



Title	Biosynthesis of GPI anchored proteins, its deficiencies and treatment
Author(s)	Murakami, Yoshiko
Citation	Journal of Human Genetics. 2025, 10, p. 293
Version Type	AM
URL	https://hdl.handle.net/11094/102797
rights	
Note	

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Biosynthesis of GPI anchored proteins, its deficiencies and treatment.

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Abstract

Glycosylphosphatidylinositol (GPI) anchoring is a widely conserved post-translational modification in eukaryotes, in which various proteins—such as receptors, cell adhesion molecules, and complement regulatory proteins—are modified with a GPI moiety and tethered to the cell membrane. GPI anchors are synthesized in the endoplasmic reticulum (ER), where they are attached to newly translated proteins. These GPI-anchored proteins (GPI-APs) then undergo structural remodeling and are transported to the cell surface. To date, approximately 30 gene products have been identified as essential for the GPI biosynthetic and remodeling pathways. In addition to paroxysmal nocturnal hemoglobinuria (PNH), a well-characterized acquired hematologic disorder caused by somatic mutations in GPI biosynthesis genes, an increasing number of inherited GPI deficiencies (IGDs) have recently been reported. These congenital disorders are typically caused by hypomorphic mutations in GPI biosynthetic genes and present with neurological abnormalities. In this review, we provide an overview of the biosynthetic pathway of GPI anchors in mammalian cells and the genetic disorders resulting from its dysfunction. We also discuss emerging therapeutic approaches currently under investigation, including gene therapy, which hold promise for improving clinical outcomes in patients with IGD.

Introduction

In eukaryotic cells, a group of proteins known as GPI-anchored proteins (GPI-APs) are expressed on the cell surface, where they are attached to the cell membrane via a glycolipid called glycosyl-phosphatidyl-inositol (GPI). The basic structure of GPI consists of phosphatidylinositol (PI), glucosamine (GlcN), three mannose (Man), and two ethanolamine phosphate (EtNP). This structure is highly conserved among all eukaryotes and is synthesized through multiple steps (Figure 1) (1).

In mammals, more than 150 kinds of GPI-APs have been identified to date. These proteins include enzymes, receptors, adhesion molecules, and complement regulatory factors, playing crucial roles in various biological processes such as development, neurodevelopment, immune function, and fertilization. In the absence of GPI, none of these proteins can be expressed on the cell surface; instead, most are either degraded within the cell or secreted without GPI attachment. Consequently, knockout mice lacking *Piga*, a gene essential for the first step of GPI biosynthesis, exhibit embryonic lethality (2). Therefore, a patient with GPI deficiency is usually a partial deficiency. Inherited GPI deficiency (IGD) is classified as a congenital disorder of glycosylation (CDG), and *PIGA* deficiency is referred to as *PIGA*-CDG because both pathways use the common donors, such as UDP- N-acetylglucosamine (GlcNAc), Dolichol-phosphate-mannose (DolP-Man) and GDP-mannose (GDP-Man) (3). However, we propose classifying IGD as a subtype of CDG and adopting the term *PIGA*-IGD accordingly.

GPI biosynthesis and remodeling

1) GPI biosynthesis

GPI-APs are synthesized in the endoplasmic reticulum (ER), where the precursor protein and GPI anchor are produced separately(1) (Figure2). The first step of GPI biosynthesis involves the transfer of GlcNAc to phosphatidylinositol (PI) to generate GlcNAc-PI. This reaction is catalyzed by the GPI GlcNAc transferase (GPI-GnT) complex, comprising eight proteins with a ring structure as predicted by AlphaFold3 (4): the catalytic subunit *PIGA*, a member of glycosyltransferase family 4 (GT4) (5), along with *PIGC*, *PIGH*, *PIGP*, *PIGQ*, *PIGY*, *DPM2* and *ARV1*(4, 6, 7). The initial two steps of GPI biosynthesis occur on the cytoplasmic side of the ER membrane. Subsequently, the intermediate is translocated into the luminal side of the ER, a process mediated by *CLPTM1L* (8), followed by the addition of an acyl group from acyl-CoA to the inositol ring of GlcN-PI by *PIGW* (9). This acylation step is necessary for efficient GPI biosynthesis and for the addition of the terminal EtNP group, which facilitates protein attachment. Interestingly, the phosphatidylinositol (PI) moiety of mammalian GPI anchors predominantly adopts an alkylacyl form, and this type of lipid has been shown to be derived from peroxisomes (10). However, its physiological significance remains unknown.

Mannose residues are then sequentially added: the first mannose (Man1) by the *PIGM*/*PIGX* complex (11, 12), the second mannose (Man2) by *PIGV* (13), and the third mannose (Man3) by *PIGB* (14), using Dol-P-Man as the donor substrate. Subsequently, EtNP side chains are attached to each mannose by distinct enzymes: *PIGN* (Man1) (12),

PIGG/PIGF (Man2) (13, 14), and PIGO/PIGF (Man3) (15), in the order illustrated in Figure 2. In specific tissues or cell types, an additional mannose (Man4) may be incorporated by PIGZ (16).

The attachment of GPI to the protein is carried out by a GPI transamidase complex (GPIT) composed of five proteins: the catalytic subunit PIGK, together with PIGS, PIGT, PIGU, and GPAA1 (15, 16). This complex recognizes the C-terminal GPI attachment signal peptide of the precursor protein, cleaves it, and covalently links the amino group of the terminal EtNP of the GPI anchor to the newly exposed carboxyl group of the protein. The amino acid residue of a precursor protein to which GPI is attached is termed the ω -site. Although there is no strict consensus sequence for the GPI-attachment signal, several common features have been identified: small amino acid residues (e.g., Gly, Ala, or Ser) at the ω - and $\omega+2$ positions; a hydrophilic stretch of 6–10 amino acids beginning at $\omega+3$; and a subsequent hydrophobic region of approximately 10–20 amino acids extending to the C-terminus (17). Recently, the structure of the GPIT complex was predicted through computational modeling by David Baker and colleagues (18), a recipient of the Nobel Prize, and was subsequently validated by cryo-electron microscopy (cryo-EM), confirming the high accuracy of the predicted model (19, 20).

The genes involved in the early steps of GPI biosynthesis within the ER are collectively termed phosphatidylinositol glycan (PIG) genes and are named alphabetically based on the order of discovery. Genes responsible for subsequent modifications of GPI-APs are designated as post-GPI attachment to proteins (PGAP) genes (1) (Figure 2).

2) Remodeling of GPI-Anchored Proteins

Deacylation of Inositol

After the GPI anchor is attached to the protein by GPIT, acyl chain linked to the inositol moiety is removed in the ER by PGAP1(21), a protein containing a lipase motif (GxSxG). In cells lacking functional PGAP1, the transport of GPI-APs from the ER to the Golgi apparatus is significantly delayed. Moreover, these proteins fail to undergo proper remodeling in the Golgi and are expressed on the cell surface in an abnormal form, retaining the acyl group. Consequently, they become resistant to cleavage by phosphatidylinositol-specific phospholipase C (PIPLC) in *in vitro* assays. Similarly, PGAP1-deficient cells are thought to exhibit resistance to cleavage by lipases and other processing enzymes, which may contribute to physiological abnormalities. GPI-APs are characterized by their ability to be shed from the cell surface, a process that can regulate signal transduction both positively and negatively. Several pairs of GPI-APs and GPI-cleaving/processing enzymes, collectively referred to as GPIases, are responsible for this

shedding (22-24). Recently, the structure of PGAP1 in complex with a GPI-AP was elucidated using cryo-electron microscopy (cryo-EM), providing detailed insights into the product–enzyme interaction (25).

GPI Glycan Remodeling (Removal of Side Chain EtNP)

Subsequently, still within the ER, EtNP linked to the second mannose (Man2) is removed by PGAP5 (also known as MPPE1) (26). PGAP5 is a protein containing a metal-dependent phosphoesterase motif and facilitates the transport of GPI-APs to the ER exit sites. There, GPI-APs interact with cargo receptors, specifically members of the p24 family of proteins, and are subsequently transported to the Golgi via COPII-coated vesicular trafficking. In PGAP5-deficient cells, similar to PGAP1-deficient cells, the transport of GPI-APs to the Golgi is delayed. However, after lipid remodeling in the Golgi, GPI-APs are expressed on the cell surface in an abnormal form, retaining the EtNP linked to Man2 (26).

Fatty Acid Remodeling

This process occurs in the Golgi apparatus and is essential for the proper localization of GPI-APs to lipid rafts. First, the unsaturated fatty acids, primarily arachidonic acid (C20:4), attached to the sn-2 position of the lipid moiety are removed by PGAP3 (27). Subsequently, saturated fatty acids, such as stearic acid (C18:0), are added. PGAP2 is critical for this second step; however, it is considered a regulatory factor rather than a direct enzymatic actor (28). To date, no specific enzyme responsible for this acylation step has been identified in mammals.

In PGAP3-deficient cells, GPI-APs are expressed on the cell surface without undergoing proper remodeling and fail to localize to lipid rafts (27). In PGAP2-deficient cells, GPI-APs are transported to the cell surface in the form of lyso-GPIs, lacking fatty acids at the sn-2 position. These altered GPI-APs are released from the cell surface and are cleaved by lipases, such as GPI-specific phospholipase D (GPIPLD), resulting in a marked reduction in GPI-AP levels on the cell surface (28).

PGAP6/TMEM8A and PGAP3 belong to the CREST superfamily of hydrolases. PGAP6 also functions as a GPI-specific phospholipase A2. Unlike PGAP3, however, PGAP6 is expressed on the surface of certain cells. One of its targets is CRIPTO-1, a GPI-anchored co-receptor of TGF β receptors. Upon removal of one acyl chain by PGAP6, CRIPTO-1 is converted to a lysoform, released from the cell surface, and subsequently cleaved by GPIPLD. This shedding of CRIPTO-1 is essential for embryogenesis (24).

Side chain modification

In many GPI-anchored proteins (GPI-APs), a β -N-acetylgalactosamine (β GalNAc) residue is added to Man1 as a side chain following fatty acid remodeling (Figure 1). This modification is catalyzed by PGAP4 (also known as TMEM246), a Golgi-resident, GPI-specific GalNAc transferase (29). In some GPI-APs, this side chain is further elongated by the addition of galactose (Gal) or by sequential addition of Gal and sialic acid (Sia). Gal elongation is mediated by B3GALT4, a Golgi-resident Gal transferase that is also known to synthesize GM1 from GM2, GA1 from GA2, and GD1 from GD2 during glycosphingolipid biosynthesis (30). PGAP4 is widely expressed across various tissues and organs, with particularly high expression levels in the brain; however, the physiological function of the GalNAc side chains remains unknown.

Paroxysmal Nocturnal Hemoglobinuria (PNH), an Acquired GPI Deficiency

PNH is a hematologic disorder characterized by three main clinical features: hemolytic anemia, bone marrow failure, and deep vein thrombosis. Diagnosis is based on episodes of hemolytic attacks and flow cytometric analysis of peripheral blood, which reveals a population of blood cells with reduced or absent expression of GPI-APs.

PNH arises from an acquired somatic pathogenic variant in the *PIGA* gene in one or more hematopoietic stem cells, resulting in the expansion of a clonal population of GPI-deficient cells. Consequently, red blood cells lacking GPI-anchored complement regulatory proteins, such as CD59 and decay-accelerating factor (DAF), become vulnerable to complement-mediated lysis, leading to hemolytic anemia (31, 32). Among the multiple genes involved in GPI biosynthesis, *PIGA* is responsible for nearly all cases of PNH. This is primarily because *PIGA* is located on the X chromosome; following X-chromosome inactivation during early development, a single somatic pathogenic variant is sufficient to cause GPI deficiency, explaining the equal incidence of PNH in males and females.

Treatment strategies mainly aim to reduce hemolysis, prevent thrombosis, and manage symptoms such as fatigue and anemia. The first approved therapy for PNH was a terminal complement inhibitor, an anti-C5 antibody. Since then, several proximal and terminal complement inhibitors have been approved, all showing marked clinical improvements in patients with PNH (33).

Recently, next-generation sequencing has identified rare forms of PNH caused by pathogenic variants in *PIGT*, *PIGB*, and *PIGV*. In these cases, an inherited pathogenic

variant in one allele is followed by a somatic event in hematopoietic stem cells: a microdeletion including *PIGT* localized on chromosome 20q, a copy-neutral loss of heterozygosity (CN-LOH) including *PIGB* on chromosome 15q, and a somatic pathogenic variant in *MPL* preceded by CN-LOH in cis including *PIGV* on chromosome 1p, respectively, leading to disease manifestation (34-36). Notably, *PIGT*- and *PIGB*-PNH presented with autoinflammatory symptoms in addition to the classical features of PNH. In *PIGT*-deficient cells, GPI is synthesized but cannot function as a protein anchor. Instead, free GPI molecules are transported to and expressed on the cell surface, where they can be detected using the T5 4E10 antibody. These free GPIs enhance complement activation, leading to autoinflammatory responses (34). Similarly, GPI intermediates accumulate on the cell surface of *PIGB*-deficient cells, also contributing to autoinflammation. Although these forms are rare, additional PNH-causing genes may be identified in the future.

Inherited GPI deficiency (IGD) usually results in partial GPI deficiency, as complete GPI deficiency is lethal (2). IGD follows an autosomal recessive inheritance pattern, with affected individuals being either homozygous or compound heterozygous for pathogenic variants in GPI biosynthesis genes. Theoretically, *PIGA*-IGD should be the most common form of IGD, as a single pathogenic variant on the X chromosome could lead to disease manifestation, similar to PNH. However, reported cases show that heterozygous females are asymptomatic, and only males exhibit clinical symptoms.

History of Inherited GPI Deficiency (IGD)

1) The First Identified Case of *PIGM*-IGD

A collaborative study with researchers from the UK analyzed two families comprising three affected individuals, all of whom exhibited a significant reduction in GPI-APs on granulocytes, while the reduction on red blood cells was relatively mild, resulting in the absence of hemolytic episodes. Instead, the primary clinical manifestations were epileptic seizures and portal vein thrombosis. Given that fibroblasts from the patients also demonstrated a marked decrease in GPI-APs, IGD was suspected. Analysis of B lymphoblasts derived from the patients identified *PIGM* as the responsible gene. *PIGM* encodes the enzyme responsible for adding Man1 during GPI biosynthesis. In both families, a single nucleotide pathogenic variant in the promoter region of *PIGM* disrupted the binding site for the transcription factor Sp1, leading to a severe reduction in *PIGM* promoter activity in homozygous patients. Sp1 is known to recruit transcriptional coactivators involved in histone acetylation. Administration of sodium butyrate (Na-

butyrate), a histone deacetylase (HDAC) inhibitor, induced forced histone acetylation, restored *PIGM* expression, and fully recovered GPI-AP expression on patient-derived B lymphoblasts. Remarkably, Na-butyrate treatment in patients led to the resolution of their intractable seizures (37, 38). Unlike other reported cases of IGD, these patients did not display facial dysmorphism, congenital malformations, or intellectual disability. It is speculated that during fetal development, other transcriptional factors may have compensated for the reduced *PIGM* expression. Nevertheless, compared to other forms of IGD, the reduction of GPI-APs in blood cells and fibroblasts was more pronounced in these patients, suggesting that enhanced local complement activation due to diminished GPI-AP expression on vascular endothelial cells may contribute to thrombus formation. Additionally, an additional patient carrying the same promoter variant in *PIGM* was reported (39). Recently, *PIGM*-IGDs caused by pathogenic variants in the coding region of *PIGM* have been reported, and they exhibit similar symptoms to those observed in other IGDs (40).

2) Hyperphosphatasia with Mental Retardation Syndrome (Mabry Syndrome)

For many years, a recessively inherited disorder characterized by elevated alkaline phosphatase (ALP) levels and intellectual disability had been recognized, with several cases reported in Europe, and was subsequently named Mabry Syndrome (41).

In 2010, German researchers performed exome sequencing using next-generation sequencing (NGS) technology and identified a pathogenic variant in the coding region of *PIGV* in patients with Mabry Syndrome. Collaborative research with our team confirmed that this variant led to reduced *PIGV* enzymatic activity (42). Subsequent collaborations with the same research group resulted in the identification of additional cases of *PIGO*-, *PGAP2*-, and *PGAP3*- IGD (43-45), all presenting with similar clinical features. Through our own screening efforts, we also discovered Japan's first case of *PIGO*-IGD (46) and subsequently identified a novel case of *PIGW*-IGD (47). With the increasing availability and application of NGS, deficiencies have now been reported in 24 out of the 30 known genes involved in the GPI biosynthetic pathway, revealing that not all cases of IGD are associated with hyperphosphatasia.

Inherited GPI Deficiency (IGD)

IGD is a recessive hereditary disorder caused by pathogenic variants in genes involved in GPI biosynthesis, characterized primarily by neurological symptoms such as intellectual disability, delayed motor development, and epilepsy (48-51) (Figure2). To date,

approximately 700 cases of IGD involving 24 genes have been reported both in Japan and internationally (Table 1).

IGD can be classified into three groups based on the impact on GPI-APs:

1) Group 1: Reduction in GPI-AP expression on the cell surface

This group includes defects in most *PIG* genes (except *PIGG*) and in *PGAP2* among the *PGAP* genes. Pathogenic variants in *PIG* genes impair the production of mature GPI anchors or the attachment of GPI to proteins, resulting in decreased cell surface expression of GPI-APs. Because these variants are typically hypomorphic, proteins with weaker GPI-attachment signals exhibit a greater reduction in expression when GPI anchors are limited, leading to symptom manifestation. Given that many patients harboring biallelic variants in *ARV1* present symptoms common to IGD, such as early infantile epileptic encephalopathy, a potential association with IGD was suggested (52). Subsequent analyses revealed that ARV1 is a component of GPI-GnT (4).

In *PGAP2*-IGD, GPI-APs are transported to the cell surface as lysolipids, then released and cleaved by lipases, leading to a reduction in GPI-AP levels (44).

The primary clinical features are neurological, including intellectual disability, delayed motor development, and seizures. Severe cases may present additional congenital anomalies, such as facial dysmorphism, hypoplasia of the distal phalanges and nails, hearing loss, congenital heart defects, Hirschsprung's disease, anorectal malformations, cerebellar atrophy, cerebral atrophy, white matter degeneration, and delayed myelination. Phenotypic severity appears to correlate more with the degree of residual enzyme activity than with the specific gene involved. Severe forms of IGD (*PIGA*-, *PIGL*-, *PIGW*-, *PIGV*-, *PIGN*-IGD) have been reported to overlap clinically with Fryns syndrome (53-57), characterized by congenital diaphragmatic hernia, developmental delay, facial dysmorphism, hypoplastic nails, and multi-organ anomalies. Some cases of *PIGL*-IGD overlap with CHIME syndrome, featuring colobomas, congenital heart defects, ichthyosiform dermatosis, intellectual disability, and ear anomalies (58-64), all associated with the p.Leu167Pro variant. In addition, some severe IGD cases (*PIGN*-, *PIGB*-, *PIGF*-IGD) have been found in DOORS syndrome (deafness, onychodystrophy, osteodystrophy, mental retardation, and seizures), which is known to be caused by pathogenic variants in the *TBC1D24* gene (65, 66).

Among Group 1, *PIGW*-, *PIGV*-, *PIGB*-, *PIGO*-IGD show hyperphosphatasia (66-69), whereas defects in other *PIG* genes usually result in normal or mildly elevated serum ALP levels (53, 57). Hyperphosphatasia results from incomplete GPI-anchor synthesis, where ALP precursors, cleaved by GPIT, fail to attach to the cell surface and are

subsequently secreted into the serum. Structural studies of GPIT indicate that precursor protein binding triggers a conformational activation of GPIT (22). GPI intermediates at later biosynthetic stages may further enhance this activity (70). PGAP2-IGD also exhibits hyperphosphatasia, as ALP expressed in the lysoform is readily released from the cell surface (44). In contrast, deficiencies in GPIT components (PIGK-, PIGT-, PIGS-, PIGU-, GPAA1-IGD) prevent cleavage of the GPI attachment signal, leading to intracellular degradation of ALP and reduced serum ALP levels (70-75).

2) Group 2: Normal expression levels of GPI-APs but with abnormal GPI structures

This group includes PGAP1- and PGAP3-IGD. In PGAP1-IGD (76), GPI-APs are expressed on the cell surface with the inositol acyl group intact, rendering them resistant to lipases and impairing physiological shedding, which may contribute to neurological abnormalities. *PGAP1*-knockout mice display otocephaly and perinatal lethality (77).

In PGAP3-IGD, GPI-APs fail to undergo proper lipid remodeling, leading to impaired localization to lipid rafts and disruption of signal transduction. PGAP3-IGD is associated with hyperphosphatasia (78), though the exact mechanism remains unclear; possible explanations include increased susceptibility to cleavage by lipases or proteases due to aberrant membrane localization. Clinical features mainly involve neurological symptoms, including intellectual disability, motor developmental delay, seizures, microcephaly, ataxia, and sleep disturbances.

3) Group 3: PIGG-IGD

PIGG encodes the enzyme responsible for transferring EtNP to Man2 during GPI biosynthesis. This EtNP moiety is subsequently removed by PGAP5 in the ER after GPI attachment to proteins. In *PIGG*-knockout cells, GPI-APs such as CD59 and DAF are expressed at normal levels with seemingly normal structures, and the physiological role of PIGG remained unclear. However, the increasing number of PIGG-IGD cases, presenting with severe neurological phenotypes such as intractable epilepsy and developmental delay (79), has highlighted its critical role in neurodevelopment. Notably, GPI anchors typically attach to proteins via EtNP linked to Man3. However, in *PIGO*- or *PIGB*-KO cells—lacking EtNP addition to Man3 or Man3 itself, respectively—residual expression of CD59 (~10%) persists. Double KO of *PIGG* eliminates CD59 expression (80), demonstrating that the EtNP added by *PIGG* to Man2 can compensate for the missing canonical structure. This novel GPI structure was also detected in wild type cells, which was confirmed by mass spectrometry (80). Among neuronal GPI-APs, CD73 and NetrinG2 were found to utilize this novel structure. Reduced expression of these proteins

likely underlies the neurological symptoms observed in PIGG-IGD. Indeed, fibroblasts from PIGG-IGD patients showed reduced CD73 expression (79). CD73 not only catalyzes the conversion of AMP to adenosine but also facilitates the hydrolysis of FAD (flavin adenine dinucleotide) to FMN (flavin mononucleotide), which is then dephosphorylated to riboflavin by ALP, allowing cellular uptake of vitamin B₂ (81). In GPI-deficient cells, provision of FAD alone results in intracellular vitamin B₂ deficiency and mitochondrial dysfunction, consistent with mitochondrial abnormalities observed in severe PIGG-IGD cases (79). Additionally, the Emm blood group antigen has been identified on normal erythrocytes and recently, cases of hemolytic transfusion reaction due to anti-Emm antibodies were traced to the patients with complete PIGG deficiency. It was revealed that the Emm antigen corresponds to free GPI bearing EtNP attached to Man2 (82), demonstrating the presence of free GPI on normal red blood cells.

Diagnosis of IGD: Symptoms and general clinical examinations

1) Symptoms (51)

Intellectual disability is constantly present, even in mild cases. As severity increases, additional symptoms may appear, including:

- Motor developmental delay
- Epilepsy (Some severe cases develop early infantile epileptic encephalopathies, such as: Ohtahara Syndrome and West Syndrome)
- Facial abnormalities (e.g., tent-shaped mouth)
- Hearing loss
- Hirschsprung's disease and other intestinal malformations
- Congenital diaphragmatic hernia (overlap with Fryns syndrome)
- Congenital heart defects
- Ichthyosis and other skin abnormalities
- Ocular abnormality and visual impairment

2) Clinical examinations

- Some patients exhibit abnormal serum ALP levels (either hyperphosphatasia or hypophosphatasia)

3) MRI Findings in Severe Cases (51)

Characteristic brain MRI features include:

- High signal intensity in the brainstem and midbrain on diffusion-weighted (DWI) and T2-weighted imaging
- Myelination disorder
- Progressive cerebellar atrophy
- Cerebral white matter degeneration after birth

4) Special examinations

- Flow cytometry analysis of granulocytes to evaluate CD16b, a GPI-AP, expression for Group1-IGD.
- For Group2- and 3-IGD, as well as definitive diagnoses for Group1-IGD, exome sequencing or targeted gene panel analysis is necessary.
(Both examinations are covered by insurance in Japan)

In some cases, functional studies to confirm the pathogenicity of variants are required. Mutant genes are transfected into cell lines deficient in the corresponding gene, and GPI-AP expression recovery is measured compared to the wild-type gene. The level of recovery correlates well with clinical severity (69).

Treatment of IGD

1) Supplement therapy

- The uptake of vitamins B₆ and B₁ into cells requires dephosphorylation of their active phosphorylated forms by tissue-nonspecific alkaline phosphatase (TNAP), a GPI-AP. IGD patients often suffer from intracellular deficiencies of vitamins B₆ and B₁. In particular, vitamin B₆ deficiency may impair GABA synthesis and trigger seizures (83). Vitamin B₆ (pyridoxine) supplementation has been effective in some cases (46, 84).
- In addition to TNAP, CD73, another GPI-AP, is necessary to the uptake vitamin B₂ into the cell (81). Vitamin B₂ plays a crucial role in mitochondrial energy production, and riboflavin supplementation is expected to be beneficial.
- Folate receptors (FOLR1, FOLR2), also GPI-APs, are required for the transcytosis of folic acid from the blood into the cerebrospinal fluid (85). Some IGD patients suffer from central folate deficiency, which cause seizures, developmental delay and cerebellar atrophy. Administration of folinic acid is expected to be effective.

2) Development of Gene Therapy

- We established a murine model of IGD harboring a patient-derived p.Thr130Asn variant in *Pigo*. This model faithfully recapitulates key clinical manifestations observed in human IGD, including hyperphosphatasia, growth retardation, reduced survival, tremors, and spontaneous seizures (86).
- Administration of an adeno-associated virus (AAV)-mediated genome editing therapy, homology-independent targeted integration assisted with a low level of transgene expression (HITI-TE), in the PIGO-IGD model resulted in substantial improvement of neuronal phenotypes and amelioration of growth impairment (86)(Figure 3).
- To further advance clinical applicability, AAV-mediated gene replacement therapy was performed in a central nervous system (CNS)-specific *Piga* knockout mouse model (PIGA-IGD), demonstrating significant attenuation of neurological symptoms (87). These findings suggest that neurological abnormalities associated with IGD are reversible if therapeutic intervention is initiated in the early postnatal period.

Conclusions

Inherited GPI deficiencies are associated with a wide spectrum of clinical manifestations; however, early diagnosis and appropriate therapeutic intervention have the potential to prevent disease progression. Ongoing research is focused on the development of curative gene therapies for clinical implementation, in parallel with efforts to integrate early diagnostic strategies into newborn mass screening programs.

Conflict of Interest

There are no conflicts of interest to declare.

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Figure legend

Figure 1

Structure of GPI anchored protein

The core structure of the GPI anchor is conserved across all eukaryotes, whereas modifications of the GPI side chain vary depending on the species, tissue, or cell type.

Figure 2

Biosynthesis and transport of GPI-anchored protein

(Refer to the main text.)

Figure 3

Gene therapy using genome editing has improved almost all phenotypes

A. AAV expressing Cas9 and AAV containing normal PIGO cDNA and guide RNA-were administered via intravenous injection into newborn *Pigo*^{b/b} and *Pigo*^{b/-} mice. **B.** The plasma ALP level was significantly decreased in HITI-TE-treated *Pigo*^{b/-} mice at 4 months of age. **C.** HITI-TE treatment rescued the growth defect in both male and female *Pigo*^{b/b} or *Pigo*^{b/-} mice. **D.** All untreated *Pigo*^{b/b} mice exhibited hindlimb clasping, whereas none of HITI-TE-treated *Pigo*^{b/b} mice showed hindlimb clasping. **E.** Tremor was significantly milder in treated *Pigo*^{b/b} mice than in untreated *Pigo*^{b/b} mice. **F.** The results of the hanging test in HITI-TE-treated *Pigo*^{b/b} mice showed clear improvements, suggesting that the low muscle strength and/or poor motor coordination were ameliorated. Figure reproduced and modified from Kuwayama et al., *Nature Communications* 13, 3107 (2022), licensed under CC BY 4.0.

Table 1

Various Inherited GPI deficiencies

Figure 1

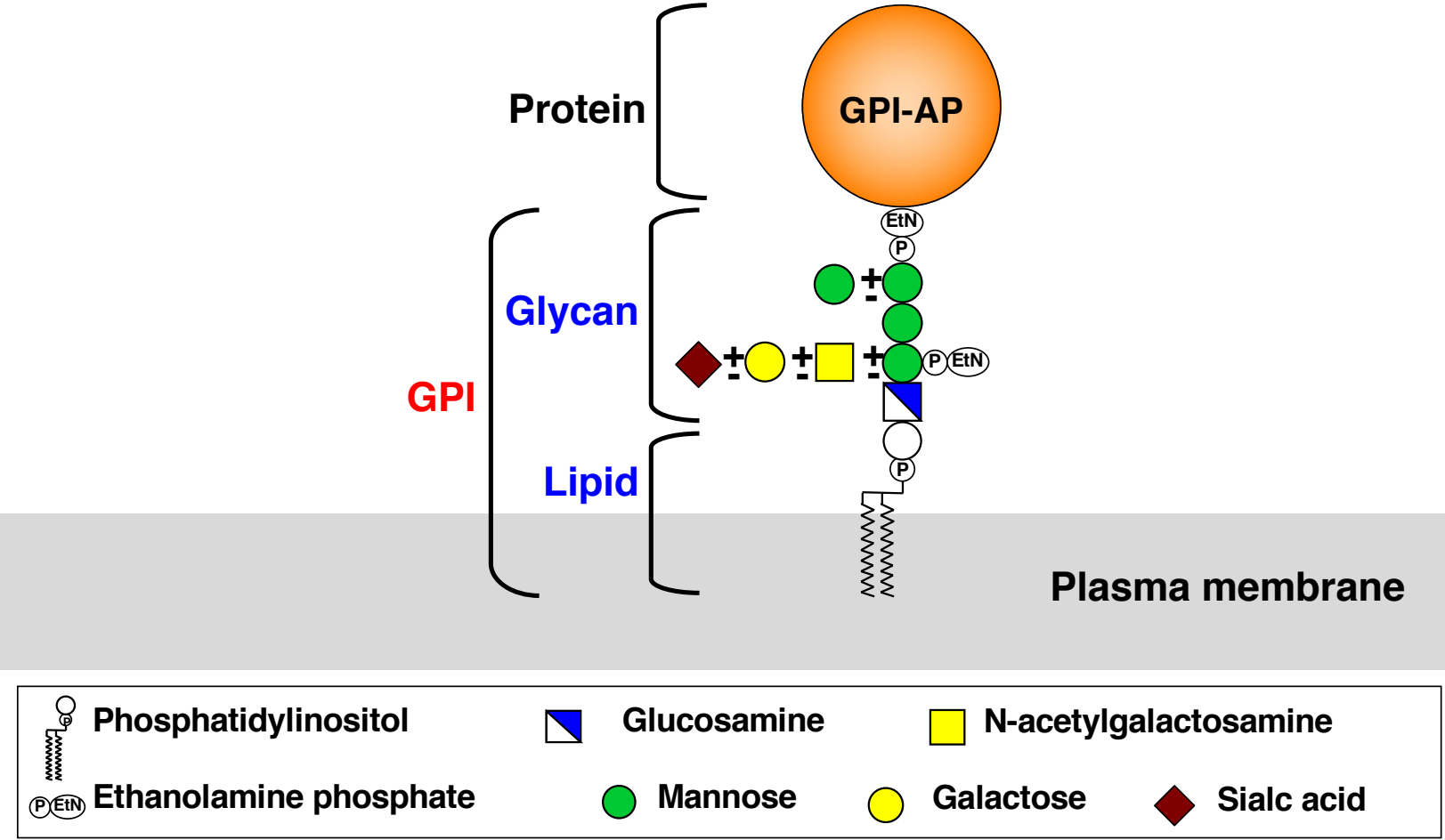


Figure 2

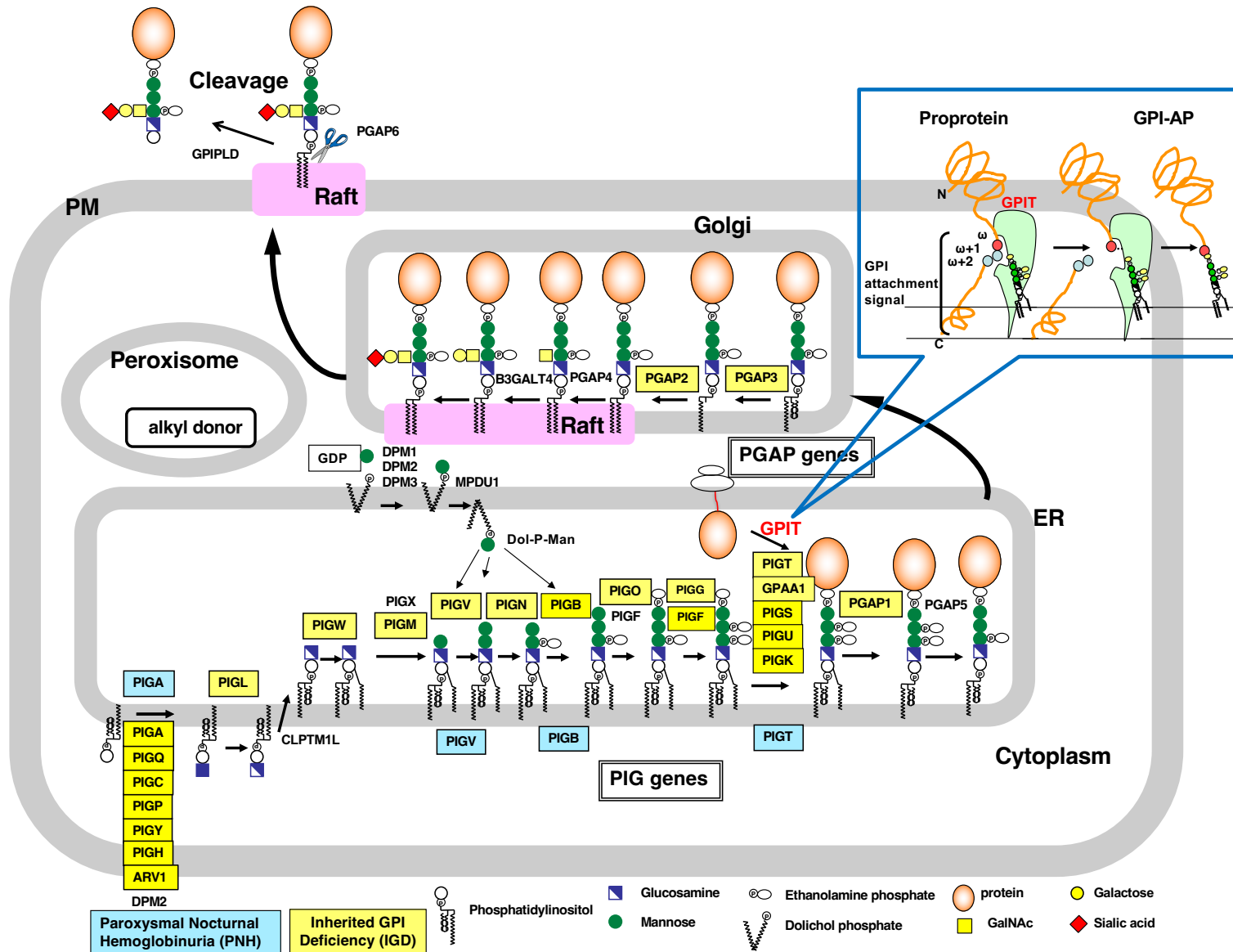


Figure 3

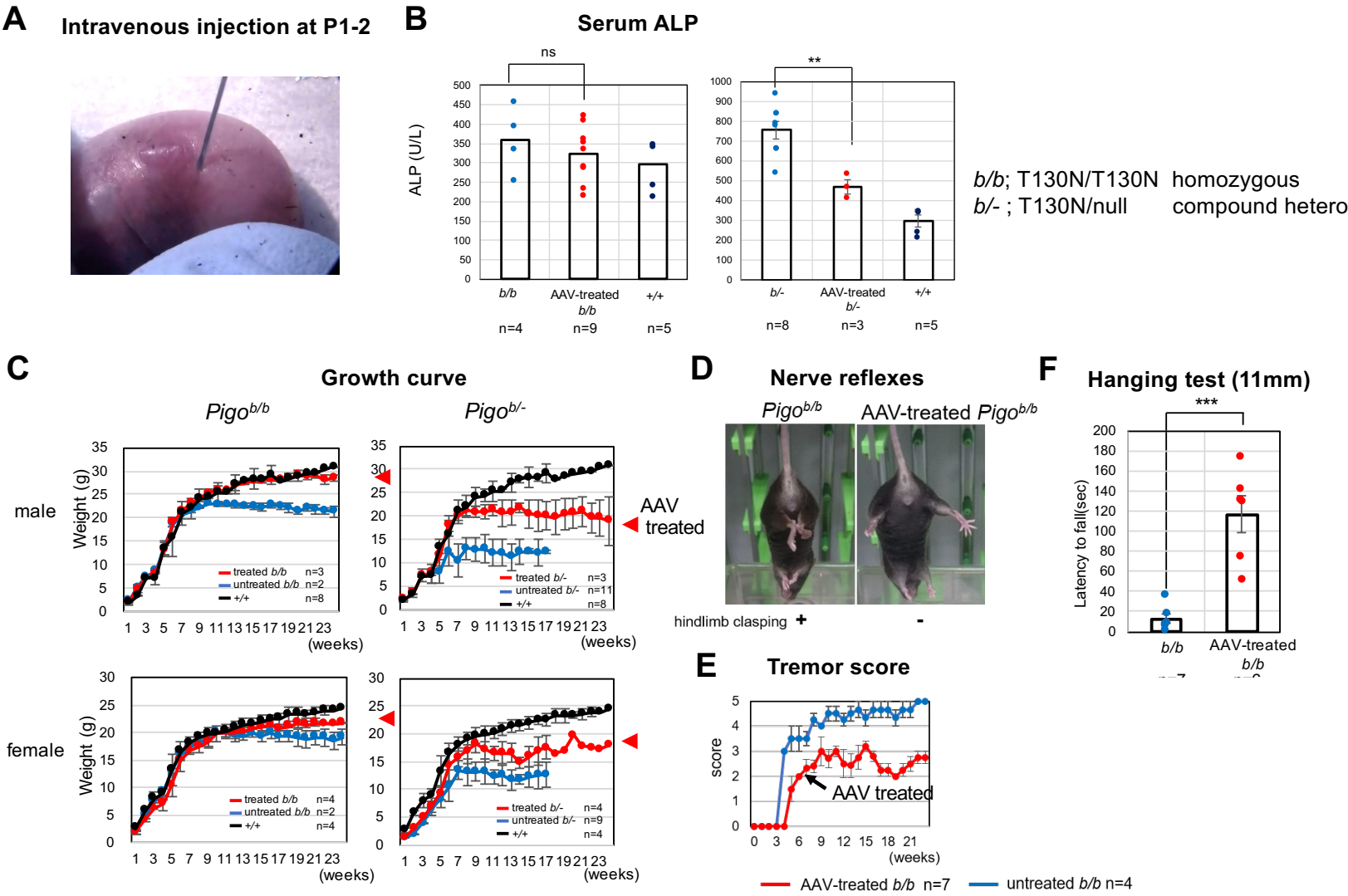


Table 1 Various Inherited GPI deficiencies

Step	-IGD	Chromosome	Gene No.	OMIM #	Alternative name #	Overlap	Number of Pt *	In Japan *	Function	First report	Ref.
1 (GPI-GnT)	PIGA	Xp	NM_002641	GPIBD4	MCAHS2	Fryns syn.	101	15	GlcNAcT	2012	53
	PIGC	1q	NM_153747	GPIBD16			28	0	GlcNAcT	2016	88
	PIGQ	16q	NM_004204	GPIBD19			12	0	GlcNAcT	2014	89
	PIGP	21q	NM_153682	GPIBD14			6	0	GlcNAcT	2017	90
	PIGY	4q	NM_001042616	GPIBD12	HPMRS6		4	0	GlcNAcT	2015	91
	PIGH	14q	NM_004569	GPIBD17			8	0	GlcNAcT	2018	92
	ARV1	1q	NM_022786	GPIBD23			26	0	GlcNAcT	2020	52
2	PIGL	17p	NM_004278	GPIBD5		CHIME syn. Fryns syn.	22	4	Deacetylase	2012	54, 58-64
4	PIGW	17q	NM_178517	GPIBD11	HPMRS5	Fryns syn.	24	4	AcylT	2013	47,55
5	PIGM	1q	NM_145167	GPIBD1			12	0	ManT1	2006	36, 39
6	PIGV	1p	NM_001202554	GPIBD2	HPMRS1	Fryns syn.	29	5	ManT2	2010	42,56
7	PIGN	18q	NM_176787	GPIBD3	MCAHS1.	Fryns syn.	120	9	EtNPT1	2011	57
8	PIGB	15q	NM_004855	GPIBD20			22	1	ManT3	2019	66
9	PIGF	2p	NM_002643	GPIBD24			2	0	EtNPT2/3	2020	65
	PIGO	9p	NM_032634	GPIBD6	HPMRS2		26	16	EtNPT3	2012	43,84
10	PIGG	4p	NM_001127178	GPIBD13			41	2	EtNPT2	2016	79
11 (GPIT)	PIGK	1p	NM_005482	GPIBD22			16	1	GPIT	2020	71
	PIGS	17p	NM_033198	GPIBD18			14	1	GPIT	2018	73
	PIGT	20q	NM_015937	GPIBD7	MCAHS3		58	6	GPIT	2013	72
	PIGU	20q	NM_080476	GPIBD21			5	0	GPIT	2019	74
	GPAA1	8q	NM_003801	GPIBD15			17	0	GPIT	2017	75
12	PGAP1	2q	NM_024989	GPIBD9			28	0	Deacylase	2014	76
14	PGAP3	17q	NM_033419	GPIBD10	HPMRS4		66	2	GPI PLA2	2014	45,78
15	PGAP2	11p	NM_001256240	GPIBD8	HPMRS3		24	0	AcylT	2013	44
Total number							711	66			

IGD: Inherited GPI Deficiency, GPI-GnT; GPI GlcNAc transferase, GPIT; GPI transamidase

GPIBD : GPI biosynthesis defect, MCAHS : multiple congenital anomalies hypotonia seizures syndrome, HPMR : hyperphosphatasia mental retardation (Mabry syndrome)

CHIME : coloboma heart defect ichthyosiform dermatosis mental retardation and ear anomalies, DOORS : deafness, onychodystrophy, osteodystrophy, mental retardation, and seizures.

Fryns syndrome : congenital diaphragmatic hernia, developmental delay, facial dysmorphism, hypoplastic nails, and multi-organ anomalies.

GlcNAcT:GlcNAc transferase, AcIT: Acyl transferase, ManT: Mannose transferase, EtNPT: Ethanolamine phosphate transferase, GPI PLA2: GPI Phospholipase A2

* ; include both published and unpublished as of March 2025