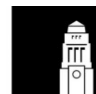

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Mutations in GPAA1, encoding a GPI transamidase complex protein, cause developmental delay, epilepsy, cerebellar atrophy and osteopenia.

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Running title (40 characters): GPAA1 mutations cause epilepsy and osteopenia.

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Abstract

Approximately one in every 200 mammalian proteins is anchored to the cell membrane through a glycosylphosphatidylinositol (GPI) anchor. These proteins play important roles notably in neurological development and function. To date, more than 20 genes have been implicated in the biogenesis of GPI-anchored proteins. GPAA1 (glycosylphosphatidylinositol anchor attachment 1 protein) is an essential component of the transamidase complex along with PIGK, PIGS, PIGT and PIGU (Phosphatidylinositol-glycan biosynthesis class K, S, T and U proteins). This complex orchestrates the attachment of the GPI anchor to the carboxyl terminus of precursor proteins in the endoplasmic reticulum. Here we report bi-allelic mutations in GPAA1 in ten individuals from five families. Using whole exome sequencing, we identified two frameshift mutations (c.981_993del [p.Gln327Hisfs*102] and c.920delG [p.Gly307Alafs*11]), one intronic splicing mutation (c.1164+5C>T) and six missense mutations (c.152C>T [p.Ser51Leu], c.160_161delinsAA [p.Ala54Asn], c.527G>C [p.Trp176Ser], c.869T>C [p.Leu290Pro], c.872T>C [p.Leu291Pro] and c.1165G>C [p.Ala389Pro]). Most individuals presented with global developmental delay, hypotonia, early-onset seizures, cerebellar atrophy, and osteopenia. The splicing mutation was found to decrease GPAA1 mRNA. Moreover, flow cytometry analysis on five available individual samples showed that several GPI-anchored proteins had decreased cell surface abundance in leukocytes (FLAER, CD16, CD59) or fibroblasts (CD73, CD109). Transduction of fibroblasts with a lentivirus encoding the wild-type protein partially rescued the GPI-anchored protein deficiency. These findings highlight the role of the transamidase complex in the development and function of the cerebellum and the skeletal system.

Main text

Initially identified more than three decades ago[1], glycophosphatidylinositol (GPI) anchor proteins (AP) are emerging as a structurally diverse group of membrane-bound glycoproteins with important roles in cellular processes such as embryogenesis, fertilization and neurogenesis. It is estimated that there are about 150 GPI-APs in the human proteome.

The attachment of a GPI anchor to a protein is a conserved posttranslational modification which occurs in the endoplasmic reticulum (ER) and requires at least twelve reactions. Proteins bearing the GPI-anchor exit the ER and are shuttled to the Golgi where they undergo fatty acid modification before being transported to the plasma membrane. Crucial in the GPI biosynthetic pathway are a group of enzymes encoded by five genes (PIGK [MIM:605087], PIGS [MIM: 610271], PIGT [MIM: 610272], GPAA1 [MIM: 603048], and PIGU [MIM: 608528]) forming the GPI transamidase complex. These proteins mediate coupling of the synthesized GPI-anchor to mature proteins with a carboxyl terminal GPI-attachment signal peptide[2] in two principal steps; the cleavage of a C-terminal propeptide from the substrate protein and the formation of an amide bond between the C-terminal residue (ω-site) of the substrate protein and a phosphoethanolamine group of the GPI lipid anchor[2]. Among the five known subunits of the GPI lipid anchor transamidase complex, GPAA1 was the first to be discovered[3; 4] but its physiological role was only demonstrated recently[5]. This protein is composed of an N-terminal transmembrane domain, a luminal region and a hydrophobic region containing six transmembrane domains in the C-terminus. Recently, Eisenhaber et al. demonstrated that the luminal region of GPAA1 is similar to M28-type peptidases and that it may have one Zn-binding site like some of the M28 family peptidases. This protein was thus suggested to be a catalyst in the second

step to complete the transamidation[5]. Several inherited disorders are known to be caused by GPI biosynthesis defects[6]. However, mutations in GPAA1 have yet to be reported in diseases other than cancer[7-10]. Here, we present functional characterization of GPAA1 mutations in ten individuals with GPAA1 deficiency who manifest clinical features consistent with other inherited GPI-anchor deficiencies, including developmental delay, hypotonia and seizures.

Ten individuals from five unrelated families with bi-allelic GPAA1 (Gene bank: NM_003801.3) mutations were included in this study. Individuals were identified by a search in the Baylor Genetics Laboratory proband whole exome sequencing database, the DECIPHER database[11], Genematcher[12], and contacting collaborators. Informed consent was obtained from all families according to protocols approved by the Institutional Review Board at the relevant institutions. Most individuals had global developmental delay including late or absent independent walking and speech abilities. Among the more severely affected individuals, six individuals from families 1, 2, 3 and 5 are unable to walk independently at age 6 and up, and the individual from family 2 has not acquired speech as of age 6. Hypotonia was identified in all cases at an early age. Seizures were present in seven individuals; cerebellar atrophy was found in seven individuals (see MRI in figure 1B) and osteopenia also in five individuals. Further clinical features are summarized in table 1, table S1, and additional MRI images of family 4 can be found in figures S1 to S4.

Exome sequencing showed compound heterozygous mutations in the two siblings of family 1 (Hispanic origin from the USA), a missense mutation c.872T>C [p.Leu291Pro] inherited from the father and a frameshift mutation c.981_993del [p.Gln327Hisfs*102] which is from the mother (Figure 1). Individual 2 (II-2 of family 2 in figure 1, a white family, from the USA) has a missense mutation c.152C>T [p.Ser51Leu] from the mother and an

intronic splicing mutation c.1164+5C>T from the father. He has an unaffected sibling who carries only the c.152C>T [p.Ser51Leu] mutation but no splicing mutation. Two affected siblings from an Egyptian-origin family (family 3) have a frameshift mutation c.920delG [p.Gly307Alafs*11] and missense mutation c.1165G>C [p.Ala389Pro]. A homozygous missense mutation c.527G>C [p.Trp176Ser] was found in two Pakistani siblings from family 4 and a distant cousin, all of whose parents are consanguineous. Two affected siblings from family 5 (a Finnish family) had compound heterozygous mutations, a missense mutation c.869T>C [p.Leu290Pro] inherited from the father and a missense mutation c.160_161delinsAA [p.Ala54Asn] inherited from the mother. DNA from two unaffected siblings was not available for sequencing. These mutations were either not seen in ExAC or noted at very low frequency without homozygotes (Table 2). See figure 1 for the pedigrees and figure 2 for the location of the mutations and the conservation of the substituted amino acids.

Frameshift or splicing mutations that introduce premature stop codons before the last 50 nucleotides of the penultimate exon usually trigger nonsense-mediated mRNA decay (NMD). To determine if the mutations identified in our cohort triggered NMD, we performed real-time PCR on both fibroblasts and lymphoblastoid cell lines (LCLs) of individuals 1a and 1b (II-1 and II-2 of family 1 in figure 1) with the frameshift mutation (c.981_993del) which introduces a stop codon at amino acid 428 (p.Gln327Hisfs*102). We observed a decrease in GPAA1 mRNA up to 50% in both fibroblasts and LCLs (Figure 3A, B). Similarly, individual 2 (II-2 of family 2 in figure 1) with the intronic splicing mutation c.1164+5C>T also has decreased mRNA expression of GPAA1 in LCLs (Figure 3B).

To assess whether these mutations could affect the level of GPI-AP found at the cell surface, we performed flow cytometry analysis on whole blood and LCLs of affected

individuals from families 1, 2 and 4 (samples from families 3 and 5 were not available). Our results show that all tested individuals have GPI-AP deficiency. As shown in Figure 3C, individuals 1a and 1b (II-1 and II-2 of family 1 in figure 1) and 2 (II-2 of family 2 in figure 1) from two families presented with lower level of total GPI-AP in granulocytes as measured by a decrease in fluorescence-labeled aerolysin (FLAER) staining, while lower levels of CD16 were observed only in individuals 2 (II-2 of family 2 in figure 1), 4a and 4b (VII-1 and VII-2 of family 4 in figure 1). In all LCLs examined, cell surface staining also showed decreased FLAER abundance (Figure 3D). In addition, a moderate decrease in CD24 was observed in all individual cell lines (Figure S5).

Since fresh blood samples were not available for the affected individuals in family 3, we also tested the effect of the mutations by transfecting plasmids encoding mutant cDNAs driven by a weak promoter with only TATA box in cells knocked-out for GPAA1. GPAA1-deficient HAP1 cells were generated by CRISPR-Cas9 targeting of the gene. We then cloned human GPAA1 isoform 1 from a Hep3B cDNA library, and ligated to generate C-terminal tagged wild-type and variant pME hGPAA1 HA, then generated by site-directed mutagenesis the different variants. The pME promoter is the SRα promoter (SV40 early promoter + HTLV LTR, enhancer)[13]. We also subcloned the constructs in plasmids with an intermediate strength promoter (pTK using the herpes simplex virus thymidine kinase, or HSV-TK, promoter), and a weak promoter (pTA using the TATA box from the HSV-TK promoter). The pTA promoter activity is similar to that of a promoter-less plasmid. The promoter activities are estimated as pTA:pTK:pME = 1:60:6000.

These results show a decreased activity compared to the wild-type protein only for the p.Ser51Leu and the p.Ala389Pro variants (Figure 4A). In this overexpression experiment, the other variants (p.Trp176Ser and p.Leu291Pro) possibly have sufficient residual

activities to provide a rescue. Similar results were obtained after transfection with plasmids encoding the cDNAs under the control of stronger promoters (see Figures S6 and S7). Western blotting of cell lysates after transfection of cells with a strong promoter (pME) indicates that the p.Ala389Pro variant, located in the second transmembrane domain, leads to protein instability (Figure 4B). This could possibly be due to inadequate incorporation of the protein into the ER membrane.

We also assessed GPI-AP abundance on the cell surface of skin fibroblasts derived from individuals 1a, 1b (II-1 and II-2 of family 1 in figure 1) and 2 (II-2 of family 2 in figure 1) using FLAER, CD73 and CD109 as GPI cell surface markers (Figure S8). Since individuals 1a and individual 2 had a significant downregulation in CD109 and CD73, respectively, we carried out rescue assays of these GPI-APs on fibroblasts of these individuals using a GPAA1-encoding Lv105 lentiviral vector. As shown in Figure 5, CD109 in individual 1a fibroblasts was completely rescued, and a partial rescue of CD73 was observed in individual 2 fibroblasts. Collectively, these findings demonstrate that individuals with GPAA1 mutations have GPI-AP deficiency, which likely causes their clinical manifestations. The protein with the p.Trp176Ser variant might cause a milder biochemical effect since when overexpressed, no decreased activity was noted.

There is clinical overlap between these individuals and those with other GPI biosynthesis defects. Inherited mutations in PIGA [MIM: 311770][14-16], PIGM [MIM: 610273][17], PIGL [MIM: 605947][18], PIGW [MIM: 610275][19], PIGV [MIM: 610274][20; 21], PIGO [MIM: 610274][22; 23], PIGN [MIM: 606097][24], PIGC [MIM: 601730][25], PIGP [MIM: 605938][26] and PIGG [MIM: 616918][27] were identified as causing GPI-biosynthesis disorders. Similar disorders can also be caused by mutations in PGAP genes (Post GPI Attachment to Proteins, notably

PGAP1 [MIM: 611655][28], PGAP2 [MIM: 615187][29; 30] and PGAP3 [MIM: 611801][31]), which code for a family of proteins involved in structural remodeling of the GPI anchor after its attachment to proteins. The phenotypes of the individuals with GPAA1 mutations in the present study overlap with those identified in other GPI-biosynthesis disorders, such as the epilepsy, hypotonia and developmental delay[23; 32; 33], as well as the progressive cerebellar atrophy in individuals with PIGT and PIGN mutations[24; 34]. The rate and severity of the progressive atrophy is comparable to that seen with PIGT and PIGN deficiency, although the finding is is rarer in PIGN deficiency. The only reported GPI-transamidase complex gene associated with GPI deficiency is PIGT, which causes multiple congenital anomalies-hypotonia-seizures syndrome[4; 32; 33; 35], characterized by hypotonia, developmental delay, seizures, dysmorphisms, and malformations of the heart, urinary tract and gastrointestinal system. With relevance to the condition we describe here, most of these individuals also have osteopenia.

All the individuals we describe have normal plasma and/or serum alkaline phosphatase (ALP) levels (Table 1), which can be perturbed in other GPI-AP-synthesis-related disorders. Previous data suggested that the elevated serum ALP observed in some GPI-biosynthesis defects could be explained by cleavage of the signal peptide and secretion of soluble ALP because of abnormal GPI structure.[36] Since GPAA1 is a component of the GPI transamidase complex, mutations in this gene leading to decreased activity might lead to a decrease in serum ALP. This was not observed in our cohort, perhaps because the remaining enzymatic activity is sufficient to maintain normal serum ALP. This hypothesis is supported by the fact that no individual with bi-allelic loss-of-function mutations was identified. With regards to the mechanism leading to osteopenia, perhaps

decreased surface expression of ALP on osteoblasts affects its function and secondarily leads to the osteopenia seen in these individuals.

To date, GPI-transamidase subunits have been proposed to function as oncogenes in breast and bladder cancer[7-9]. In addition, GPAA1 mRNA was also found to be overexpressed, correlating with increased GPAA1 protein accumulation, in samples from individuals with colorectal cancer[10]. It is thought that an overexpression of GPI-biosynthesis genes might lead to increased uPAR (a GPI-anchored protein) which in turn can lead to increased invasiveness and growth of tumor cells[37].

Limited therapies are available for GPI-AP deficiency. Since alkaline phosphatase is essential to allow pyridoxal-phosphate to pass through the blood-brain barrier and pyridoxine passes through the blood-brain barrier and is converted to pyridoxal-phosphate, which is important for GABA synthesis, pyridoxine supplementation sometimes helps control the seizures in individuals with GPI-biosynthesis defects including some patients with PIGO and PIGV mutations[38; 39]. This potential treatment has not yet been tested in our cohort. Future studies of GPAA1 deficiency, such as in mouse models, could allow further pathophysiological studies to explore the cerebellar and bone manifestations, and explore therapeutic avenues.

In summary, we report biallelic mutations in GPAA1 in ten individuals presenting global developmental delay, hypotonia, early-onset seizurescerebellar atrophy., and osteopenia. Moreover, with functional studies, we demonstrate that these individuals have low cell surface GPI-AP levels demonstrating that those mutations cause a defect in the biosynthesis of GPI anchored proteins. Thus, our work expands the group of GPI-

biosynthesis disorders and contributes to the understanding of the role of the GPI transamidase complex in health and development.

Supplemental Data

Supplemental Data include supplemental text, one table, eight figures and can be found with this article online.

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Web Resources

ExAC Browser, http://exac.broadinstitute.org/

GenBank, http://www.ncbi.nlm.nih.gov/genbank/

OMIM, http://www.omim.org/

UniProt, http://www.uniprot.org/uniprot/

References

1. Kinoshita, T. (2014). Biosynthesis and deficiencies of glycosylphosphatidylinositol. Proc Jpn Acad Ser B Phys Biol Sci 90, 130-143.
2. Kinoshita, T. (2014). Enzymatic mechanism of GPI anchor attachment clarified. Cell Cycle 13, 1838-1839.
3. Hiroi, Y., Komuro, I., Matsushita, I., Aburatani, H., Hosoda, T., Nakahori, Y., Medof, M.E., and Yazaki, Y. (1998). Assignment of the human GPAA1 gene, which encodes a product required for the attachment of glycosylphosphatidylinositols to proteins, at 8q24. Genomics 54, 354-355.
4. Skauli, N., Wallace, S., Chiang, S.C., Baroy, T., Holmgren, A., Stray-Pedersen, A., Bryceson, Y.T., Stromme, P., Frengen, E., and Misceo, D. (2016). Novel PIGT Variant in Two Brothers: Expansion of the Multiple Congenital Anomalies-Hypotonia Seizures Syndrome 3 Phenotype. Genes (Basel) 7.
5. Eisenhaber, B., Eisenhaber, S., Kwang, T.Y., Gruber, G., and Eisenhaber, F. (2014). Transamidase subunit GAA1/GPAA1 is a M28 family metallo-peptide-synthetase that catalyzes the peptide bond formation between the substrate protein's omega-site and the GPI lipid anchor's phosphoethanolamine. Cell Cycle 13, 1912-1917.
6. Ng, B.G., and Freeze, H.H. (2015). Human genetic disorders involving glycosylphosphatidylinositol (GPI) anchors and glycosphingolipids (GSL). J Inherit Metab Dis 38, 171-178.
7. Nagpal, J.K., Dasgupta, S., Jadallah, S., Chae, Y.K., Ratovitski, E.A., Toubaji, A., Netto, G.J., Eagle, T., Nissan, A., Sidransky, D., et al. (2008). Profiling the expression pattern of GPI transamidase complex subunits in human cancer. Mod Pathol 21, 979-991.
8. Zhao, P., Nairn, A.V., Hester, S., Moremen, K.W., O'Regan, R.M., Oprea, G., Wells, L., Pierce, M., and Abbott, K.L. (2012). Proteomic identification of glycosylphosphatidylinositol anchor-dependent membrane proteins elevated in breast carcinoma. J Biol Chem 287, 25230-25240.
9. Wu, G., Guo, Z., Chatterjee, A., Huang, X., Rubin, E., Wu, F., Mambo, E., Chang, X., Osada, M., Sook Kim, M., et al. (2006). Overexpression of glycosylphosphatidylinositol (GPI) transamidase subunits phosphatidylinositol glycan class T and/or GPI anchor attachment 1 induces tumorigenesis and contributes to invasion in human breast cancer. Cancer Res 66, 9829-9836.
10. Chen, G., Li, S.Y., Cai, H.Y., and Zuo, F.Y. (2014). Enhanced expression and significance of glycosylphosphatidylinositol anchor attachment protein 1 in colorectal cancer. Genet Mol Res 13, 499-507.
11. Firth, H.V., Richards, S.M., Bevan, A.P., Clayton, S., Corpas, M., Rajan, D., Van Vooren, S., Moreau, Y., Pettett, R.M., and Carter, N.P. (2009). DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet 84, 524-533.
12. Sobreira, N., Schiettecatte, F., Valle, D., and Hamosh, A. (2015). GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Human mutation 36, 928-930.
13. Takebe, Y., Seiki, M., Fujisawa, J., Hoy, P., Yokota, K., Arai, K., Yoshida, M., and Arai, N. (1988). SR alpha promoter: an efficient and versatile mammalian cDNA expression system

composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat. Mol Cell Biol 8, 466-472.
14. Johnston, J.J., Gropman, A.L., Sapp, J.C., Teer, J.K., Martin, J.M., Liu, C.F., Yuan, X., Ye, Z., Cheng, L., Brodsky, R.A., et al. (2012). The phenotype of a germline mutation in PIGA: the gene somatically mutated in paroxysmal nocturnal hemoglobinuria. Am J Hum Genet 90, 295-300.
15. Swoboda, K.J., Margraf, R.L., Carey, J.C., Zhou, H., Newcomb, T.M., Coonrod, E., Durtschi, J., Mallempati, K., Kumanovics, A., Katz, B.E., et al. (2014). A novel germline PIGA mutation in Ferro-Cerebro-Cutaneous syndrome: a neurodegenerative X-linked epileptic encephalopathy with systemic iron-overload. Am J Med Genet A 164A, 17-28.
16. van der Crabben, S.N., Harakalova, M., Brilstra, E.H., van Berkestijn, F.M., Hofstede, F.C., van Vught, A.J., Cuppen, E., Kloosterman, W., Ploos van Amstel, H.K., van Haaften, G., et al. (2014). Expanding the spectrum of phenotypes associated with germline PIGA mutations: a child with developmental delay, accelerated linear growth, facial dysmorphisms, elevated alkaline phosphatase, and progressive CNS abnormalities. Am J Med Genet A 164A, 29-35.
17. Almeida, A.M., Murakami, Y., Layton, D.M., Hillmen, P., Sellick, G.S., Maeda, Y., Richards, S., Patterson, S., Kotsianidis, I., Mollica, L., et al. (2006). Hypomorphic promoter mutation in PIGM causes inherited glycosylphosphatidylinositol deficiency. Nature medicine 12, 846-851.
18. Ng, B.G., Hackmann, K., Jones, M.A., Eroshkin, A.M., He, P., Wiliams, R., Bhide, S., Cantagrel, V., Gleeson, J.G., Paller, A.S., et al. (2012). Mutations in the glycosylphosphatidylinositol gene PIGL cause CHIME syndrome. Am J Hum Genet 90, 685-688.
19. Chiyonobu, T., Inoue, N., Morimoto, M., Kinoshita, T., and Murakami, Y. (2014). Glycosylphosphatidylinositol (GPI) anchor deficiency caused by mutations in PIGW is associated with West syndrome and hyperphosphatasia with mental retardation syndrome. Journal of medical genetics 51, 203-207.
20. Krawitz, P.M., Schweiger, M.R., Rodelsperger, C., Marcelis, C., Kolsch, U., Meisel, C., Stephani, F., Kinoshita, T., Murakami, Y., Bauer, S., et al. (2010). Identity-by-descent filtering of exome sequence data identifies PIGV mutations in hyperphosphatasia mental retardation syndrome. Nat Genet 42, 827-829.
21. Horn, D., Krawitz, P., Mannhardt, A., Korenke, G.C., and Meinecke, P. (2011). Hyperphosphatasia-mental retardation syndrome due to PIGV mutations: expanded clinical spectrum. American journal of medical genetics Part A 155A, 1917-1922.
22. Krawitz, P.M., Murakami, Y., Hecht, J., Kruger, U., Holder, S.E., Mortier, G.R., Delle Chiaie, B., De Baere, E., Thompson, M.D., Roscioli, T., et al. (2012). Mutations in PIGO, a member of the GPI-anchor-synthesis pathway, cause hyperphosphatasia with mental retardation. Am J Hum Genet 91, 146-151.
23. Nakamura, K., Osaka, H., Murakami, Y., Anzai, R., Nishiyama, K., Kodera, H., Nakashima, M., Tsurusaki, Y., Miyake, N., Kinoshita, T., et al. (2014). PIGO mutations in intractable epilepsy and severe developmental delay with mild elevation of alkaline phosphatase levels. Epilepsia 55, e13-17.
24. Ohba, C., Okamoto, N., Murakami, Y., Suzuki, Y., Tsurusaki, Y., Nakashima, M., Miyake, N., Tanaka, F., Kinoshita, T., Matsumoto, N., et al. (2014). PIGN mutations cause congenital anomalies, developmental delay, hypotonia, epilepsy, and progressive cerebellar atrophy. Neurogenetics 15, 85-92.
25. Edvardson, S., Murakami, Y., Nguyen, T.T., Shahrour, M., St-Denis, A., Shaag, A., Damseh, N., Le Deist, F., Bryceson, Y., Abu-Libdeh, B., et al. (2017). Mutations in the

phosphatidylinositol glycan C (PIGC) gene are associated with epilepsy and intellectual disability. Journal of medical genetics 54, 196-201.
26. Johnstone, D.L., Nguyen, T.T., Murakami, Y., Kernohan, K.D., Tetreault, M., Goldsmith, C., Doja, A., Wagner, J.D., Huang, L., Hartley, T., et al. (2017). Compound heterozygous mutations in the gene PIGP are associated with early infantile epileptic encephalopathy. Hum Mol Genet.
27. Makrythanasis, P., Kato, M., Zaki, M.S., Saitsu, H., Nakamura, K., Santoni, F.A., Miyatake, S., Nakashima, M., Issa, M.Y., Guipponi, M., et al. (2016). Pathogenic Variants in PIGG Cause Intellectual Disability with Seizures and Hypotonia. Am J Hum Genet 98, 615-626.
28. Murakami, Y., Tawamie, H., Maeda, Y., Buttner, C., Buchert, R., Radwan, F., Schaffer, S., Sticht, H., Aigner, M., Reis, A., et al. (2014). Null mutation in PGAP1 impairing Gpi-anchor maturation in patients with intellectual disability and encephalopathy. PLoS Genet 10, e1004320.
29. Hansen, L., Tawamie, H., Murakami, Y., Mang, Y., ur Rehman, S., Buchert, R., Schaffer, S., Muhammad, S., Bak, M., Nothen, M.M., et al. (2013). Hypomorphic mutations in PGAP2, encoding a GPI-anchor-remodeling protein, cause autosomal-recessive intellectual disability. Am J Hum Genet 92, 575-583.
30. Krawitz, P.M., Murakami, Y., Riess, A., Hietala, M., Kruger, U., Zhu, N., Kinoshita, T., Mundlos, S., Hecht, J., Robinson, P.N., et al. (2013). PGAP2 mutations, affecting the GPI-anchor-synthesis pathway, cause hyperphosphatasia with mental retardation syndrome. Am J Hum Genet 92, 584-589.
31. Howard, M.F., Murakami, Y., Pagnamenta, A.T., Daumer-Haas, C., Fischer, B., Hecht, J., Keays, D.A., Knight, S.J., Kolsch, U., Kruger, U., et al. (2014). Mutations in PGAP3 impair GPI-anchor maturation, causing a subtype of hyperphosphatasia with mental retardation. Am J Hum Genet 94, 278-287.
32. Nakashima, M., Kashii, H., Murakami, Y., Kato, M., Tsurusaki, Y., Miyake, N., Kubota, M., Kinoshita, T., Saitsu, H., and Matsumoto, N. (2014). Novel compound heterozygous PIGT mutations caused multiple congenital anomalies-hypotonia-seizures syndrome 3. Neurogenetics 15, 193-200.
33. Kvarnung, M., Nilsson, D., Lindstrand, A., Korenke, G.C., Chiang, S.C., Blennow, E., Bergmann, M., Stodberg, T., Makitie, O., Anderlid, B.M., et al. (2013). A novel intellectual disability syndrome caused by GPI anchor deficiency due to homozygous mutations in PIGT. Journal of medical genetics 50, 521-528.
34. Maydan, G., Noyman, I., Har-Zahav, A., Neriah, Z.B., Pasmanik-Chor, M., Yeheskel, A., Albin-Kaplanski, A., Maya, I., Magal, N., Birk, E., et al. (2011). Multiple congenital anomalies-hypotonia-seizures syndrome is caused by a mutation in PIGN. J Med Genet 48, 383-389.
35. Pagnamenta, A.T., Murakami, Y., Taylor, J.M., Anzilotti, C., Howard, M.F., Miller, V., Johnson, D.S., Tadros, S., Mansour, S., Temple, I.K., et al. (2017). Analysis of exome data for 4293 trios suggests GPI-anchor biogenesis defects are a rare cause of developmental disorders. Eur J Hum Genet.
36. Murakami, Y., Kanzawa, N., Saito, K., Krawitz, P.M., Mundlos, S., Robinson, P.N., Karadimitris, A., Maeda, Y., and Kinoshita, T. (2012). Mechanism for release of alkaline phosphatase caused by glycosylphosphatidylinositol deficiency in patients with hyperphosphatasia mental retardation syndrome. The Journal of biological chemistry 287, 6318-6325.
37. Smith, H.W., and Marshall, C.J. (2010). Regulation of cell signalling by uPAR. Nature reviews Molecular cell biology 11, 23-36.

38. Thompson, M.D., Killoran, A., Percy, M.E., Nezarati, M., Cole, D.E., and Hwang, P.A. (2006). Hyperphosphatasia with neurologic deficit: a pyridoxine-responsive seizure disorder? Pediatric neurology 34, 303-307.
39. Kuki, I., Takahashi, Y., Okazaki, S., Kawawaki, H., Ehara, E., Inoue, N., Kinoshita, T., and Murakami, Y. (2013). Vitamin B6-responsive epilepsy due to inherited GPI deficiency. Neurology 81, 1467-1469.

Figure titles and legends

Figure 1. A) Pedigrees of five families with GPAA1 mutations in the study. B) MRI of individual 4b (VII-2 of family 4) at 25 months. T1 sagittal image shows marked reduction in cerebellar volume (arrow). C) Photographs of, starting from the top right, individuals 1a (age 15), 1b (age 10), 3a (age 10), 3b (age 3), and 5 (age 25). A slightly broad and prominent root of the nose is noted in individuals 1b, 3b and 5.

Figure 2. A) Location of the protein variants on the GPAA1 protein (for the splicing mutation, the site of the exon-exon junction is shown). B) Amino acid conservation in vertebrates of the amino acids affected by substitution mutations.

Figure 3. GPAA1 expression by Real time-PCR in individuals from families 1 and 2. RNA extractions from fibroblasts (A) and LCLs (B) of individuals 1a, 1b and 2 were subjected to qRT-PCR. Figures show the results normalized to a reference gene (TBP for fibroblast and GAPDH for LCLs) from quadruplicate experiments. C) GPI-AP abundance in granulocytes of individuals from familly 1, 2 and 4. Fresh blood samples as well as healthy controls were fixed with 10% formaldehyde, red blood cells were lysed in 0.1% Triton X-100 and the samples were stained with GPI-AP markers (FLAER, CD16) for 1 hour at room temperature. Non-specific binding was washed before analyzing by BD FACScanto II system. The figure shows representative results from experiments done in triplicate. D) GPI-AP abundance in LCLs of individuals from familly 1, 2 and 4. LCLs were established by Epstein-Barr virus immortalization of peripheral blood mononuclear cells (PMBC). Cells were stained with a GPI-AP marker (FLAER) for 1 hour at room temperature. Non-specific binding was washed before analysing the cell surface abundance of GPI-APs by BD FACScanto II system. The figure shows representative results from experiments done in triplicate.

Figure 4. A) Rescue of GPAA1 knockout cells. GPAA1-knockout cells were transiently transfected with a plasmid encoding wild-type or variant GPAA1 under the control of a weak promoter (pTA plasmid) by electroporation. Transfection efficiency was monitored by luciferase assay, and flow cytometry analysis was performed 2 days after transfection. These results show a slightly decreased labelling only for the p.Ser51Leu and the p.Ala389Pro variants (fewer cells with a strong signal), possibly because the residual activities for the other variants are sufficient to provide a rescue in this experiment. B) The p.Ala389Pro variant leads to protein instability. HEK293 cells were transiently transfected with various GPAA1 cDNAs and proteins were analyzed by Western Blotting using an anti-HA antibody (Cell Signaling). Intensities of the bands were normalized with the loading control (GAPDH), and luciferase activities used for evaluating transfection efficiencies. Western blot for the tagged protein indicates that p.Ala389Pro, in the second transmembrane domain, leads to protein instability.

Figure 5. Lentivirus rescue assays in fibroblasts from individuals 1a and 2. Skin fibroblasts derived from individuals 1a and 2 were transduced with GPAA1-expressing-Lv105 lentivirus or empty vector-lentivirus, then transduced, and non-transduced cells were stained with CD109 and CD73 for 1 hour at room temperature. The non-specific binding was washed before analysing the cell surface abundance of GPI-APs by BD FACScanto II system. The figure shows representative results from experiments done in triplicate.

1 **Tables**

2 Table 1. Key clinical features. More details can be found in the supplement.

Individual	1a	1b	2	3a	3b	4a	4b	4c	5a	5b
Gender	F	M	M	F	F	F	M	F	M	M
Age at last assessment (yr)	15	10	6y	10.7	3.8	8	5	4	30	25
Height, cm	152.4 (8th %ile)	127 (4th %ile)	103.2 (59th %ile) at 4 y	125 (1st %ile, -2.3 SD)	95 (15th %ile)	109 (1st %ile, -3.3 SD)	98 (1st %ile, -2.3 SD)	93 (1st %ile -2.2 SD)	165 (– 2 SD)	167 (– 1.8 SD)
Weight, kg	54.4 (58th %ile)	27.8 (16th %ile)	15.4 (30th %ile)	35 (49th %ile)	14 (19th %ile)	23.6 (27th %ile)	17 (23rd %ile)	15 (31st %ile)	70 (48th%ile)	62.4 (31st %ile)
OFC, cm	53 (14th %ile)	52 (27th %ile)	52 (87th %ile)	49.5 (1st %ile, -2.3 SD)	48 (19th %ile)	51 (31st %ile)	48.6 (4th %ile)	48 (18th %ile)	57,2 (50th %ile)	57,2 (50th %ile)
Developmental delay/ Intellectual disability[a]	++	++	++	++	++	++	++	+	++	++
Hypotonia	+	+	+	+	+	+	+	++	+	+
Seizures	GTC, myoclonic, atonic	GTC	GTC	GTC	GTC, myoclonic	No	No	No	GTC, myoclonic, atonic, absence	GTC, myoclonic
Cerebellar atrophy	+	+	- at age 1	+	+	+	+	+	+	+
Nystagmus	+	+	- but cortical visual impairment	+	+	+	-	++	+	+

Dysarthria	NA	+	NA	+	NA	NA	NA	+	+	+
Dysmetria	+	+	NA	NA	NA	NA	+	+	+	+
Ataxic gait	+	+	NA	NA	NA	+	+	++	+	+
Spasticity	-	-	-	+	NA	+	+	+	-	-
Osteopenia	+	+	+	+ (Z=-2.2)	+ (Z=-2.0)	+	+	+	NA	NA
Dysmorphisms	None	Slightly broad root of the nose	Bitemporal narrowing with prominent forehead, anterevetednares	Bitemporal narrowing, widely spaced eyes, prominent ears	Slightly broad root of the nose	Bitemporal narrowing, prominent forehead, anterevetednares,	Bitemporal narrowing, prominent forehead, anterevetednares	Narrow prominent forehead antevertednares	None	Slightly broad root of the nose
Plasma alkaline phosphatase (U/L)	155 (Nl)	143 (Nl)	223 (Nl)	355 (Nl)	624 (Nl)	176 (Nl)	183 (Nl)	201 (Nl)	**Serum** alkaline phosphatase 465 (275-875 U/L)(Nl)	180 (Nl)

[a]Legend: For the intellectual disability, + refers to mild, ++ to moderate, +++ to severe.; NA, not available; Nl, normal; GTC, generalized tonic-clonic seizure; OFC, occipitofrontal circumference; F, female; M, male; %ile, percentile; SD, standard deviation from the mean.

Table 2. Genetic description of the GPAA1 variants.

Family	Genomic variant (hg19)	cDNA variant (NM_003801.3)	Protein variant	Inheritance	ExAC minor allele frequency
1	chr8:g.145139374T>C	c.872T>C	p.Leu291Pro	Compound heterozygous	Not present
	chr8:g.145139483_145139495del	c.981_993del	p.Gln327Hisfs*102	Compound heterozygous	Not present
2	chr8:g.145138104C>T	c.152C>T	p.Ser51Leu	Compound heterozygous	Not present
	chr8:g.145139783C>T	c.1164+5C>T	Splicing	Compound heterozygous	1.661e-05 total, 0.0001226 in South Asians. No homozygotes detected.
3	chr8:g.145139422del	c.920delG	p.Gly307Alafs*11	Compound heterozygous	Not present
	chr8:g.145139946 G>C	c.1165G>C	p.Ala389Pro	Compound heterozygous	8.681e-06 total, 7.592e-05 in South Asians. No homozygotes detected.
4	chr8:g.145138854G>C	c.527G>C	p.Trp176Ser	Homozygous	1.658e-05 total, 0.0001211 in South Asians. No homozygotes detected.

5	chr8:g.145138112_145138113delinsAA	c.160_161delinsAA	p.Ala54Asn	Compound heterozygous	Not present
	chr8:g.145139371T>C	c.869T>C	p.Leu290Pro	Compound heterozygous	Not present

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