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Inherited glycosylphosphatidyl inositol deficiency: a treatable CDG

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Summary

Tethering to cell membrane through attachment to the complex glycolipid anchor glycosylphosphatidyl inositol (GPI) is a mode of protein expression highly conserved in eukaryotes. The evolutionary purpose of such an elaborate way of expressing proteins is not clear and neither is the functional role of GPI itself. GPI-anchored proteins (GPI-AP) serve a variety of functions that include adhesion, receptors, signal transduction and complement activation. GPI biosynthesis, a process that is accomplished in at least 9 steps and involves several proteins, some with enzymatic activity, would be expected to be a fertile ground for development of inherited, autosomal recessive disorders. However, until recently, paroxysmal nocturnal haemoglobinuria, a rare haematological disorder caused by somatic mutations in the X-linked *PIGA* gene, was the only genetic disorder affecting GPI biosynthesis. Here we review the clinical spectrum, biochemical defect and genetic pathogenesis of inherited GPI deficiency, the first described form of inherited, autosomal recessive disorder of GPI biosynthesis and outline the molecular basis of targeted therapy for this condition.

1. Introduction

Glycan modification of secretory and surface proteins includes N-linked glycosylation and O- and C-mannosylation[1-4]; attachment to the glycosylphosphatidyl inositol (GPI) anchor and expression on the cell surface as GPI-anchored proteins (GPI-AP) constitutes an additional way of protein glycan modification[5-8]. In this respect, inherited disorders of the formation and function of GPI would constitute a form of congenital disorder of glycosylation (CDG) [9]. In this review, before elaborating on the clinical manifestations, biochemical defects and molecular genetics and pathogenesis of the GPI anchor deficiencies, a brief overview of its structure, biosynthesis and function is given.

2. Structure, biosynthesis and modifications of the GPI anchor

The core backbone structure, EtNP-6Man α 1-2Man α 1-6Man α 1-4GlcN α 1-6*myo*Inositol-phospholipid (EtNP:ethanolamine phosphate; Man: mannose; and GlcN: glucosamine), is conserved in all eukaryotes, from parasites and fungi to mammals[5-8]. However, several species- and tissue-specific differences in the modification of this core structure exist. Here we will discuss the structure, modifications and the protein products required for the biosynthesis of the mammalian GPI which takes place in at least 9 steps.

2.1 Biosynthesis of mature GPI

GPI biosynthesis (recently reviewed in [6,7]) starts on the cytoplasmic side of endoplasmic reticulum (ER) and involves the transfer of GlcNAc from UDP-GlcNAc to PI (*Step 1*). This reaction is catalysed by a multi-subunit GPI-GlcNAc transferase (GPI-GnT) comprising 6 proteins (PIG-A, PIG-C, PIG-H, PIG-P, PIG-Q, PIG-Y) all of which appear essential for this step [6,7]. PIG-A provides the catalytic function of GPI-GnT but the functions of the other five subunits are not known. DPM2, a subunit of the Dol-P-Man synthase required for mannosylation, also appears to cooperate with and enhance the activity of GPI-GnT (see Figure 1 for overview of the GPI biosynthetic pathway).

In *step 2*, GlcNAc-PI is de-*N*-acetylated by the action of PIG-L, a GlcNAc-de-*N*-acetylase generating thus GlcN-PI which in turn is flipped across to face the luminal side of ER (*step 3*) where the synthesis of GPI continues [6,7]. The responsible flippase remains uncharacterised.

In *step 4*, by the action of the acyltransferase PIG-W, an acyl chain is transferred from acyl-CoA to the inositol ring of PI to form GlcN-(acyl)PI.

In the next 2 steps, Man residues are added sequentially to GlcN-(acyl)PI generating Man-Man-GlcN-(acyl)PI. The PIG-M/PIG-X complex is the GPI-MTI α 1-4 mannosyltransferase required for the addition of Man-1 (*step 5*) [6,7]. PIG-M is the catalytic subunit of GPI-MTI while PIG-X, although devoid of catalytic activity, through its stabilizing effect on PIG-M is essential for mannosylation. PIG-V is the GPI-MTII α 1-6 mannosyltransferase responsible for *step 6*, i.e., addition of Man-2 [6,7].

In *step 7*, PIG-N, a GPI-ethanolamine phosphate transferase I (GPI-ETI), mediates EtNP modification of Man-1 generating Man-(EtNP)Man-GlcN-(acyl)PI[6,7].

Subsequently, in *step 8*, through the action of PIG-B, an α 1-2 mannosyltransferase (GPI-MTIII), Man-3 is added to form Man-Man-(EtNP)Man-GlcN-(acyl)PI[6,7].

In all 3 mannosylation steps, Dol-P-Man is the mannose donor[6,7]. Synthesis of Dol-P-Man requires Dol-P-Man synthase, a 3 subunit complex consisting of DPM1, DPM2 and DPM3[10]. DPM1 is the catalytic subunit while DPM2 & 3 serve to stabilise DPM1.

In the final *step 9*, through the action of GPI-ETIII, a complex of PIG-O and PIG-F, the terminal EtNP which is crucial for protein attachment to GPI is added to Man-3 generating thus the mature GPI structure EtNP-Man-Man-(EtNP)Man-GlcN-(acyl)PI. Both PIG-F and PIG-O are essential for the function of GPI-ETIII, with PIG-F required for the stability of the complex and PIG-O providing the catalytic activity[6,7].

In mammalian GPI another two modifications of the core EtNP-Man-Man-(EtNP)Man-GlcN-(acyl)PI structure are possible. First, EtNP modification of Man-2 (*step 10*) by the action of PIG-G/PIG-F GPI-ETII complex is often observed[6,7]. As for GPI-ETIII, PIG-G (GPI7) is the catalytic subunit stabilized by PIG-F. A second possible modification taking place after *step 8* and found mainly in the brain, is the addition of a 4th mannose mediated by PIG-Z (SMP3), an α 1-2 mannosyltransferase. The resulting intermediate containing four mannoses, is then EtNP-modified at Man-3 (*step 9*) generating thus another form of mature GPI anchor [6,7].

2.2. Post-translational attachment of proteins to GPI

Attachment of a given protein to any of the 3 mature forms of GPI described above has 2 structural requirements: the presence of an N-terminus ER-targeting motif and of a C-terminus domain which includes all the information required for attachment to GPI. The latter involves the creation of an amidic bond between the so called ω amino acid in the protein C-terminus and the EtNP attached to Man-3[6,7]. This reaction (*step 11*) is mediated by the multi-subunit, membrane-bound GPI transamidase comprising 5 proteins, all essential for the function of the transamidase: PIG-K which provides the catalytic activity, GAA1, PIG-S, PIG-T and PIG-U[6,7].

2.3. Post-protein attachment modification of the GPI anchor

While still in the ER and after attachment of the protein to the GPI, the acyl chain attached to the inositol moiety is removed by the action of PGAP1, a deacylase[11]. Subsequent transport of GPI-AP to Golgi via secretory vesicles requires a group of p24 family proteins[12,13]. While in the Golgi, fatty acid (FA) remodelling of PI takes place. Specifically, the usually unsaturated FA at position sn2 of PI in GPI precursors is converted, through the action of PGAP3, to lyso-GPI-AP intermediate; subsequently, a saturated FA (usually stearic acid) is added in a reaction requiring PGAP2[14]. The presence of saturated FA in both sn1 and sn2 positions of PI are thought to be required for stable association of the GPI anchor with the outer leaflet of the cell membrane and their incorporation into lipid rafts (see below)[14]. It must be noted that, exceptionally, in human erythrocyte GPI-AP such as CD59 neither the inositol ring acyl

chain is removed nor the unsaturated FA is replaced by saturated ones at position sn2[6,15]. This ensures the presence of 3 FA chains stabilising GPI-AP on the surface of erythrocytes during their long life.

3. Biological significance of the GPI anchor

Although GPI-AP serve a variety of functions (receptors, adhesion, enzymes, complement regulation, signal transduction), the evolutionary purpose of using a mechanism as elaborate as GPI linkage for cell surface protein expression is not clear but its importance is underscored by the fact that complete disruption of GPI biosynthesis is embryonic lethal in mice [16,17] and conditional lethal in yeast [18,19].

GPI along with glycosphingolipids and cholesterol are enriched in the specialised membrane micro-domains called rafts, the relatively rigid platforms on which through interaction of surface and cytoplasmic proteins several signal transduction pathways are triggered and vesicular trafficking is organised[20-22]. Recent evidence also suggests that GPI-linkage is required for many proteins in order to assume their fully functional conformation[23]. GPI-anchorage might also be important for apical targeting of proteins in polarised cells such as those of the intestinal epithelium[24]. Another potentially important function of GPI might be related to its susceptibility to phospholipase C and D (PI-PLC and -PLD) cleavage and release of the anchored protein. For example, Notum, a Wnt pathway inhibitor possessing PI-PLC activity, can cleave and release the GPI-anchored glypicans which in their soluble form may function as inhibitors of Wnt interaction with its receptor[25]. Finally, aerolysin, a

pore-forming toxin from *Aeromonas hydrophilia*, requires binding to surface GPI for its cell-killing activity[26].

4. Genetic disorders of GPI deficiency

The observation that complete disruption of GPI biosynthesis in *pig-a*^{-/-} mice results in embryonic lethality[16,17] whereas in vitro, cells lacking expression of GPI as a result of various biosynthetic blocks are not growth-restricted[27], would predict that GPI deficiency as a genetic nosological entity could emerge either through acquired i.e., somatic mutations in certain tissues or cells (as in paroxysmal nocturnal haemoglobinuria - PNH) or through inherited mutations that allow reduced but sufficient for survival synthesis of GPI (as in inherited GPI deficiency - IGD).

5. Acquired GPI deficiency: PNH

Given the complexity and the large number of genes and their protein products required for the biosynthesis of GPI, it was rather surprising that until recently, the only human disorder associated with GPI deficiency was PNH, a rare haematological disorder with dual pathogenesis[28]. The first pathogenetic requirement in PNH is a somatic mutation affecting the X-linked *PIGA* gene in a single haemopoietic stem cell (HSC) and its progeny, disrupting thus the first step in the GPI biosynthesis[29-31]. A second process, believed to be a T-cell mediated autoimmune attack against normal but not GPI-deficient HSC, allows survival and expansion of the latter resulting in the production of mature blood cells lacking

synthesis of GPI and expression of GPI-AP[28,32]. The clinical consequences are intravascular haemolysis as a result of erythrocytes lacking expression of the GPI-linked complement activation inhibitor CD59, propensity to splanchnic and cerebral vein thromboses also possibly linked to CD59 deficiency in erythrocytes and platelets, and finally bone marrow failure and cytopenia as a result of the GPI+ HSC being the targets of auto-aggressive T cells[33,34].

6. Inherited GPI deficiency

6.1. Clinical spectrum

Over a decade ago we studied two unrelated consanguineous families (Family 1 of Middle Eastern and Family 2 of Turkish origin) in which the index cases presented with abdominal vein thrombosis by the age of two years[35]. Both children subsequently developed persistent absence seizures. A younger sibling in Family 2 also developed absence seizures but thrombosis was prevented because prophylactic anticoagulation was instituted following pre-symptomatic diagnosis. Notably, none of the affected children exhibited clinical evidence of significant intravascular haemolysis or bone marrow failure and cytopenia, and extensive imaging revealed no evidence of cerebral venous thrombosis or other vascular abnormalities. The parents and unaffected siblings were asymptomatic. With the exception of an intermittently low-positive Ham test suggesting mild deficiency of the GPI-linked complement inhibitor CD59[36], acquired and inherited causes of thrombophilia were not found.

6.2. GPI surface expression in IGD

Staining with FLAER, an inactivated form of aerolysin and GPI-AP-specific mAb showed that the expression pattern of GPI-linked proteins and GPI itself on blood cells in IGD is distinct from that of patients with PNH[35,37] (Figure 2). The erythrocytes of the children with IGD had near normal expression of CD59 with a small proportion of cells (<5%) being completely deficient. A similar pattern was seen in platelets. This is different to the bi- or tri-modal pattern of CD59 expression in PNH representing populations with normal, complete or intermediate loss of GPI biosynthesis respectively. By contrast, expression of GPI on the surface of granulocytes in IGD was severely reduced in an unimodal fashion whilst it is usually bi-modal in PNH.

Highlighting the cell- and tissue-dependent variability of its severity, GPI deficiency in primary fibroblasts from patients with IGD was moderate rather complete.

6.3. Addition of Man-1 to the GlcN-(acyl)PI precursor is blocked in IGD

By the use of substrate labeling of patient-derived EBV-transformed B cell lines, glycolipid extraction and high performance thin layer chromatography the biochemical defect in IGD was pinpointed in the addition of Man-1 to GlcN-(acyl)PI. As mentioned above, this step is catalysed by the GPI-MTI comprising PIG-M, a multiple membrane domain protein containing a luminal DXD motif characteristic of many glycosyltransferases[38] and PIG-X.

6.4. *PIG-M* is the disease gene in IGD

In parallel genetic studies that included homozygosity mapping using microsatellite markers flanking almost all genes involved in GPI biosynthesis, *PIGM* was identified as one of the candidate genes in the affected children of both families. *PIGM* was confirmed as the disease gene in IGD as its transfection into GPI- B cell lines from affected children restored biosynthesis of GPI and expression of GPI-AP on the cell surface.

6.5. IGD is caused by a hypomorphic promoter mutation

Screening for mutations in the single-exon *PIGM* revealed that surprisingly the same C>G substitution at position -270 from ATG in the promoter of *PIG-M* was present in both families and segregated with the phenotype[35]. The same mutation was found in blood cells and fibroblasts in patients with IGD but it was not present in any of control individuals from the patients' ethnic background.

Conforming to a typical recessive trait this promoter mutation resulted in a drastically reduced *PIGM* mRNA in affected B-cell lines to approximately 1% of normal and about half of normal in heterozygous cell lines.

Bioinformatics analysis predicted that the -270C>G transversion disrupted a GC-rich binding motif for the generic transcription factor (TF) Sp1. It is notable that this GC-rich box is the most highly conserved amongst all other predicted TF binding motifs in the promoter of *PIGM* attesting thus to its functional importance. By a combination of transactivation and electro-mobility shift assays these predictions were confirmed and it was

found that the C>G transversion severely reduced but not completely abrogated the transcriptional activity of the *PIGM* promoter. This along the fact that in some cells and tissues in patients with IGD expression of GPI was not completely absent means that the -270C>G transversion is a hypomorphic mutation whose impact on transcription varies from tissue to tissue. In addition it was determined that full activity of the *PIGM* promoter requires a region of 2kb upstream of ATG[35].

6.6. The transcription factor *Sp1*

Sp1 is a ubiquitously expressed transcription factor (TF) that belongs to a wider family of *Sp* proteins, structurally characterised by the presence of 3 carboxyterminal, DNA binding zinc finger (ZnF) motifs [39-42].

A large number of in vitro studies have shown that *Sp1* can be critical for efficient transcription of many house-keeping and inducible genes. *Sp1* has been shown to enhance transcription through a variety of mechanisms that include direct interactions with elements of the TFIID complex of the basic transcriptional complex[41,43], interactions with other TF such as GATA-1[44-46] , and recruitment of histone modifying enzymes such as histone acetyltransferases [47] and deacetylases [48] (HAT and HDAC respectively). In addition, the function of *Sp1* depends on its ability to form tetramer homopolymers arranged in stacks and can be altered by its covalent modification, e.g., phosphorylation and acetylation (reviewed in [40-42]).

All these interactions require binding of *Sp1* through its ZnF motifs to a GC-rich DNA element (motif) found in the proximal promoters of all genes; GC-rich boxes are also found in distal promoters, enhancers and

locus control regions[40-42]. Sp3, another Sp TF binds to the same motif with almost equal affinity[40-42].

6.7. *Sp1 and gene-specific histone hypoacetylation in IGD*

One feature of the function of Sp1 that has attracted considerable interest is its ability to control transcriptional activity through recruitment to the promoter of histone modifying enzymes, especially HAT and HDAC: in most cases the HAT activity prevails and favours transcription.

Another interesting aspect of Sp1 and its binding element is their role in mediating the effects of HDAC inhibition by butyrate and other HDAC inhibitors. Although HDAC inhibition leads to widespread increase in histone acetylation it only impacts on the transcription rates of a minority (<5-10%) of genes [49-51]; and for a few amongst these genes, enhanced transcription as a result of HDAC inhibition and histone hyperacetylation, requires binding of Sp1 to its proximal promoter elements (i.e., GC-rich box) [52-59]. However, it is not known whether these butyrate responsive elements (BRE) are also critical for the maintenance of histone acetylation during baseline physiological transcription.

Using chromatin immunoprecipitation assays it was shown that in the presence of the homozygous C>G mutation, histone 4 (H4) at the native promoter of *PIGM* in patient B cell lines was hypoacetylated but as it would be expected of a housekeeping gene, acetylated in the parental B cell lines[37] implying that the Sp1 binding motif mutated in IGD is important for maintenance of histone acetylation at the promoter of *PIGM* and thus its transcriptional activity.

However, despite disruption of the critical proximal promoter GC-rich motif, butyrate could still enhance activity of the mutated promoter (although less so in comparison to the WT promoter) suggesting the presence of additional BRE in the promoter of *PIGM*. These, although not adequate to sustain adequate baseline transcription, these BRE could be required for the increased activity of the mutated promoter after HDAC inhibition[37]. Software analysis indicated the presence of another 3 GC-rich boxes upstream of the mutated motif (Fig 1), suggesting that Sp1 binding is required for the butyrate effect on the mutated promoter. This was confirmed in reporter experiments in which when binding of Sp1 to DNA was disrupted by mithramycin, the butyrate-mediated enhancing effect was dramatically reduced for the mutated as well as the WT promoter[37]. Furthermore, exposure of the patients B cell lines to butyrate resulted in restoration of H4 acetylation in the native mutated *PIGM* promoter, a 20-fold increase of the *PIGM* mRNA levels and complete restoration of GPI biosynthesis as shown by normal expression levels of GPI-linked proteins on the cell surface[37].

6.8. HDAC inhibition as a treatment for IGD

In view of these results we treated a child with IGD who suffered chronic intractable seizures and severe disability with butyrate. This resulted in the progressive increase of blood cell *PIGM* RNA levels from ~1% to 60% and also a progressive increase in the expression of GPI-linked molecules on the surface of granulocytes; more importantly, 2 weeks after commencement of butyrate, the child became seizure-free for the first time in 12 years [37].

Therefore, the *PIGM* mutation causing IGD results in significant perturbation of the epigenetic landscape in the promoter of *PIGM* and eventually in a clinical phenotype directly-dependent on the biosynthesis of GPI and cell surface expression of GPI-AP.

7. A tentative model of transcriptional control of *PIGM* by Sp1 in health and disease

Considering these findings we propose a model of transcriptional control of *PIGM* by Sp1 (Figure 3) whereby during baseline transcription of *PIGM*, binding of Sp1 to the proximal promoter GC-rich box is associated with co-recruitment of HAT and HDAC. Presumably in this context, HAT prevails over HDAC activity ensuring histone acetylation and normal transcriptional output. Similar arrangements might occur in the upstream GC-rich boxes but their role may not be significant in baseline transcription. In IGD, disruption of the proximal GC-rich box by the C>G mutation significantly reduces transcriptional output in a cell- and tissue-dependent manner while Sp1 binding to upstream GC-rich boxes is preserved. In the mutated promoter, during butyrate treatment, selective HDAC inhibition leaves HAT activity unopposed resulting in significant restoration of histone acetylation and thus of transcriptional activity and GPI biosynthesis.

8. IGD and other disorders of glycosylation

The topology of the assembly of GPI (i.e., first on the cytoplasmic and then luminal side of the ER) has many similarities with that of the N-glycan assembly; the two pathways also share 3 sugar donors, namely GlcNac-UDP, Man-GDP and Dol-P-Man and possibly the same flippase[10].

Therefore, it would be expected that defects in the synthesis of these 4 molecules would impact on both protein glycosylation and GPI modification. Thus far, only defects of Dol-P-Man, the mannose donor required for N-glycan assembly, O- and C-mannosylation of proteins as well as of GPI have been reported. Specifically, in CDG1e, in which synthesis of Dol-P-Man is impaired as a result of mutations in *DPM1*, reduced expression of GPI-AP in patient fibroblasts has been documented[60,61]. However, because the small amount of Dol-P-Man synthesized in CDG1e is preferentially used in the mannosylation of GPI[62,63] the observed deficiency is mild. Instead, the shunting of Dol-P-Man away from the glycosylation pathways leaves protein glycosylation severely disrupted. This leads to the serious clinical phenotype of CDG1e (intractable seizures, encephalopathy, microcephaly, dysmorphism but no thrombosis)[60,61] which closely resembles that of other CDG disorders[9,64,65] rather than IGD. In addition, because PIGM catalyzes the α 1-4 mannosylation of GlcN-(acyl)PI, a linkage not encountered in other mannosylated lipids or proteins[9,65], selective GPI deficiency is sufficient to account for the phenotype in IGD.

9. IGD: questions and perspectives

9.1. A common ancestral mutation?

From the genetic viewpoint an interesting question is whether the disruption of the same biosynthetic step involving the same gene and identification of exactly of the same mutation in ethnically different families with IGD has occurred by chance or it reflects a mutation in a common, distant ancestor. Arguing against the latter, microsatellite- and

SNP-based haplotypes encompassing PIG-M were clearly disparate[35], suggesting that the mutation arose in the two families independently and by chance.

9.2. Mechanism of cell- and tissue-dependent GPI expression variability

A second question pertains to the mechanism responsible for the cell- and tissue-dependent variability in GPI synthesis in IGD as exemplified by the nearly normal expression of GPI in erythrocytes and its severe deficiency in granulocytes. One possibility is that transcription of *PIGM* might be regulated by erythroid-specific TF which can maintain adequate transcriptional activity even in the presence of the mutated core promoter Sp1 binding motif. Preliminary work suggests that GATA-1, an erythroid/megakaryocyte-specific TF[66] might play a critical role in this process (Almeida *et al*, unpublished data).

9.3. Mechanism of thrombosis and epilepsy

At the moment we can only speculate on the pathogenesis of seizures and thrombosis in IGD and the role of specific GPI-AP in these processes. The generation of a *pigm* c>g knock-in mouse model will help greatly investigations towards this direction.

9.4. IGD caused by mutations in other genes of the GPI biosynthetic pathways?

The paradigm of PIG-M-related IGD suggests that probably every gene essential for GPI assembly suffering hypomorphic (e.g., promoter or missense) mutations could lead to inherited deficiency of GPI. However,

this would be expected to be a very rare event occurring in the context of consanguinity.

9.5. HDAC inhibition as a means to enhance transcription and activity of house-keeping genes associated with human pathology

Because *PIGM* is a housekeeping gene and part of a ubiquitous biosynthetic pathway it might be expected that other genes required for GPI biosynthesis may be subject to the same type of Sp1-dependent transcriptional control as *PIGM*. Extending this notion further, it could be surmised that Sp1 and its control of histone acetylation are also important for the transcription of genes that are part of other house-keeping, enzyme-based pathways. If so, characterisation of Sp1-binding motifs/BRE required for transcriptional control of genes with these characteristics could offer new therapeutic opportunities for inherited disorders of other ubiquitous metabolic pathways such as glycolysis and in fact disorders of glycosylation. Often in these autosomal recessive disorders, the molecular pathology comprises missense mutations which allow for residual enzymatic activity. Increased transcription and protein production induced by HDAC inhibitors might translate to increased enzymatic activity and thus amelioration of the cellular and clinical phenotype.

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Figure legends**Figure 1. The biosynthetic pathway of GPI**

GPI biosynthesis starts on the cytoplasmic face of ER with the fatty acid chains of phosphatidylinositol embedded in the ER lipid bilayer. Step 1 is blocked in PNH as a result of mutations in *PIGA*. Through the action of a flippase biosynthesis continues in the luminal side of ER. Step 5, i.e., addition of Man-1 is blocked in IGD due to mutations in *PIGM*. Dol-P-Man, addition of Man-4, and lipid modification of GPI are not shown.

Figure 2. Expression of GPI in IGD and PNH.

A. In patients with IGD, expression of the GPI-AP CD59 is near normal contrasting with the bi- or tri-modal pattern in PNH. Expression of CD59 on erythrocytes from the parents of patients with IGD is normal. (FLAER is not suitable for erythrocyte staining). **B.** Expression of GPI and GPI-AP (CD59 and CD24) in granulocytes. Note the unimodally severe deficiency of GPI expression in patients with IGD and the typically bimodal pattern in patients with PNH. The expression pattern in parents of patients with IGD is identical to normal. **C.** Dot blot showing GPI and GPI-AP co-expression on fibroblasts from a normal donor and a patient with IGD. Expression of GPI in IGD is reduced but not completely deficient.

Figure 3. A tentative model of transcriptional control of PIG-M by Sp1 in health and disease.

A. Baseline transcriptional activity of the *PIGM* promoter depends on the RNA polymerase II transcriptional machinery and the open configuration

of acetylated chromatin. Sp1 might regulate both processes by interacting with the Pol II complex and by ensuring histone acetylation through its ability to recruit histone modifying enzymes, i.e., acetyl-transferases (HAT) and de-acetylases (HDAC). Under normal circumstances, the balance favours HAT activity and thus acetylation of histones. **B.** In IGD, mutation in the core promoter Sp1 binding motif prevents Sp1 binding resulting in the interruption of its interaction with the Pol II complex and loss of its ability to recruit HAT/HDAC. The ensuing chromatin de-acetylation and condensation dramatically but not completely reduces transcriptional activity of the *PIGM* promoter. **C.** In the presence of butyrate, a HDAC inhibitor, HDAC recruited to the upstream Sp1 binding motifs are inhibited leaving activity of HAT unopposed and thus restoration of histone acetylation and transcriptional activity.

Reference List

1. P.Ghosh, N.M.Dahms, and S.Kornfeld, Mannose 6-phosphate receptors: new twists in the tale, *Nat Rev Mol Cell Biol* 4 (2003) 202.
2. A.Helenius and M.Aebi, ROLES OF N-LINKED GLYCANS IN THE ENDOPLASMIC RETICULUM, *Annual Review of Biochemistry* 73 (2004) 1019.
3. K.Ohtsubo and J.D.Marsh, Glycosylation in Cellular Mechanisms of Health and Disease, *Cell* 126 (2006) 855.
4. L.W.Ruddock and M.Molinari, N-glycan processing in ER quality control, *J Cell Sci.* 119 (2006) 4373.
5. M.A.J.Ferguson and A.F.Williams, Cell-Surface Anchoring of Proteins Via Glycosyl-Phosphatidylinositol Structures, *Annual Review of Biochemistry* 57 (1988) 285.
6. T.Kinoshita, M.Fujita, and Y.Maeda, Biosynthesis, remodeling and functions of mammalian GPI-anchored proteins: recent progress, *J Biochem.* (2008).
7. P.Orlean and A.K.Menon, Thematic review series: lipid posttranslational modifications. GPI anchoring of protein in yeast and mammalian cells, or: how we learned to stop worrying and love glycosphospholipids, *J Lipid Res* 48 (2007) 993.
8. M.G.Paulick and C.R.Bertozi, The glycosylphosphatidylinositol anchor: a complex membrane-anchoring structure for proteins, *Biochemistry* 47 (2008) 6991.
9. J.Jaeken and G.Matthijs, Congenital disorders of glycosylation: a rapidly expanding disease family, *Annu. Rev Genomics Hum. Genet.* 8 (2007) 261.
10. Y.Maeda and T.Kinoshita, Dolichol-phosphate mannose synthase: structure, function and regulation, *Biochim. Biophys. Acta* 1780 (2008) 861.
11. S.Tanaka, Y.Maeda, Y.Tashima, and T.Kinoshita, Inositol deacylation of glycosylphosphatidylinositol-anchored proteins is mediated by mammalian PGAP1 and yeast Bst1p, *J Biol Chem.* 279 (2004) 14256.
12. S.Mayor and H.Riezman, Sorting GPI-anchored proteins, *Nat Rev Mol Cell Biol* 5 (2004) 110.

13. S.Takida, Y.Maeda, and T.Kinoshita, Mammalian GPI-anchored proteins require p24 proteins for their efficient transport from the ER to the plasma membrane, *Biochem. J* 409 (2008) 555.
14. Y.Maeda, Y.Tashima, T.Houjou, M.Fujita, Yoko-o T, Y.Jigami, R.Taguchi, and T.Kinoshita, Fatty acid remodeling of GPI-anchored proteins is required for their raft association, *Mol Biol Cell* 18 (2007) 1497.
15. P.M.Rudd, B.P.Morgan, M.R.Wormald, D.J.Harvey, C.W.van den Berg, S.J.Davis, M.A.Ferguson, and R.A.Dwek, The glycosylation of the complement regulatory protein, human erythrocyte CD59, *Adv. Exp. Med Biol* 435 (1998) 153.
16. K.Kawagoe, D.Kitamura, M.Okabe, I.Taniuchi, M.Ikawa, T.Watanabe, T.Kinoshita, and J.Takeda, Glycosylphosphatidylinositol-anchor-deficient mice: implications for clonal dominance of mutant cells in paroxysmal nocturnal hemoglobinuria, *Blood* 87 (1996) 3600.
17. G.Tremml, C.Dominguez, V.Rosti, Z.Zhang, P.P.Pandolfi, P.Keller, and M.Bessler, Increased sensitivity to complement and a decreased red blood cell life span in mice mosaic for a nonfunctional Piga gene, *Blood* 94 (1999) 2945.
18. S.D.Leidich, D.A.Drapp, and P.Orlean, A conditionally lethal yeast mutant blocked at the first step in glycosyl phosphatidylinositol anchor synthesis, *J Biol Chem.* 269 (1994) 10193.
19. P.Orlean, S.D.Leidich, D.A.Drapp, and P.Colussi, Isolation of temperature-sensitive yeast GPI-anchoring mutants, *Braz. J Med Biol Res* 27 (1994) 145.
20. S.Munro, Lipid Rafts: Elusive or Illusive?, *Cell* 115 (2003) 377.
21. S.Mukherjee and F.R.Maxfield, MEMBRANE DOMAINS, *Annual Review of Cell and Developmental Biology* 20 (2004) 839.
22. P.Sharma, R.Varma, R.C.Sarasij, Ira, K.Gousset, G.Krishnamoorthy, M.Rao, and S.Mayor, Nanoscale organization of multiple GPI-anchored proteins in living cell membranes, *Cell* 116 (2004) 577.
23. P.E.T.E.BUTIKOFER, T.A.T.I.MALHERBE, M.O.N.I.BOSCHUNG, and I.S.A.B.RODITI, GPI-anchored proteins: now you see 'em, now you don't, *FASEB J.* 15 (2001) 545.
24. S.Schuck and K.Simons, Controversy fuels trafficking of GPI-anchored proteins, *J. Cell Biol.* 172 (2006) 963.

25. A.Traister, W.Shi, and J.Filmus, Mammalian Notum induces the release of glypicans and other GPI-anchored proteins from the cell surface, *Biochem. J* (2007).
26. D.B.Diep, K.L.Nelson, S.M.Raja, E.N.Pleshak, and J.T.Buckley, Glycosylphosphatidylinositol anchors of membrane glycoproteins are binding determinants for the channel-forming toxin aerolysin, *J Biol Chem.* 273 (1998) 2355.
27. Y.Maeda, H.Ashida, and T.Kinoshita, CHO Glycosylation Mutants: GPI Anchor, in: F.Minoru (Ed.), *Methods in Enzymology Glycomics*, Academic Press, 2006, pp.182-205.
28. L.Luzzatto and M.Bessler, The dual pathogenesis of paroxysmal nocturnal hemoglobinuria, *Curr. Opin. Hematol.* 3 (1996) 101.
29. L.Luzzatto, M.Bessler, and B.Rotoli, Somatic mutations in paroxysmal nocturnal hemoglobinuria: a blessing in disguise?, *Cell* 88 (1997) 1.
30. W.F.Rosse and R.E.Ware, The molecular basis of paroxysmal nocturnal hemoglobinuria, *Blood* 86 (1995) 3277.
31. J.Takeda, T.Miyata, K.Kawagoe, Y.Iida, Y.Endo, T.Fujita, M.Takahashi, T.Kitani, and T.Kinoshita, Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria, *Cell* 73 (1993) 703.
32. A.Karadimitris and L.Luzzatto, The cellular pathogenesis of paroxysmal nocturnal haemoglobinuria, *Leukemia* 15 (2001) 1148.
33. P.Hillmen, S.M.Lewis, M.Bessler, L.Luzzatto, and J.V.Dacie, Natural history of paroxysmal nocturnal hemoglobinuria, *N. Engl. J. Med.* 333 (1995) 1253.
34. G.Socie, J.Y.Mary, A.de Gramont, B.Rio, M.Leporrier, C.Rose, P.Heudier, H.Rochant, J.Y.Cahn, and E.Gluckman, Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. French Society of Haematology, *Lancet* 348 (1996) 573.
35. A.M.Almeida, Y.Murakami, D.M.Layton, P.Hillmen, G.S.Sellick, Y.Maeda, S.Richards, S.Patterson, I.Kotsianidis, L.Mollica, D.H.Crawford, A.Baker, M.Ferguson, I.Roberts, R.Houlston, T.Kinoshita, and A.Karadimitris, Hypomorphic promoter mutation in PIGM causes inherited glycosylphosphatidylinositol deficiency, *Nat. Med.* 12 (2006) 846.
36. M.H.Holguin, L.R.Fredrick, N.J.Bernshaw, L.A.Wilcox, and C.J.Parker, Isolation and characterization of a membrane

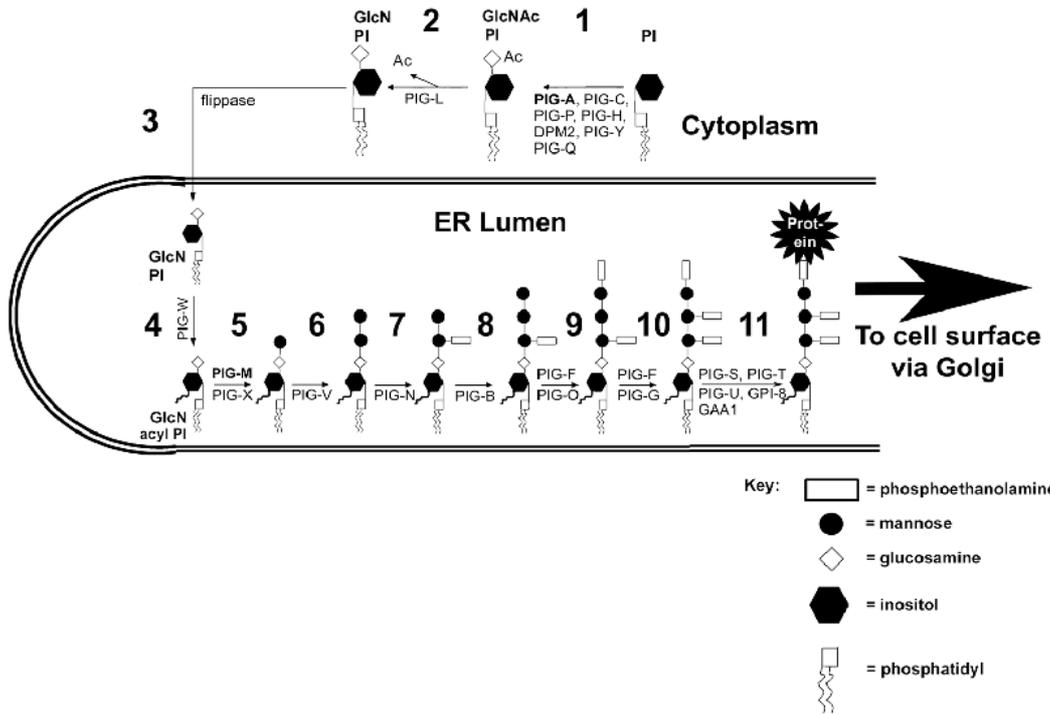
protein from normal human erythrocytes that inhibits reactive lysis of the erythrocytes of paroxysmal nocturnal hemoglobinuria, *J Clin. Invest* 84 (1989) 7.

37. A.M.Almeida, Y.Murakami, A.Baker, Y.Maeda, I.A.Roberts, T.Kinoshita, D.M.Layton, and A.Karadimitris, Targeted therapy for inherited GPI deficiency, *N Engl J Med* 356 (2007) 1641.
38. Y.Maeda, R.Watanabe, C.L.Harris, Y.Hong, K.Ohishi, K.Kinoshita, and T.Kinoshita, PIG-M transfers the first mannose to glycosylphosphatidylinositol on the luminal side of the ER, *EMBO J* 20 (2001) 250.
39. I.Wierstra, Sp1: emerging roles--beyond constitutive activation of TATA-less housekeeping genes, *Biochem. Biophys. Res Commun.* 372 (2008) 1.
40. L.Li, S.He, J.M.Sun, and J.R.Davie, Gene regulation by Sp1 and Sp3, *Biochem. Cell Biol.* 82 (2004) 460.
41. P.Bouwman and S.Philipsen, Regulation of the activity of Sp1-related transcription factors, *Mol. Cell Endocrinol.* 195 (2002) 27.
42. G.Suske, The Sp-family of transcription factors, *Gene* 238 (1999) 291.
43. A.Emili, J.Greenblatt, and C.J.Ingles, Species-specific interaction of the glutamine-rich activation domains of Sp1 with the TATA box-binding protein, *Mol. Cell Biol.* 14 (1994) 1582.
44. M.Merika and S.H.Orkin, Functional synergy and physical interactions of the erythroid transcription factor GATA-1 with the Kruppel family proteins Sp1 and EKLF, *Mol. Cell Biol.* 15 (1995) 2437.
45. M.Furusawa, T.Taira, S.M.Iguchi-Ariga, and H.Ariga, Molecular cloning of the mouse AMY-1 gene and identification of the synergistic activation of the AMY-1 promoter by GATA-1 and Sp1, *Genomics* 81 (2003) 221.
46. K.Chin, N.Oda, K.Shen, and C.T.Noguchi, Regulation of transcription of the human erythropoietin receptor gene by proteins binding to GATA-1 and Sp1 motifs, *Nucleic Acids Res.* 23 (1995) 3041.
47. H.Xiao, T.Hasegawa, and K.Isobe, p300 collaborates with Sp1 and Sp3 in p21(waf1/cip1) promoter activation induced by histone deacetylase inhibitor, *J Biol. Chem.* 275 (2000) 1371.
48. A.Doetzlhofer, H.Rotheneder, G.Lagger, M.Koranda, V.Kurtev, G.Brosch, E.Wintersberger, and C.Seiser, Histone

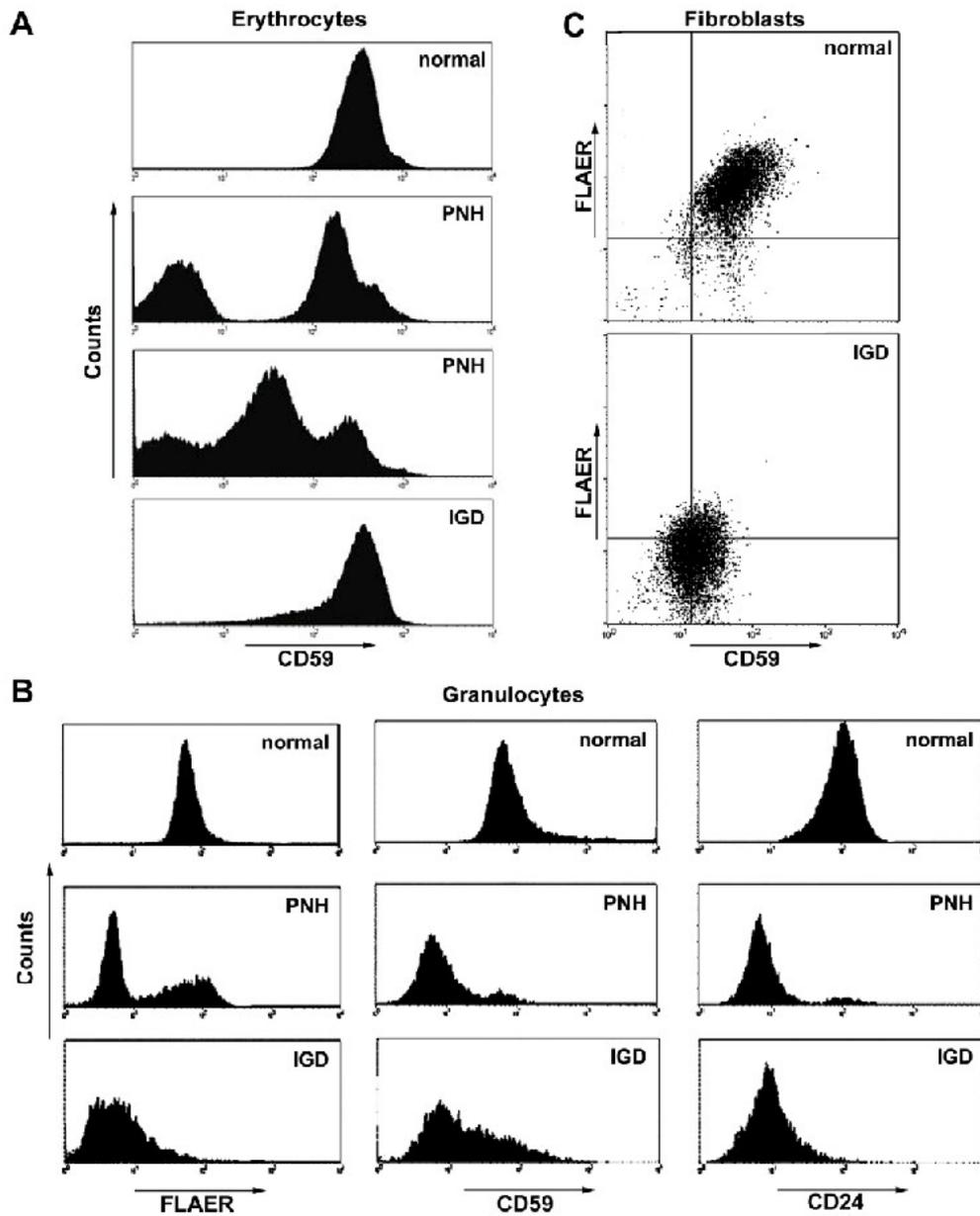
deacetylase 1 can repress transcription by binding to Sp1, *Mol. Cell Biol.* 19 (1999) 5504.

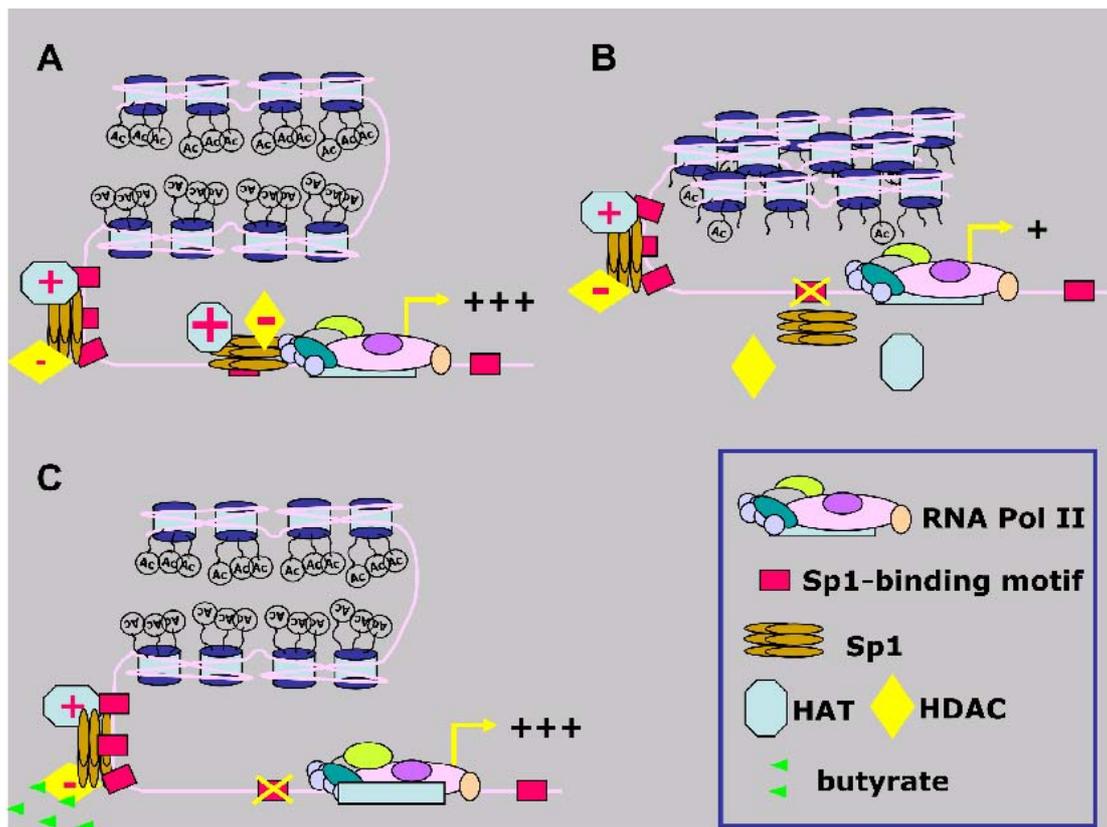
49. S.G.Gray, C.N.Qian, K.Furge, X.Guo, and B.T.Teh, Microarray profiling of the effects of histone deacetylase inhibitors on gene expression in cancer cell lines, *Int. J Oncol.* 24 (2004) 773.
50. J.M.Mariadason, G.A.Corner, and L.H.Augenlicht, Genetic Reprogramming in Pathways of Colonic Cell Maturation Induced by Short Chain Fatty Acids: Comparison with Trichostatin A, Sulindac, and Curcumin and Implications for Chemoprevention of Colon Cancer, *Cancer Res* 60 (2000) 4561.
51. L.C.Van, S.Emiliani, and E.Verdin, The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation, *Gene Expr.* 5 (1996) 245.
52. N.Camarero, A.Nadal, M.J.Barrero, D.Haro, and P.F.Marrero, Histone deacetylase inhibitors stimulate mitochondrial HMG-CoA synthase gene expression via a promoter proximal Sp1 site, *Nucleic Acids Res.* 31 (2003) 1693.
53. J.R.Davie, Inhibition of histone deacetylase activity by butyrate, *J Nutr.* 133 (2003) 2485S.
54. P.E.Di, M.D.Cappellini, R.Mazzucchelli, V.Moriondo, D.Mologni, P.B.Zanone, and A.Riva, A point mutation affecting an SP1 binding site in the promoter of the ferrochelatase gene impairs gene transcription and causes erythropoietic protoporphyria, *Exp. Hematol.* 33 (2005) 584.
55. Y.Gan, Y.H.Shen, J.Wang, X.Wang, B.Utama, J.Wang, and X.L.Wang, Role of histone deacetylation in cell-specific expression of endothelial nitric-oxide synthase, *J Biol. Chem.* 280 (2005) 16467.
56. B.Guyot, K.Murai, Y.Fujiwara, V.Valverde-Garduno, M.Hammett, S.Wells, N.Dear, S.H.Orkin, C.Porcher, and P.Vyas, Characterization of a megakaryocyte-specific enhancer of the key hemopoietic transcription factor GATA1, *J Biol. Chem.* 281 (2006) 13733.
57. L.Han, J.Lu, L.Pan, X.Wang, Y.Shao, S.Han, and B.Huang, Histone acetyltransferase p300 regulates the transcription of human erythroid-specific 5-aminolevulinatase synthase gene, *Biochem. Biophys. Res. Commun.* 348 (2006) 799.
58. E.Steiner, K.Holzmann, C.Pirker, L.Elbling, M.Micksche, and W.Berger, SP-transcription factors are involved in basal MVP promoter activity and its stimulation by HDAC inhibitors, *Biochem. Biophys. Res. Commun.* 317 (2004) 235.

59. J.Ye, D.Shedd, and G.Miller, An Sp1 response element in the Kaposi's sarcoma-associated herpesvirus open reading frame 50 promoter mediates lytic cycle induction by butyrate, *J Virol.* 79 (2005) 1397.
60. T.Imbach, B.Schenk, E.Schollen, P.Burda, A.Stutz, S.Grunewald, N.M.Bailie, M.D.King, J.Jaeken, G.Matthijs, E.G.Berger, M.Aebi, and T.Hennet, Deficiency of dolichol-phosphate-mannose synthase-1 causes congenital disorder of glycosylation type Ie, *J Clin. Invest* 105 (2000) 233.
61. S.Kim, V.Westphal, G.Srikrishna, D.P.Mehta, S.Peterson, J.Filiano, P.S.Karnes, M.C.Patterson, and H.H.Freeze, Dolichol phosphate mannose synthase (DPM1) mutations define congenital disorder of glycosylation Ie (CDG-Ie), *J Clin. Invest* 105 (2000) 191.
62. P.Orlean, Congenital disorders of glycosylation caused by defects in mannose addition during N-linked oligosaccharide assembly, *J Clin. Invest* 105 (2000) 131.
63. L.J.Thomas, M.Urakaze, R.DeGasperi, T.Kamitani, E.Sugiyama, H.M.Chang, C.D.Warren, and E.T.Yeh, Differential expression of glycosylphosphatidylinositol-anchored proteins in a murine T cell hybridoma mutant producing limiting amounts of the glycolipid core. Implications for paroxysmal nocturnal hemoglobinuria, *J Clin. Invest* 89 (1992) 1172.
64. E.A.Eklund and H.H.Freeze, The congenital disorders of glycosylation: a multifaceted group of syndromes, *NeuroRx.* 3 (2006) 254.
65. J.Jaeken and G.Matthijs, Congenital disorders of glycosylation, *Annu. Rev Genomics Hum. Genet.* 2 (2001) 129.
66. A.B.Cantor and S.H.Orkin, Transcriptional regulation of erythropoiesis: an affair involving multiple partners, *Oncogene* 21 (2002) 3368.



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