

ARTICLE

Cerebral visual impairment and intellectual disability caused by *PGAP1* variants

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Homozygous variants in *PGAP1* (post-GPI attachment to proteins 1) have recently been identified in two families with developmental delay, seizures and/or spasticity. *PGAP1* is a member of the glycosylphosphatidylinositol anchor biosynthesis and remodeling pathway and defects in this pathway are a subclass of congenital disorders of glycosylation. Here we performed whole-exome sequencing in an individual with cerebral visual impairment (CVI), intellectual disability (ID), and factor XII deficiency and revealed compound heterozygous variants in *PGAP1*, c.274_276del (p.(Pro92del)) and c.921_925del (p.(Lys308Asnfs*25)). Subsequently, *PGAP1*-deficient Chinese hamster ovary (CHO)-cell lines were transfected with either mutant or wild-type constructs and their sensitivity to phosphatidylinositol-specific phospholipase C (PI-PLC) treatment was measured. The mutant constructs could not rescue the *PGAP1*-deficient CHO cell lines resistance to PI-PLC treatment. In addition, lymphoblastoid cell lines (LCLs) of the affected individual showed no sensitivity to PI-PLC treatment, whereas the LCLs of the heterozygous carrier parents were partially resistant. In conclusion, we report novel *PGAP1* variants in a boy with CVI and ID and a proven functional loss of *PGAP1* and show, to our knowledge, for the first time this genetic association with CVI.

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INTRODUCTION

Cerebral visual impairment (CVI) accounts for 27% of the visual impairment in children, and is due to a disorder in projection and/or interpretation of the visual input in the brain.^{1–3} Acquired damages, for example, due to preterm birth, are well known causes of CVI, whereas genetic factors are largely unidentified.⁴ Several chromosomal aberrations have been associated with CVI, such as Phelan–McDermid syndrome (22q13.3 deletion) and 1p36 microdeletion syndrome.⁵ Moreover, single-gene disorders can be implicated in CVI, and, recently, we identified *de novo* variants in *NR2F1* as a cause of CVI.⁶ In addition, in several congenital disorders of glycosylation (CDG type 1a, type 1q and type 1v) CVI has been reported.^{4,7–9} Glycosylation disorders are caused by a defect in the glycosylation of glycoproteins and glycolipids, and one subclass is the defect in glycosylphosphatidylinositol (GPI) anchor glycosylation.¹⁰

GPI anchor many cell-surface proteins with various functions, so called GPI-anchored proteins (GPI-APs), to the membrane of eukaryotic cells.^{11–13} GPI is synthesized in the endoplasmic reticulum, transferred to the proteins, and remodeled. During the biosynthesis of GPI anchors, an acyl chain is linked to the inositol. This acyl chain is a transient structure and is necessary for efficient completion of later steps in GPI biosynthesis. After the attachment of GPI to the protein, this acyl chain is removed by *PGAP1* (post-GPI attachment to proteins 1), the first step of the remodeling phase.¹⁴ Subsequently, the GPI anchor is transported from the endoplasmic reticulum to the plasma membrane through the Golgi apparatus and further remodeled. When the inositol-linked acyl chain is not removed, this transport is

delayed,¹⁴ but the presence of this acyl chain does not affect the cell-surface expression of GPI-APs.¹⁵

Several genes known to be involved in the GPI anchor biosynthesis (*PIGA*, *PIGL*, *PIGN*, *PIGT*, *PIGV*, *PIGO*, *PIGW* and *PIGQ*) and modeling (*PGAP2* and *PGAP3*) are implicated in X-linked and autosomal recessive intellectual disability disorders.^{16–26} Additional features such as seizures, congenital abnormalities and facial dysmorphisms are commonly present. Moreover, CVI has been reported in individuals with *PIGA*, *PIGN* and *PIGT* variants, suggesting that the GPI anchor biosynthesis is important in the development of the visual areas of the brain.^{18,19,27,28}

Recently, two families with developmental delay, seizures and/or spasticity were reported with homozygous variants in *PGAP1*.^{15,29} However, *PGAP1* variants have not to date been associated with CVI.

Here we report an individual with CVI and intellectual disability (ID) and variants in *PGAP1*, thereby showing the association between *PGAP1* and CVI.

MATERIALS AND METHODS

Case report

The boy was the second child of healthy unrelated parents. He was born after a normal pregnancy by a planned caesarian section because of a difficult delivery of the previous child. His birth weight was 3550 g (70th centile) at a gestational age of 38 weeks and Apgar scores were 9 and 10, after 1 and 5 min, respectively. During the neonatal period, he was hypotonic and there were feeding difficulties. His development was delayed as he started walking and speaking around the age of 2–2½ years. At the age of 8 years 3 months, his total IQ was 49 (Wechsler Intelligence Scale for Children-III). He was very social in his

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behavior. There were no signs of epilepsy and an MRI of the brain was normal at age 5 years. Hearing was normal, but ophthalmological examination at 7 years revealed CVI with a visual acuity of 0.3 (Landolt C). He had strabismus divergences and nystagmus. Slit lamp examination and funduscopy were normal. He looked only shortly at a target and he had more difficulties with recognition of objects in clutter (crowding) than was expected for his age.

He had mild asthma and his APTT was prolonged due to a factor XII deficiency (factor XII activity <1%). His mother and sister also had decreased factor XII activity (40% and 22%, respectively), with a normal APTT. There were no signs of immunodeficiency.

Physical examination at 7 years 10 months showed a height of 131 cm (50th centile) and head circumference of 52.5 cm (50th centile). Facial dysmorphisms consisted of upward slanting palpebral fissures, deep-set eyes, large ear lobes and prominent helices and antihelices (Figure 1). His teeth showed extra mamelons, and diminished enamel was noted. Previous genetic studies, consisting of *FMR1* gene, *AIP* gene, array CGH and metabolic studies (including transferrin glycosylation assay) showed normal results. This study was approved by the Ethics Committee of the Radboud University Medical Center (Commissie Mensgebonden Onderzoek, regio Arnhem-Nijmegen), and written informed consent was obtained.

Whole-exome sequencing (WES)

WES in this individual (CVI10) was performed as part of a larger study to identify the genetic causes of CVI.⁶ WES was performed by the Baylor-Hopkins Center for Mendelian Genomics, using methods reported previously (Supplementary Material and Methods, whole-exome sequencing).^{6,30} The results were analyzed for *de novo* variants, homozygous variants, compound heterozygous variants and hemizygous variants as described in de Ligt *et al.*³¹ Truncating variants, splice site variants and missense variants predicted to be pathogenic were validated by Sanger sequencing in the affected subject and his parents. The variants identified have been submitted to the Leiden Open Variation Database LOVD database (<http://databases.lovd.nl/>, individual #00025011).

Functional analysis using CHO cells

PGAP1-deficient CHO cells (C10)¹⁴ were transiently transfected with wild-type or mutant pMEFLAG-h*PGAP1* by electroporation as reported previously (Supplementary Material and Methods, Functional analysis using CHO cells).¹⁵ Four days after transfection, cells were treated with or without PI-PLC. Surface expression of GPI-APs was determined by staining cells with mouse anti-human CD59 (5H8), -human DAF (IA10), -hamster uPAR (5D6) antibodies and analyzed by flow cytometry using Flowjo software (Tommy Digital Inc., Tokyo, Japan).

PI-PLC treatment and FACS analysis

Heparin blood samples were collected from the affected individual and his unaffected parents and lymphoblastoid cell lines (LCLs) were generated (Supplementary Material and Methods, PI-PLC treatment and FACS analysis). Four days after transfection, cells were treated with or without PI-PLC as reported previously.¹⁵ Surface expression of GPI-APs was determined by staining cells with mouse anti-human CD59 (5H8), -human DAF (IA10), -human CD48 (BJ40) antibodies and analyzed by flow cytometry using Flowjo software.

RESULTS

WES

WES in the affected individual and the parents was performed with an average coverage of 126×. After applying the above mentioned prioritizations step only two variants in *PGAP1* remained, a paternal inherited chr2.hg19:g.197784746_197784748del; c.274_276del; p.(Pro92del) and a maternal inherited chr2.hg19:g.197761857_197761861del; c.921_925del; p.(Lys308Asnfs*25) (MIM 611655, RefSeq accession number NM_024989.3; Supplementary Material, Supplementary Table S1). The variants were validated by Sanger sequencing, and an additional maternal inherited variant was identified, chr2.hg19:g.197761868C>T; c.914C>T; p.(Ala305Val), which

was previously filtered out due to quality settings (Supplementary Material, Supplementary Figure S1). In addition, the exome results were screened for rare variants (<1% occurrence) in the coding regions of *F12* (MIM 610619, RefSeq accession number NM_000505.3). A paternal inherited heterozygous variant was identified and validated with Sanger sequencing chr5.hg19:g.176831388G>A; c.827G>A; p.(Trp276*).

Functional analysis using CHO cells

Under the presence of PI-PLC, structurally normal GPI-APs, without inositol-linked acyl chain, are cleaved from the cell membrane.³² CHO cells deficient for *PGAP1* were used to investigate the expected structural abnormalities of the GPI anchors by testing the sensitivity of GPI-APs to PI-PLC. Wild-type *PGAP1* and the p.(Ala305Val) rescued the sensitivity for PI-PLC (Figure 1 and Supplementary Figure S2). However, transfection with empty vector, the p.(Pro92del) mutant or p.(Lys308Asnfs*25) mutant, did not increase the sensitivity for PI-PLC (Supplementary Figure S3).

PI-PLC treatment and FACS analysis

In addition, the LCLs of the affected individual and parents were analyzed for PI-PLC resistance. The LCLs of the affected individual were completely resistant to PI-PLC treatment, whereas the LCLs of the parents were partially resistant (Supplementary Figure S4).

DISCUSSION

Here we present an individual with CVI, ID, minor facial dysmorphisms and factor XII deficiency. WES revealed compound heterozygous variants in *PGAP1*, c.274_276del (p.(Pro92del)), c.914C>T (p.(Ala305Val)) and c.921_925del (p.(Lys308Asnfs*25)). To investigate the functional impact of the variants, PI-PLC sensitivity assay was performed in *PGAP1*-deficient CHO cells. The c.914C>T (p.(Ala305Val)) mutant rescued the PI-PLC sensitivity in *PGAP1*-deficient CHO cells, similar to wild-type *PGAP1*. Therefore, this variant was considered to have no functional impact. The c.274_276del (p.(Pro92del)) and c.921_925del (p.(Lys308Asnfs*25)) mutant constructs, however, were not able to rescue the sensitivity, indicating a functional loss of the cDNA constructs. In addition, the PI-PLC sensitivity was investigated in LCLs derived from the affected individual and parents. The LCLs of the affected individual were resistant to PI-PLC, indicating that the GPI-APs are structurally abnormal, whereas the parents showed a partial resistance. These results are in line with the reported family with *PGAP1* variants by Murakami *et al.*¹⁵ The other family reported by Novarino *et al.*²⁹ was not tested. Besides a developmental delay, the phenotype is variable. The reported siblings by Murakami *et al.* had severe developmental delay with seizures, stereotypic movements and brain atrophy (Table 1). The siblings reported by Novarino *et al.* had spasticity and structural brain abnormalities. In the individual described in this study, no seizures or brain abnormalities were present, but he had impaired vision and CVI. No ophthalmological examination was performed in the Murakami siblings. In the Novarino siblings, no 'ophthalmological signs' were present, but whether complete ophthalmological examination had been performed remained unclear. Depending on the genetic background of the mouse strain used, *PGAP1* deficiency in mice could lead to various phenotypes, ranging from a normal structural brain to forebrain abnormalities, including holoprosencephaly.^{33–36} In the mutant phenotypically abnormal mice, Wnt signaling and Nodal signaling were reported to be affected.^{35,36} However, the precise role of *PGAP1* in the function and development of the brain and more specific of the visual system remains to be elucidated.

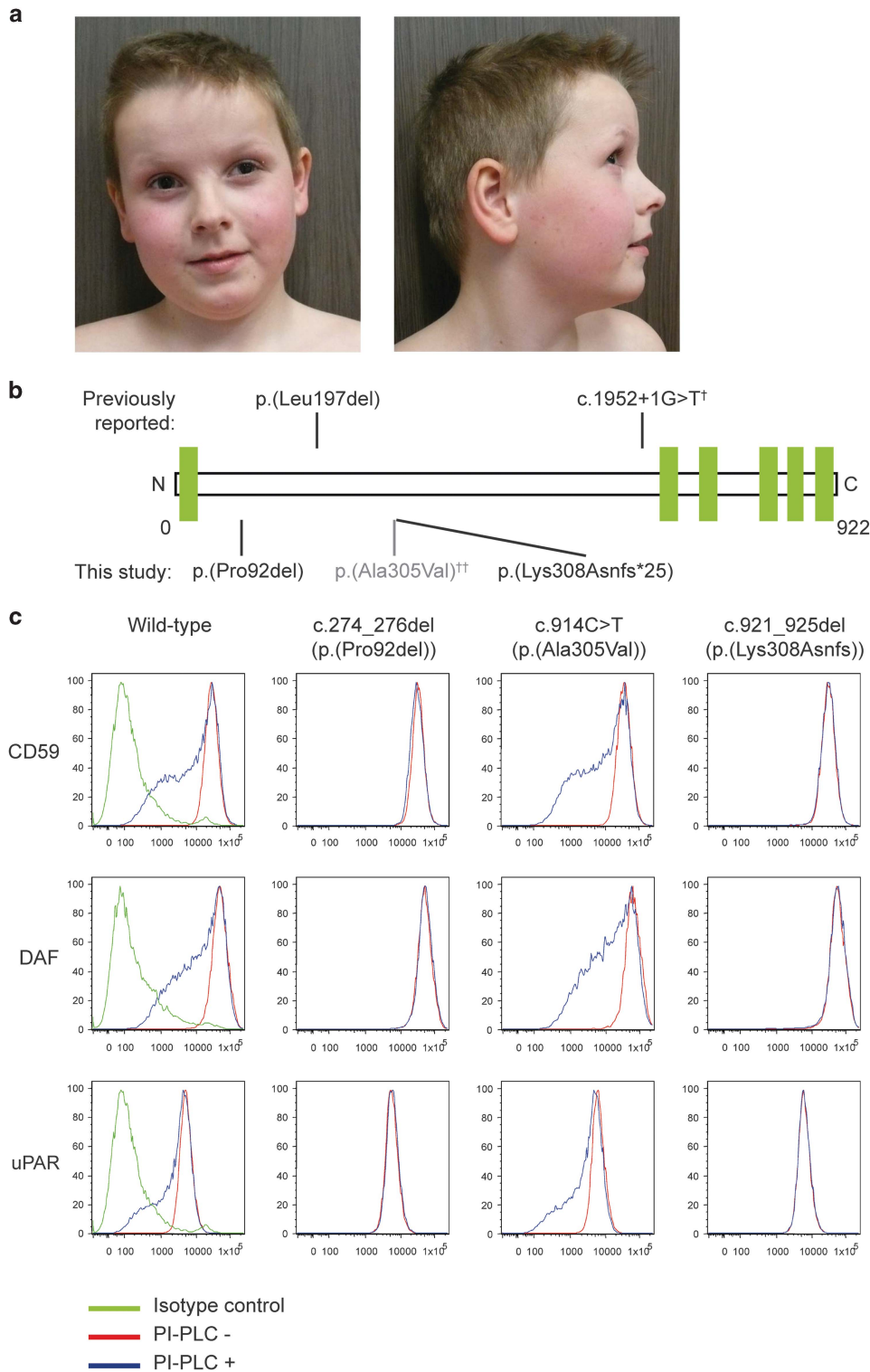


Figure 1 Phenotype of the affected individual and the functional analysis of the variants. **(a)** Photographs of the affected individual, note the upward slanting palpebral fissures, deep-set eyes, large ear lobes and prominent helices and antihelices. **(b)** Schematic representation of *PGAP1*-containing transmembrane domains (in green). The positions of the variants identified in this study and in the previous reports are indicated. †For this variant the effect on amino acid sequence was not studied. ††Considered as a variant without functional impact. **(c)** CHO cells deficient for *PGAP1* were used to investigate the expected structural abnormalities of the GPI anchors by testing the sensitivity of GPI-APs to PI-PLC. Wild-type *PGAP1* and the construct containing the variant c.914C>T (p.(Ala305Val)) rescued the sensitivity for PI-PLC strongly suggesting that this variant is benign. The *PGAP1* constructs containing the c.274_276del (p.(Pro92del)) and c.921_925del (p.(Lys308Asnfs*25)) variants did not increase the sensitivity for PI-PLC, suggesting that both are causal.

Table 1 Phenotype of individuals with *PGAP1* variants

	<i>This study</i>	<i>Murakami III-2</i>	<i>Murakami III-3</i>	<i>Novarino 1241-IV-3</i>	<i>Novarino 1241-IV-4</i>
Variant identified (Hg 19)	c.274_276del (p.(Pro92del)) and c.921_925del (p.(Lys308Asnfs*25))	c.589_591del (p.(Leu197del)) and c.589_591del (p.(Leu197del))	c.589_591del (p.(Leu197del)) and c.589_591del (p.(Leu197del))	c.1952+1G>T and c.1952+1G>T	c.1952+1G>T and c.1952+1G>T
Gender	M	F	M	M	M
Birth weight	3550 g (70th centile)	Normal	Normal	NA	NA
Gestational age (weeks)	38	Normal	Normal	NA	NA
Age at investigations (years+months)	7+10	4+5	2+9	6+6	0+9
Height	131 cm (50th centile)	96 cm (25th centile)	Normal	NA	NA
OFC	52.5 cm (50th centile)	46 cm (<5th centile)	47 cm (<5th centile)	NA	NA
Delayed development	+	+	+	+	+
Walking independently (years+months)	2+6	4+5	–	–	–
First words (years+months)	2+6	–	–	NA	NA
Hypotonia	+	+	+	NA	NA
Brain imaging (MRI/CT)	Normal (MRI)	Pronounced brain atrophy (CT)	NP	Prominent cortical sulci and widened sylvian fissures (MRI)	Corpus callosum agnesis, vermian hypoplasia, defective myelination (MRI)
Hearing investigation	Normal	NP	NP	NA	NA
Ophthalmological examination	CVI, strabismus, nystagmus	NP	NP	–	–
Other abnormalities	Factor XII deficiency	Stereotypic movements, seizures	Stereotypic movements	Spasticity	Spasticity, distended abdomen
Dysmorphism	Upward slanting palpebral fissures, deep-set eyes, large ear lobes, prominent helices and antihelices, teeth showed extra mamelons with diminished enamel	Large ears, flattenend nasal bridge	Large ears, flattenend nasal bridge	NA	NA

Abbreviations: NA, not available; NP, not performed.

One marker of CDG syndromes can be the abnormal glycosylation of transferrin.³⁷ However, in the subclass of GPI anchor glycosylation defects, no abnormal transferrin is detectable, making this marker unreliable in diagnosing GPI anchor glycosylation defects, such as those caused by variants in *PGAP1*.

One other feature of CDG syndromes is the deficiency of coagulation factors.³⁷ However, factor XII deficiency has not been reported so far, neither has a direct functional link between factor XII and GPI-APs been made. Factor XII deficiency can be caused by variants in *F12*; a heterozygous variant can lead to an intermediated level of factor XII activity, whereas homozygous or compound heterozygous variants lead to almost no activity (<1%).^{38,39} The current individual had a factor XII activity <1%, and a paternal inherited loss of function variant in *F12*, c.827G>A (p.(Trp276*)), but his father had a normal factor XII activity. The mother had an intermediate factor XII activity, but no variant could be identified in her exome results. Whether the variants in *PGAP1* are related to the factor XII deficiency is yet unclear.

Two other GPI anchor-modeling proteins, *PGAP2* and *PGAP3*, are implicated in hyperphosphatasia with mental retardation syndrome, also named Mabry syndrome.^{22–24} These individuals showed, in addition to ID, seizures, typical facial dysmorphisms and an increased alkaline phosphatase (ALP). This increase is due to diminished GPI-APs on the cell surface, resulting in less binding of ALP to the cell membrane and more ALP in the plasma.^{22–24,40,41} ALP was not

measured in the *PGAP1*-affected individuals, but in *PGAP1*-deficient cells, no diminished cell-surface expression of GPI-APs was measured, making elevated ALP levels less likely.¹⁵ In addition, the typical facial dysmorphisms of Mabry syndrome, consisting of apparent hypertelorism, long palpebral fissures, short nose with broad nasal bridge and tip and tented upper lip vermillion, were not present in the here presented individual.

In conclusion, we identified novel *PGAP1* variants in an intellectual disabled boy with a proven functional loss of *PGAP1* and showed for the first time the genetic association with cerebral visual impairment. Additional affected individuals will be required to gain better insights into pathophysiology and acquire knowledge of the clinical spectrum of this novel disorder.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on European Journal of Human Genetics website (<http://www.nature.com/ejhg>)