A step closer in defining glycosylphosphatidylinositol anchored proteins role in health and glycosylation disorders

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ABSTRACT

Glycosylphosphatidylinositol anchored proteins (GPI-APs) represent a class of soluble proteins attached to the external leaflet of the plasma membrane by a post-translation modification, the GPI anchor. The 28 genes currently involved in the synthesis and remodelling of the GPI anchor add to the ever-growing class of congenital glycosylation disorders. Recent advances in next generation sequencing technology have led to the discovery of Mabry disease and CHIME syndrome genetic aetiology. Moreover, with each described mutation known phenotypes expand and new ones emerge without clear genotype-phenotype correlation. A protein database search was made for human GPI-APs with defined pathology to help building-up a physio-pathological mechanism from a clinical perspective. GPI-APs function in vitamin-B6 and folate transport, nucleotide metabolism and lipid homeostasis. Defining GPI-APs role in disease bears significant clinical implications.

1. Introduction

In 1970, Mabry et al. made the first observation of the Hyperphosphatasia with mental retardation syndrome (HPMRS) with the following findings: severe mental retardation, seizures, various neurologic abnormalities, and elevated serum levels of alkaline phosphatase. With recent advances in the next generation sequencing technology both hypomorphic and loss of function mutations of the genes involved in synthesis or remodelling of the GPI-APs have been described. This helped expanding HPMRS clinical phenotype to multi-system involvement. PIGA gene defect, previously associated with paroxysmal nocturnal haemoglobinuria (PNH), was also attributed multiple congenital anomalies, hypotonia and seizures X-linked syndrome. Moreover, PNH was also described in a PIGT gene mutation. Other clinical phenotypes associated with molecular defects in the biosynthesis of the GPI anchor include: CHIME syndrome (coloboma, heart defects, ichthyosiform dermatosis, intellectual disability, and either ear defects or epilepsy), MCAHS syndrome (multiple congenital anomalies, hypotonia and seizures, type 1–3) and early-onset epileptic encephalopathies (Table 1).

Pending on the cell polarisation type, the GPI-anchor is required for trafficking proteins to certain domains of the plasma membrane. There, the GPI-APs oligomerize in the rich cholesterol/sphingolipid areas by saturated fatty acids interactions within the GPI anchor and with other molecules building up “signalling platforms”. Several studies showed that GPI-APs oligomerize/cluster at the cell surface of different cell types such as fibroblasts, immune T and epithelial cells [1–5]. Importantly, GPI-AP clustering at the cell surface is cholesterol-sensitive and dynamically regulated by the cortical cytoskeleton. Crucial for their biological role, GPI-AP clustering at the membrane rafts permits interaction with side partners such as enzymes, adaptors, co-factors and scaffolding proteins, initiating spatio-temporal compartmentalization and context-specific activation of downstream signalling cascades [6, 7].

The role of the GPI-APs at the plasma membrane is even more complex, considering their recruitment to exosomes, release in the extracellular environment by proteolysis or further trafficking via endocytic pathway [9–11]. Broadly, a defect in the later stages of biosynthesis / remodelling of the GPI anchor results in shedding of the soluble protein in the extracellular environment, with or without the abnormal GPI signal; in defective early GPI anchor biosynthesis, the soluble protein will be subjected to intracellular degradation [12]. This mechanism explains high plasma alkaline phosphatase levels in patients with HPMRS [13].

Abnormal surface expression of the GPI-APs was demonstrated by transfection of patients' DNA to GPI-AP defective cell lines. In vitro functional analysis has shown variable degrees of GPI-APs restoration compared to wild gene transfection [14–23].
| Clinical presentation | HPMRS (6 types) | CHIME | MCAHS 1 OMIM # 614080 | MCAHS 2 OMIM # 300868 |
|-----------------------|----------------|-------|----------------------|----------------------|
| Inheritance           | AR             | AR    | AR                   | X-linked             |
| Dysmorphism           | Secondary microcephaly | Brachycephaly | Macrocephaly | Macrosomia at birth |
|                       | Plagiocephaly  | Hypertelorism | Macrocephaly at birth | Accelerated linear growth |
|                       | Coronal synostosis | Broad, flat nasal root | Poor growth | Obesity |
| Midface hypoplasia    | Short philtrum | Coarse facial features | Light hair | Microcephaly |
| Prognathism           | Full lips      | Bi-temporal narrowing | Micrognathia | Coarse facies |
| Hypertelorism         | Anomalus dention | Depressed nasal bridge | Overfolded helix | Overfolded helices |
| Long palpebral fisses | Cleft palate   | High arched palate with wide | Upslanting palpebral fissures | Downturned corners of the mouth |
| Broad nasal bridge    | Epicritic folds | alveolar ridge | Wideness of the palpebral fissures | Triangular mouth |
| Broad nasal tip       | Macrosephaly   | Frontal bossing | Gingival hyperplasia | Gingival hyperplasia |
| Short philtrum        | Long philtrum  | Long philtrum | Micrognathia | Micrognathia |
| Large flesh nose      | Micromegaly    | Large, fleshy ears | Small mouth | Small mouth |
| Downturned mouth      | Overfolded helices | Overfolded helices | Depressed nasal bridge | Depressed nasal bridge |
| Corners               | Low-set ears   | Low-set ears | Widely spaced teeth | Widely spaced teeth |
| Tented mouth          | Posteriorly rotated ears | Cupped ears | Pointed teeth | Pointed teeth |
| Cleft palate          | Large, fleshy ears | Depressed nasal bridge | Short, reverse position | Short neck |

Cardiovascular

| ASD | VSD | Tetralogy of Fallot | Transposition of great arteries | Peripheral pulmonary stenosis |
|-----|-----|---------------------|-------------------------------|-------------------------------|
| ASD | ASD | Over-riding aorta   | Hypoplastic pulmonary trunk   | Non-compacting cardiomyopathy |

Respiratory

| Lung hypoplasia (in some patients) | Diaphragmatic hernia |
|-----------------------------------|----------------------|
| Respiratory drive requiring tracheostomy and assisted ventilation. | |
| Obstructive apnoea                |                      |

Gastrointestinal

| Upper GI dysmotility | Feeding difficulties requiring tube feeding |
|----------------------|------------------------------------------|
| Congenital diaphragmatic hernia | Gastroesophageal reflux |
| Intestinal malrotation | Hepatemalgia |
| Hirschsprung disease | Intestinal malrotation |
| Anorectal abnormalities (anal stenosis, atresia antevestibular fistula, anteriorly displaced anus) | |

Genitourinary

| Anal stenosis or atresia | Dysplastic kidney |
|--------------------------|------------------|
| Multicystic kidneys      |                  |
| Unilateral hydrenephrosis |                |
| Duplicated collecting system | Microphallus, cryptorchidism |

Skeletal

| Hip dysplasia | Pectus excavatum |
|---------------|-----------------|
| Narrow inferior iliacs | Fifth finger clinodactyly |
| Fifth finger | Hip dysplasia |
| Joint contractures | |

Skin, nail, hair

| Migratory ichthyosiform dermatosis | Meckel's diverticulum |
|-----------------------------------|----------------------|
| Seborrheic dermatitis | Linear plaque-like scales |
| Ichthyosis | Pigmentation abnormalities |
| Hypoplastic nails, anonychia | Hypoplastic nails |

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chemically with high plasma and cerebrospinal fluid PLP levels [25, 26]. This abnormal distribution could be explained by a normal maternal-dependent trans-placental B6 transport but deficient cellular uptake. Unable to convert the PLP to pyridoxal, the TNAP-deficient patient becomes intracellularly B6 depleted. Applying metabolomics, Cruz et al. aimed to identify the biochemical changes associated with B6-deficient metabolism in several mouse models with null TNAP, heterozygote and normal TNAP expressing brain tissues. The amplitude of the concentration variation between the TNAP-knockout mice and the other genotypes grouped together reached −40% for GABA, −80% for adenosine and +450% for cystathionine [27]. Dysregulation of the transulfuration pathway and of purinergic signalling represents the biochemical expression of the TNAP deficient brain. The CSF PLP levels were showed to have a negative age correlation [28]. This seems to follow the postnatal decline in TNAP expression, with an expected steep increment in patients with GIAP-deficiency.

The pathways of 1-carbon metabolism, transulfuration and glutathione synthesis are critical for nucleotide synthesis, DNA and histone methylation, and antioxidant defense. PLP acts as co-factor of 5 enzymes in these metabolic pathways: cystathionine β-synthase (CBS), cystathionine γ-lyase (CGL), cytoplasmic and mitochondrial serine hydroxymethyltransferase (cSHMT and mSHMT), and glycine decarboxylase (GDC) in the mitochondria (see Fig. 1).

The transulfuration pathway acts in sulfur amino acid metabolism contributing to the regulation of cellular homocysteine, cysteine production (required for synthesis of proteins, glutathione and taurine) and generation of H2S for signalling functions. Kinetic studies, on animals and humans, have shown that although PLP serves as coenzyme for both CBS and CGL enzymes, CGL is more sensitive to B6-depletion. In humans, have shown that although PLP serves as coenzyme for both CBS and CGL enzymes, CGL is more sensitive to B6-depletion.
vitamin B-6 restriction. Interestingly, they have also found an increase in plasma glutathione by approximate 40% [30]. Lima et al. explain, in their experiment on rats, that cellular and plasma cystathionine concentrations increase in B6 deficiency mainly due to the bottleneck caused by reduced CGL activity. The increase in substrate concentration (i.e. cystathionine) yields a nearly proportional increase in $v/V_{max}$ due to the high Michaelis-Menten constant of CGL enzyme. This allows cysteine concentrations, cysteine flux, and net transsulfuration flux to be maintained in mild to moderate vitamin B-6 deficiency [31].

Mathematical models, based on experimentally determined kinetic parameters and known regulatory mechanisms, are increasingly used to explain how biological pathways work. Moreover, they can help identifying unexpected system properties or behaviour. Frederik et al., based their mathematical model on the structure and function of the 1-carbon and glutathione metabolism. They modelled vitamin B6 deficiency by reducing the $V_{max}$ of the PLP dependent enzymes, thus simulating a range of vitamin B6 deficiencies. Firstly, they found that cystathionine was by far the most sensitive biomarker for vitamin B-6 deficiency. Secondly, they simulated B6 deficiency induced oxidative stress by increasing H2O2 concentration and confirmed the

Table 2
GPI-APs function and physio-pathologic roles.

| GPI-AP          | Other post-translational modifications | Function                  | Pathology†                  | Tissue specificity† |
|-----------------|----------------------------------------|---------------------------|----------------------------|---------------------|
| TNAP            | N-glycosylated                         | Phosphatase, nucleotidase | Hypophosphatasia            | Widely expressed   |
|                 | Phosphorlated                          |                           |                            |                     |
|                 | Dist sulphide bond                     |                           |                            |                     |
| FOLR1           | N-glycosylated                         | Folate transport across choroid plexus | Cerebral folate deficiency | Choroid plexus Kidney Lung Cerebellum |
|                 | Dist sulphide bond                     |                           |                            |                     |
| S’ ecto-nucleotidase | N-glycosylated                         | Ecto-nucleotidase         | Defective purinergic signalling | Widely expressed   |
|                 | Dist sulphide bond                     |                           |                            |                     |
| GPIHBP1         | N-glycosylated                         | Lipoprotein lipase transport | Hyperlipoproteinemia 1D | Adipose tissue Lung |
|                 | Dist sulphide bond                     |                           |                            |                     |
| Vanin 1         | N-glycosylated                         | Inflammatory response and innate immunity | Impaired cyto-protective role | Endothelial cell |
|                 | Dist sulphide bond                     |                           |                            |                     |
| Carboxy-peptidase M | N-glycosylated                         | Peptidase                 | Not yet defined            | Lung Kidney Adipose tissue Female tissues |
|                 | Dist sulphide bond                     |                           |                            |                     |
| Urokinase plasminogen activator receptor | N-glycosylated                         | Cell surface fibrinolysis | Role in development of focal segmental glomerulosclerosis | Bone marrow Immune tissue Lung |
|                 | Dist sulphide bond                     |                           |                            |                     |
| Via interactome |                                         |                           | Rolandoide epilepsy, developmental verbal dyspraxia and perisylvian polymicrogyria (via SRPX2) |                     |
|                 |                                         |                           | Developmental verbal dyspraxia (via FOXP2) |                     |
|                 |                                         |                           | Congenital symmetrical cornification of the palms and soles, with ichthyosis (via SLURP1) |                     |
|                 |                                         |                           | Immunodeficiency-35 (via TYK2) |                     |
| Alpha and Beta tectorin | N-glycosylated                         | Tectorial membrane component | Non-syndromic deafness (DFNB21) | Ear |
|                 | Dist sulphide bond                     |                           | Deafness (DFNA12)          | Oocyte Bone marrow and immune system |
| Otouncorin      | N-glycosylated                         | Interface between epithelia and tectorial membrane | Non-syndromic deafness (DFNB22) | Ear |
|                 | Dist sulphide bond                     |                           |                            | Bone marrow and immune system |
| CD59            | N-glycosylated                         | Potent inhibitor of the complement membrane attack complex action | Hemolytic anemia, CD59 mediated, with or without immune-mediated polyneuropathy | Widely expressed |
|                 | O-glycosylated                         |                           |                            |                     |
|                 | Dist sulphide bond                     |                           |                            |                     |
| CD55            | Dist sulphide bond                     | Regulates complement activation | Paroxysmal nocturnal haemoglobinuria | Widely expressed |
|                 | N-glycosylated                         |                           | Complement hyperactivation, angiopathic thrombosis, protein-losing enteropathy (CHAPLE) |                     |
| Prion protein   | N-glycosylated                         | Its primary physiological function is unclear. | Paroxysmal nocturnal haemoglobinuria | Brain |
|                 | Dist sulphide bond                     |                           |                            |                     |

* http://uniprot.org
† http://omim.org

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70
experimental results that showed increased plasma glutathione (GSH) in humans and hepatic GSH in dietary B6 deficient rats. Interestingly, the effect was independent of B6 deficiency; when B6 deficiency was corrected but oxidative stress remained GSH response was nearly identical [32].

Vitamin B-6 deficiency can be expected to interfere with the metabolic functions that depend critically on SHMT, which catalyses the interconversion of glycine to serine and of THF to CH2-THF in cytoplasm and the mitochondria (see figure). Insufficient CH2-THF, 1-carbon donor for thymidylate synthesis, leads to uracil mis-incorporation into DNA and subsequent strand breaks. CH2-THF is also substrate for the synthesis of 5MTHF, the primary methyl group donor for the methionine cycle that controls DNA and histone methylation and purine synthesis. In rats, vitamin B6 deficiency was found to lower both enzymes abundance. Several experimental studies have shown that SHMT depletion induces glycine auxotrophy. The mathematical model explained this effect. The normal balance between glycine and serine in the absence of SHMT only occurred if there was input of glycine into the system. When glycine input was set to zero, in the absence of SHMT, many metabolites and reaction rates were significantly reduced: the GDC reaction was reduced to nearly zero, the thymidylate synthase reaction was reduced to 59%, and the rate of export of 1-carbon units from the mitochondria was reduced to 29%. Interestingly, setting the serine input to zero had little effect suggesting that glycine input can completely make up for the lack of serine, but not vice versa [32]. Vanessa et al. targeted metabolomics analysis of 1C metabolites in HepG2 cells showed that methionine cycle flux is maintained by directing 1C units to the re-methylation process rather than purine synthesis explaining higher THF and lower CH2-THF levels in B6 deficient conditions [33].

The presence of CBS in human plasma was confirmed by in-silico search of the proteome database and experimentally evidenced by Krijt et al. [34]. Patients with mild GPI-AP defects that maintain a high PLP plasma level could stimulate CBS activity and lower the homocysteine level within this compartment.

In mammals, 70–80% of the vitamin B6 is located in the skeletal muscle, where it functions as co-factor for glycogen phosphorylase [35]. Further studies are required to appreciate the relation between altered glycogen phosphorylase activity, accumulation of cytoplasmic PAS positive material and clinical hypotonia in GPI-AP defects [19, 36].

3.2. Folate receptor (FOLR)

FOLR defects are associated with neurodegeneration due to cerebral folate transport deficiency. Biochemically, patients present low 5-methyltetrahydrofolate (5MTHF) levels in CSF with normal peripheral folate status. FOLR1 and FOLR2 are anchored at the plasma membrane by the GPI anchor, whereas FOLR3 is secreted due to the lack of a signal sequence for GPI-anchor attachment [37]. Within the brain FOLR1 is almost selectively expressed in the choroid plexus. Its high binding affinity for folates perfectly matches the physiological 5MTHF concentration in human plasma. Grapp et al. demonstrate a basolateral to
apical sorting of the GPI-anchored FOLR1 that explains receptor-mediated endocytosis and transcytosis of the FOLR1 - SMTFH complex across the choroid plexus. Then, FOLR1-containing exosomes circulate in the CSF, cross the ependymal cell layer and are distributed in the brain parenchyma [38]. Replacing the GPI-anchor of the FOLR with a trans-membrane domain, in a mouse model, results in endocytosis via clathrin-coated pits and altered intracellular destination of the FOLR with reduced folate transport [39]. In foetal life, FOLR1 is involved in neural tube closure, development of pharyngeal arches and the secondary heart field [40].

3.3. Ecto 5' nucleotidase (5NTE, CD73)

Purinergic signalling effects are mediated by extracellular nucleotides and nucleosides in virtually all tissues. In the central nervous system, nucleotides mediate neurotransmission and neuron-glia interactions. Elsewhere, they are involved in smooth muscle (e.g. vascular and gut) and myocardial contractility, endocrine secretion, immune response modulation, control of leukocyte trafficking and platelets aggregation at vascular injury sites. Purinergic signalling regulates retinal neurotransmission, blood flow and intraocular pressure. In the inner ear, purine-receptors modulate fluid homeostasis, cochlear blood flow, hearing sensitivity and development. In addition to acute signalling events, there is increasing awareness of purines and pyrimidines potent long-term trophic roles [41–43].

A network of 8 ectoenzyme families, including 5NTE, TNAP and other alkaline or acid phosphatases, govern the duration and magnitude of purinergic signalling by hydrolyzing nucleotides to their corresponding nucleosides. 5NTE hydrolyses a variety of nucleoside 5'-monophosphates including CMP, UMP, IMP, and GMP, whereby AMP generally is the most effectively hydrolysed. The resulted adenosine will act on purinergic receptors (e.g. P1) before being salvage by the cells. Therefore, 5NTE contributes to the termination of the purinergic signalling. Moreover, due to its age-dependent expression, 5NTE has an important recycling role as de-novo synthesis of nucleotides in the adult brain is limited.

It is well-established that adenosine homeostasis is disrupted both in animal models and human epilepsies [44]. Increased 5NTE expression was found in the dentate gyrus of resective surgery specimens from temporal lobe epilepsy patients. Consistent with this data, increased 5NTE activity was also found in a number of rodent models of epilepsy [45–47]. More recently, genetic variants of both 5NTE and ADK were associated with the development of post-traumatic epilepsy in a study involving samples from 162 patients [48]. Thus, increased expression of 5NTE in the epileptic brain might be a compensatory response to inhibit synaptic transmission [44]. Starting from the premise that brain adenosine rises 100-fold under ischemic conditions, Chu et al. demonstrated that 5NTE-knockout mouse astrocyte cultures did not evoke adenosine production or obtained a limited adenosine response in mixed astrocyte-neurons cultures [49].

Wurm et al. demonstrate that adenosine or uracil nucleotides are able to restore purinergic signalling in a 5NTE deficient retina mouse model [50]. Paget et al. conclude that a high level of 5NTE in fibroblast lysate might cause intracellular nucleotide deficiency secondary to decreased salvage in patients presenting with developmental delay, seizures, ataxia, recurrent infections and severe language deficit. Uridine supplementation led to a remarkable improvement in speech and development as well as decreased seizure activity [51]. The anticonvulsant role of uridine, and of nucleotide salvage pathway, is clearly demonstrated in epileptic encephalopathy caused by an inherited defect in de-novo pyrimidine biosynthesis [52].

3.4. GPI-anchored high-density lipoprotein-binding protein 1 (GPIHBP1)

GPIHBP1 is an endothelial cell GPI-AP mainly expressed on the luminal face of capillaries in brown adipose tissue, heart, lung, and liver [55]. GPIHBP1 plays a major role in transporting lipoprotein lipase (LPL) from the subendothelial spaces to the capillary lumen [56]. GPIHBP1 knockout mice cannot transport lipoprotein lipase to the capillary lumen, resulting in mis-localization of lipoprotein lipase within tissues, defective lipolysis of triglyceride-rich lipoproteins and chylomicronemia. The immobilization of LPL in the interstitial space will result in normal tissue stores with very low plasma levels [57]. Few patients with homozgyous missense mutations in GPIHBP1 have been reported having a similar phenotype with LPL deficiency [58, 59]. However, defective localisation of GPIHBP1 may have a variable impact on the lipid profile as increased triglyceride uptake by the liver compensates, to a degree, for the loss of LPL-mediated triglyceride delivery to peripheral tissues [60]. Markedly elevated triglycerides and cholesterol with absent post-heparin lipoprotein lipase activity suggesting LPL deficiency was already documented in two patients with GPI-AP defects, one carrying missense PIGA germline mutation and another with a homozygous variant disrupting the PIGH start-codon [61, 62].

3.5. Vanin 1 (VNN1)

VNN 1 is a pantetheinase highly expressed in the liver, gut, and kidney, where hydrolyses pantetheine into pantothenic acid (vitamin B5) and cysteamine, a potent antioxidant [63]. Cysteamine modulates cysteine, cystine and GSH levels, playing a significant role in regulating cellular redox status. Indeed, lacking tissue cysteamine, the VNN1 knockout mice displays down-regulated inflammatory response to oxidative stress [64]. Moreover, clinical data support VNN1 biomarker candidacy in auto-inflammatory processes and early diagnosis of tissue injury [65–68]. Pantothenic acid, generated by VNN1 activity, is further recycled to coenzyme A (CoA) through a series of five synthetic reactions. CoA has a major role in mitochondrial and peroxisomal lipid metabolism with neurodegenerative consequences [69, 70]. Thus, VNN1 serves as link between inflammation and metabolism.

Peroxisome Proliferator Activated Receptors (PPAR) are a transcription factor family that modulate acyl-CoA synthesis, VNN1 and GPIHBP1 gene expression. Their role can be explored from a therapeutic perspective [71]. Known PPAR agonists, such as fibrates, thiazolidinediones, certain non-steroidal anti-inflammatory drugs (e.g. ibuprofen and indomethacin) and glitazars target PPAR to exercise their metabolic role in hyperlipidaemia, insulin resistance and inflammation modulation in asthma.

3.6. Carboxypeptidase M (CPM)

CPM belongs to the large family of the carboxypeptidases which remove C-terminal amino acids from peptides and proteins, contributing to complement regulation, pro-hormone and neuropeptide processing at the cell surface, and coagulation-fibrinolysis [72]. Its role in human disease is yet to be defined.

3.7. Urokinase plasminogen activator receptor (UPAR)

UPAR was originally identified as the membrane receptor of the serine protease urokinase, thereby implicated in the plasminogen activation and the coagulation cascade. To date, more than 40 proteins, soluble plasma ligands and cell membrane lateral partners, are known to directly interact with UPAR. For example, factor XII and high molecular weight kinin-free kinogen link UPAR with the contact system (which consists of factor XII, kinogen and pre-kallikrein), complement and fibrinolysis system. The fact that UPAR interacts with members of three major families of membrane receptors i.e. G protein-coupled receptors, tyrosine kinases, and integrins implies that the actual number of components constituting UPAR's interacome is high. Indeed, UPAR's expression was found to be up-regulated in various malignancies, inflammatory and infectious conditions. Moreover, UPAR has been found to have a role central nervous system, promoting neuro-
repair following ischemic brain injury or language development. Mutations in SRPX2 gene, which acts as an UPAR ligand, have been linked to bilateral perisylvian polymicrogyria, Rolandic epilepsy, speech dyspraxia and cognitive disability [73–75].

Paroxysmal nocturnal haemoglobinuria (PNH) is a clonal bone marrow disorder caused by PIGA gene mutation in a hematopoietic stem cell. Deficiency of the GPI-anchored complement regulators CD55 and CD59 on red cells surface leads to intravascular haemolysis upon complement activation. However, in patients with PNH, thrombosis is the most serious clinical complication with a predilection for the hepatic, abdominal and cerebral veins. Sloord and al. showed that GPI-anchored UPAR is decreased or absent on the surface of granulocytes and platelets of patients with PNH while a soluble UPAR, lacking its GPI anchor, is found increased in patient’s plasma. Moreover, serum soluble UPAR concentrations correlated with the size of the PNH clone and were highest in patients who later developed thrombosis [76]. Propensity to venous thrombosis was also described by Almeida et al. in three patients with a PIGM hypomorphic promoter mutation and severe clinical phenotype [77].

Mal de Meleda is a rare skin disorder caused by SLURP1 gene mutations, in which affected individuals present palmoplantar keratoderma and ichthyotic changes elsewhere. Human Ly-6/UPAR molecules are a superfamily composed of two subfamilies; one is the GPI-anchor membrane bound proteins (such as CD59, GPHIPBP1 and UPAR) and the other are secreted proteins without the GPI-anchor such as SLURP1 (Secreted Ly6/UPAR Related Protein1). Patients with a clinical diagnosis of CHIME syndrome, and PIGL proven mutations, are usually described having migratory ichthyosiform dermatosis and palmoplantar hyperkeratosis [78–81]. Moreover, Morren and al. also described a patient with PIGO mutations and psychomotor disability, epilepsy, palmoplantar keratoderma, hyperphosphatasia and platelet dysfuncion [82]. The biochemical basis explaining this dermatologic phenotype in patients with GPI-AP defects remains to be established.

3.8. Non-collagenous glycoproteins of the acellular gels of the inner ear - alpha / beta tectorin and otoancorin

The mammalian inner ear consists of the cochlea, the organ of hearing, and the vestibule, which is responsible for balance. The apical surface of each sensory organ is covered by an acellular gel - the tectorial membrane, de

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4. Discussion

Genetic mutations disrupting biosynthesis or GPI-APs remodelling represent an emerging class of congenital glycosylation disorders. Clinically, the pervasive neurologic disability associates multi-system involvement. Estimated to more than 150, these glycosylated proteins play a wide variety of physiological roles in immune response regulation, cellular and intercellular signalling, cell adhesion and migration, neural and embryonic development, and cell metabolism.

The aim of this research was to identify potentially disrupted metabolic pathways, helping the clinician in the pursuit of early diagnosis and management. A combined database search and literature review was employed to identify GPI-APs with known pathology and treatment. GPI-APs have an established role in vitamin-B6, folate and LPL transport. Moreover, cellular signalling is disrupted when SNTE, tectorin / otoancorin and UPAR’s expression at the plasma membrane is impaired.

Patients with TNAP mutations that cause a severe phenotype, present pyridoxine-responsive seizures, undetectable alkaline phosphatase (ALP) and high plasma and CSF PLP. Other, milder presentations, do not associated seizures but persistent low ALP and high plasma PLP across childhood and puberty. Persistent high PLP levels may explain low homocysteine secondary to plasma CBS activation. However, age-dependent CSF PLP decline may be more abrupt and is expected to reach deficient range. Clinical data supports low ALP levels in patients with biosynthetic GPI-APs defects. High ALP plasma levels were also reported in patients with GPI-AP anchor remodelling defects, due to shedding of the protein from the plasma membrane. Other GPI-AP phenotypes describe normal or fluctuant ALP.

The hallmark of defective PLP transport across blood-brain barrier and cellular membranes is high cystathionine in both CSF and plasma. Intracellular B6 deficiency will disturb transsulfuration pathway, 1-carbon and nucleotide metabolism. Mitochondrial and nuclear DNA replication, energy metabolism and cell signalling are predictable consequences. Defective 1-carbon metabolism will directly decrease 5MTHF, as shown in other B6-deficient disorders, directing the flow towards re-methylation rather than nucleotide biosynthesis. This will worsen the neural folate metabolism caused by defective folate transport as FOLR1 is also affected.

Multi-antiepileptic resistant seizures were virtually described in all patients with GPI-APs defects. High dose pyridoxine was attempted in patients with HPMR phenotype with clinical benefit [85–87]. However, combined pyridoxine and folic acid supplementation to overcome the transport defects has not been reported yet. Furthermore, abnormal expression of the SNTE at the plasma membranes disrupts nucleotide salvage pathway and purinergic signalling. Uridine supplementation is a potential therapeutic tool in rescuing both pathways as it acts on P2Y4/6 purinergic receptors.

Hyperlipidemia is already shown to be associated with GPI-APs defects, however the onset may be dependent on the lipid metabolism regulatory complexity. The VNN role in coenzyme-A biosynthesis and disease is yet to be defined.

GPI-AP gene defects will only modify the protein expression targeted to the plasma membrane by the GPI-anchor. However, most GPI-AP are also described in trans-membrane and soluble form (intra or extra-cellular) without the attached GPI-anchor. Their role and expression need to be considered together with tissue specificity when attempting to resolve genotype-phenotype correlations. For example, complex regulation across cell types and tissues govern coagulation or complement activation and lipid homeostasis. A systems biology approach, using mathematical modelling, can provide a significant insight into GPI-APs role in modulating particular cellular functions.

Multiple post-translational modifications implicate GPI-APs in the pathophysiology of other types of glycosylation disorders and beyond. Modified plasma membrane lipid composition, in lysosomal disorders, disturbs GPI-APs membrane distribution in neurons [88]. Moreover, peroxisomal biogenesis defects were shown to perturb GPI anchor remodelling [89]. Therefore, defining GPI-APs role in disease bears significant clinical implications.

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Ligand binding to GPCR does not necessarily lead to G protein activation, but it can also inhibit G protein activation, depending on the specific GPCR and the signal transduction pathway involved. In some cases, ligand binding to GPCRs can also lead to changes in cell morphology, migration, and proliferation. Therefore, understanding the mechanisms by which ligands interact with GPCRs and modulate their functions is crucial for the development of new therapeutic strategies. In conclusion, the study of GPCR signaling is an essential field of research with broad applications in medicine and pharmacology.
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