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Expanding the clinical and molecular characteristics of PIGT-CDG, a disorder of glycosylphosphatidylinositol anchors

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ABSTRACT

PIGT-CDG, an autosomal recessive syndromic intellectual disability disorder of glycosylphosphatidylinositol (GPI) anchors, was recently described in two independent kindreds [Multiple Congenital Anomalies-Hypotonia-Seizures Syndrome 3 (OMIM, #615398)]. *PIGT* encodes phosphatidylinositol-glycan biosynthesis class T, a subunit of the heteropentameric transamidase complex that facilitates the transfer of GPI to proteins. GPI facilitates attachment (anchoring) of proteins to cell membranes. We describe, at ages 7 and 6 years, two children of non-consanguineous parents; they had hypotonia, severe global developmental delay, and intractable seizures along with endocrine, ophthalmologic, skeletal, hearing, and cardiac anomalies. Exome sequencing revealed that both siblings had compound heterozygous variants in *PIGT* (NM_015937.5), i.e., c.918dupC, a novel duplication leading to a frameshift, and c.1342C > T encoding a previously described missense variant. Flow cytometry studies showed decreased surface expression of GPI-anchored proteins on granulocytes, consistent with findings in previous cases. These siblings further delineate the clinical spectrum of PIGT-CDG, reemphasize the neuro-ophthalmologic presentation, clarify the endocrine features, and add hypermobility, low CSF albumin quotient, and hearing loss to the phenotypic spectrum. Our results emphasize that GPI anchor-related congenital disorders of glycosylation (CDGs) should be considered in subjects with early onset severe seizure disorders and dysmorphic facial features, even in the presence of a normal carbohydrate-deficient transferrin pattern and N-glycan profiling. Currently available screening for CDGs will not reliably detect this family of disorders, and our case reaffirms that the use of flow cytometry and genetic testing is essential for diagnosis in this group of disorders.

1. Introduction

Glycosylphosphatidylinositol (GPI) anchors are a group of glycolipids composed of a glycan core, a phosphoethanolamine linker, and a phospholipid tail [1,2]. GPI anchors are attached during posttranslational modification to the C-terminus of certain proteins destined to attach to the outer leaflet of the cell membrane and face the extracellular environment. This permits these proteins to participate in processes such as signal transduction and the immune response [1,3].

In humans, the biosynthesis and attachment of GPI anchors to proteins occurs in the endoplasmic reticulum and Golgi and involves 11 steps and protein products of at least 27 genes [4]. To date, inherited

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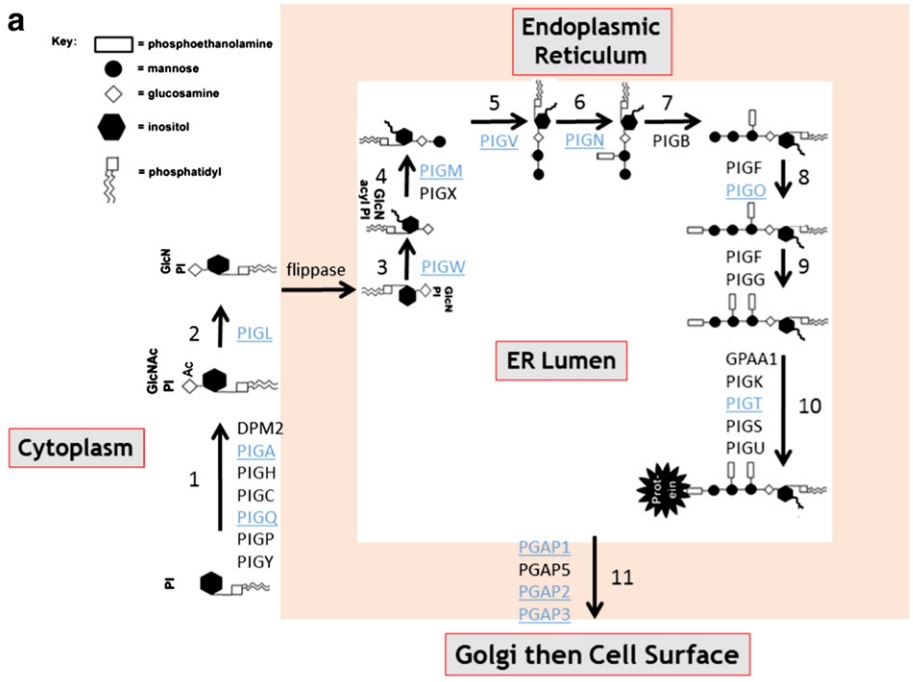
loss-of-function mutations in twelve of these genes have been implicated in human disease (Fig. 1a) [5–21]. Inherited congenital deficiencies in GPI anchor biosynthesis and attachment comprise a subset of congenital disorders of glycosylation (CDGs) and cause a spectrum of symptoms in humans, including seizures, intellectual disabilities, and congenital anomalies. These findings are present in most of the GPI anchor disorders (Fig. 1b).

PIGT encodes phosphatidylinositol-glycan biosynthesis class T, a subunit of the heteropentameric GPI transamidase complex that facilitates the attachment of GPI anchors to proteins [22]. Recently, variants in *PIGT* were identified in two unrelated families with recessively inherited Multiple Congenital Anomalies-Hypotonia-Seizures Syndrome 3 (OMIM, #615398) [11,23].

Here we describe a third independent family with PIGT-CDG affecting two siblings who presented with seizures, intellectual disability, and congenital anomalies. The siblings were admitted to the National Institutes of Health (NIH) Clinical Center and enrolled in the NIH Undiagnosed Diseases Program protocol #76-HG-0238 [24,25]. Exome sequencing identified biallelic variants in *PIGT* (NM_015937.5), and molecular, protein, and flow cytometry studies verified pathogenicity of the variants. Our results expand the clinical spectrum of PIGT-CDG, verify the pathogenicity of a novel mutation, and underline the importance



b GPI anchor presentations

Finding	PIGA*	PIGQ	PIGL	PIGM	PIGN	PIGO	PIGT*	PIGV	PGAP1	PGAP2	PGAP3	PIGW
Multiple Congenital Anomalies	X		X		X	X	X	X		X		X
Macrocephaly					X		X					
Abnormal MRI		X					X		X		X	
Coloboma			X									
Down gaze Ophthalmoplegia							X					
Hearing Impairment								X		X		
Developmental delay/ Intellectual disability		X	X		X		X	X	X	X	X	X
Restrictive Cardiomyopathy/Congenital heart defects			X				X			X		
Abnormal Lung anatomy							X					
Inverted nipples							X					
Hypotonia	X				X		X		X	X	X	
Seizures	X		X	X	X	X	X	X	X		X	X
Hyperphosphatasia		X	X			X		X		X	X	X
Nephrocalcinosis							X					
FTT/Feeding problems					X							
Thrombosis	X			X								
Hemolytic Anemia	X											
Frequent Infections					X							
Ichthyosis			X									
Hypoplastic nails						X		X		X		
Skeletal abnormalities	X						X					
GU Anomalies/Renal cysts							X					

Fig. 1. GPI anchor pathway and phenotypic findings of GPI anchor disorders. (a) The 11 steps of mammalian GPI anchor biosynthesis and protein attachment. The genes associated with each step are labeled. Underlined genes have been associated with congenital human disease. Note *PIGT* in step 10. (b) Summary of the clinical features described in cases of congenital GPI anchor biosynthesis disorders. The most common features are highlighted in blue. * Note that somatic mutations in *PIGA* and *PIGT* in hematopoietic stem cells can cause paroxysmal nocturnal hemoglobinuria manifesting with hemolytic anemia, bone marrow failure, thrombosis, and smooth muscle dystonia. This is distinct from the clinical entity caused by germline mutations in these two genes. Additionally, acquired mutations in the GPI transamidase complex subunits have been implicated in human cancers.