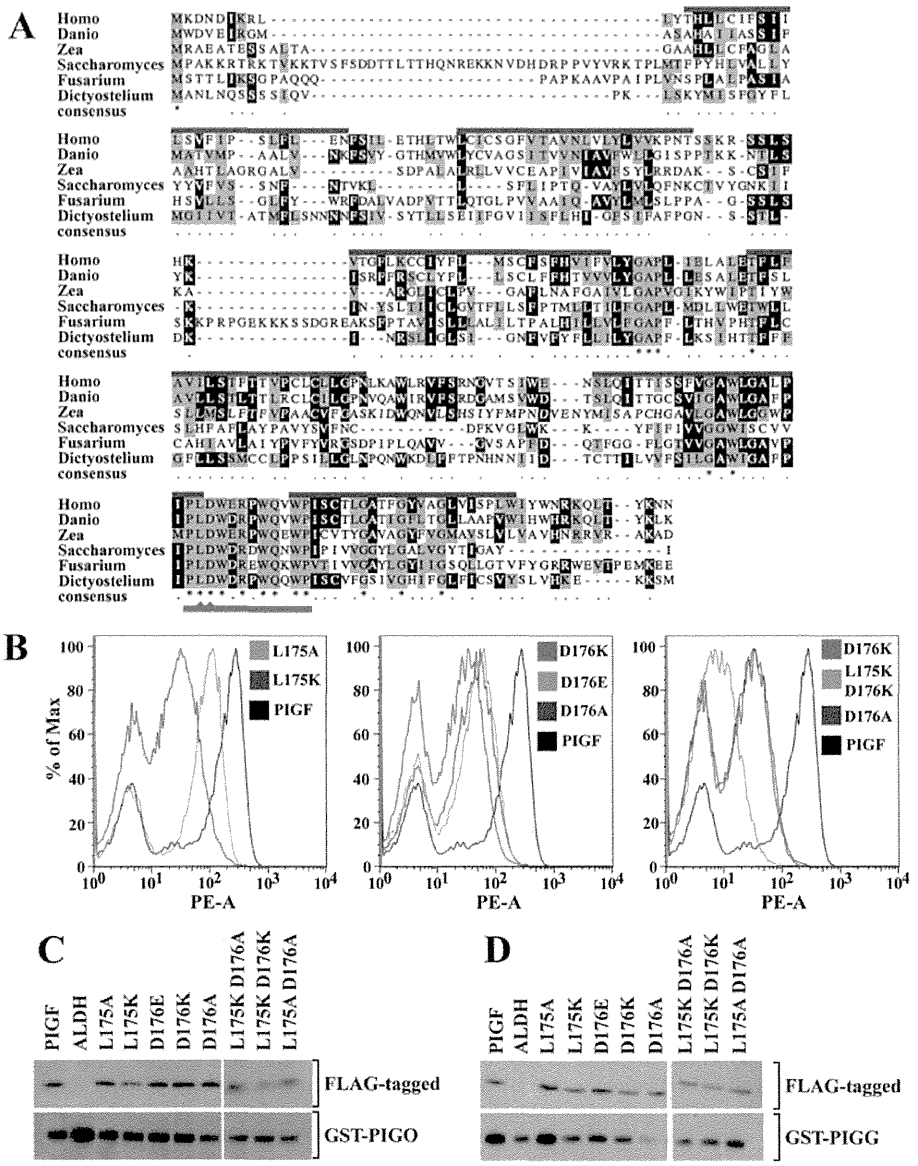


**Figure 4 Evidence of functional motif in PIGF**

(**A**) Alignment of amino acid sequences of PIGF homologues from *Homo sapiens* (gi|48146117), *Danio rerio* (gi|45387713), *Zea mays* (gi|525344664), *Saccharomyces cerevisiae* (gi|849212), *Fusarium pseudograminearum* (gi|408397024) and *Dictyostelium discoideum* (gi|66809485). Purple bars indicate predicted TM regions. Red bar indicates conserved motif with $Leu^{175}$ and $Asp^{176}$ indicated by triangles. (**B**) PIGFs containing point mutations analysed for their ability to restore Thy-1 expression in class F cells using FACS analysis. (**C** and **D**) CHO-K1 cells transiently co-expressing PIGFs containing point mutations and GST–PIGO (**C**) or GST–PIGG (**D**) were lysed in 1 % digitonin. The GST-tagged proteins in the supernatant were precipitated using glutathione beads. GST-tagged (bottom panels) and co-precipitated FLAG-tagged proteins (top panels) were detected by Western blot analysis using anti-GST and anti-FLAG respectively.

this scenario further, we took advantage of another well-studied model organism of GPI biosynthesis, *T. brucei*. *T. brucei* is a protozoan parasite responsible for African sleeping sickness in human and nagana in livestock. GPI anchors in this parasite are not modified by additional EtNP residues and there is only one protein-anchoring EtNP attached to the third mannose. As such, we could only identify a PIGO homologue in the *T. brucei* genome and homologues of PIGN and PIGG are absent. If PIGF functions solely to control the relative activities of PIGG and PIGO, there is no reason for PIGF to exist in the *T. brucei* genome. It was therefore surprising that we could identify TbPIGF and demonstrate its interaction with TbPIGO (see Figure 5). Our new data suggest that evolution of PIGF is not necessarily dependent on the presence of PIGG. Taken together with the finding that PIGF functions beyond its stabilizing roles in the formation of heterodimers, we propose that PIGF plays a more fundamental role in the biosynthesis of GPI.

There are several possibilities for the additional functions of PIGF. Since it is involved in the transfer of EtNP from PE to a GPI intermediate, it is possible that PIGF is involved in the

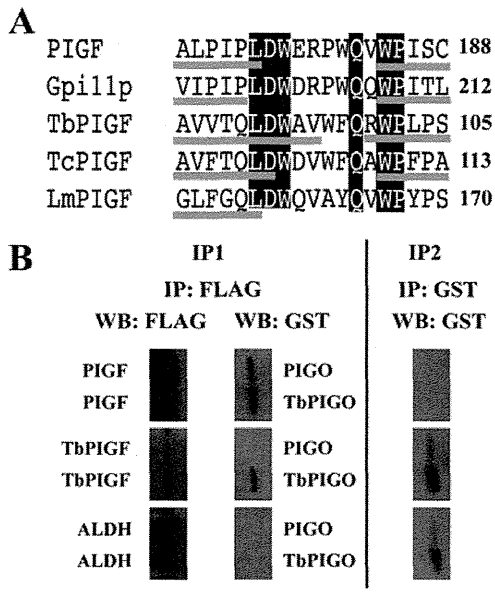


**Figure 5 PIGF and PIGO homologues in *T. brucei***

(**A**) Comparison of conserved motifs from kinetoplastids with those from human (PIGF) and yeast (Gpi11p). TbPIGF (gi|71748704), TcPIGF (gi|71410645), and LmPIGF (gi|321399857: 612828-613430) indicate newly identified PIGF homologues from *T. brucei*, *Trypanosoma cruzi* and *Leishmania major*. (**B**) CHO-K1 cells transiently expressing FLAG-tagged PIGF, ALDH or TbPIGF were co-transfected with either GST-tagged TbPIGO or PIGO. Cells were lysed in 1% digitonin and the FLAG-tagged proteins in the supernatant were precipitated by anti-FLAG beads (IP1). FLAG-tagged proteins and co-precipitated GST-tagged proteins were detected by Western blot analysis using anti-GST and anti-FLAG respectively. Unbound GST-tagged proteins were immunoprecipitated by glutathione beads and detected by Western blotting using anti-GST antibody (IP2).

recruitment of these substrates to the catalytic subunit (i.e. PIGG or PIGO). We demonstrated that the aspartic acid residue in the last hydrophilic loop of PIGF is highly conserved. It is tempting to speculate that the conserved aspartic acid residue interacts with the positively charged amino group on either PE or GPI core glycan GlcN. Future studies are needed to determine its exact role in GPI biosynthesis at the molecular level.

Another focus of future research should be further analysis of heterodimer formation at the molecular level. Because both PIGF-ΔN3 and PIGF-C3 can bind to PIGG, one logical possibility is that PIGF binds to PIGG through the region between TM3 and TM4. Unfortunately, attempts to express further truncated PIGF mutants failed possibly due to the loss of protein stability. Therefore we cannot exclude an alternative possibility that two distinct binding motifs exist in PIGF for PIGG binding and one of them is sufficient for the binding of PIGF to PIGG. Whereas our current study could not pinpoint the precise amino acid residues of PIGF involved in heterodimer formation, our data demonstrate that PIGF contains two distinct binding domains: one C-terminal domain for PIGO and a second domain elsewhere for PIGG. Therefore it may be important to reconsider the possibility that PIGO, PIGG and PIGF form a heterotrimeric complex that allows efficient sequential additions of EtNP to the third and second mannose residues. Finally, we also know little about motifs in PIGO and PIGG involved in PIGF binding and more detailed studies are needed to determine the significance of interactions among these three proteins.

## AUTHOR CONTRIBUTION

Matthew Stokes and Yasu Morita designed and performed experiments, analysed data and wrote the paper. Yoshiko Murakami, Yusuke Maeda and Taroh Kinoshita contributed to experimental design and data analysis.

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RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

# Mutations in *PIGL* in a patient with Mabry syndrome

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Mabry syndrome, hyperphosphatasia mental retardation syndrome (HPMRS), is an autosomal recessive disease characterized by increased serum levels of alkaline phosphatase (ALP), severe developmental delay, intellectual disability, and seizures. Recent studies have revealed mutations in *PIGV*, *PIGW*, *PIGO*, *PGAP2*, and *PGAP3* (genes that encode molecules of the glycosylphosphatidylinositol (GPI)-anchor biosynthesis pathway) in patients with HPMRS. We performed whole-exome sequencing of a patient with severe intellectual disability, distinctive facial appearance, fragile nails, and persistent increased serum levels of ALP. The result revealed a compound heterozygote with a 13-bp deletion in exon 1 (c.36_48del) and a two-base deletion in exon 2 (c.254_255del) in phosphatidylinositol glycan anchor, class L (*PIGL*) that caused frameshifts resulting in premature terminations. The 13-bp deletion was inherited from the father, and the two-base deletion was inherited from the mother. Expressing c.36_48del or c.254_255del cDNA with an HA-tag at the C- or N-terminus in *PIGL*-deficient CHO cells only partially restored the surface expression of GPI-anchored proteins (GPI-APs). Nonsynonymous changes or frameshift mutations in *PIGL* have been identified in patients with CHIME syndrome, a rare autosomal recessive disorder characterized by colobomas, congenital heart defects, early onset migratory ichthyosiform dermatosis, intellectual disability, and ear abnormalities. Our patient did not have colobomas, congenital heart defects, or early onset migratory ichthyosiform dermatosis and hence was diagnosed with HPMRS, and not CHIME syndrome. These results suggest that frameshift mutations that result in premature termination in *PIGL* cause a phenotype that is consistent with HPMRS.



**Key words:** glycosylphosphatidylinositol anchor; Mabry syndrome; hyperphosphatasia mental retardation syndrome; genetic testing

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## INTRODUCTION

In 1970, Mabry et al. reported three siblings with increased serum levels of alkaline phosphatase (ALP), severe developmental delay, intellectual disability, and seizures [Mabry et al., 1970]. Subsequently, a condition displaying the aforementioned symptoms was referred to as hyperphosphatasia with mental retardation (HPMR) syndrome or Mabry syndrome [Krawitz et al., 2010]. Other clinical features included distinctive facial features such as hypertelorism, a

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broad nasal bridge, long palpebral fissures, and a tented mouth, as well as some degree of brachytelephalangy [Horn et al., 2011]. Variable neurological abnormalities, including seizures and muscular hypotonia, were also associated with this condition. Extensive biochemical analysis showed that hyperphosphatasia was unlikely to be the result of increased activity of osteoblastic cells or hepatobiliary dysfunction, and the causes of HPMRS were unknown [Kruse et al., 1988].

In 2010, Krawitz et al. performed whole-exome sequencing of three siblings with HPMRS (OMIM 239300) and identified homozygous or compound heterozygous mutations in *PIGV* in a total of six families with HPMRS [Krawitz et al., 2010]. *PIGV* encodes a molecule that acts as the second mannosyltransferase in the glycosylphosphatidylinositol (GPI) anchor biosynthesis pathway. Subsequently, mutations in *PIGO, PGAP2, PGAP3*, and *PIGW*, which are also involved in GPI biosynthesis, have been identified in individuals with HPMRS (OMIM 614749, OMIM 614207, OMIM 615716, and OMIM 610275, respectively) [Krawitz et al., 2012, 2013; Hansen et al., 2013; Chiyonobu et al., 2014; Howard et al., 2014]. In this study, we performed whole-exome sequencing of a family with HPMRS but without mutations in *PIGV* and *PIGO* and identified compound heterozygous mutations in *PIGL*.

## MATERIAL AND METHODS

### Exome Sequencing

DNA was extracted using a standard protocol from blood samples of an affected female and her parents. Control DNA was obtained from 192 healthy Japanese individuals. This study was approved by the Ethics Committee of Tohoku University School of Medicine. We obtained informed consent and specific consent for photographs from the parents of the affected individual.

Exome sequencing was conducted on an individual with HPMRS. Targeted enrichment was performed using the SureSelect Human All Exon V2 kit. Exon-enriched DNA libraries were sequenced on the Illumina Hiseq 2000 for 101 bp. Burrows-Wheeler Aligner (BWA) was used to align the sequence reads to the human genome (hg19); all parameters of BWA were kept at the default settings. Following removal of duplicates from the alignments, realignment around known indels, recalibration, and SNP/indel calling were performed with Genome Analysis Toolkit (GATK, 1.5) [McKenna et al., 2010]. ANNOVAR was used for the annotation against the RefSeq database and dbSNP [Wang et al., 2010].

### Sanger Sequencing

Each exon with flanking intronic sequences in *PIGV, PIGO, PIGN, PIGB*, and *PIGL* was amplified with primers based on GenBank sequences (NM_017837.2, NM_152850.3, NM_176787.4, NM_004855.4, and NM_004278.3, respectively). The M13 reverse or forward sequence was added to the 5′ end of the polymerase chain reaction (PCR) primers for use as sequencing primers. PCR was performed in 15 μl of solution containing 67 mM Tris–HCl (pH 8.8), 6.7 mM $MgCl_2$, 17 mM $NH_4SO_4$, 6.7 μM EDTA, 10 mM β-ME, 1.5 mM dNTPs, 10% (v/v) DMSO, 1 μM of each primer, 25 ng genomic DNA and 1 U of Taq DNA polymerase. The reaction consisted of 37 cycles of denaturation at 94°C for 20 sec, annealing at the indicated temperature for 30 sec and extension at 72°C for 30 sec. PCR products were purified using MultiScreen PCR plates (Millipore, Billerica, MA). The purified products were sequenced on an ABI 3500xL Genetic Analyzer (Applied Biosystems, Foster City, CA).

For subcloning, PCR products of exon 1 and 2 in *PIGL* were subcloned using a pTOPO TA cloning kit (Invitrogen, Carlsbad, CA) and transformed in TOP10F competent cells (Invitrogen). Plasmids were purified from each colony and sequenced.

### Flow Cytometry

Surface expression of GPI-anchored proteins (GPI-APs) of granulocytes was determined by staining cells with PE-conjugated mouse anti-human CD59 (H19), anti-human DAF (IA10), anti-human CD24 (ML5), anti-human CD16 (3G8) antibodies, each isotype IgG (BD Biosciences, Franklin Lakes, NJ) and Alexa 488-conjugated inactivated aerolysin (FLAER; Protox Biotech, Canada). CD59, DAF, CD24, and CD16 are GPI-APs and FLAER binds to surface GPI-APs. The surface expression was then analyzed using a flow cytometer (Canto II; BD Biosciences) and FlowJo software (Tommy Digital, Inc., Tokyo, Japan).

### Functional Analysis

*PIGL*-deficient CHO cells (M2S2) [Nakamura et al., 1997] were transiently transfected with *PIGL* cDNA that was driven by the strong SRα promoter (pME hPIGL-HA or pME HA-hPIGL). Two days later, cells were stained with anti-CD59, anti-DAF and FLAER and analyzed by flow cytometry. Lysates were separated using SDS–PAGE and Western blotting was performed.

## RESULTS

### Clinical Report

The patient was the first child of healthy and non-consanguineous Japanese parents. She was born at 33 weeks and 3 days of gestation by caesarean because of maternal infection. Her weight and height at birth were 2,510 g and 51 cm, respectively. She was treated with oxygen inhalation for several days because of transient tachypnea. She was suspected as having Beckwith–Wiedemann syndrome because of hypotonia, a large tongue, separation of the rectus abdominis muscle, and coarse facial features.

At 4 months of age, the patient's serum levels of ALP were found to be extremely high at 4,394 IU/L (Normal, 395–1,289 IU/L) and she was referred to our hospital. A radiograph of her hands showed a slight delay of bone maturation and slight dilation of the ulnar metaphysis. Alpha D3 administration was started at 5 months and ended at 1 year and 8 months of age. Laboratory investigations for inborn errors of metabolism, including toluidine blue staining in a urine sample, glycosaminoglycan in the urine, and lysosome enzymes, were normal. Karyotyping analysis on cultured leukocytes and subtelomere MLPA, FISH for 17p11.2 detecting Smith–Magenis syndrome, and array CGH analysis were all normal.

Twitching of the extremities with epileptic discharge on EEG started at 4 months of age and required anti-convulsant therapy. Brain CT and MRI showed dilatation of the bilateral lateral ven-

tricles, third and fourth ventricles and sub-arachnoid space, as well as hypoplasia of the cerebellar vermis. She was suspected as having mild deafness (right: 30 dB and left 45 dB).

At 1 year and 10 months, her weight and height were 7.9 kg (−2 SD) and 80 cm (−1 SD), respectively. Her craniofacial features included midface hypoplasia, hypertelorism, long palpebral fissures, strabismus, depressed nasal bridge, anteverted nostrils, tented upper lip vermilion, full cheeks, a high palate, and ear anomalies (Fig. 1A). Teeth eruption was not observed. In the extremities, the 2nd and 3rd digits were overlapping. The terminal phalanges of the hands and feet were short with hypoplastic nails. Her development was as follows: head control at 5 months, crawling at 1 year and 7 months, sitting at 2 year and 3 months, and walking while holding onto something at 2 years and 7 months. At 3 years and 6 months, she was unable to walk independently, and speech development was markedly delayed. ALP remained persistently elevated (3,000–4,500 U/L, Fig. 1B).

## Molecular and Biochemical Analysis

Mutation screening for genes in GPI biosynthesis showed that no mutations were identifiable by Sanger sequencing analysis in any exons coding for *PIGV, PIGO, PIGN,* and *PIGB.* We then performed exome sequencing with a sample from the proband. Using the sequencing analysis pipeline from BWA and GATK, we identified approximately 8,883 nonsynonymous, nonsense, splicing site variations, coding insertions, and/or deletions (indels) per individual. Filtering steps using variant databases (dbSNP132, the 1,000 Genome Project database and ESP 5,400) resulted in the identification of 216 variants. Two frameshift mutations in *PIGL* (c.36_48del [p.Leu13Alafs*11] and c.254_255del [p.Glu86Aspfs*2]) were detected as variants in genes that participate in the GPI-anchor biosynthesis (hsa00563) and the GPI-anchor biosynthesis (hsa00563)/metabolic pathway (hsa00110) in KEGG pathway, respectively. Sanger sequencing validated the heterozygous state of the two variants in *PIGL* (Fig. 2A). Two variants resulted in a premature termination and it was assumed that truncated proteins were produced (Fig. 2B).

Subcloning of PCR products of exons 1 and 2 from parental samples followed by sequencing showed that the father was heterozygous for c.36_48del and the mother was heterozygous for c.254_255del in *PIGL*, thus confirming the compound heterozygosity in the affected individual (Fig. 2A).

The c.36_48del and c.254_255del in *PIGL* were not reported in dbSNP132, the 1,000 Genome Project database or in ESP 5,400. c.254_255del, and not c.36_48del, was reported in one in 1,000 Japanese exomes (http://www.genome.med.kyoto-u.ac.jp/SnpDB/) and identified in 1 in 192 of our in-house Japanese controls in a hetrozygous manner, suggesting that c.254_255del could be identified at a frequency of 1:200–1:1000 in Japanese people.

These genomic analyses suggested that the patient has *PIGL*-deficiency, which is one of the inherited GPI deficiencies. More than 100 mammalian proteins are modified by a GPI anchor at their C terminus [Krawitz et al., 2010]. The expression of GPI-APs have been decreased in other inherited GPI deficiencies [Maydan et al., 2011; Chiyonobu et al., 2014; Howard et al., 2014]. To examine if the GPI-APs decreased in cells from our patient, peripheral blood cells were analyzed by flow cytometry. Surface expression of various GPI-APs on the granulocytes from the patient (Fig. 3, thick lines) was severely decreased (2% of the control in CD16 expression) compared with that from the normal samples (dotted lines).

*PIGL*-deficient CHO cells (M2S2) [Nakamura et al., 1997] were transiently transfected with wild type (Fig. 4, dotted line),

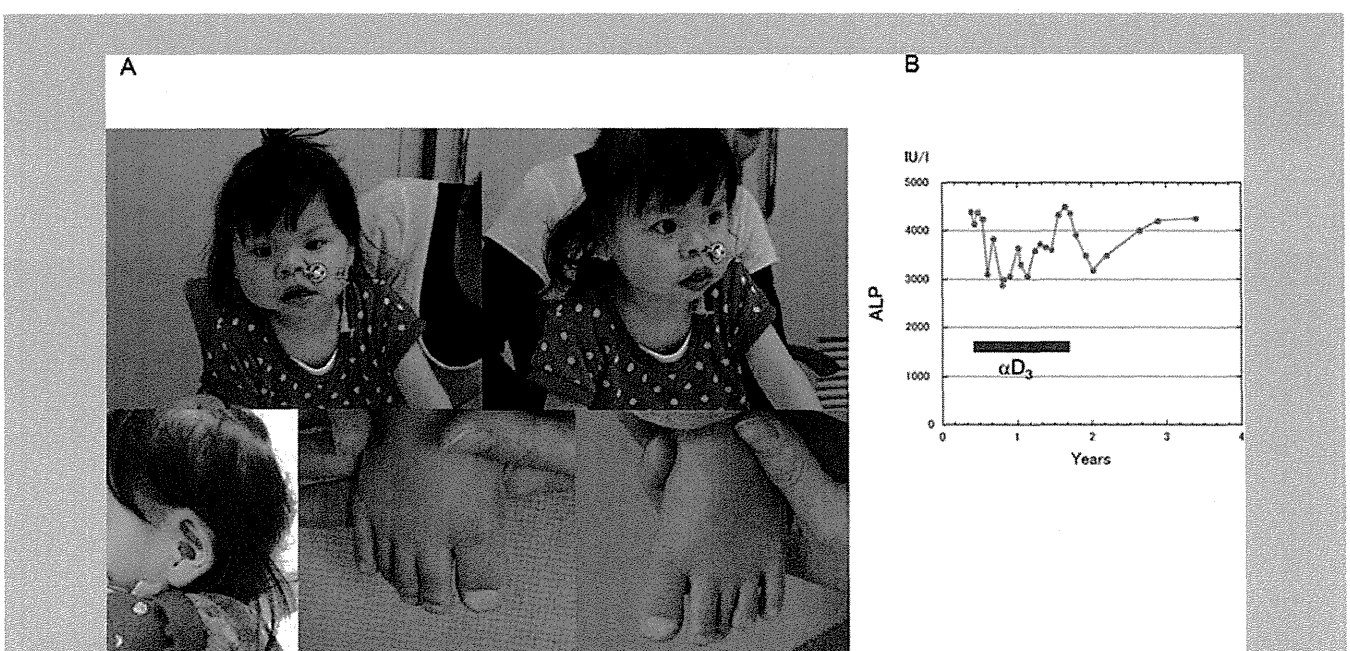


**FIG. 1. Clinical manifestation of the proband. A: Photos of the girl at 2 years and 7 months of age. B: Serum levels of ALP in the patient.**

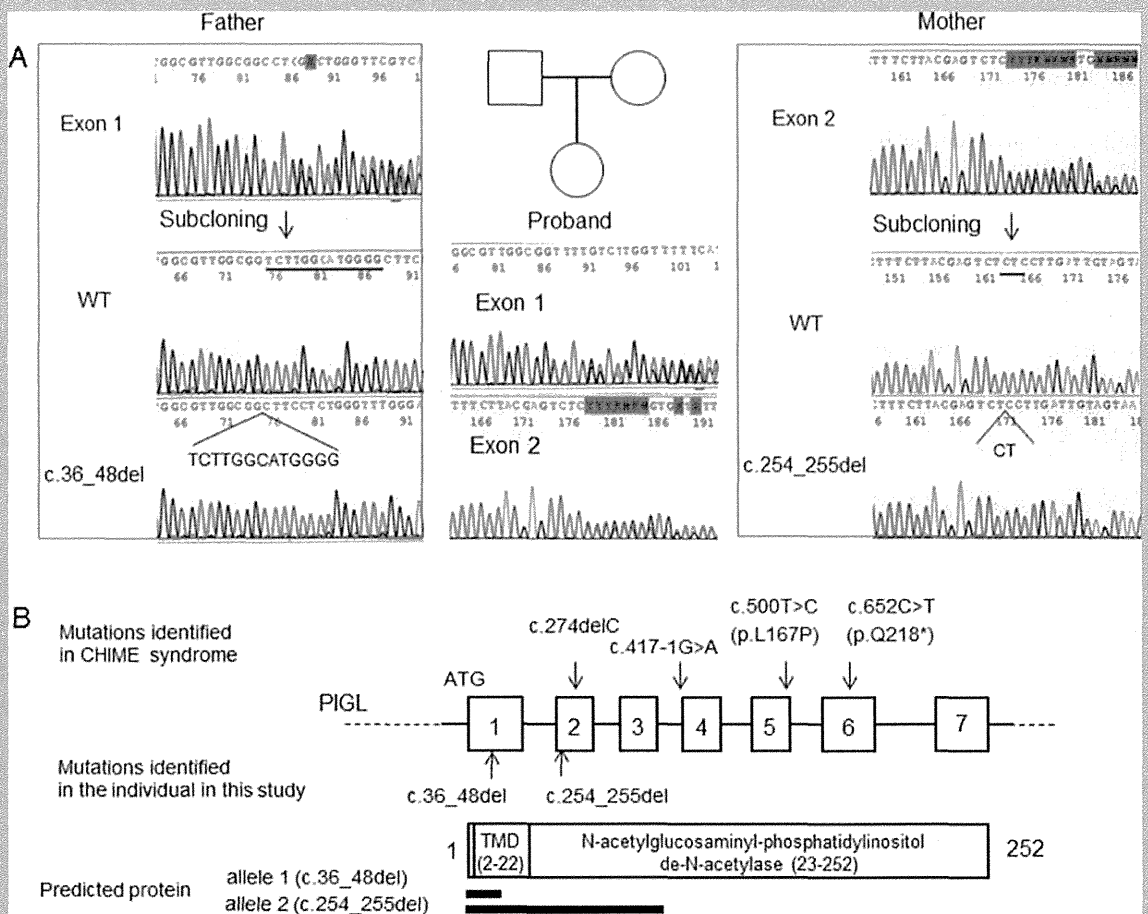


FIG. 2. Sanger sequencing of the family. A: Mutation analysis of the individual and her parents. B: The genomic structure of *PIGL* and mutations identified in CHIME syndrome and this study.

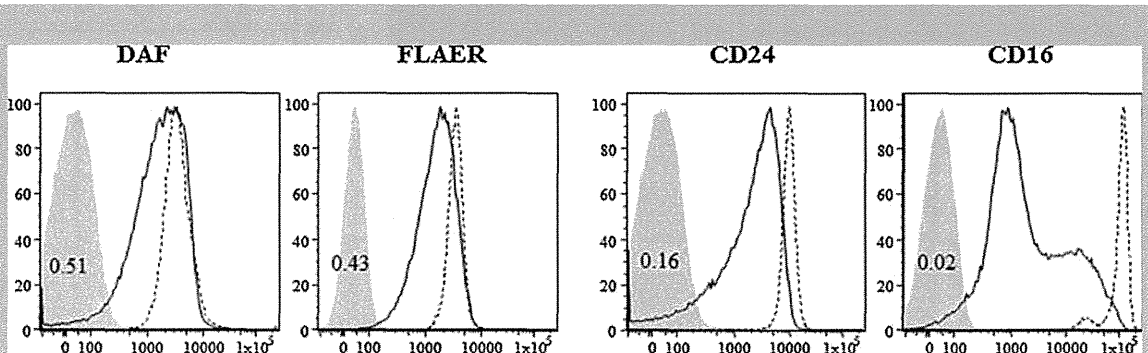


FIG. 3. Flow cytometry of granulocytes. Surface expression of various GPI-APs on the granulocytes from the patient (thick lines) was severely decreased compared with that from the normal samples (dotted lines). Light shadows are isotype controls. The value of the mean fluorescent intensity of each sample against normal is shown in each panel.

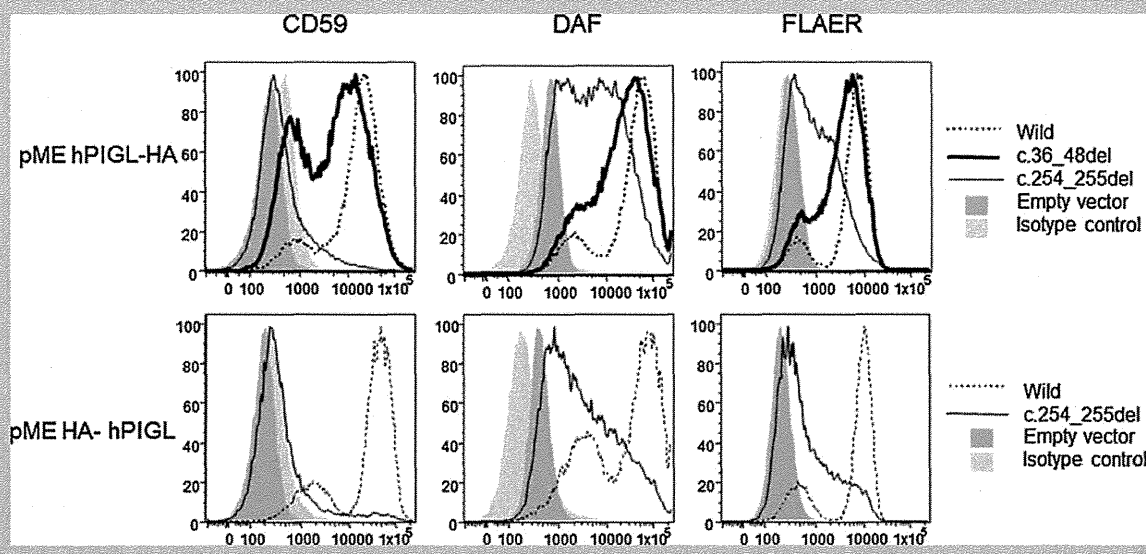


**FIG. 4. PIGL-deficient CHO cells (M2S2) [Nakamura et al., 1997] were transiently transfected with wild type (dotted line), the c.36_48del mutant (thick line), or the c.254_255del mutant (thin line) PIGL cDNA that was driven by the strong SRα promoter (pME hPIGL-HA or pME HA-hPIGL) with an HA-tag at the C-(upper panels) or N-terminus (lower panels). Two days later, cells were stained with anti-CD59, anti-DAF and FLAER and analyzed by flow cytometry. The gray shadow denotes empty vector transfections; light gray shadows are isotype controls.**

c.36_48del mutant (thick line) or c.254_255del mutant (thin line) *PIGL* cDNA with an HA-tag at the C-terminus (upper panels) or at the N-terminus (lower panels). Both mutants could only partially restore the surface expression of GPI-APs. The activity of the c.254_255del mutant was severely affected, and the N-terminal tagged construct had almost no activity. These results suggested that the remaining activity was not due to the truncated proteins. Faint bands (* and **) could be detected in the lysate of C-terminally tagged c.36_48 mutant transfected cells (Fig. 5A), which corresponded to the isoforms starting at the downstream methionines (2 and 3 of Fig. 5B) that showed residual PIGL activity. No band could be detected from the lysate of the c.256_255 mutant tagged at either terminus (data not shown).

## DISCUSSION

We report on the case of a girl with distinctive facial features, severe intellectual disability, and persistent increased serum levels of ALP who was diagnosed with HPMRS. Whole-exome sequencing identified two frameshift mutations in *PIGL*, which were inherited from the father and mother, suggesting that *PIGL* mutations are responsible for HPMRS.

Many eukaryotic cell surface proteins are bound to the cell membrane by a GPI-anchor. More than 20 different gene products are involved in GPI biosynthesis [Fujita and Kinoshita 2012]. Recent studies revealed that genetic defects in various components of the GPI-anchor biosynthesis pathway cause inherited GPI deficiencies. Somatic mutations in *PIGA* in hematopoietic stem cells cause paroxysmal nocturnal hemoglobinuria [Ware et al., 1994]. A promoter mutation in *PIGM* causes portal venous thrombosis and absence seizures [Almeida et al., 2006]. Germline mutations in *PIGN* and *PIGA* cause congenital anomalies with hypotonia and seizures [Maydan et al., 2011; Johnston et al., 2012]. Germline mutations in *PIGV*, *PIGO*, *PGAP2*, *PGAP3*, and *PIGW* have been identified in individuals with HPMRS [Krawitz et al., 2010, 2012, 2013; Chiyonobu et al., 2014; Howard et al., 2014]. Recently, mutations in *PIGT* have been identified in a family with intellectual disability [Kvarnung et al., 2013]. Thus, the clinical spectrum of disorders caused by the GPI-anchor deficiency has expanded.

*PIGL* encodes a 252 amino acid endoplasmic reticulum (ER) protein, located on the ER membrane with one transmembrane region and most of the protein located on the cytoplasmic side [Nakamura et al., 1997]. PIGL is involved in the second step of GPI biosynthesis, which is de-N-acetylation of N-acetylglucosaminyl-phosphatidylinositol (GlcNAc-PI). Following de-N-acetylation, glucosaminyl-phosphatidylinositol (GlcN-PI) flips to the luminal side of ER where GlcN-PI undergoes further extensions followed by its transfer to acceptor proteins [Nakamura et al., 1997; Watanabe et al., 1999]. Mutations in *PIGL* have been identified in CHIME syndrome, an autosomal recessive disorder characterized by colobomas, congenital heart defects, early onset migratory ichthyosiform dermatosis, intellectual disability, and ear anomalies, including conductive hearing loss [Ng et al., 2012]. Our patient manifested severe intellectual disability, seizures, ear anomalies, and feeding difficulties, which overlapped with symptoms of CHIME syndrome [Ng et al., 2012]. However, our patient did not have colobomas, congenital heart defects, early onset migratory ichthyosiform, or genitourinary abnormalities. Inherited GPI

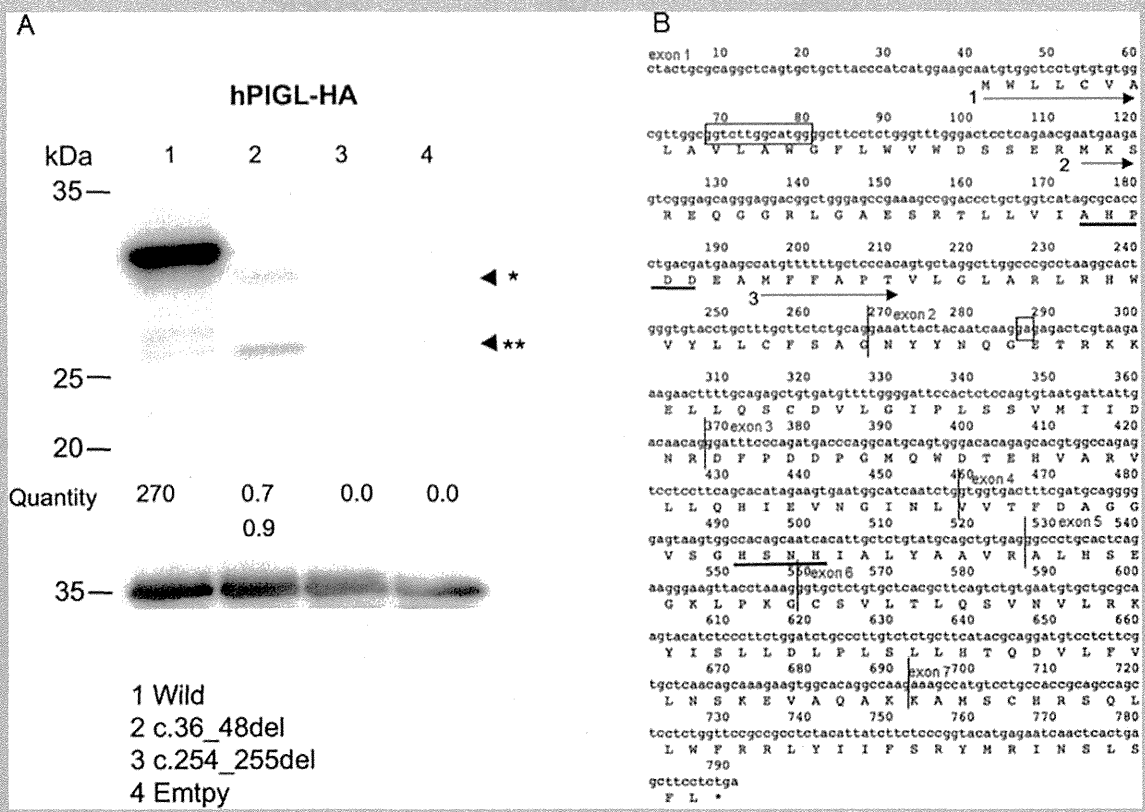


**FIG. 5. Functional analysis. A: Lysates were separated by SDS–PAGE and western blotting was performed. Faint bands (* and **) could be detected from the lysate of the C-terminally tagged c.36_48 mutant transfected cells, which corresponded to the isoform starting from the down stream methionines (2 and 3 in Fig. 5B) that showed residual *PIGL* activity. No band could be detected from the lysate of the c.256_255 mutant tagged at either terminus. (Normalized with the intensities of GAPDH, the loading control, and luciferase activities used for evaluating transfection efficiencies). B: The sequence of *PIGL* cDNA; thick lines, conserved motifs; numbered arrows, three translation initiation sites; boxes, deletions in this patient.**

deficiencies caused by a defect in the GPI-biosynthesis genes should show similar symptoms that result from the decreased expression of various GPI-APs on the cell surface. The severity depends on the amount of the GPI-anchor produced in ER, and the individual's genetic background may also have some influence on variations. Clinical manifestations in our patient were more similar to those in individuals with HPMRS, including hypertelorism, long palpebral fissures, broad nasal tip, tented upper lip, brachytelephalangy, severe intellectual disability and persistent hyperphosphatasia (Table I) [Horn et al., 2011]. These results suggest that frameshift mutations in 5′ terminus of *PIGL* cause HPMRS. It is possible that *PIGL* mutations identified in patients with CHIME syndrome might have higher residual activities.

Brain CT and MRI of our patient showed cerebellar atrophy. Frontotemporal atrophy and cerebellar hypoplasia have been shown in patients with *PIGT* mutations [Kvarnung et al., 2013]. Cerebral and cerebellar atrophy have been reported in a patient with *PIGO* mutation [Nakamura et al., 2014] and in patients with *PIGN* mutations [Maydan et al., 2011; Ohba et al., 2014], suggesting that cerebral atrophy and cerebellar atrophy are common features in patients with inherited GPI deficiencies. It is of note that cerebral atrophy has been also observed in patients with CHIME syndrome [Shashi et al., 1995; Schnur et al., 1997].

Our previous study demonstrated that mutant CHO cells having defects in the later step genes efficiently secrete ALP into the medium, whereas most ALP produced in the early step mutants is degraded in the cells [Murakami et al., 2012] because GPI transamidase is activated through binding with a mannose-containing GPI intermediate before this enzyme complex processes the precursor proteins for release. However, there are cases that are inconsistent with these experimental results. Some *PIGA*-deficient patients showed mild elevation of ALP (412 IU/L, normal range, 39–117 IU/L) [Johnston et al., 2012]. Elevation of ALP has not been reported in previously reported patients with *PIGL* deficiencies [Ng et al., 2012]. There may be differences in in vitro culture and in vivo body conditions; however, the primary reason for these differences needs to be identified in the future.

TABLE I. Summary of Clinical Features in Our Patient and HPMRS Patients With *PIGV*, *PIGO*, *PGAP2*, *PGAP3*, and *PIGW* Mutations

| | Our study (PIGL) | PIGV | PIGO | PGAP2 | PGAP3 | PIGW |
|---|---|---|---|---|---|---|
| References | | Horn et al. [2011]; Krawitz et al. [2010, 2012] | Krawitz et al. [2012] | Krawitz et al. [2013]; Hansen et al. [2013] | Howard et al. [2014] | Chiyonobu et al. [2014] |
| Sex | 1 female | 9 females and 5 males | 3 females | 4 female and 1 male | 4 females and 2 males | 1 proband |
| Age at assessment | 1 year 10 months | 7 months–17 years | 20 months–15 years | 3.5 and 28 years | 2–17 years | ND |
| Origin | Japanese | German, Moroccan, Dutch, Polish, British, and European American | European | Finnish, Turkish, Northwestern Syria and Pakistani | Pakistani, American and Saudi–Arabian | Japanese |
| Height | −1.0 SD | normal in 13/14 | −1.4 to −4.2 SD | −0.9 to 0.6 SD | normal in 4/5 | ND |
| Weight | −2.0 SD | normal in 13/14 | +0.6 to −3.3 SD | −1.0 SD to normal | normal in 4/5 | ND |
| OFC | −1.0 SD | normal in 12/14 | +0.7 to −5.5 SD | −4.5 SD to normal | −3.0 SD to normal | ND |
| Hyperphosphatasia | + | 14/14 | 3/3 | 4/4 | 5/5 | + |
| Intellectual disability | severe | 14/14 | 3/3 | 4/4 | 5/5 | + |
| Age at walking | delayed | delayed | delayed | 5/5 | delayed unable to walk in 4/5 | ND |
| Delayed speech and language development | + | 14/14 | 3/3 | 4/5 | 5/5 (none) | + |
| Muscular hypotonia | + | 11/12 | 3/3 | 4/5 | 5/5 | ND |
| Seizures | + | 9/12 | 1/3 | 2/5 | 4/5 | + |
| Apparent hypertelorism | + | + | 3/3 | 1/2 | 5/5 | ND |
| Long palpebral fissures | + | + | 3/3 | 1/2 | | ND |
| Broad nasal tip | + | + | 3/3 | 2/2 | 5/5 | + |
| Tented upper lip vermilion | + | + | 3/3 | 1/2 | 5/5 | + |
| Brachytelephalangy | + | 14/14 | 3/3 | 1/2 | 0/5 | − |
| Anorectal abnormalities and/or constipation | − | 6/12 | 3/3 | 1/2 | ND | − |
| Aganglionic megacolon | − | 2/14 | 1/3 | 1/2 | ND | − |
| Heart defect | − | 1/14 | + | 1/2 | ND | − |
| Cleft palate | − | 3/14 | 0/3 | 1/2 | ND | − |
| Hearing impairment | + | 3/14 | 0/3 | 1/2 | ND | ND |

ND, not described.

FACS analysis of granulocytes from our patient demonstrated severely decreased expression of GPI-APs. Functional analysis using *PIGL*-defective CHO cells revealed that the isoforms starting from the downstream methionines showed residual PIGL activity. It has been reported that two well-conserved motifs are essential for PIGL activity (Fig. 5B underlines). Two faint bands corresponding to the sizes of these isoforms were detected by western blot; the bigger band had both motifs but not the N-terminal transmembrane region. These two translation start sites do not fit well with Kozak's rule; therefore, these isoforms were not detected in the wild-type cells.

Severely decreased expression of GPI-APs in granulocytes of the patient suggest that these mutations in *PIGL* are associated with decreased GPI biosynthesis. Previous studies showed that the disruption of *PIGL* caused lethality in Saccharomyces cerevisiae [Watanabe et al., 1999], suggesting that PIGL has been considered as the only enzyme to catalyze the second step of GPI biosynthesis in yeast. Although the disease mechanims remain unknown, it is possible that a truncated protein translated from the allele with c.254_255del or C-terminal protein isoforms shown in our functional analysis using CHO cells might have the minimal residual activity of PIGL.

In conclusion, we identified compound heterozygous deletions in *PIGL* in a patient with distinctive facial appearance, developmental delay, intellectual disability, brachytelephalangy, and hyperphosphatasia. The clinical features were similar to those of HPMRS caused by mutations in *PIGV*, *PIGO*, *PGAP2*, *PGAP3*, and *PIGW*. Our findings will broaden the clinical spectrum of disorders with defects in the GPI biosynthesis pathway.

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＝総　　説＝

# 知的障害とてんかんを主症状とする新しい疾患
## ―先天性GPI欠損症―

村上　良子　　木下タロウ

**要旨**　最近，先天性GPI欠損症が知能障害や乳幼児発症の難治性てんかんの原因疾患として注目を集めている．GPI（glycosyl-phosphatidyl-inositol）は150種以上のタンパク質を細胞膜につなぎとめるアンカーの役割をする糖脂質でその基本骨格は真核生物で保存されている．GPIアンカー型タンパク質の生合成とそのリモデリングに関与する遺伝子は現在までに少なくとも26個あることがわかっている．次世代シークエンサーを使ったエキソーム解析により，最近このうち12個の遺伝子を責任遺伝子とするGPI欠損症が報告されている．変異遺伝子のステップや活性の低下の程度により，症状にはバリエーションがあるが共通症状として知的障害と運動発達障害があり多くはてんかん発作を伴っている．本総説ではこの先天性GPI欠損症について，最近の知見を概説する．

**見出し語**　GPIアンカー，てんかん，知能障害，高アルカリホスファターゼ血症，ビタミンB6

## はじめに

真核生物の細胞表面にはGPI（glycosyl-phosphatidyl-inositol）と呼ばれる糖脂質によって細胞膜に結合するタンパク質のグループ（GPIアンカー型タンパク質）が発現している．GPIの基本構造はホスファチジルイノシトール（PI），グルコサミン（GlcN），3つのマンノース（Man），2つのエタノールアミンリン酸（EtNP）から成り立っており，全ての真核生物でよく保存され，複数のステップにより合成される（図1）．ほ乳類においては現在までに150種以上のGPIアンカー型タンパク質（GPI-APs）が知られており，酵素や受容体，接着因子，補体制御因子など個体発生や神経発達，免疫機能，受精等非常に重要な働きを担っている．GPIが欠損するとこれらのすべてのタンパク質は細胞表面に発現できずに多くは細胞内で破壊されてしまうので，GPI生合成の最初のステップに必要な遺伝子 *Piga* のノックアウトマウスは胎生致死になる．

## I　GPIアンカー型タンパク質（GPI-AP）の生合成とリモデリング（図2）

### 1. GPIアンカーの生合成

GPI-APは小胞体（ER）で蛋白部分とGPI部分が別々に合成される．GPI生合成の最初のステップは触媒サブユニットPIGAとPIGC，PIGH，PIGP，PIGQ，PIGY，DPM2の7個のタンパク質からなる酵素複合体によって担われている．その後10個のステップを経て完成したGPI-APに付加される．この反応は触媒サブユニットPIGKとPIGS，PIGT，PIGU，GPAA1の5個のタンパク質からなるトランスアミダーゼ複合体によって担われ，この酵素はGPI付加シグナルを持つタンパク質のC末端シグナルペプチドを認識して切断しGPIの末端のエタノールアミンのアミノ基と共有結合させる．このように小胞体でGPI-APに付加されるまでのステップに関わる遺伝子群を *PIG*（PhosphatidylInositol Glycan）genesと呼び，ほぼクローニングされた順にアルファベットの名前がついている．またその後の修飾に関係する遺伝子群をPost GPI Attachment to Proteins（*PGAP*）genesと呼んでいる[1]．

### 2. GPIアンカー型タンパク質のリモデリング

1）イノシトールの脱アシル化

GPI生合成の初期にPIGWによってGlcN-PIのイノシトールにアシル基が付加される．この反応はGPIの生合成が細胞質側から小胞体内膜側に移行した直後に起こり，その後の効率的なGPI生合成と哺乳動物においてはタンパク質と結合する末端のEtNPの付加に必要である．このアシル基はGPIアンカーがタンパク質に付加された後に小胞体でPGAP1によって除かれる．PGAP1はリパーゼモチーフ（GxSxG）を有するタンパク質で，この遺伝子の欠損細胞では小胞体からゴルジ体へのGPI-APsの輸送が大幅に遅れ，さらにゴルジ体でのリモデリングは受けず，細胞表面にはアシル基の付いた異常構造のまま発現する．そのため細菌由来のリパーゼPIPLC（phosphatidylinositol-specific-phospholipase C）による切断に抵抗性となる[2]．

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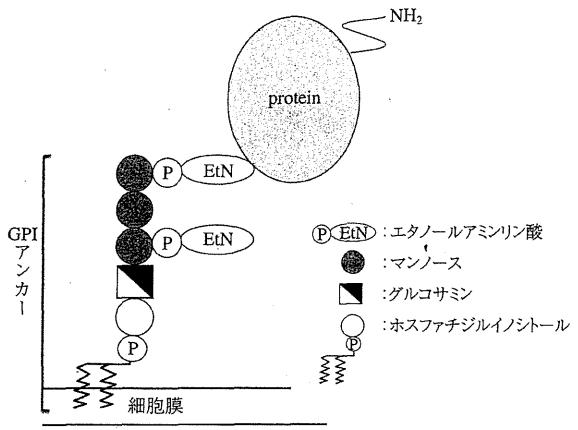


図1 GPI アンカー型タンパク質の構造

GPI の基本構造はフォスファチジルイノシトール（PI），グルコサミン（GlcN），3 つのマンノース（Man），2 つのエタノールアミンリン酸（EtNP）から成り立っており，すべての真核生物でよく保存されている．

2）GPI 糖鎖リモデリング（側鎖 EtNP の除去）

GPI 生合成過程において 3 つの Man にはそれぞれ EtNP が付加される．この反応はフォスファチジルエタノールアミンを基質とし 1 つ目の Man には PIGN が，2 つ目の Man には PIGG/PIGF 複合体が，3 つ目の Man には PIGO/PIGF 複合体が働く．このうち 2 つ目の Man に付加された EtNP は GPI アンカーがタンパク質に付加された後に小胞体で PGAP5（MPPE1）によって除かれる．PGAP5 は金属要求性のリン酸エステラーゼモチーフを有しているタンパク質で GPI-APs は PGAP5 に結合して小胞体出口部位へ運ばれさらにカーゴレセプターである p24 ファミリータンパク質と結合して小胞輸送によりゴルジ体に運ばれる．PGAP5 の欠損細胞では PGAP1 の欠損細胞と同様ゴルジ体への GPI-APs の輸送が遅れるが，ゴルジ体での脂質のリモデリングは正常に受けた後，細胞表面には EtNP が除かれない異常構造のまま発現する．

3）脂肪酸リモデリング

この反応はゴルジ体で行われ，GPI-APs が脂質ラフトに局在するために必須である．まず脂質部分の sn-2 位に付加されて

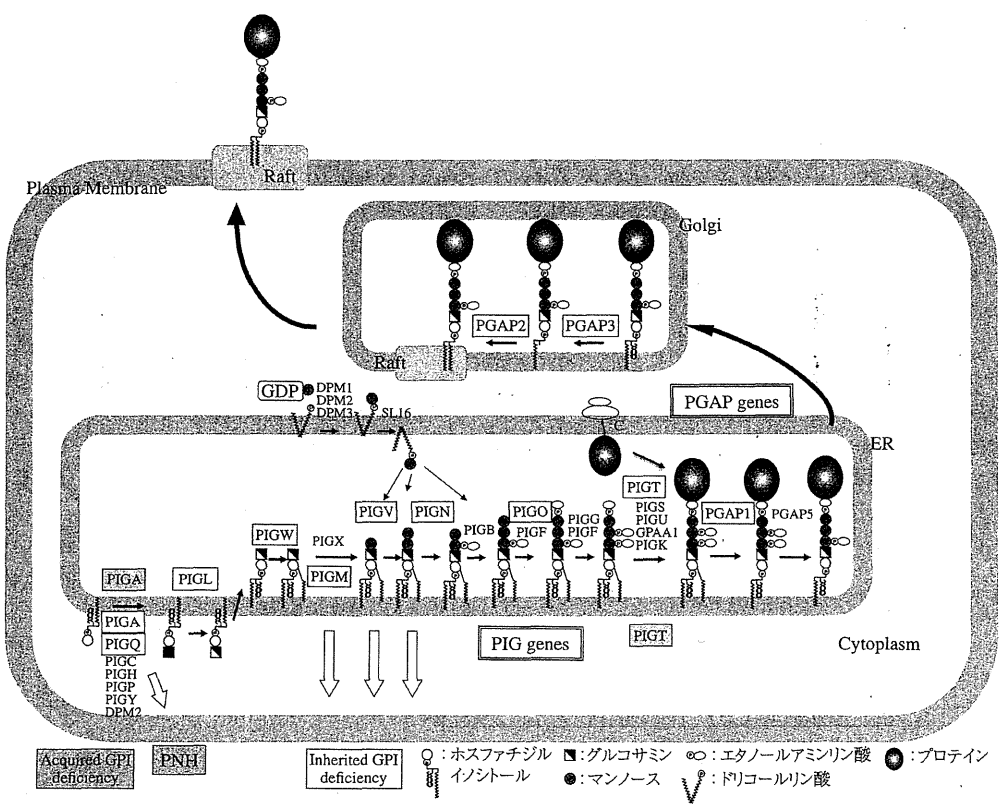


図2 GPI アンカー型タンパク質の生合成とリモデリング

GPI アンカー型タンパク質はタンパク質部分と GPI アンカー部分が小胞体（ER）において別々に合成され，GPI トランスアミダーゼ複合体が GPI 付加シグナルを持つタンパク質の C 末端シグナルペプチドを認識して切断し GPI アンカーに付加する．その後 ER およびゴルジ体で様々な修飾を受け細胞表面のラフトに局在できるようになる．白抜きの四角で囲んでいるのは現在までに報告のある先天性 GPI 欠損症，グレーの四角は後天性 GPI 欠損症（PNH）の原因遺伝子．

いる不飽和脂肪酸（アラキドン酸（C20:4）が主要）がPGAP3により除去され，飽和脂肪酸（ステアリン酸（C18:0））が付加される．この反応にはPGAP2が必須であるが，酵素ではなく制御因子であると考えられており，まだ哺乳動物で働く酵素タンパク質は同定されていない．PGAP3の欠損細胞ではGPI-APsはリモデリングを受けない異常構造のまま細胞表面に発現するが，脂質ラフトに局在できない．PGAP2の欠損細胞ではGPI-APsはsn-2位に脂肪酸が付加されないリゾ体のまま細胞表面に運ばれるが未知のリパーゼによって切断遊離され，その結果細胞表面のGPI-APsは著減する．

## Ⅱ　後天性GPI欠損症である発作性夜間ヘモグロビン尿症との相違点と共通点

発作性夜間ヘモグロビン尿症（paroxysmal nocturnal hemoglobinuria;PNH）は，溶血性貧血，骨髄不全，深部静脈血栓を3主徴とする血液疾患である．溶血発作のエピソードと末梢血のフローサイトメトリーで顆粒球および赤血球表面のGPI-APsであるCD59やDAFの発現が低下あるいは欠損している細胞集団を確認することで診断される．後天的に1個あるいは数個の造血幹細胞の*PIGA*遺伝子に突然変異が起こってGPI欠損細胞となり，クローナルに増殖することによって発症する疾患なので正常細胞とGPI欠損細胞が混在していることが特徴である．血球分化にはGPI-APsは必須ではないので，クローナルに増殖している細胞の多くは完全欠損の細胞である．この異常クローンの増殖機序については未だ完全解明には至っていない．数あるGPI生合成遺伝子のうちほとんどの患者において責任遺伝子は*PIGA*である．その理由は*PIGA*のみがX染色体上の遺伝子であり，女性も発生初期のX染色体不活化後には1回の体細胞突然変異でGPI欠損細胞になるためと考えられ，実際にPNHの男女比は等しい．最近，次世代シークエンサーを用いた解析によって*PIGT*を責任遺伝子とするPNHが見つかったが，遺伝的に1本のアレルに変異があるところに，造血幹細胞において体細胞突然変異が起こり*PIGT*周辺領域の欠損が起こって発症したものであった[3]．このように今後まれではあるが他の遺伝子を責任遺伝子とするPNHが見つかる可能性がある．

一方先天性GPI欠損症（inherited GPI deficiency;IGD）においては，完全欠損は致死になるのですべて部分欠損症である．劣性遺伝形式をとり，患者は変異遺伝子のホモ接合体あるいは，複合ヘテロ接合体である．理論的にはPNHと同様1本のX染色体の変異で発症するPIGA欠損症の割合が高いと考えられる．報告例ではヘテロの女性は無症候性であり，男性のみ罹患する[4]．

## Ⅲ　現在までに報告されている先天性GPI欠損症（IGD）

### 1. 最初に発見されたPIGM欠損症

イギリスの研究者との共同研究で解析した症例は，2家系3症例とも顆粒球ではすべてのGPI-APsが著明に減少していたが赤血球では軽微であったため溶血発作はみられず，てんかん発作と門脈血栓症が主症状であった．線維芽細胞のGPI-APsも著減していたことからIGDが疑われた[5]．患者のBリンパ芽球の解析により*PIGM*が責任遺伝子であることがわかった．PIGMは最初のマンノースを付加する酵素であるが，2家系ともに*PIGM*のプロモーターの転写因子Sp1の結合部位の1塩基の変異が原因でその結合が阻害され，ホモの変異を持つ患者では著しく*PIGM*のプロモーター活性が低下していた．Sp1はヒストンのアセチル化に関わる転写因子をリクルートする分子であるので，ヒストン脱アセチル酵素（HDAC）インヒビターであるNa butyrateの投与によって強制的にヒストンのアセチル化を起こすことによりPIGMの発現が回復しBリンパ芽球のGPI-APsの発現が完全に回復した．そこで患者にNa butyrateを投与したところ，難治のけいれん発作が治まった[6]．これらの患者には最近見つかっているIGD症例と異なり異常顔貌や奇形，知能障害などがみられない．おそらく胎生期は他の転写因子によりPIGMの発現が促進されSp1結合部位の異常による基礎的な発現低下が代償されていたのだと考えられる．一方，以下に述べる他のIGD症例に比較して血球や線維芽細胞におけるGPI-APsの低下は著明なので，血管上皮細胞のGPI-APsの発現低下等による局所の補体活性の亢進が血栓症を引き起こす原因になっている可能性がある．

### 2. Hyperphosphatasia mental retardation syndrome（Mabry syndrome）

古くから高アルカリホスファターゼ（alkaline phosphatase;ALP）血症と知能障害をきたす劣性遺伝の疾患が知られておりヨーロッパでは多くの患者が登録されていた．2010年にドイツの研究者が次世代シークエンサーによるエキソーム解析により，兄弟例に*PIGV*のcoding領域の遺伝子変異を発見し，我々との共同研究によりこの変異のためPIGVの活性が低下していることを示し責任遺伝子であることを証明した[7][8]．その後も同じグループとの共同研究により，同様の症状を示すPIGO欠損症[9]，PGAP2欠損症[10][11]，PGAP3欠損症[12]を報告した．また我々のスクリーニングにより国内初のGPI欠損症であるPIGO欠損症[13][14]，新規のPIGW欠損症[15]が見つかっている．PIGV欠損症はヨーロッパに多くすでに10家系以上が報告されているが，1つのアレルに共通の変異を持つ創始者効果のためと考えられる．これらの欠損症では共通症状として知的障害と運動発達障害，けいれん発作，顔貌異常が見られ，重症例では手指の末節骨や爪の低形成，難聴，心臓奇形，ヒルシュスプルング病，鎖肛等腸管の奇形，他の臓器の奇形，小脳萎縮，大脳萎縮・白質変性・髄鞘化の遅延などが見られる（図3）．このうちPGAP3欠損症以外の欠損症の症状については細胞表面のGPI-APsの発現の低下によって起こり，低下の程度によって表現型が異なるが責任遺伝子による差はあまりないと考えられる．一方PGAP3欠損症では，細胞表面の低下は顕著ではないと考えられGPI-APが脂質ラフトに局在できないために，シグナル伝達等の機能が阻害さ

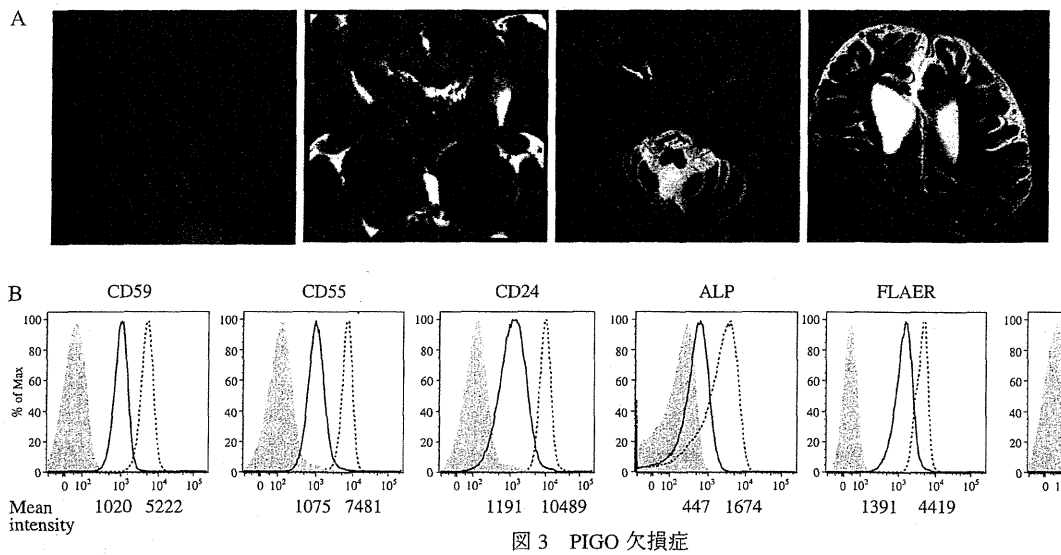


図3 PIGO欠損症

A：患者は精神発達遅滞，難治性てんかん，高アルカリフォスファターゼ血症（5959 IU/L），異常顔貌，手指末節骨の欠損（第5指），爪の低形成，ヒルシュスプルング病，ファロー四徴症をきたしていた．4歳時の手指のX線写真，4カ月時の頭部MRI（T2強調画像）では脳幹部における高シグナルが特徴的で，進行性に小脳萎縮と大脳の白質変性が見られた（共に9歳時の画像）．

B：顆粒球のFACS解析－GPIアンカー型蛋白質の発現の低下が見られた．

点線：正常コントロール，実線：患者，数値：平均蛍光強度（タンパク質発現量を示す）

（文献13）より改変）

れて起こっている可能性がある．知的障害，運動発達障害と軽いけいれん発作，時に小頭症が主症状で，PGAP3の活性がほとんどない重症例であっても発達障害は重度であるが他の臓器の奇形は伴わず手指の異常もない[12]．

**3．高ALP血症の機序**

責任遺伝子により機序が異なる．

1）小胞体からの分泌―PIGV，PIGO，PIGW欠損症の場合（図4）

前述したようにPIGKを触媒コンポーネントとするGPIトランスアミダーゼ複合体はGPI付加シグナルを持つ前駆タンパク質のC末端のシグナルペプチドを認識しそのωサイトで切断して酵素－基質中間体を形成し，完成したGPIアンカーに付加する．その切断のためにはトランスアミダーゼの活性化が必要でそれはGPIアンカーの複合体への結合によって起こると考えられている．その結合には少なくともマンノース1つとそれにエタノールアミンが付いていることが必要である．すなわち*PIGM*のステップより前の初期の生合成遺伝子の欠損で蓄積するGPIアンカー中間体はマンノースが付いていないので複合体に結合できず，GPI-APであるALPのシグナルペプチドの切断が起こらないためそのまま細胞内で破壊され（小胞体関連分解によると考えられる），効率よく分泌されない．一方*PIGO*等*PIGV*以降の遺伝子の欠損ではシグナルペプチドの切断が効率よく起こるが，GPIアンカーが完成されていないので付加できずそのまま分泌経路に乗って分泌され，高ALP血症になる[16]．ただしPIGN欠損症では1つ目のマンノースにEtNPが付かないが，以降の生合成は最終ステップまでゆっくり進み効率は悪いものの，シグナルペプチド切断とGPIへの付加が起こるので高ALP血症を呈さない．しかし，細胞表面のGPI-APsの発現は低下し，アンカーの構造もEtNP側鎖を欠いている．またPIGWはPIGMの前のステップでイノシトールにアシル基を付加する酵素であるが，変異によりアシル基がつかない場合，その後の反応はゆっくり進むがPIGOが担う末端のEtNPを付加する反応が起こらない．このとき蓄積するのは，PIGO欠損症で蓄積するGPI中間体のアシル基のない構造であるため，シグナルペプチド切断と分泌が起こり，高ALP血症を呈する．

逆にGPIトランスアミダーゼ複合体の必須成分であるPIGTの欠損症では切断は全く起こらず，すべてが細胞内で破壊されるため低ALP血症をきたす[17]．

2）細胞表面からの遊離―PGAP2とPGAP3欠損症の場合（図5）

前述したようにPGAP2はゴルジ体での脂質部分のリモデリング反応の際にPGAP3で除かれた不飽和脂肪酸の代わりにsn-2の位置に飽和脂肪酸を付加する働きをするタンパク質であるので，PGAP2欠損症ではGPI-APは脂肪酸を1本しか持たないリゾ体のまま細胞表面に運ばれて血清中に遊離され，細胞表面のGPI-APsの低下と高ALP血症を起こす[10)11)]．したがってPGAP2欠損症は上記のPIGV欠損症等GPI生合成遺伝子の欠損症と同様の表現型を示す．細胞表面からの遊離のメカニズムは明らかになっていないが，培養液中ではホスホリパーゼD（PLD）で切断された構造で存在するので細胞膜上あるいは遊離してからPLDによって切断されると考えら

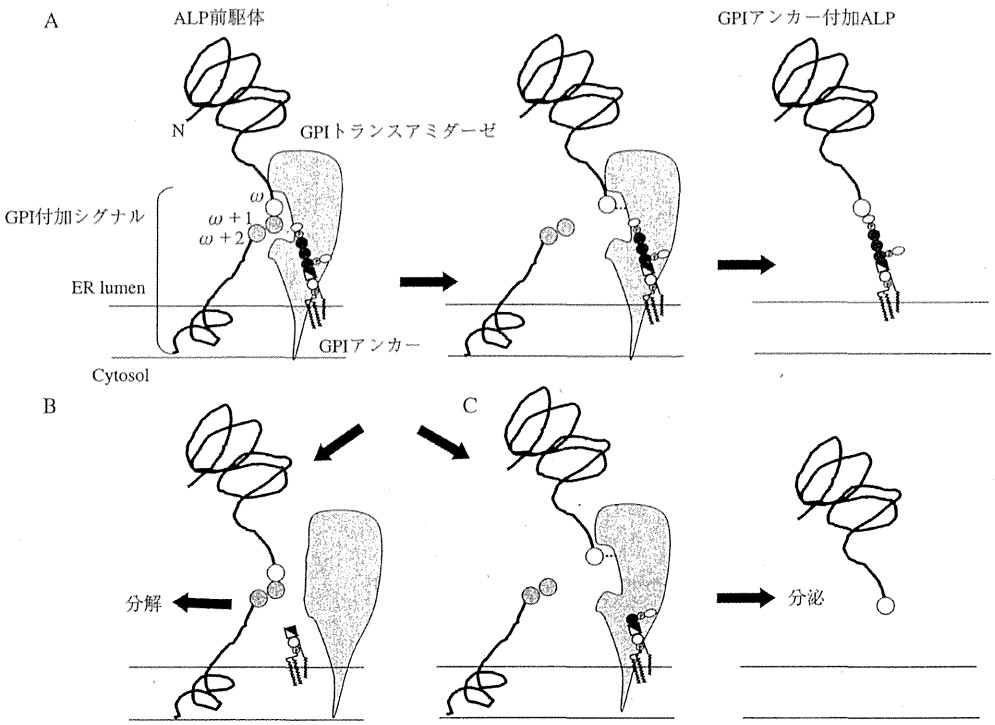


図4 高ALP血症の機序

GPIトランスアミダーゼ複合体はGPI付加シグナルを持つタンパク質のC末端のシグナルペプチドを認識しそのωサイト（切断を受けるアミノ酸）で切断して酵素－基質中間体を形成し，完成したGPIアンカーに付加する（A）．初期の生合成遺伝子やGPIトランスアミダーゼの欠損ではGPIアンカー型タンパク質であるALPのシグナルペプチドの切断が起こらないためそのまま細胞内で分解される（B）が，生合成の後期ステップの遺伝子の欠損ではシグナルペプチドの切断が効率よく起こるが，GPIアンカーが完成されていないので付加できずそのまま分泌経路に乗って分泌され，高ALP血症になる（C）．

れている．一方PGAP3欠損症ではGPI-APsはリモデリングを受けない不飽和脂肪酸を含んだ脂質部分のまま細胞表面に運ばれるので脂質ラフトに局在できない．実際PGAP3欠損症やPgap3ノックアウトマウスでは高ALP血症になることは観察されるが培養細胞では確認できず，機序は明らかになっていない．GPI局在が異常であるために生体ではリパーゼやプロテアーゼに切断されやすくなっている可能性もある．

**4. 高ALP血症をきたさない先天性GPI欠損症（IGD）**

1）GPI生合成遺伝子の欠損症

前述したPIGM欠損症を除くと現在までにPIGA，PIGQ，PIGL，PIGN，PIGT[4)17)～24)]欠損症が報告されている．これらはすべてGPIアンカーの生合成に関わる遺伝子なので患者の症状は細胞表面のGPI-APsの発現低下によって起こっていると考えられる．すなわちMabry syndromeと共通な症状として知的障害と運動発達障害，けいれん発作が見られ，重症例では顔貌異常，多臓器の奇形，小脳萎縮，大脳萎縮・白質変性・髄鞘化の遅延などが見られる．手指の奇形は今のところ報告されていない．PIGT欠損症では前述したように低ALP血症を来し，腸管骨の短縮や頭蓋骨の早期癒合などの骨の異常を呈する．このうちPIGN欠損症は完全欠損であっても1つ目のマンノースにEtNPが付かない異常構造のまま細胞表面に少量のGPI-APが発現する．今後症例数が増加してPIGN欠損症に特徴的な症状が明らかになるようであれば，1つ目のマンノースについたEtNPの機能的意義が解明される可能性がある．

2）PGAP1欠損症

前述のようにPGAP1欠損細胞ではGPI-APsは細胞表面にはイノシトールのアシル基が残り，脂質のリモデリングを受けない異常構造のまま発現するが，発現の低下は見られない．PGAP1ノックアウトマウスでは耳頭症（otocephaly）あるいは全前脳胞症（holoprosencephaly）といった頭部の形成異常を来たし生直後に死亡するが，まれに形成異常を示さず育つものは雄性不妊を呈する．形成異常はWntやNodalシグナルの異常，雄性不妊は細胞膜からのGPI-APsの遊離の障害が関係していると考えられている[25)26)]．一方最近PAGP1欠損症の1家系が見つかり，患者ではほとんどPGAP1の活性が認められないにもかかわらず，症状は知的障害と軽症のてんかんを呈し，顔貌は正常で奇形等も見られなかった[27)]．マウスの表現型は系統によって差が見られているので，今後症例数が増えれば特徴的な症状が明らかになると考えられる．

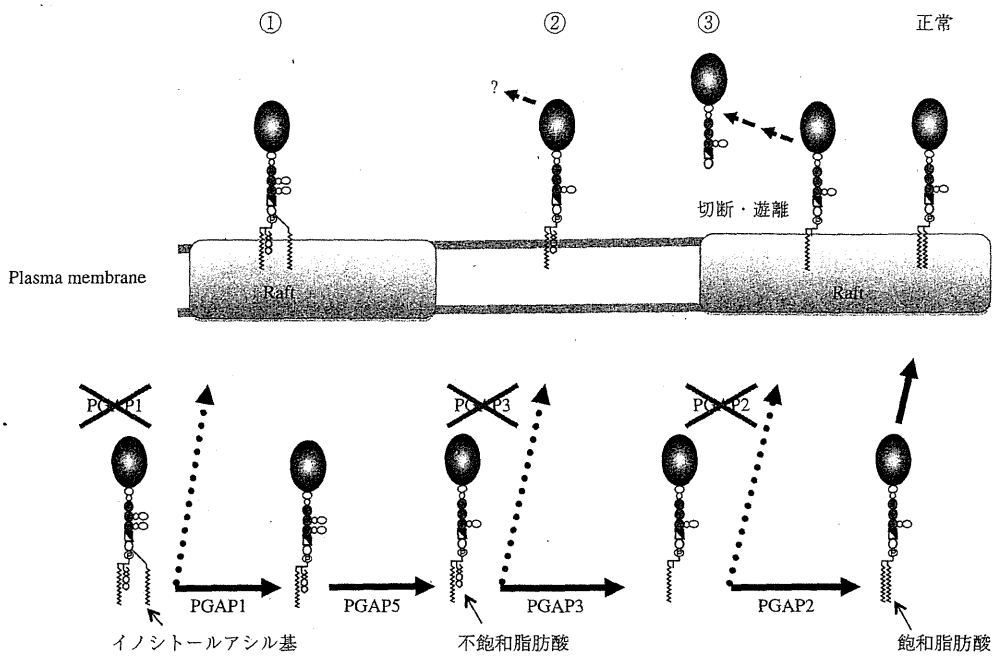


図5 GPI-APsのリモデリング

小胞体でタンパク質付加後にイノシトールのアシル基がPGAP1によって除かれる．PGAP1の欠損細胞ではGPI-APsはゴルジ体でのリモデリングは受けず，細胞表面にはアシル基の付いた異常構造のまま発現する（①）．ゴルジ体では，脂質部分のsn-2位に付加されている不飽和脂肪酸がPGAP3により除去され，PGAP2が関係する反応で飽和脂肪酸が付加される．PGAP3の欠損細胞ではGPI-APsはリモデリングを受けない異常構造のまま細胞表面に発現するが，脂質ラフトに局在できない（②）．PGAP2の欠損細胞ではGPI-APsはsn-2位に脂肪酸が付加されないリゾ体のまま細胞表面に運ばれるが未知のリパーゼによって切断遊離され，その結果細胞表面のGPI-APsは著減する（③）．

## Ⅳ 症状の特徴（表1）と検査所見

GPI生合成遺伝子の欠損症ではその症状は活性の低下の程度，すなわちGPIアンカーの量に依存すると考えている．各ステップの遺伝子欠損CHO細胞に変異遺伝子を導入して正常遺伝子と比べてどの程度GPI-APsの発現を回復できるかをみる機能解析の結果は，重症度とよく相関する．最も軽症例でも知的障害は必発である．重症度が増すに従って，運動発達障害，てんかん，テント状の口などの異常顔貌，難聴，Hirschsprung病などの腸の奇形，心奇形等多臓器の奇形，時に魚鱗癬などの皮膚症状，目の異常等が見られる．乳児早期発症のてんかん性脳症である大田原症候群や早期ミオクロニー脳症，West症候群を呈する場合もある[15)20)21)]．また重症例では脳MRIで脳幹部や中脳に拡散強調やT2強調画像で高信号をきたすことが特徴である．また小脳萎縮や大脳白質変性が生後も進行する（図3A）[13)20)]．

検査所見

1）血清ALP値

高ALP血症を伴う発達障害はIGDである可能性が高い．アイソザイムは骨型，肝型共に上昇する．高ALP血症の有無は変異遺伝子のステップによることは先に述べた．このグループの患者では手指，足趾の末節骨や爪の低形成が特徴的であるが，高ALP血症が比較的軽度なPGAP3欠損症とPIGW欠損症では見られていない．一方トランスアミダーゼのコンポーネントであるPIGT欠損症ではALPが細胞内で破壊されてしまうため低ALP血症をきたし，共通の症状に加えて骨の形成異常を示す．

2）顆粒球のフローサイトメトリー（図3B）

末梢血のフローサイトメトリーで顆粒球に発現するGPI-APであるCD16の発現量の低下がIGD診断の決め手になる．重症の場合にはCD24やFLAER（GPI-APに結合するエロリジン毒素の細胞溶解能欠失変異体を蛍光ラベルしたもの）染色性等も顆粒球では低下するが，リンパ球や赤血球のGPI-APsの低下は見られない．

## Ⅴ てんかん発作の発症機序と治療

てんかんの発症には神経細胞に発現するGPI-APであるALPの欠損が関係している場合があると考えられる．ALPのアイソザイムのうち組織非特異的（tissue non-specific）ALP（TNAP）の遺伝子異常は低ALP血症を来し骨の形成異常とてんかん発作を主症状とする．TNAPノックアウトマウスにおけるてんかん発作の機序が詳細に報告されている[28)]．すなわ

表1　先天性GPI欠損症の報告例と症状のまとめ

| Affected Gene | | PIGA (11)[a] | PIGQ (1) | PIGL (6) | PIGW (1) | PIGM (3) | PIGV (15) | PIGN (9) | PIGO (6) | PIGT (4) | PGAP1 (2) | PGAP2 (6) | PGAP3 (5) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MIM | | 300868 | 605754 | 280000 | 610275 | 610293 | 239300 | 614080 | 614749 | 610272 | 611655 | 614207 | 611801 |
| Clinical diagnosis | | Ohtahara, West syndrome, MCAHS2 | Ohtahara syndrome | CHIME syndrome | West syndrome HPMRS | | HPMRS1 | MCAHS1 | EOEE HPMRS2 | MCAHS3 | ID | HPMRS3 | HPMRS |
| Age at assessment | | Died (5/11) | Died at 2Y | | 7M | Died (1/3) | 7M-17Y | Died (6/9) | 0-15Y | Died at 2Y (1/4) | 4Y, 2Y | 3.5-28Y | 4-17Y |
| Neurological disorder | | (11/11) | (1/1) | (4/4) | (1/1) | (3/3) | (14/14) | (9/9) | (6/6) | (4/4) | (2/2) | (6/6) | (5/5) |
| Global developmental delay | HP:0001263[b] | Severe (9/11) | (1/1) | (4/4) | (1/1) | (1/3) | (14/14) | (9/9) | (6/6) | (4/4) | (2/2) | (6/6) | (5/5) |
| Motor delay | HP:0001270 | Severe (9/11) | (1/1) | (4/4) | (1/1) | (1/3) | (9/9) | (9/9) | (6/6) | (4/4) | (2/2) | (5/5) | Severe (5/5) |
| Delayed speech | HP:0000750 | No speech (11/11) | | (4/4) | | | (9/9) | (9/9) | (6/6) | (4/4) | (2/2) | (5/6) | None (5/5) |
| Microcephaly | HP:0000252 | (2/6) <3rd centile | | (0/6) | (0/1) | | (0/9) | (0/9) | (1/6) −5.5 SD | (0/4) | (2/2) | (1/6) −4.5 SD | (3/5) −3 SD |
| Muscular hypotonia | HP:0001252 | (8/11) | | | (1/1) | | (9/12) | (9/9) | (6/6) | (4/4) | (2/2) | (5/5) | (5/5) |
| Seizures | HP:0001250 | (11/11) | (1/1) | (4/4) | (1/1) | (3/3) | (11/12) | (9/9) | (4/6) | (4/4) | (1/2) | (3/6) | (4/5) |
| Type | | Myoclonic (5/11) | | | Tonic spasm | Absence | | Complex partial | Tonic clonic, Partial seizure, Vitamin B6 dependent | Generalized toinic clonic, Myoclonic Absence seizure | Absence | Absence Myoclonic Tonic-clonic | Generalized toinic clonic, Myoclonic |
| Tremor | HP:0001337 | (1/3) | | | | | | (7/9) | | | (0/2) | | |
| Nystagmus | HP:0000639 | | | | | | | (7/9) | | (4/4) | (0/2) | | |
| Hearing impairment | HP:0000365 | (2/3) | | | (0/1) | | (3/10) | | (1/6) | | (0/2) | (1/6) | (0/5) |
| Eye abnormality | HP:0000504 | Cortical blind (3/3) | | Coloboma (4/4) | (0/1) | (0/3) | | Cortical blind (1/2) | | Cortical blind (4/4) | (0/2) | | |
| Abnormal facial features | | (9/11) | | (4/4) | (1/1) | (0/3) | (14/14) | (9/9) | (6/6) | (4/4) | (2/2) | (2/6) | (5/5) |
| Hypertelorism | HP:0000316 | (5/7) | | (4/4) | (1/1) | | | (1/9) | (4/6) | | | (1/6) | (5/5) |
| Long palpebral fissures | HP:0000637 | (5/7) | | | | | | | (4/6) | | | (1/6) | |
| Broad nasal bridge | HP:0000431 | (5/7) | | (4/4) | (1/1) | | | (1/9) | (4/6) | (4/4) | (2/2) | (2/6) | (5/5) |
| Broad nasal tip | HP:0000455 | (2/2) | | | (1/1) | | | | (4/6) | | | (1/6) | (5/5) |
| Tented upper lip | HP:0010804 | (5/7) | | | (1/1) | | | (3/9) | (6/6) | | | (2/6) | (5/5) |
| Micrognathia | HP:0000347 | (2/6) | | | | | | (4/9) | | | | | |
| Cleft palate | HP:0000175 | | | (2/4) | (0/1) | | (1/10) | (0/9) | (1/3) | | | (1/6) | (2/5) |
| Abnormal skeletal features | | | | | (0/1) | (0/3) | | | | (4/4) | (0/2) | | (0/5) |
| Craniosynostosis | HP:0001363 | | | | | | | (2/7) | (1/6) | (2/4) | | | |
| Short arms | HP:0009824 | | | | | | | | | (4/4) | | | |
| Scoliosis | HP:0002650 | | | | | | (1/5) | | | (2/4) | | | |
| Reduced mineralisation | HP:0004348 | | | | | | | | | (4/4) | | | |
| Delayed bone age | HP:0003799 | | | | | | (1/5) | | | (4/4) | | | |
| Brachytelephalangy | HP:0009882 | (0/11) | | (0/4) | (0/1) | | (13/14) | (1/9) | (4/6) | | | (1/6) | (0/5) |
| Teeth abnormality | HP:0000164 | (6/11) | | (4/4) | (0/1) | | | | | (2/2) | | | |
| Joint contracture | HP:0003121 | (8/11) | | | (0/1) | | | | | | | | |
| Other organ anomalies | | | | | (0/1) | (0/3) | | | | (4/4) | (0/2) | | (0/5) |
| Anorectal abnormalities | (anal stenosis) HP:0002025 | | | (0/4) | | | (6/12) | (2/9) | (3/6) | | | (1/6) | |
| Aganglionic megacolon | HP:0002251 | | | (0/4) | | | (2/14) | (0/9) | (2/6) | | | (1/6) | |
| Heart defect | HP:0001631 | ASD (2/11) PDA (1/11) | | VSD (1/4), TOF (1/4), TGA (1/4) | | | ASD (1/14) | ASD (2/7), PDA (2/7), PFO (3/7), PS (1/7) | TOF (1/6) ASD, PS (1/6) | PDA (1/4) | | ASD (1/6) | |
| Vesicoureteral reflex or Anomalies in Urinary tract | HP:0000079 | (2/4) | | (1/1) | | | (2/5) | (4/9) | | (4/4) | | | |
| Skin abnormalities | | | | | (0/1) | (0/3) | | | | | (0/2) | | |
| Deep plantar groove | HP:0001869 | | | | | | | (6/7) | | | | | |
| Skin psoriasis, Ichthyosis | HP:0008064 | (3/11) | | (4/4) | | | | | | | | | |
| Clinical Test | | | | | | | | | | | | | |
| Decreased expression of GPI-APs on patients' cell | | (8/8) PMN | | (1/1) B lymphoblast, Fibroblast | (1/1) PMN | (2/2) PMN | (2/2) PMN | (2/2) Fibroblast (2/2) PMN | (2/2) PMN | (2/2) PMN | (0/2) B lymphoblast PIPLC resistant | | (1/2) PMN |
| Hyperphoshatasia | HP:0003155 | Fluctuating mild elevation (4/9) | | | (1/1) | (0/3) | (14/14) | (0/2) | (4/6) | (4/4) Hypophosphatasia | (0/2) | (4/4) | (5/5) |
| MRI abnormality | | | | | | (0/3) | | | | (3/4) | CT, Brain atrophy | | |
| Thin corpus callosum | HP:0002079 | (6/11) | | | | | | (1/4) | | | | | (1/5) |
| White matter immaturity | HP:0002500 | (9/11) | (1/1) | | (1/1) | | | (2/4) | (1/1) | | | | |
| Restricted diffusion pattern | | | | | (1/1) | | | | (1/1) | | | | |
| Cerebellar atrophy | HP:0001272 | (3/11) | | | | | | (1/4) | (1/1) | (2/4) | | | |
| Others | | Hepatosplenomegaly (4/4), Iron overload (4/4) | | | | Portal vein thrombosis (2/3) | | Large ears (5/7) | | | Large ears (2/2) | | |
| Reference | | 4), 18), 19), 20) | 21) | 22) | 15) | 5), 6) | 7), 8) | 23), 24) | 9), 13), 14) | 17) | 27) | 10), 11) | 12) |

MCAHS: multiple congenital anomalies-hypotonia-seizures syndrome, CHIME: colobomas of the eye, heart defect, ichthyosiform dermatosis, mental retardation, and ear defects, HPMRS: hyperphosphatasia mental retardation syndrome, ID: intellectual disability, ASD: atrial septum defect, PDA: patent ductus arteriosus, VSD: ventricular septal defect, TOF: tetralogy of Fallot, TGA: Transposition of great arteries, PS: pulmonary stenosis, PMN: polymorphonuclear leukocyte

a) Numbers in parentheses: number of affected indivisuals, b) Human Phenotype Ontology ID

表2 先天性GPI欠損症の臨床症状

| |
|---|
| 多くは知的障害，運動発達障害，てんかん発作を伴う．（時に家族性に見られる） |
| 新生児期，乳児期早期発症の難治性てんかん（大田原症候群・West症候群） |
| 顔貌異常（両眼解離・幅の広い鼻梁・長い眼裂・テント状の口・口唇，口蓋裂） |
| 手指，足趾の異常（末節骨の短縮・爪の欠損，低形成） |
| その他の奇形（肛門，直腸の異常・Hirschsprung病・水腎症・心奇形など） |
| 難聴・眼，視力の異常 |
| 皮膚の異常（魚鱗癬など） |
| 筋緊張の低下，四肢の短縮，関節拘縮 |
| 高アルカリフォスファターゼ血症 |
| 低アルカリフォスファターゼ血症 |

ちALPは細胞表面で，ピリドキサールリン酸を脱リン酸化して細胞内に取り込める形のピリドキサールにし，細胞内に入ったピリドキサールは再びリン酸化されてピリドキサールリン酸となり，抑制性ニューロンにおいてγ-アミノ酪酸（GABA）合成酵素の補酵素として働く．細胞膜上にALPが発現しないと細胞内のピリドキサールリン酸が不足しGABA合成が抑制される結果けいれん発作がおこると考えられる．実際，細胞内のピリドキサールを補うために，患者にビタミンB6（ピリドキシン）の投与を行ったところけいれん発作が消失した[13]．一方ビタミンB6が効かない症例もあり，ALP以外のGPI-APsも多く神経細胞には発現しているので，それらの発現低下がてんかん発作に関与していると考えられる．

## Ⅵ 治療の可能性

多くの症例は，大脳の白質変性や小脳萎縮が進行し，発語の消失など退行を示す．将来的には生後すぐに血液検査でスクリーニングして診断し，GPIアンカー生合成を促進する薬を投与すれば，けいれん発作等，神経症状の進行を止めることができる可能性がある．前述したように患者の遺伝子異常はほとんどが部分欠損症であり，変異を持ったcDNAでも強いプロモーターで発現させると，多くの場合正常のcDNAと同様にGPI-APsの発現を回復させることができる．今後化合物のライブラリーを用いてハイスループットにGPIアンカー生合成を促進できる化合物をスクリーニングする系を作りたいと考えている．また症状の原因となっているGPI-APが同定できれば，補充療法も可能になる．前述したビタミンB6による治療もその一つであり，葉酸受容体がGPI-APであることから，フォリン酸の投与が奏功する可能性もある．

## Ⅶ 今後の展望

表2にあげた患者を対象に海外，国内の多くの症例を集積し，詳細な症状の観察と検査所見をもとに疾患概念を確立させると共に診断基準，国内外共通の患者データーベースを作成することを目標としている．

GPI生合成遺伝子の欠損症では，変異による活性低下の程度によって症状にバリエーションがある．GPIアンカーの量が制限されているときには，それに付加されるタンパク質の優先度はタンパク質のC末端のGPI付加シグナルの配列に依るとされている．すなわち少しの活性の低下で発現が減少するタンパク質と下がりにくいタンパク質がある．活性がどこまで下がるとどのような症状がでるのか，その症状はどのGPI-APsの低下に起因するのか，そのタンパク質の生理的な機能は何なのか？ iPS細胞を使った細胞レベルの解析やモデルマウスの解析により神経症状の機序の解明を目指したいと考えている．

著者の利益相反：本論文発表内容に関連して開示すべき事項なし．

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