compared with all other genotypes (biallelic missense and mixed genotypes combined). A summary of the clinical features of the 21 presented cases and 40 literature cases grouped according to genotype is presented in Supplemental Table 2.

Antenatally, polyhydramnios was observed in 19% of cases always in association with severe congenital anomalies. Among the 16 cases with biallelic-truncating variants, there were 2 third-trimester intrauterine deaths and 6 second-trimester pregnancy terminations owing to severe congenital anomalies, which included CDH, abdominal wall defects, cardiac defects, hypoplastic pulmonary trunk, renal dysplasia, hyperechogenic kidneys, cleft lip, cleft palate, distal digital hypoplasia, and cystic hygromas.

Among 55 cases surviving to birth, a significant proportion of cases were in >90th centile for birthweight (38%) and 11 of 33 (33%) had a birth head circumference in >90th centile. Neonatal observations included hypotonia, feeding difficulties, and abnormal movements (tremor, spasms, oculomotor movements). Of the 8 cases with biallelic-truncating variants surviving to birth, 3 died in the first 8 days of life and a further 2 cases died at 39 days and 2 years 4 months, respectively. The remaining 3 cases were alive aged 4 months, 18 months, and 10 years, respectively, at their reported last observations.[13,19]

Congenital anomalies were present in 43% of the cases and were significantly more common in cases with biallelic-truncating variants than other genotypes (RR = 2.8 [95% CI = 1.6–4.6]). Omphalocele and exomphalos were exclusively seen in cases with biallelic-truncating variants. CDH and orofacial clefting were very strongly associated with biallelic-truncating variants (RR = 19.3 [2.6–144.5] and 26.4 [3.6–191.4], respectively).

Congenital cardiac anomalies were more common in cases with biallelic-truncating variants than other genotypes (RR = 3.3 [1.6–7.0]). Cardiac anomalies associated with biallelic-truncating variants included ventricular septal defects and conotruncal defects, including tetralogy of Fallot and right ventricular and pulmonary trunk hypoplasia/stenosis. Among biallelic missense and mixed genotypes, cardiac anomalies tended to be less severe and included patent foramen ovale, atrial septal defects, and persistent ductus arteriosus and a single case of tetralogy of Fallot.

Renal tract anomalies were found in 26% of the cases. Dilatation of the renal pelvis and/or ureters was the most common anomaly. Cystic, echogenic, or dysplastic kidneys and partial duplications of the kidney or collecting systems were also observed. There was not a statistically significantly difference in the prevalence or types of renal tract anomalies

between the two groups of genotypes. Anogenital anomalies were found in 17% of the patients. Micropenis and cryptorchidism were the most common genital anomalies. Anal stenosis/atresia was found in 3 cases. In total, 34 cases had no congenital renal or anogenital anomalies, 31 of which had biallelic missense or mixed genotypes.

Dysmorphic facial features were reported in all cases with biallelic-truncating variants but were also common in other genotypes (64%). Variable abnormal morphology of the ears was especially common (54.5%), with ears described as dysplastic, unfolded, large, or asymmetric. Neonates were described as having coarse facial features. The most consistent features in children were a small nose with anteverted nares and an open mouth. Distal digital hypoplasia—ranging from small nails to hypoplastic distal phalanges—was observed in association with all types of genotypes but was more strongly associated with biallelic-truncating variants (RR = 2.8 [1.5–5.2]).

Gastroesophageal reflux, swallowing problems, or feeding difficulties were common (52.5%), and some cases had permanent feeding tubes. Consistent with previous reports, most cases had normal serum alkaline phosphatase levels (96%).

Neurologic manifestations

Nearly all individuals were reported to be hypotonic (95.5%). All individuals who were old enough to be assessed had developmental delay. Most of our presented cases had severe to profound developmental delay (90%) and most individuals were nonverbal (78%, including 6 individuals aged >10 years at the time of assessment). Motor skills were more variable and ranged from inability to sit with no head control to being able to walk and feed oneself.

The siblings, patients 3-I and 3-II, were the most mildly affected. At age 28 years, patient 3-II had speech and language delay, had a moderate learning disability, was able to speak in sentences and copy writing, was independent with self-care tasks, and was employed as a trainee chef. His brother patient 3-I at age 19 years had severe learning disability but was independent with self-care tasks and able to cook under supervision.

Nearly all cases surviving the neonatal period had seizures (98%). Among our presented cases, the mean age of seizure onset was 14 months (range: 2 days to 11 years) and multiple seizure types were observed: generalized tonic-clonic, tonic, myoclonic, focal, atonic, absence, startle, gelastic, and epileptic spasms. Febrile seizures (50%) and reports of status epilepticus (39%) were common. Seizure frequency varied from 20 per day to having only had 2 seizures by age 11 years (Table 1). There were several reports of regression or stagnation of development with the onset of seizures, but for many, developmental delay predated the onset of seizures.

Among all cases, movement disorders were common (38%) and included dyskinesia, dystonia, stereotypies, paresis, ataxia, and gait abnormalities. Nystagmus or abnormal eye movements were also common (64%). Ptosis and strabismus were also observed in few cases.

Brain abnormalities

Brain abnormalities were observed in 72% of the cases. Common findings included cerebral volume loss (57%), cerebellar volume loss (48%), hypomyelination (28%), and hypoplasia of the corpus callosum (23%). Among our presented cases, patients 2, 8, 9, and 11 had progressive cerebral and/or cerebellar atrophy presented across serial magnetic resonance imaging scans (Table 1). The overall prevalence of reported brain abnormalities therefore is potentially an underestimate because several cases with reported normal brain imaging underwent brain imaging once and in the first few months/years of life.

Genotype–phenotype correlation

Cases with biallelic-truncating *PIGN* variants had a significantly increased relative risk of polyhydramnios, congenital anomalies, distal digital hypoplasia, and dysmorphism compared with those with other genotypes. There were 16 cases with biallelic-truncating *PIGN* variants: 10 had a Fryns syndrome phenotype, 1 had an MCAHS1 phenotype, and 3 had neurologic phenotypes (the remaining 2 cases were fetuses with congenital anomalies but insufficient clinical details were presented to classify between Fryns syndrome or MCAHS1 phenotypes). Biallelic-truncating *PIGN* variants were also correlated with an increased risk of prenatal and neonatal death. Of the 16 cases with biallelic-truncating *PIGN* variants, 2 died in utero, 6 pregnancies were terminated, and 4 cases died in the first 2 months of life (Table 1; Supplemental Table 2).

A total of 45 cases had biallelic missense or mixed genotypes. In total, 32 (71%) had a neurologic phenotype, 12 (27%) had an MCAHS1 phenotype, and 1 (patient 1, who is homozygous for the p.[Ser893Arg] functionally null missense variant) had a Fryns syndrome phenotype. Cases in this group had complete penetrance for developmental delay, near-complete penetrance for seizures and hypotonia, and frequent but incompletely penetrant nystagmus, feeding difficulties, movement disorders, and brain abnormalities. The presence of dysmorphic facial features, ears, and digits in this group was variable. There was no difference in the relative risk for neurologic manifestations between this group and those with biallelic-truncating variants. Congenital anomalies were generally fewer in number and less severe in this group, and there were no observed cases of CDH, abdominal wall defects, or cleft lip/palate except for case 1.

Discussion

With the 21 patients presented in this paper, the number of individuals described in the literature with biallelic *PIGN* variants is 61. For congenital anomalies, there is a continuous spectrum of severity from Fryns syndrome phenotypes through MCAHS1 phenotypes to cases who have mainly neurologic manifestations without visceral congenital anomalies. The boundaries between the categories are ill-defined, and the existence of distinct categories in the published literature is secondary to how patients were historically ascertained and described. Patients with Fryns syndrome often do not survive to birth or die in the early neonatal period, sometimes before presenting or being diagnosed with the characteristic neurologic features of MCAHS1 (hypotonia, seizures, and movement disorders). Patients with MCAHS1 typically have fewer and less severe congenital

anomalies than Fryns syndrome, albeit affecting the same organ systems and therefore survive longer to manifest neurologic features. The expanding clinical use of genomic sequencing has identified cases with biallelic *PIGN* variants who share the neurologic features of MCAHS1 but lack the visceral congenital anomalies.

There is a wide spectrum of neurologic manifestations, and these are not obviously correlated with the genotype or the presence/absence of congenital anomalies. Seizure frequencies ranged from 2 seizures by age 11 to refractory epilepsy. Developmental delay was severe to profound for the majority, but we have expanded the spectrum describing 2 adults (patients 3-I and 3-II) who are more mildly affected. Bayat et al[27] have described the epileptology of cases with biallelic variants in *PIGN*, observing that for most, abnormal development predates the onset of seizures and a majority of patients have epileptic encephalopathy (ie, seizures and/or interictal electroencephalogram abnormalities negatively affecting development), but few have intellectual disability and epilepsy but no evidence of epileptic encephalopathy.

Biallelic-truncating genotypes were significantly more strongly associated with congenital anomalies, particularly CDH, abdominal wall defects, and cleft lip/palate, than biallelic-missense and mixed genotypes. For example, the nonsense variant p.Lys232Ter was observed in a homozygous state in a patient with Fryns syndrome[11] and observed in 3 unrelated individuals with neurologic phenotypes (patients 6, 8, and 3) in trans with 3 different missense variants. Furthermore, support for this correlation is provided by the observation that affected siblings have similar phenotypes (patients: 3-I and 3-II and 5-I and 5-II and literature cases[5,9,11,13,14]). In addition, patient 1, who had a Fryns syndrome phenotype, had a deceased sibling who also had a Fryns syndrome phenotype and was found posthumously to have the same homozygous *PIGN* loss-of-function (LoF) variants (data not presented). However, Khayat et al[4] reported a possible exception, which was a child homozygous for the missense variant c.755A>T, p.Asp252Val with a neurologic phenotype who had a deceased sibling with CDH; although, the genotype of the deceased sibling was not assessed.

We have grouped and analyzed biallelic-truncating genotypes as a proxy for genotypes that would cause a total LoF and biallelic-missense and mixed genotypes as a proxy for genotypes that may retain some protein function. It is a limitation of this study that we were able to perform functional analyses only for the most common variants, but our functional analyses add support to the hypothesis that combined effects of variants on total residual PIGN function are related to the clinical severity of the phenotype with regard to congenital anomalies. We have shown that p.Ser893Arg is a LoF variant. It was observed in a homozygous state in a patient with a Fryns syndrome phenotype (patient 1). It was also observed in 2 patients with neurologic phenotypes (patients 9 and 18) in trans with p.Leu311Trp, which we have shown to have some residual function. The hypomorphic p.Leu311Trp variant has been observed in 11 cases in total, only 1 of whom had mild congenital anomalies (duplicated urinary collecting system and splenic duplication [patient 16]). The remaining 10 had neurologic phenotypes.

Among the 61 cases, there are a few exceptions to the described phenotype–genotype correlations. There are 3 cases with biallelic-truncating genotypes and no congenital anomalies: a 10-year-old female homozygous for the splice variant c.1434+5G>A who had a neurologic phenotype[19] and 2 siblings with neurologic phenotypes and coarse facial features who also had c.1434+5G>A but in trans with the nonsense variant p.Tyr780Ter.[13] The c.1434+5G>A variant may be predicted to cause skipping of exon 16, which retains the reading frame but leads to deletion of 61 amino acids from the PigN domain. It is possible therefore that protein could be expressed from alleles containing this variant and could retain some function, possibly explaining the absence of congenital anomalies in these 3 cases. In addition, c.1434+5G> A variant has been previously reported in multiple affected individuals in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000264636.18) and in literature[11,13,19] from independent submitters.

It is possible that different phenotypes, eg, congenital anomalies and seizures, could be caused by the effect of variants on different functions of PIGN. For example, in addition to its role in GPI-anchor preassembly, Ihara et al[35] recently showed that PIGN has a second, independent and evolutionarily conserved function in protein quality control. They reported that several proteins in *C. elegans* pign-1 mutants failed to be secreted and formed abnormal aggregates in the endoplasmic reticulum.

The presence of a functionally null missense variant p.Ser893Arg in the final exon just outside of the PigN functional domain (amino acid 430–884) suggests a possible undescribed functionally important region (Figure 1). Amino acid 893 and the preceding 10 amino acids (883–893) are highly conserved and lie in the last cytoplasmic loop (880–894) between the penultimate and the last of the 15 transmembrane regions (uniprot.org). Missense variants of uncertain significance have been reported in this region in ClinVar (p.Ile892Val, p.Ser891Gly, p.Ser891Asn, p.Thr890Ala, p.Ser884Ile, p.Gly883Ser) (ncbi.nlm.nih.gov/clinvar/) and in literature (p.Ile888Thr).[20]

Since Almeida et al[36] discovered the first human syndromic IGD in 2006, variants in 23 out of the 27 genes involved in GPI biosynthesis and GPI-AP maturation have been identified in cases of IGD.[2] Similar to *PIGN*, the phenotypes of individuals with biallelic variants in *PIGO* and males with hemizygous variants in *PIGA* have been shown to range from mild neurologic phenotypes to severe infantile lethality and include intellectual disability (which ranges from mild to profound) and variable penetrance of dysmorphic facial features, distal digital hypoplasia, congenital anomalies, hypotonia, seizures, and brain abnormalities. CDH has been reported in a male fetus with a hemizygous *PIGA* variant, and phenotypic severity has been shown to be related to residual functional protein activity in *PIGO* cases.[37,38]

Conclusion

Biallelic variants in *PIGN* are associated with a spectrum of phenotypes from Fryns syndrome through MCAHS1 to cases who have mainly neurologic manifestations without visceral congenital anomalies. Genotypes predicted to result in a total LoF were significantly more strongly associated with congenital anomalies than the genotypes that may retain some functional protein. Neurologic manifestations are present in all described cases

who survive the neonatal period. We have presented functional work that shows that the recurrent variants p.Ser893Arg and p.Leu311Trp are LoF and hypomorphic variants, respectively, and comparing the phenotypes of cases who have one or both of these variants provided evidence for the genotype–phenotype correlation identified. Observations of similar phenotypes between pairs of siblings in this analysis also corroborate this finding.

We anticipate that this work will be relevant to *PIGN* variant interpretation and the counseling of patient families. Although we have described genotype–phenotype correlations with regard to total loss vs partial retention of PIGN function, it is not possible to predict a priori that missense variants will have residual PIGN function or that splice variants will cause LoF. Indeed, the case exceptions to the genotype–phenotype correlation that we have discussed exemplify this problem, which is universal to variant interpretation. For the interpretation of missense variants, part of this difficulty arises from an incomplete biological understanding of protein function, eg, we have identified a possible undescribed functionally important region in the last exon of *PIGN* (amino acids 883–893).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Data Availability

Please contact the corresponding author for access to available data.

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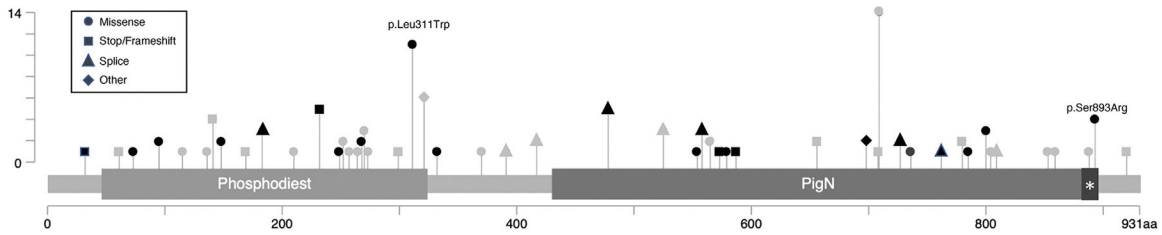

Figure 1. Location, type, and observed frequency of variants within *PIGN*.

PIGN gene is depicted by a light gray bar with amino acid (aa) positions denoted along the *x*-axis. Number of observed alleles within the 61 cases is presented on the y-axis. The missense variants p.Leu311Trp and p.Ser893Arg for which we present functional analyses are labeled. Phosphodiest indicates type 1 phosphodiesterase functional domain (aa 46–324). PigN indicates PigN functional domain (aa 430–884). * indicates highly conserved region of possible functional importance (aa 883–893) (see Discussion). Circles indicate missense variants. Squares indicate premature stop codons and frameshifting variants. Triangles indicate splice variants. Diamonds indicate other variants (synonymous and inframe deletion variants). Black symbols indicate variants from our cohort. Gray symbols indicate variants from the literature. Exon deletions are not depicted (https://www.cbioportal.org/mutation_mapper)[34].

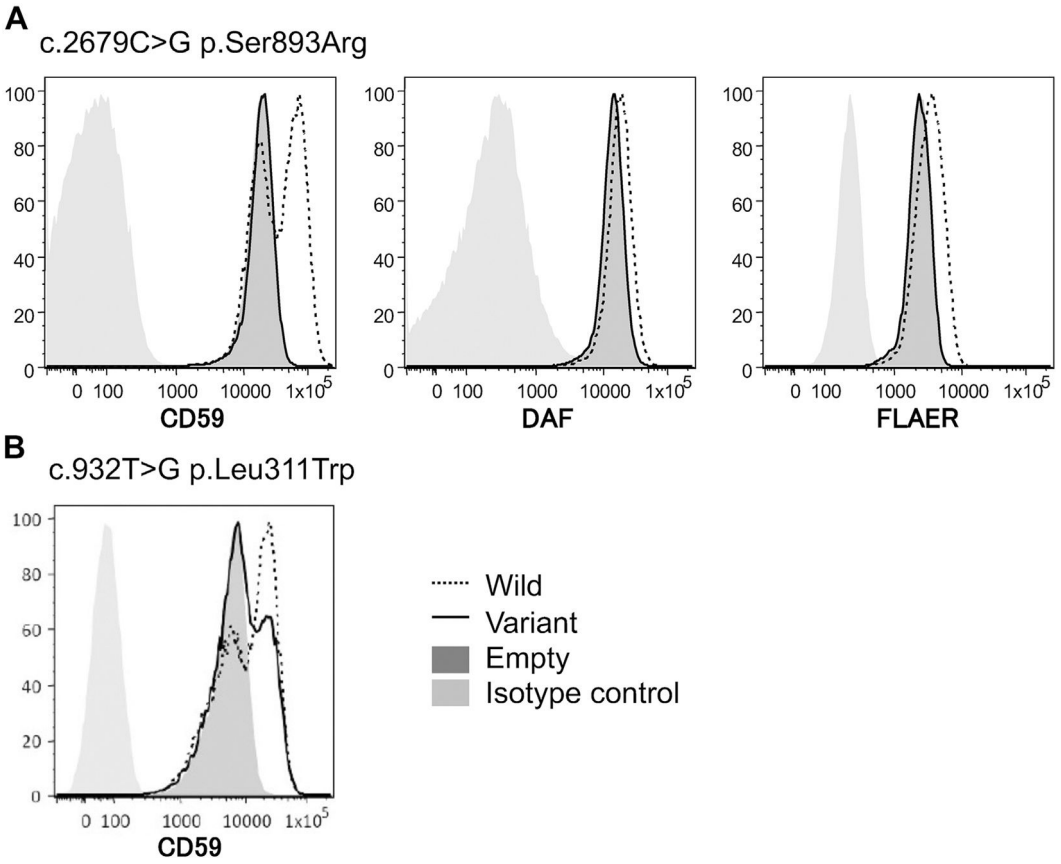

Figure 2. Functional analysis.

PIGN-null HEK293 cells were generated and transfected with human wild-type or variant complementary DNA under a strong promoter (pME)-driven expression vector. A. Restoration of cell surface expression of CD59, DAF, and FLAER by wild-type and p.Ser893Arg variant *PIGN* was evaluated using flow cytometry. The variant p.Ser893Arg failed to restore expression, indicating that it is a null variant. B. Restoration of cell surface expression of CD59 by wild-type and p.Leu311Trp variant *PIGN* was evaluated using flow cytometry (reproduced with permission from Pagnamenta et al[7]). The variant p.Leu311Trp did not restore CD59 expression as efficiently as the wild-type construct, indicating that the variant results in reduced PIGN activity. FLAER, fluorescein-labeled proaerolysin.

Table 1

Clinical findings of 21 patients with biaLLeLic PIGN variants

Patient(DDD Id)	1	2(261191)	3-I(303643)	3-II(306247)	4(294274)	5-I(273015)	5-II	6(259633)[a]	7[a]	8[a]	9[a]	10[a]	11[a]	12[a]	13	14[a]	15	16[a]	17[a]	18[a]	19[a]
Variant (c.) NM_176787.5	c.[2679C>G]; c.[2679C>G]	c.[548_549+6 del]; [283C >T]	c.[804G>C]; [932T >G]	c.[804G>C]; [932T >G]	c.[548_549+6 del]; [2091_2093 del]	c.[932T>G]; [2180+1G >T]	c.[932T>G]; [2180+1G >T]	c.[694A>T]; [932T >G]	c.[2354G>A]; [746A>G]	c.[694A>T]; [2399G>A]	c.[932T>G]; [2679C>G]	c. [1735C>T]; [94delA]	c.[218T>G]; [1759C>T]	c.[2284–1G>C]; [2091_2093delTGT]	c.[1575–2A>T]; [2207T>G]	c. [1434+5G>A]; [1660G>A]	c.[996T>G]; [1717dupA]	c.[932T>G]; [443G>T]	c.[932T>G]; [1674+1G>C]	c. [2679C>G]; [932T>G],	c.[932T>G]; [443G>T]
Variant (p.) NP_789744.1	p. [Ser893Arg]; [Ser893Arg]	p. [Ala149_Gly212]; [Arg95Trp]	p. [Trp268Cys]; [Leu311Trp]	p. [Trp268Cys]; [Leu311Trp]	p. [Ala149_Gly212]; [Val698del]	p.[Leu311Trp]; [Tyr728Metfs*7, Gly727Alafs*9]	p.[Leu311Trp]; [Tyr728Metfs*7, Gly727Alafs*9]	p. [Lys232Ter]; [Leu311Trp]	p.[Arg785His]; [Tyr249Cis]	p. [Lys232Ter]; [Gly800Glu]	p. [Leu311Trp]; [Ser893Arg]	p. [Pro579Ser]; [Met32fs]	p.[Ile73Ser]; [Arg587Ter]	p.[?];[Val698del]	p.[?]; [Leu736Trp]	p.[?]; [Gly554Arg]	p.[Ile332Met]; [Thr573Asnfs]	p. [Leu311Trp]; [p.Gly148Val]	p. [Leu311Trp]; [?]	p. [Ser893Arg]; [Leu311Trp]	p. [Leu311Trp]; [Gly148Val]
Variant type	ms; ms	ms; spl	ms; ms	ms; ms	spl; id	ms; spl	ms; spl	ms; st	ms; ms	ms; st	ms; ms	ms; fs	ms; st	spl; id	ms; spl	ms; spl	ms; fs	ms; ms	ms; spl	ms; ms	ms; ms
Test performed	GPI-anchor gene panel	DDD trio exome	DDD trio exome	DDD trio exome	DDD trio exome	DDD trio exome	Familial variants	DDD trio exome	Exome, analysis of ID panel	450 gene panel for ID	Trio exome	Trio exome	Exome	Undeclared	Undeclared	Trio exome	Trio exome	Undeclared	Undeclared	Undeclared	Undeclared
Biparental inheritance	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Confirmed	Undeclared	Undeclared	Confirmed	Confirmed	Undeclared	Undeclared	Undeclared	Undeclared
Age	Died at 32 d	11 y	19.4 y	28.5 y	13 y	30 y	28 y	5.4 y	3 y	Died at 15 y	9 y	5 y	20 y	4 y	12 y	14 y	16 mo	2 y	6 y 5 mo	18 mo	11 y
Sex	F	M	M	M	M	M	F	F	F	M	M	M	F	M	F	F	F	F	F	M	F
Polyhydramnios	+	-	-	-	-	-	-	/	-	-	-	-	-	/	/	/	-	-	-	/	/
Birthweight >90th centile	-	/	-	-	-	-	-	+	-	/	-	-	+	-	/	-	+	-	-	+	+
Birth head circ. >90th centile	-	/	-	/	/	+	-	/	-	/	-	-	-	/	/	-	+	+	-	+	+
Congenital anomalies	+	-	-	-	+	-	-	-	+	-	-	-	+	-	-	-	-	+	-	-	-
Diaphragmatic hernia	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Abdominal wall defects	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cardiac	ToF	-	-	-	-	-	-	-	-	-	-	-	ToF	-	-	-	-	-	-	-	-
Renal and ureters	L kidney cysts	-	-	-	R kidney cyst	-	-	-	Hydronephrosis, ectopic pyelum	-	-	-	-	-	-	-	-	Duplicated urinary collecting system	-	-	-
Anogenital	Anal atresia	-	-	-	Cryptorchidism	-	-	-	-	-	-	-	-	/	/	/	/	/	-	/	
Cleft lip and/or palate	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-
Distal digital hypoplasia	+	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-	-
Dysmorphic facial features	Coarse, hypertelorism, sparse medial eyebrows, flat nasal bridge, broad nasal tip	Long eyelids, pupils not round, small pointed nose, plagiocephaly, high anterior hairline	Short chin, broad forehead, round face	Bitemporal narrowing, short palpebral fissures, downturned corners of mouth, prominent frontal incisors	-	Epicanthic folds prominent philtral pillars, long face	Long face, prominent forehead	-	Frontal bossing, strabismus (left esotropia), telecanthus, small nose anteverted nares, low columella, flat philtrum	Ptosis, high arched palate	Open mouth with triangular shape, malpositioned teeth, small nose, anteverted nares	Epicanthic folds, open mouth with triangular shape	Microcephaly, flat occiput, high arched palate, opened mouth, small nose	-	-	Up slanting narrow palpebral fissures, ptosis, small nose anteverted nares, high insertion of columella, open mouth with triangular shape	-	-	Protruding tongue, coarse facial features, high frontal hairline, large tongue, wide mouth, open mouth	-	-
Dysmorphic ears	Dysplastic	Asymmetric and simple	-	-	-	-	-	-	-	Large and unfolded	Dumpy	Large everted	Dysmorphic	-	-	-	-	-	-	-	-
Other findings	T4 butterfly vertebra	Bilateral inguinal hernias, severe constipation, failure to thrive, scoliosis	-	-	Pectus excavatum	Scoliosis	-	-	Congenital dysplasia of nails, congenital hypoplasia of right acetabulum	-	-	-	Scoliosis, coxa valga	-	-	Scoliosis	-	-	Scoliosis, flatfoot	-	-
Feeding difficulty/GERD	/	-	-	-	GERD	Feeding difficulty,	-	-	-	Feeding tube from 13 y	-	-	-	-	-	-	+	+	-	-	-

Patient(DDD Id)	1	2(261191)	3-I(303643)	3-II(306247)	4(294274)	5-I(273015)	5-II	6(259633)[a]	7[a]	8[a]	9[a]	10[a]	11[a]	12[a]	13	14[a]	15	16[a]	17[a]	18[a]	19[a]
						frequent vomiting															
Hypotonia	+	+	+	+	-	+	+	+	+	+	/	+	/	/	+	/	+	+	+	+	+
Nystagmus/abn. eye movements	-	+	-	-	+	+	+	-	-	/	+	-	-	-	-	-	-	+	+	-	+
Seizures (onset)	+	+ (6 wk)	+ (9 mo)	+ (4 mo)	+ (11 y)	+ (neonate)	+ (21 mo)	+	+ (4 mo)	+ (4 mo)	+ (18 mo)	+ (20 mo)	+ (7 mo)	+ (5 mo)	+ (8–10 mo)	+ (9 mo)	+ (2–3 mo)	Uncertain (1 mo)	+ (11 mo)	+ (3 mo)	+ (2 d)
Seizure types	/	TS, GTC, SE	FebS, absence, GTC, MS	FebS, absence	/	Absence, TS, FoS, GTC, Lennox-Gastaut diagnosis, SE at age 5 y	FoS, AS, absence, GTC, SE	/	GTC, eyeM, ES, SE	FoS, FSSG, MS, SE, FebS	FebS, FSSG, AS, AA, gelastic	TS, FoS, eyeM, SE, AS, FebS	MS, TS, GTC, FebS, SE	GTC, FoS, AA, FebS	TS, AS	GTC, AS, FebS	MS, ES, FoS	ES	MS, SS	MS, TS, SS	MS, FoS, FebS, GTC, Todd's paresis
Developmental delay	/	+++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Developmental regression	/	-	+	-	-	-	+	-	-	-	+	-	+	-	+	+	-	-	-	+	-
Movement/ behavioral disorders	/	Choreoathetoid movements, roving eye movement	-	-	-	Side-to-side head movements	Repetitive protrusion of tongue and chin quivering as infant, repetitive movements	Extra pyramidal dyskinesia	-	-	Ataxia, mild right hemiparesis, impulsive behavior	+	Bilateral spastic-dystonic palsy	-	-	-	Dystonia	Multiple dyskinesias, oropharyngeal movements	ADHD, stereotypies, dystonia	-	Stereotypies (swaying)
Brain abnormalities	+	+	/	/	-	+	+	+	-	+	+	+	+	+	-	+	+	-	-	+	-
Cerebral volume loss	+	+	/	/	-	+	+	+	-	+	+	+	+	-	-	+	-	-	-	-	-
Cerebellar volume loss	+	+	/	/	-	+	+	+	-	+	+	-	-	+	-	-	-	-	-	-	-
Hypoplasia of corpus callosum	+	+	/	/	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-
Hypomyelination	/	+	/	/	-	-	-	-	-	+	+	+	-	-	-	-	-	-	-	+	-
Elevated serum ALP	-	-	/	/	-	-	-	-	/	/	-	-	/	-		-	/	-	-	-	-
Phenotype	FS	NE	NE	NE	MC	NE	NE	NE	MC	NE	NE	NE	MC	NE	NE	NE	NE	MC	NE	NE	NE

"+" indicates present; "−" indicates absent; "/" indicates unknown. In the developmental delay row, "+++" indicates severe, "++" indicates moderate, and "+" indicates mild.

AA, atypical absence; *abn*, abnormal; *ADHD*, attention deficit hyperactivity disorder; *ALP*, alkaline phosphatase; *AS*, atonic seizure; circ, circumference; *DDD*, Deciphering Developmental Disorders; *ES*, epileptic spasms; *eyeM*, eye myoclonus; *F*, female; *FebS*, febrile seizure; *FoS*, focal seizure; *fs*, frameshift; *FS*, Fryns syndrome phenotype; *FSSG*, focal with secondary generalization; *GERD*, gastroesophageal reflux disease; *GTC*, generalized tonic-clonic seizure; *ID*, intellectual disability; *Id*, identifier; *id*, inframe deletion; *L*, left; *M*, male; *MC*, multiple congenital anomalies-hypotonia-seizures syndrome 1; *MS*, myoclonic seizure; ms, missense; *NE*, neurologic phenotype; *R*, right; *SE*, status epilepticus; *spl*, splicing; *SS*, startle seizure; *st*, stop; *ToF*, tetralogy of Fallot; *TS*, tonic seizure.

[a]Published cases for which we present additional new clinical details.

Table 2

Prevalence of clinical features by genotype

Clinical Feature	All Genotypes		Biallelic Missense		Mixed Missense and Truncating		Biallelic Truncating		Biallelic Truncating vs Other Genotypes	
	n/N	%	*n/N*	%	*n/N*	%	*n/N*	%	RR	95% CI
Polyhydramnios	7/37	19	1/11	9	0/17	0	6/9	67	18.7	(2.6–135.1)
Birthweight >90th centile	17/45	38	10/19	53	5/16	31	2/10	20	0.5	(0.1–1.7)
Birth head circumference >90th centile	11/33	33	6/13	46	3/12	25	2/8	25	0.7	(0.2–2.6)
Congenital anomalies	26/60	43	9/20	45	4/24	17	13/16	81	2.8	(1.6–4.6)
Congenital diaphragmatic hernia	8/60	13	1/20	5	0/24	0	7/16	44	19.3	(2.6–144.5)
Abdominal wall defects	5/59	8	0/20	0	0/24	0	5/15	33	*i*	
Cardiac	17/59	29	7/20	35	1/24	4	9/15	60	3.3	(1.6–7.0)
Renal and ureters	15/58	26	7/20	35	2/24	8	6/14	43	2.1	(0.9–4.9)
Anogenital	8/48	17	4/17	24	1/19	5	3/12	25	1.8	(0.5–6.4)
Cleft lip and/or palate	10/59	17	1/20	5	0/24	0	9/15	60	26.4	(3.6–191.4)
Distal digital hypoplasia	19/49	39	6/18	33	4/19	21	9/12	75	2.8	(1.5–5.2)
Dysmorphic facial features	39/55	71	12/20	60	16/24	67	11/11	100	1.6	(1.3–2.0)
Dysmorphic ears	24/44	55	11/18	61	6/18	33	7/8	88	1.9	(1.2–2.9)
Feeding difficulty/GERD	21/40	53	8/17	47	8/18	44	5/5	100	2.2	(1.5–3.1)
Hypotonia	43/45	96	19/19	100	19/21	90	5/5	100	1.1	(1.0–1.1)
Nystagmus/abnormal eye movements	27/42	64	13/20	65	10/18	56	4/4	100	1.7	(1.3–2.1)
Seizure	47/48	98	18/19	95	25/25	100	4/4	100	1.0	(1.0–1.1)
Developmental delay	48/48	100	19/19	100	25/25	100	4/4	100	1.0	
Movement disorders	16/42	38	6/18	33	8/19	42	2/5	40	1.1	(0.3–3.3)
Brain abnormalities	33/46	72	9/13	69	19/25	76	5/8	63	0.8	(0.5–1.5)
Cerebral volume loss	25/44	57	8/13	62	15/25	60	2/6	33	0.6	(0.2–1.8)
Cerebellar volume loss	21/44	48	6/13	46	11/25	44	4/6	67	1.5	(0.8–2.9)
Hypoplasia of corpus callosum	10/44	23	2/13	15	6/25	24	2/6	33	1.6	(0.4–5.7)
Hypomyelination	12/43	28	5/12	42	6/25	24	1/6	17	0.6	(0.1–3.6)
Elevated serum alkaline phosphatase	1/25	4	0/6	0	1/16	6	0/3	0	0.0	

Prevalence of clinical features among all genotypes, all patients (21 presented patients and 40 literature cases); biallelic missense, cases with biallelic missense variants; biallelic truncating, cases with biallelic-truncating variants (intragenic deletions, premature stop codons, frameshifting indels, and splice variants); mixed missense and truncating, cases with a missense and truncating variant in trans. The

relative risk (and 95% CI) for clinical features in cases with biallelic-truncating genotypes compared with other genotypes (biallelic missense and mixed genotypes combined) are presented in the last 2 columns.

GERD, gastroesophageal reflux disease; *i*, incalculable as clinical feature exclusively observed with biallelic truncating genotypes; *RR*, relative risk.