Role of *PIGM* and *PIGX* in glycosylphosphatidylinositol biosynthesis and human health (Review)

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Abstract. Glycosylphosphatidylinositol-glycan (GPI) is an anchor to specific cell surface proteins known as GPI-anchored proteins (APs) that are localized in lipid rafts and may act as cell co-receptors, enzymes and adhesion molecules. The present review investigated the significance of GPI biosynthesis class phosphatidylinositol-glycan *(PIG)M* and *PIGX* in GPI synthesis and their implications in human health conditions. *PIGM* encodes GPI-mannosyltransferase I (MT-I) enzyme that adds the first mannose to the GPI core structure. *PIGX* encodes the regulatory subunit of GPI-MT-I. The present review summarizes characteristics of the coding sequences of *PIGM* and *PIGX*, and their expression in humans, as well as the relevance of GPI-MT-I and the regulatory subunit in maintaining the presence of GPI-APs on the cell surface and their secretion. In addition, the association of *PIGM* mutations with paroxysmal nocturnal hemoglobinuria and certain types of GPI-deficiency disease and the altered expression of *PIGM* and *PIGX* in cancer were also reviewed. In addition, their interaction with other proteins was described, suggesting a complex role in cell biology. *PIGM* and *PIGX* are critical genes for GPI synthesis. Understanding gene and protein regulation may provide valuable insights into the role of GPI-APs in cellular processes.

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Contents

1. Introduction

Glycosylphosphatidylinositol (GPI) serves as a glycolipid anchor for numerous cell surface proteins in eukaryotes (1). In humans, ~150 GPI-anchored proteins (APs) have been identified, displaying roles such as enzymatic activity, antigen presentation, co-receptor engagement, adhesion and involvement in immune responses (1-3).

The core structure of GPI remains highly conserved across diverse organisms, featuring a PI molecule firmly integrated into the cell membrane. This structure is followed by glycans, namely glucosamine (GlcN) and three mannoses (Man) culminating in a terminal ethanolamine phosphate (EtNP) group. The latter binds covalently to the C-terminal end of the target protein (1,4). Variations in GPI backbone, involving modifications in EtNP and various glycan side branches, depend on the organism, cell type and specific protein (1,4). Notably, in mammalian cells, Man1 undergoes modification with an EtNP side chain and Man4 may attach to Man3 via α1,2-linkage (4,5).

To date, 30 genes have been identified encoding proteins implicated in GPI synthesis (4,6). These genes belong to the phosphatidylinositol-glycan biosynthesis class (*PIG*) and the post-GPI attachment to proteins (*PGAP*) families (3). The initiation of glycolipid biosynthesis occurs on the cytoplasmic side of the endoplasmic reticulum (ER). GPI N-acetylglucosamine transferase (GPI-GnT) catalyzes transfer of N-acetylglucosamine (GlcNAc) glycan to the PI molecule within the ER membrane, yielding GlcNAc-PI (1). Subsequent steps involve deacetylation, translocation to the ER lumen and

acylation of inositol resulting in GlcN-acyl-PI and sequential addition of three Man. The addition of Man1 in ER is orchestrated by GPI-mannosyltransferase (MT)-I, anchored in the ER membrane and composed of the proteins PIGM and PIGX (Fig. 1) (1), where PIGM serves a functional role and PIGX as the stabilizing protein for PIGM enzyme (1).

Following GPI synthesis, GPI-MT-II encoded by *PIGV* links Man2 to Man-GlcN-acyl-PI, while GPI-MT-III encoded by *PIGB* adds a third Man yielding the glycolipid Man-Man-Man3-GlcN-acyl-PI (Fig. 1) (1,7). In addition, an EtNP molecule is integrated into the glycan, contributing to formation of the GPI core (1,7). The addition of Man4 as a side chain to Man3 is facilitated by GPI-MT-IV, encoded by *PIGZ* (1,7). Subsequently, the GPI molecule can bind to proteins featuring a C-terminal GPI attachment hydrophobic signal peptide, mediated by the GPI transamidase enzyme complex (GPI-TA) bound to the EtNP molecule (1). Synthesis proceeds in the Golgi apparatus, where GPI undergoes further lipid remodeling and glycan modification by PGAP enzymes (1,7-9). For example, the enzyme GPI-N-acetylgalactosamine transferase (GalNAc), encoded by the *PGAP4* gene, modifies Man1 by adding a GalNAc (10). Ultimately, GPI-APs are transported to the plasma membrane via vesicular transport where they function within lipid rafts or are released into the extracellular space (8). GPI-APs exhibit diverse roles, including enzymatic activity, signaling, cell adhesion, cell wall metabolism, neuritogenesis and immune response (11).

Alterations in genes involved in GPI biosynthesis have been implicated in congenital anomalies such as multiple congenital anomalies-hypotonia-seizures, hyperphosphatasia with mental retardation and anomalies/epilepsy syndrome (3,12,13). In addition, certain types of cancer display altered expression in some *PIG* genes (14-16). Currently, little is known about the involvement of altered expression of *PIGM* and *PIGX*, genes that encode and regulate the GPI-MT-I, respectively, in human disease (17). The characteristics of the *PIGM* and *PIGX* genes and their encoded proteins are summarized in the present review, as well as the relevance of both genes in GPI synthesis and certain human health diseases, and their potential role in other biological functions.

2. Characteristics of the coding sequences of *PIGM* and *PIGX* and their expression in humans

PIGM is localized in chromosome 1q23, consists of 7,038 bp and encodes the transcript ENST00000368090.5, resulting in the protein PIGM Q9H3S5 (18). The protein consists of 423 amino acid residues (2); the predicted structure by AlphaFold DB indicates that the tertiary structure consists of 10 transmembrane α-helices that alternate with 11 lumenal and cytoplasmic domains (Fig. 2A) (19). At amino acid positions 49-51, the protein harbors a sugar-binding motif, aspartate-any residue-aspartate (DXD) situated within a hydrophilic region flanked by the first and second transmembrane domains. The DXD motif is a prevalent feature in numerous glycosyltransferases and serves a pivotal role in coordinating a manganese ion, crucial for binding to a nucleotide sugar substrate (20). Notably, mutations in the DXD motif, such as the D51A alteration in PIGM, lead to the absence of GPI-APs on the cell surface, indicating the essential role of the DXD motif in expression of GPI-APs (20). Moreover, according to the predicted structure, all lumenal domains are comprised of the amino acid residues 39-79, 162-169, 247-287, 338 and 379-384, where PIGM should exert the catalytic activity (19); to the best of our knowledge however, there is no experimental evidence regarding the functional importance of these regions. There is no predicted site for phosphorylation (2) and thus far, binding to PIGX is the only mechanism proposed to regulate catalytic activity.

PIGX is 23,630-bp long and is located on chromosome 3q29 (21). Currently, there is a total of nine known potential mRNA variants of *PIGX* resulting from alternative splicing (18). However, only two mRNA variants encode two protein isoforms (18). The protein isoform of PIGX Q8TBF5-1 is encoded by mRNA variant ENST00000392391.9, with a size of 258 amino acid residues and a mass of 28,788 Da (2). This isoform has been chosen as the canonical protein since it was first described (2,18). The amino acid residues 1-21 comprise the signal peptide that recognizes the protein as an ER membrane protein (Fig. 2B). The amino acid residues 22-230 form a soluble amino acid chain in the lumen of the ER, while the amino acid residues 231-251 are inserted in the ER membrane and amino acid residues in the C-terminal region, 252-258, are soluble in the cytoplasm (Fig. 2B) (2). *PIGX* has a non-ATG start codon and instead contains a CTG start codon that is well-conserved in mammals (18); non-ATG start codons are associated with key cellular functions such as development and stress responses (22). Regarding post-translational modifications, PIGX Q8TBF5-1 isoform harbors an N-glycosylation site at asparagine 103, phosphorylation site at serine 136 (2) and two ubiquitination sites at lysines 66 and 82 (23). By contrast, the isoform Q8TBF5-2 is encoded by the mRNA variant ENST00000296333.10 of *PIGX*. This isoform has a size of 276 amino acids and a mass of 30,974 Da, distinguishing it from the canonical sequence at positions 177-195, which contain the sequence QAGSRRMIRFRFDSFDKTI (Fig. 3) (2), and comprises the soluble chain in the lumen of the ER. As for the tertiary structure of PIGX, bioinformatic predictions in AlphaFold DB of the canonical protein (19) show that the soluble luminal amino acid chain in the ER consists of a random coil structure and β-sheets, while the transmembrane region is a single α-helix (Fig. 2B). Co-precipitation has demonstrated that PIGX is associated with PIGM (24). However, whether the transmembrane or the large luminal domain is implicated in stabilizing the PIGM protein remains unknown.

According to the Genotype-Tissue Expression project (25,26), there is mRNA expression of *PIGM* and *PIGX* in all major tissues (26,27), including the nervous system, heart, digestive system, skin and reproductive system in humans (Fig. 4A). Consistently, Proteomics DB reports the expression of both proteins (PIGX Q8TBF5-1) in the brain and the digestive and reproductive systems, but also in the breast, lung, retina, kidney and thyroid gland (Fig. 4B) (28). Notably, the expression of PIGM and PIGM has been detected in the colon, T lymphocytes, prostate and rectum (Fig. 4B) (28). These data suggest that GPI-MT-I is present in numerous types of tissues regardless of their specialized function in humans.

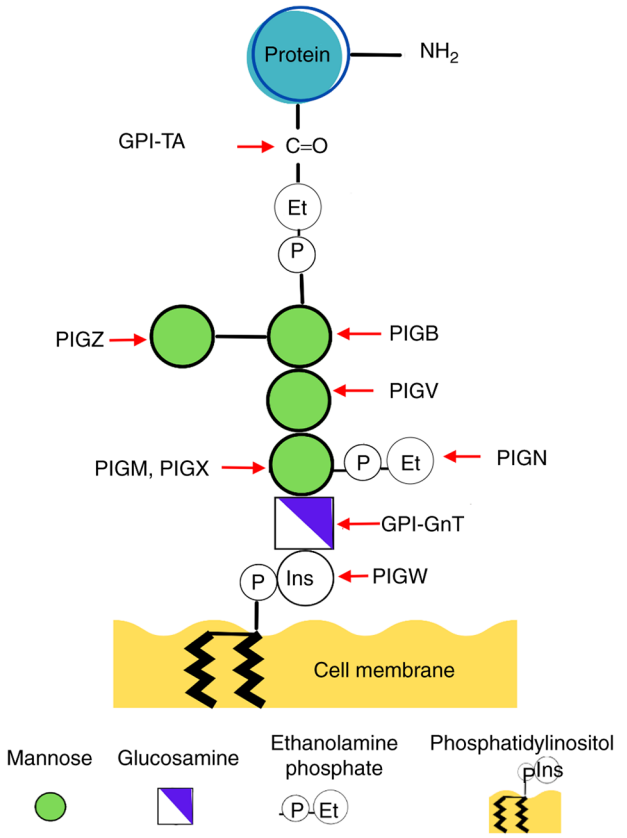

Figure 1. GPI core and genes and enzymes implicated in GPI biosynthesis. The GPI core consists of a phosphatidylinositol, a glucosamine, three mannoses and an ethanolamine phosphate that covalently links with target proteins. GPI-GnT enzyme adds glucosamine to the phosphatidylinositol; PIGM that encodes GPI-MT-I and the regulatory subunit PIGX transfer the first mannose to glucosamine. The GPI-MT-II encoded by *PIGV* transfers the second mannose, while GPI-MT-III encoded by *PIGB* binds the third mannose. The ethanolamine phosphate that binds to the third mannose covalently links to the C-terminal region of the target protein by the action of the enzyme GPI-TA. GPI, glycosylphosphatidylinositol; GnT, N-acetylglucosamine transferase; PIG, phosphatidylinositol-glycan biosynthesis class; MT, mannosyltransferase.

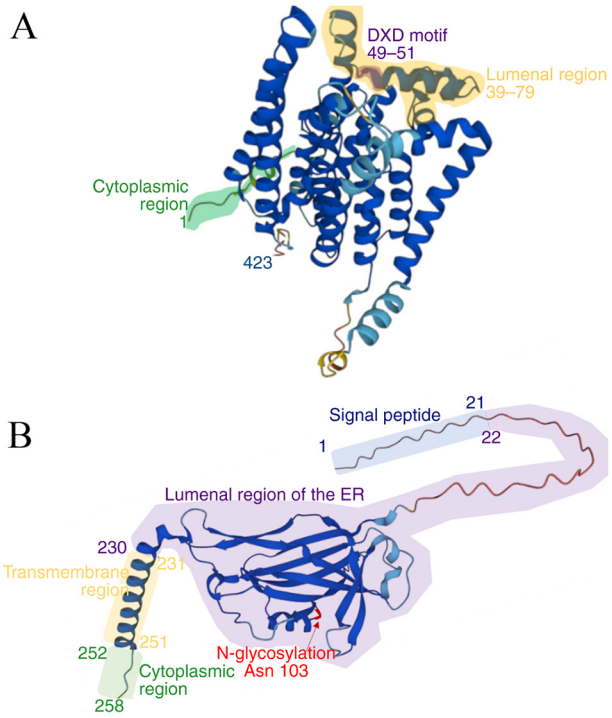

Figure 2. Tertiary structure of PIGM and PIGX. (A) PIGM structure showing the DXD motif and one predicted lumenal region. (B) PIGX structure showing the lumenal and transmembrane regions. Figures obtained and modified from (19). ER, endoplasmic reticulum; PIG, phosphatidylinositol-glycan biosynthesis class.

3. Consequences of *PIGM* and *PIGX* knockout (KO)

Mutant mammalian cells with deficiencies in genes implicated in the GPI-anchor biosynthesis have been previously reported, including those encoding GPI-GnT and GPI-TA (29), and the genes *PIGV*, *PIGB*, *PIGM* and *PIGX* (6,20,29-31). For example, *in vitro* experiments conducted using human lymphoma cells reveal that lack of *PIGM* results in elevated GlcN-acyl-PI levels and impaired surface expression of GPI-APs (Fig. 5) (20). Moreover, a recent study using a KO human cell library targeting GPI biosynthetic genes indicated that suppressing expression of specific *PIG* genes results in the absence of GPI-APs on the cell surface (6). Notably, KO of regulatory subunits of GPI-GnT leads to diminished presence of GPI-APs, while KO of catalytic subunits results in complete absence of these proteins (Fig. 5). Furthermore, elimination of GPI-AP synthesis occurs following KO of genes involved in steps subsequent to GPI-GnT activity. Regarding enzymes catalyzing the transfer of Man1 and 2, GPI-MT-I (*PIGM*) and GPI-MT-II (*PIGV*), complete removal of GPI-AP presence is observed upon *PIGM* KO, whereas residual presence of GPI-APs persists after *PIGX* KO (6). Experiments conducted in CHO cells derived from hamster adult ovaries demonstrated that defective *PIGX* leads to the accumulation of GlcN-acyl-PI, imitating the phenotype observed in *PIGM*-deficient cells (32). Additionally, the aforementioned study revealed diminished expression of protein PIGM in the absence of *PIGX*, while a 10-fold increase in expression was observed when *PIGX* was expressed. For these reasons, it is hypothesized that PIGX has a role in stabilizing PIGM (32).

Similar outcomes are associated with mutations affecting other genes involved in GPI synthesis. *PIGV* KO completely abolishes the surface expression of GPI-APs in human cells (Fig. 5). Conversely, *PIGB* KO, involved in transferring Man3, allows limited expression of GPI-APs, while *PIGZ* KO does not impact GPI-AP biosynthesis (6). In summary, the aforementioned studies demonstrate the essential role of enzymes in the initial stages of GPI synthesis, including GPI-MT-I, for expression of GPI-APs on the cell surface. Dysregulation of their expression may lead to alterations in cell surface characteristics.

4. *PIGM* and *PIGX* in human disease

Paroxysmal nocturnal hemoglobinuria (PNH). PNH represents a rare and chronic hematological disorder resulting from somatic mutations in the X-linked *PIGA* gene within hematopoietic stem cells (33). *PIGA* gene encodes the catalytic subunit of GPI-GnT (2). Consequently, hematopoietic stem cells carrying these mutations give rise to aberrant clone blood cells that lack GPI-APs, specifically CD55 and CD59. Notably,

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sp|Q8TBF5|PIGX_HUMAN        MAARVAAVRAAAWLLLGAATGLTRGPAAAFTAARSDAGIRAMCSEIILRQEVLKDGFHRD        60
sp|Q8TBF5-2|PIGX_HUMAN      MAARVAAVRAAAWLLLGAATGLTRGPAAAFTAARSDAGIRAMCSEIILRQEVLKDGFHRD        60

sp|Q8TBF5|PIGX_HUMAN        LLIKVKFGESIEDLHTCRLLIKQDIPAGLYVDPYELASLRERNITEAVMVSENFDIEAPN        120
sp|Q8TBF5-2|PIGX_HUMAN      LLIKVKFGESIEDLHTCRLLIKQDIPAGLYVDPYELASLRERNITEAVMVSENFDIEAPN        120

sp|Q8TBF5|PIGX_HUMAN        YLSKESEVLIYARRDSQCIDCFQAFLPVHCRYHRPHSEDGEASIVVNNPDLLMFCDQ---        177
sp|Q8TBF5-2|PIGX_HUMAN      YLSKESEVLIYARRDSQCIDCFQAFLPVHCRYHRPHSEDGEASIVVNNPDLLMFCDQAGS        180

sp|Q8TBF5|PIGX_HUMAN        ---------------EFPILKCWAHSEVAAPCALENEDICQWNKMKYKSVYKNVILQVPV        222
sp|Q8TBF5-2|PIGX_HUMAN      RRMIRFRFDSFDKTIEFPILKCWAHSEVAAPCALENEDICQWNKMKYKSVYKNVILQVPV        240

sp|Q8TBF5|PIGX_HUMAN        GLTVHTSLVCSVTLLITILCSTLILVAVFKYGHFSL        258
sp|Q8TBF5-2|PIGX_HUMAN      GLTVHTSLVCSVTLLITILCSTLILVAVFKYGHFSL        276
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Figure 3. Sequence alignment of PIGX isoforms. Amino acid alignment between the isoforms of PIGX Q8TBF5-1 and Q8TBF5-2 shows the differences at positions 177-195. Figure retrieved from (54). PIG, phosphatidylinositol-glycan biosynthesis class.

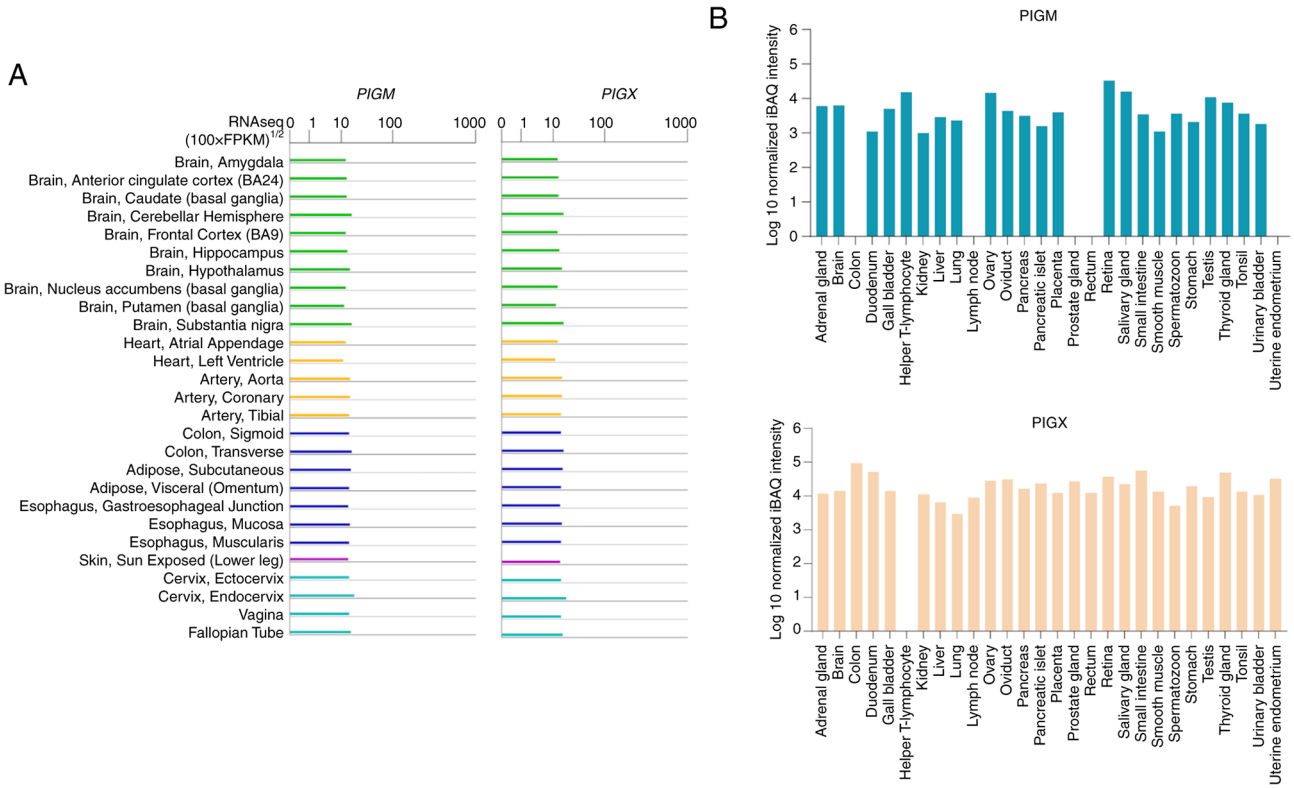

Figure 4. Expression of gene and protein of *PIGM* and *PIGX* in human tissues. (A) mRNA expression of *PIGM* and *PIGX* and (B) expression of their encoded proteins. Figure obtained from (27) and adapted from (28). PIG, phosphatidylinositol-glycan biosynthesis class.

these proteins serve crucial roles as regulatory components in the complement system (33). PNH manifests as a hematological condition marked by intravascular hemolysis, thrombosis and bone marrow failure, often resulting in cytopenia. The chronic hemolysis observed in patients with PNH is linked to the absence or deficiency of GPI-APs (33). This deficiency disrupts activation of the complement system, leading to the lysis of immune and red blood cells (33). Small PNH clones with GPI-AP deficiencies are detected in the bone marrow of patients displaying PNH-associated symptoms or in healthy individuals (34). An ultra-deep sequencing analysis of PNH small clones revealed that a patient with classic PNH harbored a *PIGM* gene deletion at 459-462, suggesting a protein change in valine 154, a transmembrane region (2). Despite the absence of reported *PIGX* mutations in patients with PNH, there are allele variants in small PNH clones without clinical relevance (34).

PIGM-associated GPI deficiency. Certain inherited GPI deficiencies are due to mutations in *PIGM*. A study of inherited GPI deficiency in two unrelated consanguineous families

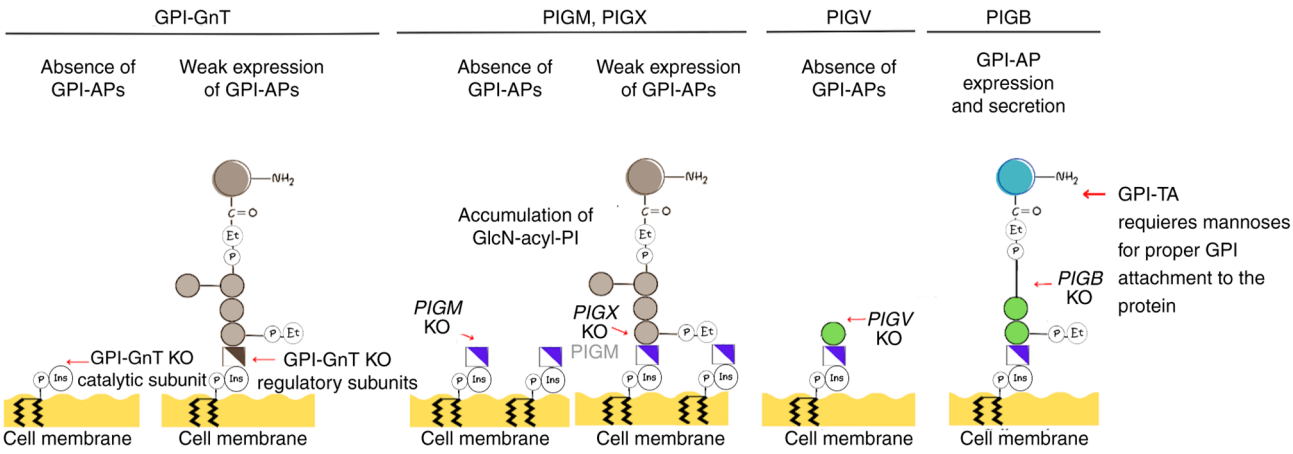

Figure 5. GPI-anchored protein expression following KO of encoding enzymes in the early stages of GPI biosynthesis. KO of the catalytic subunits of GPI-GnT produces the absence of GPI-APs on the cell surface, while knocking out the regulatory subunits leads to weak expression of the protein. Knocking out *PIGM* produces the absence of GPI-APs, whereas knocking out *PIGX* leads to low expression of PIGM protein and weak GPI-AP expression. In both cases, cells display an accumulation of glucosamine-acyl-phosphatidylinositol. KO of *PIGV* leads to the absence of GPI-APs, while the KO of *PIGB* does not affect the expression of GPI-APs in the cell surface or their secretion, suggesting the GPI-TA enzyme requires three mannoses to attach the GPI core to target proteins. GPI, glycosylphosphatidylinositol; KO, knock out; GnT, N-acetylglucosamine transferase; AP, anchored protein; PIG, phosphatidylinositol-glycan biosynthesis class; TA, transamidase enzyme complex; Et, ethanolamine; Ins, inositol; P, phosphate.

characterized by venous thrombosis and seizures indicated that a hypomorphic promoter mutation in *PIGM* causes GPI deficiency (24). Homozygosity mapping demonstrated a point mutation, 270 C→G, at the promoter of *PIGM* was associated with decreased levels of *PIGM* mRNA. Further experiments demonstrated that the point mutation disrupted binding of Sp1, an ubiquitous transcription factor, to a GC box, which is located proximal to the transcription initiation site; the point mutation led to decrease in the activity of the *PIGM* promoter. Decreased transcription of *PIGM* led to a blockage of GPI mannosylation from partial to severe deficiency of GPI (24). This mutation has also been described in GPI-inositol deficiency characterized by cerebrovascular thrombotic events (35). Molecular analysis indicated that patients were homozygous for the point mutation 270 C→G mutation and that cells displayed low mRNA expression levels compared with controls (35).

Further investigation into the mechanism underlying *PIGM* deficiency is required to elucidate the mechanism behind PNH and *PIGM* deficiency predisposing to thrombosis, a characteristic that is not observed in other GPI deficiencies (35). Additionally, patients with *PIGM*-associated GPI deficiencies do not display intravascular hemolysis (35,36). These phenotypes may be attributed to variations in *PIGM* mRNA levels and GPI expression. Specifically, the differential expression of *PIGM* occurs in patient-derived B cells compared with erythrocytes, and it is linked to distinct promoter chromatin accessibility and binding of Sp1 (36).

Cancer. According to gene expression analysis in patients with cancer, *PIGM* is upregulated in lung and other types of cancer, including glioma, skin, liver and thyroid cancer (37). In lung cancer, *PIGM*, in combination with other genes, is associated with patient survival outcomes: Patients with lung cancer and low expression of *PIGM* mRNA exhibit higher overall survival than those with high *PIGM* mRNA expression (38). Analysis in myeloma showed that high expression of *PIGM* is associated with adverse survival outcomes (37). Expression of *PIGM* is notably higher in myeloma samples compared with that in normal cells. Furthermore, there is a marked increase in *PIGM* expression in myeloma cell samples exhibiting cytogenetic aberrations such as 1q21-gain and 13q14-deletion, and a corresponding decrease in hyperdiploid myeloma cell samples. Experimental data in myeloma cells highlight the direct influence of varying *PIGM* expression on the presence of the GPI-APs CD55 and CD59 on the cell surface (37). Analysis of tumor samples revealed gene alterations in *PIGM* across either all or most metastatic sites in ependymoma, with a notable absence of these alterations at the primary site (39). The aforementioned studies demonstrate a clear association between malignancy and increased *PIGM* expression or genetic modifications in *PIGM* gene.

Certain types of cervical cancer display low expression of *PIGX* (16), and mutations in 17 genes, including *PIGX*, have been identified in young, non-smoker patients with lung cancer, displaying potentially pathogenic effects (40). In breast cancer, high levels of *PIGX* mRNA are associated with decreased survival in disease-free patients compared with those with low levels (14). *In vitro* experiments indicate that *PIGX* expression promotes the proliferation of breast cancer cells. However, *PIGM* does not affect cell proliferation, suggesting that *PIGX* promotes cancer cell proliferation independently of *PIGM* (14). Further analysis showed that protein PIGX may form a protein complex with Reticulocalbin-1 (RCN1) and RCN2 in the ER, which regulate calcium-dependent activity. Moreover, *in vitro* experiments reveal that the silencing of *PIGX*, *RCN1* or *RCN2* results in reduced expression of the genes Zic family member 1 (*ZIC1*) and EH domain containing 2 (*EHD2*), two putative tumor suppressor genes (14). *PIGX* might contribute to the promotion of cancer cell proliferation by suppressing *EHD2* and *ZIC1*. Notably, PIGX, in addition to its association with PIGM, may engage with other proteins in the ER and exhibit an autonomous functional role (14). It is currently unclear how *PIGX* affects the gene expression of *EHD2* and *ZIC1*.

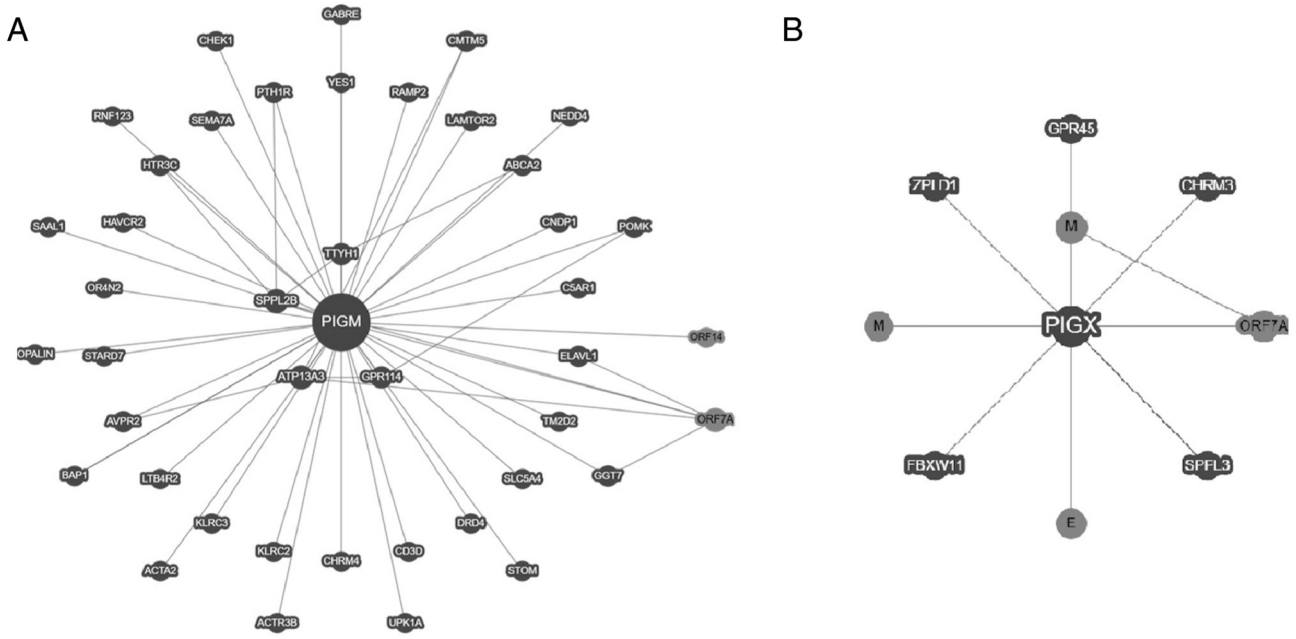

Figure 6. Interaction network between proteins with PIGM and PIGX. (A) Network of physical interaction between PIGM and 39 proteins, including viral proteins of severe acute respiratory syndrome coronavirus 2 (light gray). (B) Network of physical interaction between PIGX and six proteins. Figure obtained from (23). PIG, phosphatidylinositol-glycan biosynthesis class.

5. Key roles of *PIGM*, *PIGX* and *PIGV* in GPI-AP secretion

The role of GPI-MT-I and II in GPI synthesis and secretion has been studied in hyperphosphatasia with mental retardation syndrome (HPMRS) (41-44). This autosomal recessive syndrome is characterized by intellectual disability and elevated serum and remodeling are associated with HPMRS, with the *PIGV* gene being well-studied (41-44). *PIGV* mutations include c.53G→A, c.176T→G, c.467G→A, c.905T→C, c.1022C→A and c.1405C→T and in some instances, mutations can be biallelic (42,43). Experiments using *PIGV* with transmembrane region mutations suggest that these mutations may destabilize the protein (41).

Cells deficient in *PIGV* and *PIGB* secrete GPI-APs into the medium, accompanied by the accumulation of incomplete GPI-bearing Man (41). This phenotype is explained by the fact that secretion of alkaline phosphatase (ALP) requires GPI-TA, which, in normal cells, cleaves the C-terminal GPI attachment signal peptide and replaces it with GPI. In *PIGX*-deficient cells, where incomplete shorter GPIs lacking Man accumulate, ALP is degraded. This suggests that at least one Man residue is required for GPI-TA to cleave the GPI attachment signal. Consequently, it is hypothesized that GPI-TA recognizes incomplete GPI-bearing mannose, cleaving a hydrophobic signal peptide, resulting in secretion of soluble ALP (Fig. 5) (41). Although HPMRS is not observed in patients with a *PIGM* mutation (41), the aforementioned study suggested a key step during GPI synthesis involving the enzymes GPI-MT-I (*PIGM*), PIGX and GPI-MT-II (*PIGV*) for proper recognition of GPI-APs by GPI-TA and subsequent secretion.

Studies indicated a crucial role of GPI-TA in regulating glycolipid biosynthesis, since GPI biosynthesis is suppressed by the ER-associated degradation pathway when cells are defective in transferring the complete GPI core to proteins to prevent GPI accumulation (9,45). Biosynthesis of GPI is upregulated in ER-associated degradation-deficient cells (46). Whether the ER-associated degradation pathway is triggered in *PIGM*-deficient cells or in cells with decreased expression of *PIGM* or *PIGX* that accumulate GlcN-acyl-PI remains unclear.

6. Interaction between PIGM and PIGX and other proteins

Several studies have investigated the human interactome and its implication in human diseases (47-49). BioGRID, a public database of genetic and protein interactions (23), indicated that PIGM and PIGX proteins interact physically with other proteins (Fig. 6). Affinity capture-mass spectrometry and two-hybrid assays indicate that PIGM can bind to 39 proteins (Fig. 6A) and PIGX can bind to six (Fig. 6B). Notably, each subunit binds to different proteins. Proteins interacting with PIGM include glycoproteins, G-protein-coupled receptors in humans and the proteins ORF7, ORF14, E and M of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Fig. 6A) (23). Whether these interactions reveal a specific biological context of ER or a cross-regulation of the proteins remains unknown; G-protein-coupled receptors, glycoproteins and GPI-APs are commonly recruited in lipid rafts (50-52) and recent evidence indicates that GPI-biosynthesis is relevant for the life cycle of SARS-CoV-2 (53).

7. Conclusion

In summary, expression of *PIGM* and *PIGX* is reported in most types of cell, suggesting that GPI-APs may be present in most cells. The present review indicated that *PIGM* and *PIGX* are key genes for GPI synthesis since their absence may lead to accumulation of GPIs that lack Man in the cell and

deficiency of GPI-APs in the cell membrane or their secretion. Absence or altered expression of *PIGM* gene is associated with PNH and inherited GPI deficiency, which are characterized by thrombosis; whether this phenotype is due to altered expression of specific GPI-APs remains unclear. Altered expression of *PIGM* and *PIGX* has been reported in cancer (14,16,37,39); to the best of our knowledge, however, whether these changes may lead to altered expression of GPI-APs that drive malignant phenotype has not been explored. Transcription factor Sp1 may exert a role in the transcription of *PIGM*; to the best of our knowledge, there are no studies regarding the transcription of *PIGX*. Regulation of GPI-MT-I enzyme activity depends on the expression of *PIGX* and, to the best of our knowledge, no other mechanism has been described. However, since PIGM and PIGX may interact with other proteins independently, additional regulation may be involved. Further exploration may enable the development of targeted therapies for cancer.

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Availability of data and materials

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Authors' contributions

ATV performed the literature review and wrote the manuscript. VVR wrote and reviewed the manuscript. LMF and PMM performed the literature review, supervised the study and wrote and reviewed the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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