PIGT-CDG, a disorder of glycosylphosphatidylinositol anchors: description of fourteen novel patients and expansion of the clinical characteristics.

Allan Bayat [1γ*], Alexej Knaus [2,3,4 *], Annika Wollenberg Juul [1 *], Usha Kini [5], Elena Gardella [6,10], Agnieszka Charzewska [7], Emma Clement [8], Helle Hjalgrim [6,9,10], Dorota Hoffman-Zacharska [7], Denise Horn[2], Rachel Horton [11], Jane A Hurst [8], Dragana Josifova [12], Peter Krawitz [2,3,4], Line HG Larsen[9], Karine Lascelles [13], Ewa Obersztyn[7], Deb K Pal [14], Manuela Pendziwiat [15], Mina Ryten[12,16], Julie Vogt [17], Yvonne Weber [18], Ingo Helbig [16,19], Rikke S. Møller[9,10]

[1] Department of Pediatrics, University Hospital of Hvidovre, Hvidovre, Denmark.

[2] Institute for Medical Genetics and Human Genetics, Charité Universitätsmedizin Berlin, Berlin, Germany.

[3] Berlin-Brandenburg School for Regenerative Therapies (BSRT), Charité, Germany.

[4] Institute for Genomic Statistics and Bioinformatics, Rheinische Friedrich-Wilhelms-University of Bonn, Germany

[5] Department of Clinical Genetics, Oxford University Hospitals NHS Trust, Oxford, United Kingdom.

[6] Danish Epilepsy Centre, Dianalund, Denmark.

[7]Institute of Mother and Child, Department of Medical Genetics, Kasprzaka 17A, 01-211 Warsaw, Poland

[8] North East Thames Regional Genetics Service, Great Ormond Street Hospital for Children, London WC1N 3JH, United Kingdom.

[9] Amplexa Genetics, Odense, Denmark.

[10] Institute for Regional Health Services Research, University of Southern Denmark, Odense, Denmark.

[11] To be investigated

[12] The Guy's and St. Thomas NHS Trust, Clinical Genetics Department, Great Maze Pond, London SE1 9RT, United Kingdom.

[13] Department of Neuroscience, Evelina London Children's Hospital, St Thomas' Hospital, Westminster Bridge Road, London SE1 7EH, United Kingdom.

[14] Department of Basic and Clinical Neuroscience, Institute of Psychiatry, Psychology and Neuroscience, King's College London, London , United Kingdom.

[15] Department of Neuropediatrics University Medical Center Schleswig-Holstein Christian Albrechts University Kiel, Germany.

[16] Reta Lila Weston Research Laboratories, Department of Molecular Neuroscience, University College London, Institute of Neurology, London, United Kingdom.

[17] Clinical Genetics Unit, Birmingham Women's Hospital, Birmingham, United Kingdom.

[18] Department of Neurology and Epileptology, Hertie Institute of Clinical Brain Research, University of Tübingen, Tübingen, Germany.

[19] Division of Neurology, The Children's Hospital of Philadelphia, Philadelphia, PA, United States.

[γ] **Corresponding author**

*** Shared first authors**

Abstract

PIGT-CDG, an autosomal recessive syndromic form of a glycosylphosphatidylinositol biosynthesis defect (GPIBD) with intellectual disability, has so far only been described in seven independent families and all but one patient presented with an epileptic encephalopathy. PIGT encodes phosphatidylinositol-glycan biosynthesis class T, a subunit of the heteropentameric transamidase complex that facilitates the transfer of proteins to the GPI anchor. The GPI anchor links proteins to the cell membrane in all tissues.

We describe fourteen novel patients from nine unrelated families with homozygous (NM_015937.5: c.709G>C, p.Glu237Gln and c.550G>A; p.Glu184Lys) or compound heterozygous (c.1472T>A, p.L491H; c.1484+2T>A and c.494-2A; c.1582G>A; p.Val528Met) mutations in *PIGT*. All patients had hypotonia, severe global developmental delay and epilepsy. Epilepsy onset ranged from first day of life to 2 years of age, and the severity of the seizure disorder varied from treatable seizures to severe neonatal onset epileptic encephalopathies. Furthermore, nine patients presented with ophthalmologic anomalies and four patients had skeletal anomalies such as long slender bones, scoliosis, pectus excavatum and dysplastic distal phalanxes. Congenital fractures were only found in one patient which is a feature not described previously. In addition, abnormal body-hair distribution was observed in eight out of the fourteen patients. In this study we provide a detailed description of the phenotype of PIGT-CDG. Furthermore we investigate the onset and severity of epilepsy determined by the different genetic subtypes and our results emphasize that GPI anchor-related congenital disorders of glycosylation (CDGs) should be considered in both subjects with a developmental delay plus epilepsy and in patients with an epileptic encephalopathy 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 cases reaffirm that the use of genetic testing is essential for diagnosis in this group of disorders.

Key Words

PIGT-CDG, Congenital disorder of glycosylation, Transamidase, GPI, Exome.

Key Point Box (…)

1. Introduction

Glycosylphosphatidylinositol (GPI) is a glycolipid that is synthetized and transferred to proteins in the membrane of the endoplasmic reticulum[1]. Biogenesis of GPI anchored proteins (GPI-APs) is a conserved post-translational mechanism in eukaryotes and is important for attaching these proteins to the cell membrane, for protein sorting, trafficking, and dynamics[2]. It also plays an essential role in embryogenesis, immune responses and neurogenesis[3-6]. GPI synthesis and GPI- anchored protein (GPI-AP) modification are mediated by at least 29 genes and loss-of-function mutations in 16 of these genes may lead to neurological impairments including seizures, intellectual disability (ID), developmental delay (DD) and multiple congenital anomalies[7-22].

PIGT [MIM, 610272] encodes phosphatidylinositol-glycan biosynthesis class T, which is a subunit of the heteropentameric GPI transamidase complex that facilitates the attachment of GPI anchors to proteins[23;24]. In 2013 Kvarnung et al. reported a homozygous *PIGT* mutation in four patients from a consanguineous Turkish family with Multiple Congenital Anomalies-Hypotonia-Seizures Syndrome 3 (MCAHS3) [MIM, 615398][10]. Subsequently compound heterozygous mutations in *PIGT* were identified in six unrelated families with a similar clinical presentation[25-28]. Recently Skauli et al. presented two somalic brothers with typical features of MCAHS3, but in addition, pyramidal tract neurological signs[27]. The predominant presentation for the 12 patients described so far is that of an epileptic encephalopathy including profound ID, hypotonia, intractable seizures, cortical visual impairment, and nystagmus and/or strabismus.

Here we describe fourteen additional patients from nine unrelated families with *PIGT* mutations presenting with seizures, ID, and congenital anomalies.

2. Methods

2.1. Variant filtering and analysis.

We screened a cohort of 793 patients with various childhood-onset epilepsies for mutations in the *PIGT* gene using a previously described next generation sequencing panel[29]. The panel included targeted capture of all exons and at least 10 base pairs of flanking intronic sequence of *PIGT*. Genomic DNA from blood was extracted with standard methods, and a next-generation sequencing panel screening method was applied that was based on the Ion Torrent PGM platform. Ion AmpliSeq (kit version 2.0) or Sureselect library building methods were used with subsequent clonal amplification and enrichment on an Ion OneTouch 2 system with the Ion PGM Template OT2 200 Kit, followed by sequencing on the Ion Torrent system with the Ion PGM 200 Sequencing Kit. Variants resulting from the gene panel sequencing were reduced by allele frequency ≥2% and SNPs observed in more than 2 samples for each analyzed sample batch were filtered out. Genetic non-synonymous/splice site variants were evaluated through database searches such as dbSNP, Exome Variant Server, the Exome Aggregation Consortium database (ExAC), and HGMD Professional. One homozygous missense mutation (NM_015937.5: c.709G>C, p.Glu237Gln) that leads to an exchange of a highly conserved amino acid was predicted by SIFT, MutationTaster, and PolyPhen-2 to be pathogenic. 16 heterozygos carriers were identified in the gnomAD database of this variant and Sanger sequencing confirmed biparental inheritance.

In parallel, we ascertained additional, previously unreported *PIGT* patients through epilepsy and genetic centers in Europe and the United States. The probands and their families underwent detailed clinical examinations, review of the medical files, MRIs, and EEG investigations.

2.2. Standard protocol approvals, registrations, and patient consents.

The study was approved by the local ethics committees. All probands or, in case of minors, their parents or legal guardians gave informed consent. The clinical information has been collected from hospital journals of the patients and their family members.

3. Results

In our screening cohort of 793 patients we identified one patient with a likely pathogenic homozygous *PIGT* mutation. Furthermore, we recruited twelve additional patients with *PIGT* mutations from other research and diagnostic programs. The pedigrees of the twelve newly identified individuals with homozygous or compound heterozygous *PIGT* mutations are shown in figure 1. An overview of the clinical, MRI and genetic features of the fourteen novel patients and of the twelve previously published PIGT-CDG cases is provided in Table 1.

3.1. Phenotypic analysis.

All the fourteen patients suffered from ID/DD, either profound (patients 1-3, 7-10, and 12-14) or severe (patients 4-6 and 11), and from epilepsy with neonatal / infancy onset. The median age at seizure onset was six months (range 1 day – 18 months). All the patients had myoclonic and/or tonic seizures, often with apnea, sometimes evolving to bilateral tonic-clonic seizures. Subtle focal seizures were also described (patients 1-3, 9-10 and 12). Fever-associated seizures were reported in all patients.

The symptoms varied from profound intellectual disability and severe drug resistant, neonatal or infantile onset epilepsy (patients 1-3, 7-10 and 12-14) with recurrent episodes of convulsive status epilepticus (patients 1-2), to severe intellectual disability and treatable epilepsy with later age at onset (8-18 months) (patients 4-6 and 11).

The interictal EEG was available in 13 out of 14 patients (no data available for patient 8) and was severely abnormal in 12 of them (see table 1). One single patient had a normal EEG at epilepsy onset (patient 9) and no further EEG controls were available. In patients (1-3, 13, 14) with neonatal epilepsy onset, the EEG showed initially a burst suppression pattern and at follow up (patients 1-2) was characterized by background slowing/destructuration, with frequent multifocal spike and slow waves. Patients (7, 9, 10, 12) with early infantile epilepsy onset and severe drug resistant epilepsy might have a normal EEG at epilepsy onset (patients 9-10) and developed background slowing, focal theta-delta activity in the fronto-temporal regions or in the posterior quadrants, and frequent multifocal spike and slow waves at follow up. The EEG of patients (4-6 and 11) with treatable epilepsy with later age at onset showed only background slowing (patient 11) or background slowing with sporadic (patients 4-6) epileptiform abnormalities in the fronto-temporal regions.

A cortical visual impairment was diagnosed in six patients (patients 1, 2, 5, 6, 8 and 12). None of the patients were diagnosed with hearing loss, and they all exhibited normal alkaline phosphatase, plasma calcium, plasma phosphate and parathyroid hormone values. Congenital heart defects were identified in two individuals and both resolved spontaneously: a persistens foramin ovale (PFO) in one patient (patient 9) and the combination of an PFO and an atrial septal aneurysm in another (patient 7).

Patients 2 and 8 both died due to a pneumonia at respectively 11 and 15 months of age. Patient 3 died at six months of age following a cardiac arrest and patient 4 was operated on at the age of two years for a suprasellar adamantinomatous craniopharyngioma.

Our novel cases shared similar craniofacial features that included a high forehead with bitemporal narrowing, a depressed nasal bridge, a short anteverted nose, distinct philtrum, full cheeks and an open mouth consistent with general hypotonia. Scalp hair, eyebrows, and eyelashes were sparse in eight of the fourteen patients (patient 1, 2, 4-8 and 12) (figure 2 and supplementary figure S1). Since clinical pictures were not available it has not been possible to evaluated whether or not the remaining patients shared this feature.

Cerebral MRI was available in all fourteen patients and was abnormal in all but two patients (patients 6 and 13). MRI abnormalities included prominent cortical and subcortical volume loss with brainstem atrophy (patients 1, 2, 4, 9, and 11-12), white matter immaturity (patients 1, 2, 3), hypoplastic cerebellum (patients 4-6, 9 and 12) and an abnormal corpus callosum (patients 1 and 2) (table 1).

A detailed clinical description of all fourteen patients is available in the supplementary file S-1.

3.2. Mutational analysis

We identified twelve different mutations including three novel mutations. One of these three mutations was a missense mutation and two of the novel mutations affected a splice site. Concerning the inheritance mode, in all families the mutation was inherited in a recessive fashion from unaffected parents. All mutations were predicted to be damaging by 1 or 2 prediction tools (Polyphen2 or SIFT; supplementary file S-2). The protein positions of the different *PIGT* mutations are shown in figure 3. The mutation c.709G>C was found in a girl from Asian heritage (patient 3) and in two Bangladeshi siblings (patient 13 and 14) while the mutation was found c.1582G>A in

four Polish patients (patient 4-6 and 11). While c.709G>C has been previously published in an Afghanistani male[30] the variant c.1582G>A has not previously been published. This leads us to conclude that c.1582G>A seems to be a European variant while c.709G>A is a Middle-Eastern or Asian variant. The variant c.1582G>A was also found in the four patients classified as having a severe developmental delay with a treatable epilepsy (patients 4-6 and 11). Since this variant has also previously been described in an affected female with a developmental delay and epilepsy[27] (Table 1). We contacted Dr. Kini Usha and Dr. Rachel Horton who informed us that this patient had a global developmental delay with seizures, and that she became seizure-free with a combination of antiepileptic drugs. This suggests that the missense variant c.1582G>A could be associated with a milder phenotype.

3.3. Overall mutational landscape

Mining the available literature and databases, we were able to identify 12 previously reported *PIGT* cases (Table 1)[10;25-27;30]. From the complete dataset of 24 patients with *PIGT* mutations, 14 different mutation sites emerged:
c.250G>T (n=2); c.494-2A, (n=1); c.547A>C (n=2); c.550G>A (n=2); c.709G>C (n=4); c.918dupC (n=1); c.1079G>T (n=1); c.1096G>T (n=1); c.1342C>T (n=2); c.1472T>A (n=2); 1484+2T>A (n=2); c.1582G>A (n=6); c.1724_1725insC (n=1); c.1730dupC (n=1)

4. Discussion

So far only 12 patients from seven different families have been described with a GPI anchor deficiency due to recessive *PIGT* mutations[10;25-27;30] and all but one patient[27] presented with an epileptic encephalopathy. We describe an additional fourteen patients from nine unrelated families. The patients show a broad clinical spectrum and share several common features (Table 1). The neurological findings include a severe/profound ID, epilepsy with variable onset and severity and a severe congenital hypotonia. The symptoms varied from profound intellectual disability and severe drug resistant epilepsy with neonatal-infantile onset epilepsy (9/13 patients), to severe intellectual disability and treatable epilepsy with later age at onset (8-24 months) (4/13 patients). Based on previously published and our novel findings we conclude that the missense variant c.1582G>A seems to be associated with a milder phenotype. Ophthalmological features

including nystagmus and/or strabismus and a cortical visual impairment were also observed in nine out of fourteen patients (Table 1).

The bone and endocrine features in our patients differed from the initial five patients reported by Nakashima et al.[28] and Kvarnung et al.[10], however their features overlapped with the four patients presented by Lam et al.[25] and Skauli et al.[27]. Our patients exhibited normal alkaline phosphatase, plasma calcium, plasma phosphate and parathyroid hormone values. Bone age was only available in patient 1 and was normal. Except of the brothers presented by Skauli et al.[27] all previously described patients had reduced bone mineralization and scoliosis, features that could arise secondary to their neurologic complications. X-rays were only available in three of our patients (patient 1 and 2) and only two patients (patient 1 and 13) underwent a systematic skeletal survey. Scoliosis was found in one of our patients (patient 5), long bones with reduced mineralization were found in three patients (patient 1, 2 and 13) and in patient 13 the reduced mineralization was present already at birth (pictures not shown). Dysplastic distal phalanges were found in one patient (patient 1) (pictures not shown). Congenital fractures located at the right humeral, the right femoral diaphyses and along the midshaft of the left humerus and also subtle contour abnormality at the anterior ends of the left seventh and eighth ribs were only found in one patient (patient 13) which is a feature not described previously (pictures not shown). So far craniosynostosis has only been described in two patients by Kvarnung et al.[10] however a metopic and a saggital ridge was found in one of our patients (patient 9) and did not require surgical intervention.

One of the affected sibpairs had abnormal dentition (patient 1 and 2), similar to the patients described by Kvarnung et al.[10] which has previously been reported in one additional family. This finding could be unrelated to *PIGT*, could reflect a genotype phenotype effect, or could arise from differences in genetic background. Five patients (patient 1-2,5-6 and 12) also exhibited significant joint hypermobility which was also reported by Lam et al.[25].

Ten of the published *PIGT* deficient patients had an MRI done and in all patients cortical and cerebellar atrophy was evident. Kvarnung et al.[10] presented two patients in whom MRI at the age of 2.7 years demonstrated global atrophy with predominant vermis and cerebellar atrophy.

The patient described by Nakashima et al.[28] demonstrated progressive atrophy of the cerebral hemisphere, cerebellum and brainstem at the age of three years. The two brothers published by Skauli et al.[27] were examined respectively at 14 months and 2.8 years of age. Both demonstrated cortical atrophy and cerebellar atrophy, primarily affecting the vermis. Finally Lam et al.[25] presented MRI observations supporting that atrophy in the cerebellum starts earlier and proceeds more rapidly than atrophy elsewhere in the brain. So far published data suggests that the disease preferentially affects the cerebellum. In contrast to previously reported patients four of our patients (patients 1-3, 6-8, 10 and 14) showed no signs of cerebellar atrophy (Figure 4, pictures not available for patient 3 and 6). Patients 2, 3, 6 and 14 had the MRI done at a very early age, which could perhaps explain why the cerebellar atrophy was not yet evident, however patient 1 had an MRI done at three days (Figure 4A-B), three months (Figure 4C-D), nine month (Figure 4E-F) and four years (Figure 4G-H) of age. Based on the previously published MRI findings in PIGT patients we would have expected a cerebellar hypoplasia to be detectable at four years of age. Although cortical atrophy was evident we detected no signs of cerebellar atrophy in patient 1. This further expands the clinical spectrum of *PIGT*-CDG.

Congenital heart defects are were indentified in only two individuals and both resolved spontaneously: a persistent foramin ovale in one and a combined persistent foramin ovale and an atrial septum aneurysm in another.

The majority of the published patients had onset of febrile-induced seizures between four and six months of age followed by unprovoked and poorly controlled seizures. In the article by Kvarnung et al.[10] the age at onset of seizures was not specified. We therefore contacted Dr. Kvarnung who informed us that onset of febrile-induced seizures in their patients was around 12-18 months of age. In our cohort the onset of epileptic seizures did also include the neonatal period leading us to conclude that *PIGT*-CDG should be considered in patients with hypotonia, severe global developmental delay, and neonatal seizures. EEG recordings were severly abnormal in patients with neonatal onset epilepsy, and might also be normal or almost normal in the other patients early in life. At follow up, the most common EEG features were the slowing of the background activity, often associated with focal slowing and multifocal epileptiform abnormalities, predominant in the fronto-

temporal regions. The severity of the EEG abnormalities seemed to correlate with the severity of the phenotypes.

Our novel cases shared similar facial features with previous patients leading us to conclude that there is a common facial gestalt that includes a high forehead with bitemporal narrowing, a depressed nasal bridge, a short anteverted nose, distinct philtrum, full cheeks and an open mouth consistent with general hypotonia. Scalp hair, eyebrows, and eyelashes were sparse in eight of fourteen patients (patient 1, 2, 4-8 and 12). After reviewing the pictures of the previously described patients we believe that the patients V-1, V-2, V-4 described by Kvarnung et al.[10] and the siblings reported by Lam et al.[25] all show signs of sparse scalp hair including a high and thin anterior hairline. Abnormal distribution of hair on the body has not been described as a feature of any of the GPI anchor disorders[25;31] and the hypotrichosis as a clinical feature has not been previously described in patients with *PIGT*-CDGs. We furthermore identified hypertrichosis in patient 1 which is a possible novel feature in this disease. Therefore abnormal body-hair distribution including hypotrichosis might be a recurrent but overlooked feature of *PIGT*-CDGs.

There are no current FDA approved therapies for GPI anchor disorders, but the progressive nature of *PIGT*-CDG is attractive for therapies that slow or halt the neurologic deterioration[37]. Identification of additional *PIGT*-CDG patients should further define the clinical spectrum and assist in developing diagnostic criteria.

Conflict of interest

The authors declare no conflict of interest.

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Legends

Figure 1:

Two-generation pedigree of the nine affected families. Clinically affected family members are shown as shaded squares and circles. P - patient.

Figure 2:

Clinical pictures of patient 1 at 3½ years (A), patient 2 at 11 months (B), patient 4 at 2½ years (C), patient 5 at 7 years (D), and patient 6 at 19 months (E). Sparse scalp hair, bitemporal narrowing, a high forehead, a distinct philtrum, a tented mouth, full cheeks, a depressed nasal bridge, and a short nose in all patients is notable. Eyebrows were either straight (A, B, C) or arched (D, E).

Figure 3:

A: Locations of mutations in the PIGT gene. Novel mutations described in this study are represented in red with the patient ID in superscript. Known pathogenic mutations are shown with corresponding reference numbers.
B: Conservationanalysis. Amino acid comparison of PIGT of different species. Mutations affect highly conserved amino acids (black boxes).

Figure 4.

MRI of patient 1 and patient 2 showing sagital T1W and axial T1W. MRI exams of patient 1 were performed at three days (4A-B), three months (4C-D), nine month (4E-F) and four years of age (4G-H). MRI of patient 2 were performed only once at thirteen days of age (4I-J).
MRI at three days (4A-B) shows delayed myelination, including insufficient myelinated PLIC (posterior limb internal capsule), normal sulcations and no atrophy, at three month (4C-D) atrophy

of the splenium of the corpus callosum without growth, supratentorial atrophy of white matter, delayed myelination, including unmyelinated ALIC (anterior limb of the internal capsule) and only PLIC (posterior limb internal capsule) fully myelinated, at nine months (4E-F) somewhat progressed myelination, still delayed, resembling a three month old on T1W with a little, but still insufficient growth of corpus callosum and supratentorial atrophy of white matter, at four years (4G-H) clearly progressed myelination, but still not completed, supratentorial atrophy of white matter and corpus callosum. No atrophy of the cerebellum.

Magnetic Resonance Spectroscopy (TE:135 ms) at 3T performed in Patient 1 , nine months old , from right parietal white matter and bioccipital grey matter showed near normal N-acetylaspartate/choline, choline/creatine-ratios, no lactate or other unusal metabolites.

T1W sagittal (4I) and axial (4J) brain MRI exam of patient 2 taken at thirteen days of age revealed global delayed myelination, including insufficient myelinated PLIC (shown), normal sulcation and no atrophy.

Table 1:

Clinical features of fourteen novel PIGT-CDG cases and the clinical features in the twelve previously published PIGT-CDG cases

Supplementary figure S1:

Sparse scalp hair (A - C), hypertrichosis of the legs (D) and back (E), and delayed tooth eruption with small and widely spaced teeth (F) of patient 1.

Supplementary figure S2:

Patient 2 presented with a pectus excavatum (A), short and friable nails and slightly clinidactyly (B and C).

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