Clinical Case Report

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Case report of a child bearing a novel deleterious splicing variant in *PIGT*

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Abstract

Rationale: Trio family-based whole exome sequencing (WES) is a powerful tool in the diagnosis of rare neurodevelopmental diseases, even in patients with the unclear diagnosis. There have been previous reports of variants in the *phosphatidylinositol glycan anchor biosynthesis class T* (*PIGT*) gene associated with multiple congenital anomalies, with a total of 14 affected individuals across 8 families.

Patient concerns: An 18-month-old boy of Greek ancestry presented with global developmental delay, generalized tonic-clonic seizures, hypotonia, renal cysts, esotropia, bilateral undescended testes, bilateral vesicoureteric reflux, marked cardiac dextroposition, bilateral talipes equinovarus, and dysmorphic features.

Diagnosis: WES revealed 2 compound heterozygous variants in the *PIGT* gene, c.[494-2A>G]; [547A>C]/p.[Asp122Glyfs*35]; [Thr183Pro]. The splicing mutation was demonstrated to lead to the skipping of exon 4.

Interventions: Seizures, infections, and other main symptoms were treated.

Outcomes: The patient died at 2 years of age before the molecular diagnosis was achieved. Genetic counseling has been offered to the family.

Lessons: Most of the clinical features of the patient are in agreement with the previously described *PIGT* cases corroborating the usefulness of WES as a diagnostic tool.

Abbreviations: CNAG = National Centre of Genomic Analysis, Cp = crossing point cycle, CV = coefficient of variation, E = efficiency, GPI = glycosylphosphatidylinositol, MCAHS3 = multiple congenital anomalies-hypotonia-seizures syndrome 3, MRI = magnetic resonance imaging, PIGT = phosphatidylinositol glycan anchor biosynthesis class T, WES = whole exome sequencing.

Keywords: developmental delay, epilepsy, functional studies, hypotonia, *PIGT*, splicing

1. Introduction

Glycosylphosphatidylinositol (GPI) anchoring is a highly conserved process that enables proteins to attach to the cell surface membrane as part of the process of post-translational modification by glycosylation.[1,2] Close to 30 genes have been identified that are involved in the biosynthesis of the GPI anchors and proteins they attach to, and variants in more than a dozen of these genes have been associated with human disease.[1–4] Individuals affected by variants in component genes of this pathway have variable clinical phenotypes, with features including seizures, global developmental delay, and multiple congenital anomalies.[1,2,4,5]

Since the introduction of massively parallel sequencing technology, there has been an increase in the discovery of individuals with GPI pathway mutations. In particular, a total of 14 individuals have currently been reported in the literature with biallelic *phosphatidylinositol glycan anchor biosynthesis class T* variants (*PIGT*, MIM *610272). Phenotypic features have included difficulty feeding, hypotonia, skeletal anomalies, renal cysts, seizures, esotropia, severe developmental delay, and dysmorphic features.[1,5–10] The *PIGT* gene encodes a subunit of the GPI transamidase, which catalyzes the transfer of fully assembled GPI units to proteins in the endoplasmic reticulum.[11]

Here, we report a male infant with multiple congenital anomalies-hypotonia-seizures syndrome 3 (MCAHS3, OMIM # 615398), as a result of autosomal recessive inheritance of variants in the *PIGT* gene detected by whole exome sequencing (WES). One variant, c.547A>C, has been reported previously in 4 patients from a Turkish family[6]; the other, c.494-2A>G, is novel.

2. Material and methods

2.1. Patient data

Clinical data and samples were obtained from Sydney Children's Hospital, Randwick and the child's parents gave written

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informed consent to participate in this study, including explicit permission to share clinical and identifying information, even in online open-access journals. Institutional ethics committee approval was granted by the Prince of Wales Hospital Campus Human Research Ethics Committee, Sydney, Australia (HREC ref no 13/094) and the Ethics Committee of the Universitat de Barcelona (IRB00003099) and all methods were performed in accordance with the relevant guidelines and regulations.

Genomic DNA was obtained from the parents' peripheral blood and the proband's fibroblasts.

2.2. WES and molecular analyses

WES of the proband and his parents were performed in the National Centre of Genomic Analysis (CNAG; Barcelona, Spain) using the Illumina HiSeq-2000 platform. Exome capture was performed with Agilent SureSelect v5 (Agilent, CA). The samples were sequenced at coverage of 140×. The data were analyzed as described elsewhere.[13] The results were then filtered under de novo dominance and recessive hypotheses. Variants with a minimum allele frequency above 0.001 (under the dominant) and above 0.01 (for recessive) in the common population (according to ExAC and 1000 genomes) were excluded. Variants in genes included in DDG2P (the development disorder genotype–phenotype database[14,15]), and covered by at least 10 reads, were prioritized for validation (it should be noted that those who carried out the original DECIPHER analysis and collection of the data bear no responsibility for the further analysis or interpretation of it).

The mean coverage was of 142.49, 179.29, and 166.43 reads for the patient, father, and mother, respectively, and 91.7% to 97.7% of the target region was covered with at least 10 reads (C10). A total of 4 variants were selected for validation by Sanger sequencing. Primer sequences and polymerase chain reaction (PCR) conditions are available on request. PCR reaction, purification, and sequencing were performed as described previously.[16]

2.3. Cell culture

Patient's fibroblasts and those of 6 controls were cultured in DMEM supplemented with 10% fetal bovine serum (Gibco, Life Technologies, Carlsbad, CA) and 1% streptomycin–penicillin (Gibco, Life Technologies) and were maintained at 37°C and 5% of CO_2. Cycloheximide (Sigma-Aldrich, St. Louis, MO) treatment was applied in a concentration of 1 mg/ml in DMEM during 6 hours.

2.4. PIGT transcript analysis

RNA was extracted from confluent fibroblasts plates with the High Pure RNA Isolation Kit (Roche, Basel, Switzerland). Integrity and purity of the RNA were tested using a 1% agarose gel and 260/230 and 260/280 absorbance ratios using an ND-1000 Spectrophotometer (Nanodrop Technologies Inc; Thermo Fisher Scientific, Waltham, MA). The High-capacity complementary DNA (cDNA) Reverse Transcription kit (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA) was used to retrotranscribe up to 2 ug of RNA. Primers cPIGT-spl-F (5'-GCTGGGTAGGCGGAAGTAG-3') and cPIGT-spl-R (5'-TGGTAGCTGGTGTGGAACAA-3') were used to amplify the spliced region. The PCR fragments were separated by 2% agarose gel electrophoresis. The illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare Life Sciences, Chicago, IL) was used to isolate each specific band, the obtained purified product was manually sequenced.

2.5. Real-time PCRs

Real-time PCRs were performed in triplicate using Universal Probes Library probes (Roche), gene-specific primers and Universal Probes Mastermix (Roche), with the exception of PPIA assay (Applied Biosystems) (Supplemental Table S2, http://links.lww.com/MD/C825). The qPCR reactions were performed on a final volume of 15 μl with 10 ng of cDNA. The amplification was done using the thermocycler LightCycler 480 (Roche). Expression levels of 6 putative reference genes were tested in control and the patient's fibroblast and *GAPDH* and *PPIA* were chosen as they were the genes with the lowest coefficient of variation (<0.3% CV). The relative transcription level was calculated with the crossing point cycle (Cp) calculation using the LightCycler 480 Software (release 1.5.0) (Roche). For every assay, the efficiency (E) of the reaction was calculated from a 6 points standard curve. The intra-assay CV of all the assays at the working conditions used was lower than 1% and Cp standard deviation was smaller than 0.3.

3. Case report

3.1. Clinical report

The patient is the first child to nonconsanguineous, healthy parents of Greek ancestry. At the time of the patient's birth his mother was 27 years of age, and his father 35. The patient was first noted to have a possible bladder outlet obstruction at a 20-week antenatal morphology scan, and the pregnancy was then further complicated by polyhydramnios and the finding of shortened long bones from week 30. At 34 weeks of gestation, he was noted to have left hydronephrosis, and an amniocentesis was performed at this time. Array comparative genomic hybridization showed a normal male pattern. The patient was born via elective Caesarean section at 38 + 3 weeks of gestation. At birth, his length was 43 cm (below 3rd percentile), weight 3.21 kg (25th percentile), and head circumference 36 cm (25th percentile). Postnatal ultrasound confirmed obstructive nephropathy with renal impairment.

The patient was transferred to intensive care unit on day 4 of life with acute bladder outlet obstruction, bilateral hydronephrosis, and evidence of increasing creatinine. During this period, a head ultrasound was normal, and a spinal ultrasound revealed a tethered cord. Spinal and brain magnetic resonance imaging (MRI) were normal at 2 weeks and 5 months of age. Echocardiogram was normal apart from patent foramen ovale.

Multifocal seizures developed at 5 months, often progressing to bilateral convulsive seizures. Dysmorphic features (Fig. 1 and Table 1), global developmental delay, hypotonia, esotropia, delayed visual maturation, hypermetropia, undescended testes, bilateral talipes equinovarus, and bilateral inguinal hernias were also noted. Repeat MRI of the brain and spine were normal and renal ultrasound showed bilateral grade 5 vesicoureteric reflux, and bilateral renal cysts.

He was feeding poorly and had slow growth. Developmentally, he made some early gains but had regression from 3 months followed by a period of developmental stagnation, and some possible further regression from the age of 1 year and 9 months.

On examination, the patient had a high palate with lateral palatine ridging, plagiocephaly was open-mouthed at rest, and had low-set, posteriorly rotated ears (Fig. 1). He had pectus

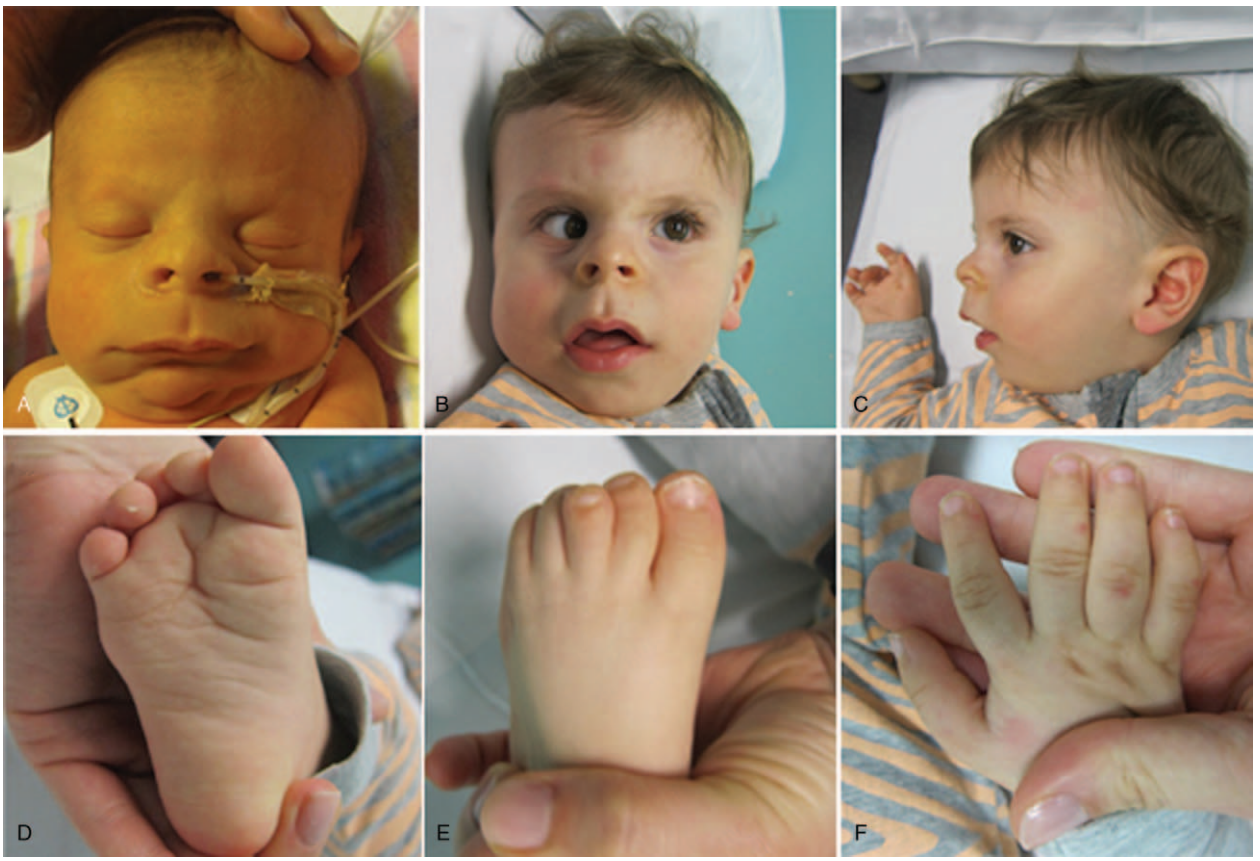

Figure 1. Most relevant features of the current patient. (A) Facies at 6 days of life. (B and C) Facies at 18 mo old. Note cupid's bow lips, full lower lip, posteriorly rotated ears, and a prominent premaxilla. (C) Deep plantar creases. (E) Small feet, third toes proximally inserted and bilateral 2-3 toe syndactyly. (F) Hand, with broad fingers and relatively large palms.

excavatum, posterior right shoulder dimple and elbow dimples, right single transverse palmar crease, broad fingers, and relatively large palms. The patient presented with bilateral 2-3 toe syndactyly and rhizomelic upper limb shortening, however, his father also had these features, suggesting that they may be unrelated to the *PIGT* variants. The patient's feet were small with the third toes proximally inserted, and he had pudgy soles with deep longitudinal creases bilaterally. Alkaline phosphatase levels were normal.

Seizures were frequent, often prolonged and intractable. He made minimal developmental progress, only achieving the ability to reach, roll, coo, and laugh. He frequently suffered aspiration pneumonia. He died aged 2 years and 3 months of respiratory failure following an adenovirus infection.

3.2. Molecular findings

We performed whole-exome sequencing on the patient and parents' genomic DNA and found 2 heterozygous mutations in the *PIGT* gene (OMIM *610272; ENSG00000124155): the c.547A>C (p.Thr183Pro), inherited from his father and the c.494-2A>G, affecting the canonical splice acceptor site of intron 3, of maternal origin. The p.Thr183Pro was previously identified in a Turkish family[6] while the c.494-2A>G was novel. The effect of this mutation was studied in the patient's fibroblasts and the skipping of *PIGT* exon 4 was verified by Sanger sequencing (Fig. 2). In control fibroblasts, 2 major *PIGT* RNA isoforms were detected, 1 including all exons (ENST00000279036) and the other lacking exons 2 and 3 (ENST00000279035). A third minor band corresponding to the isoform lacking exon 3 (ENST00000543458) was also observed. In the patient's fibroblasts, these 3 isoforms were detected, together with 3 additional ones, lacking exon 4. The mutant isoforms were moderately affected by nonsense-mediated decay, as observed in the cycloheximide-treated cells, in which the bands corresponding to the fragments without exon 4 were more intense (band 2 and 4, in Fig. 2).

To further characterize the effect of the *PIGT* variants, we analyzed *FOXC2* expression levels, previously reported to be positively correlated with those of *PIGT*.[12] In the patient's fibroblasts, we observed an increase in the expression of the *FOXC2* gene when compared with the average of the fibroblasts of 6 control individuals (Supplemental Fig. 1, http://links.lww.com/MD/C825), although the results were not conclusive.

In the WES analysis we also identified a de novo variant in the *FBN2* gene and 2 missense mutations in the *ATP10A* gene (Supplemental Table 1, http://links.lww.com/MD/C825). These changes have been interpreted as variants of unknown significance.

4. Discussion

There is an emerging syndrome associated with variants in *PIGT*. Besides global developmental delay, seizures, hypotonia, plagiocephaly, strabismus/nystagmus, which are present in all the cases for which data are available,[1,5–9] over half of the reported

Table 1

Description of clinical features observed in patients with *PIGT* variations.

	Kvarnung 2013[6]				Nakashima 2014[7]	Lam 2015[1]		Skauli 2016[8]		Pagnamenta 2017[5]			Kohashi 2018[9]	Yang 2018[10]	Current patient		
Features	**V-1**	**V-2**	**V-3**	**V-4**	**1**	**Sib 1**	**Sib 2**	**Sib1**	**Sib 2**	**1**	**2a**	**2b**	**1**	**1**	**1**	**Overall**	**%**
Sex	F	F	F	F	F	F	M	M	M	F	M	M	M	M	M	7F: 8M	88%
Consanguinity	Yes	No	No	Yes	No	Yes	No	No	No	3 out of 9 families	33%						
Neurological																	
Global developmental delay/intellectual disability	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15/15	100%
Seizures	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	15/15	100%
Cortical/cerebellar atrophy	+[1]	−	+	+	+	+	+	+	+	+	+	+	+	+	−	13/15	87%
Hypotonia	+	+	+	+	+	+	+	+	+	na	na	na	+	+	+	12/12	100%
Hearing loss	−	−	−	−	na	+	+	−	−	na	na	na	na	−	−	2/10	20%
Cortical visual impairment	+	+	+	+	+	+	+	+	+	na	na	na	na	na	+	10/15	67%
Head																	
Macrocephaly	+	+	−	−	−	−	−	−	+[3]	−	−	−	na	−	−	3/14	21%
Microcephaly	−	−	−	−	−	−	−	−	−	−	+	+	na	−	−	2/14	14%
Craniosynostosis	+	+	na	na	−	na	na	−	na	na	na	na	na	−	na[6]	2/5	40%
Plagiocephaly	+	+	+	+	na	+	+	na	na	na	na	na	na	+	+	8/8	100%
Strabismus/nystagmus	+	+	+	+	+	+	+	+	+	na	+	na	+	+	+	13/13	100%
Tooth abnormalities	+	+	+	+	−	−	−	−	−	na	na	na	na	+	−	5/11	45%
Dysmorphic facial features																	
Depressed nasal bridge	+	+	+	+	+	+	+	+	+	+	−	−	+	+	−	12/15	80%
Broad nasal root	+	+	+	+	−	−	−	−	−	+	−	−	na	+	+	7/14	50%
Anteverted nose	+	+	+	+	+	+	+	+	+	−	−	−	+	+	+	12/15	80%
High forehead	+	+	+	+	−	+	+	+	+	+	−	−	na	+	+	11/14	79%
Bitemporal narrowing	+	+	+	+	na	+	+	+	+	−	−	−	na	+	+	10/13	77%
Long philtrum	+	+	+	+	−	+	+	−[4]	−[4]	−	−	−	+	+	+	9/15	60%
Cupid bow lips	+	+	+	+	−	−	−	na	na	−	−	−	na	+	+	6/12	50%
Tented lip	na	na	na	na	+	−	−	+	+	−	−	−	+	+	+	6/11	55%
High arched palate	na	na	na	na	+	+	+	+	+	−	−	−	+	+	+	8/11	73%
Low set ears	na	na	na	na	+	na	na	na	na	+	−	−	na	+	+	4/6	67%
Upslanting palpebral fissures	na	na	na	na	+	na	na	na	na	−	−	−	na	na	−	1/5	20%
Torax																	
Pectus excavatum	+	−	−	−	−	−	+	−	−	na	na	na	+	na	+	4/11	36%
Inverted nipples	−	−	+	+	na	−	−	+	+	na	na	na	na	−	−	4/10	40%
Abnormal lung anatomy	na	na	na	+	na	na	na	na	na	na	na	na	na	na	+	2/2	100%
Musculo-skeletal																	
Skeletal abnormalities (clinodactily, syndactyly...)	+	+	+	+	+	+	+	−	−	na	na	na	+	na	+	9/12	75%
Scoliosis	+	+	−	−	+	+	+	−	−	na	na	na	−	−	−	5/12	42%
Osteopenia/delayed bone age	+	+	+	+	+	+	+	−	−	na	na	na	+	−	na	8/11	73%
Slender long bones	+	+	+	+	na	+	+	−	−	na	na	na	na	na	na	6/8	75%
Short limbs	+	+	+	+	na	−	−	−	−	na	na	na	na	na	+	5/9	56%
Congenital talipes equinovarus	−	−	−	−	−	−	−	−	+	na	na	na	na	na	+	2/10	20%
Urologic																	
Nephrocalcinosis	+	+	+	+	−[2]	−	−	−	−	+	−	−	+	−	−	6/15	40%
Ureteric dilatation	−	+	+	+	+	−	−	−	−	na	na	na	na	na	+	5/10	50%
Renal cysts and dysplasia	−	+	−	−	−	−	−	−	−	na	na	na	na	na	+	2/10	20%
Other	−	+	+	+	+	−	−	−	−	na	na	na	+	na	+[7]	6/11	55%
Endocrine																	
High plasma calcium	+	+	+	+	−	−	−	−	−	na	na	na	na	−	−	4/11	36%
High urine calcium	+	+	+	+	−	−	−	−	−	na	na	na	na	−	na	4/10	40%
Low alkaline phosphatase	+	+	+	+	+	−	−	−	−	−	+	−[5]	+	−	−	7/15	47%
Other																	
Gastroesophageal reflux	na	na	na	na	na	+	+	na	na	na	na	na	na	na	−	2/3	67%
Congenital heart defects	+	−	+	+	+	+	+	−	−	na	na	na	−	−	+	7/12	58%

The "Overall" column refers to the total of patients presenting each feature over all the patients in which this feature has been assessed. This data is also expressed in percentage of the total (%). NA: Not available or not assessed. (1) primitive Sylvian fissures, indicating a possible neuronal migration disorder; (2) urolithiasis; (3) at birth; (4) short philtrum; (5) at the lower end of the normal range; (6) he has prominent metopic suture but craniosynostosis has not been demonstrated; (7) undescended testis.

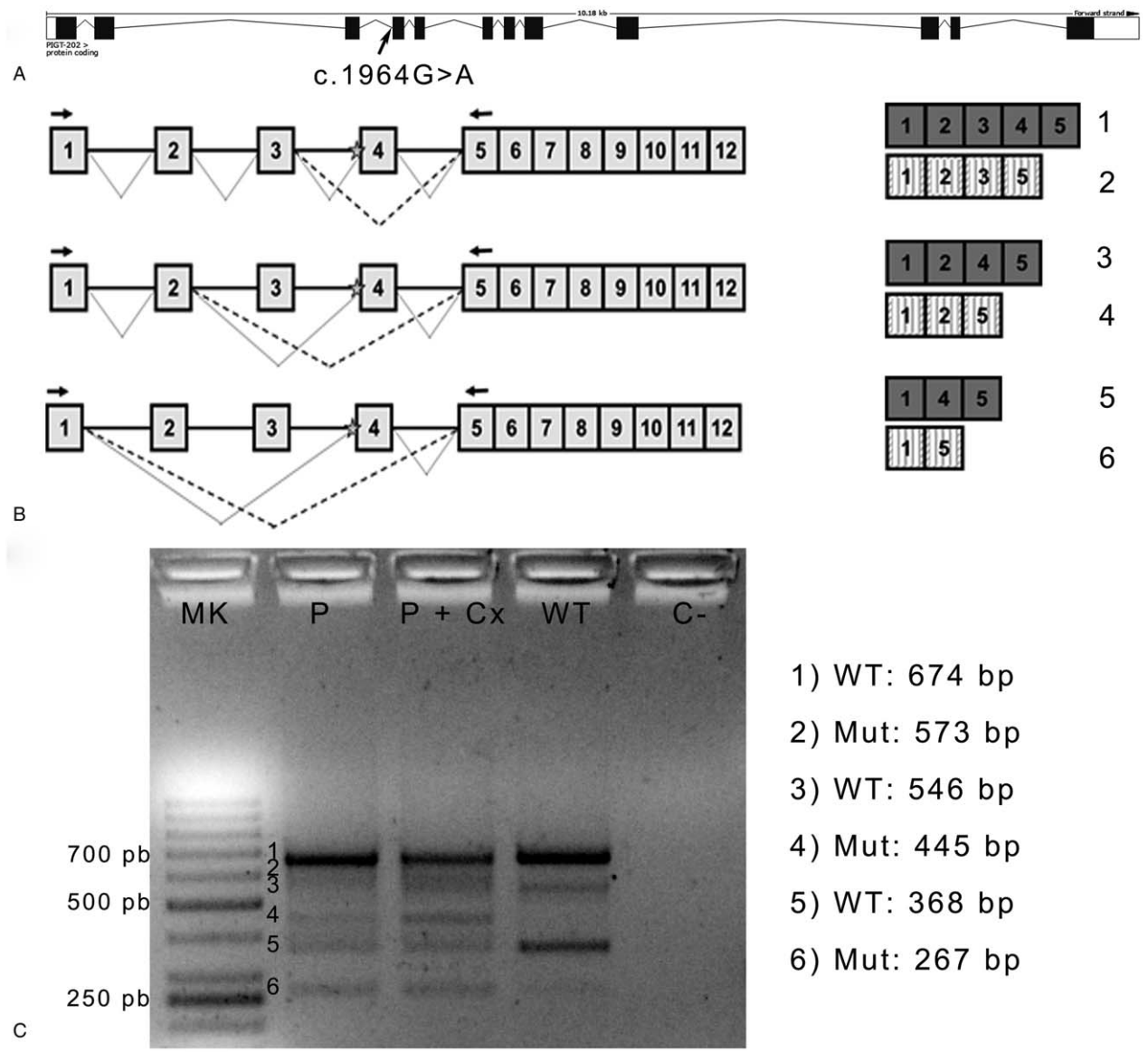

Figure 2. (A) *PIGT* gene representation (ENSG00000124155). The black arrow marks the position of the mutation (at exon 4 splicing acceptor site). (B) Schematic representation of the *PIGT* alternative transcripts and putative splicing events. (C) PCR amplification of the cDNA of the patient cells (P), the patient cells treated with Cycloheximide (P + Cx) and the control cells (WT). Mk: 100 pb molecular weight marker, (C−) PCR negative control. cDNA = complementary DNA, PCR = polymerase chain reaction, PIGT = phosphatidylinositol glycan anchor biosynthesis class T.

patients presented with cortical/cerebellar atrophy, cortical visual impairment, osteopenia, skeletal abnormalities, low alkaline phosphatase levels, anteverted nose, a high forehead, and congenital heart defects (Table 1).

At birth, most of the patients with variants in *PIGT* have been noted to feed poorly and have hypotonia (Table 1). All have gone on to develop seizures before 2 years of age, with most having their first seizure before 1 year of age. Initial presentation in most has been with generalized tonic-clonic seizures, often triggered by febrile illness.[1,5–8,10] Timing of seizures and progression to generalized tonic-clonic seizures in our patient is consistent with previous reports. Severe developmental delay and profound intellectual disability have also been reported in all of the currently known patients with *PIGT* variations (Table 1), with the exception of patient 258,094 at Pagnamenta et al,[5] presenting with a much milder phenotype. Strabismus and nystagmus have also been reported in all the assessed cases, often in conjunction with cortical visual impairment (Table 1).[1,5–8,10] In addition, the majority of these patients presented characteristic craniofacial abnormalities with plagiocephaly, high forehead and bitemporal narrowing, depressed nasal bridge and an arched palate. Cupid bow lips, long philtrum, and low set ears are also observed in around half of the patients.

Skeletal abnormalities, together with osteopenia, slender long bones or delayed bone age are common traits in the "PIGT syndrome" patients. While mineralization and ossification problems are common in these patients, low serum alkaline phosphatase level has been observed in 47% of the patients, suggesting that this is not the only reason for the ossification problems.

Our reported patient is similar to the 14 patients previously described in the literature, who had either compound heterozygous or homozygous variants in the *PIGT* gene resulting in an MCAHS3 phenotype.[1,5–9] Undescended testes, seen in this case, has not been previously reported.

Functional tests performed by us and others reinforce the pathogenic implication of the 2 *PIGT* variants described here. The missense mutation p.Thr183Pro was previously tested in a zebrafish model and was demonstrated to derogate the ability of *PIGT* mRNA to recover the gastrulation defects of pigt-depleted zebrafish.[6] Regarding the c.494-2A>G mutation, we have shown that it alters the normal *PIGT* splicing pattern, leading to the skipping of exon 4. The mutant protein is predicted to have a new reading frame from position 165 and a premature stop codon after 34 residues (p.Asp165Glyfs*34).

Previous studies in breast carcinoma showed that *PIGT* regulates *FOXC2* expression levels in 2 breast cancer cell lines.[12] We have studied *FOXC2* levels in the patient's fibroblasts but no clear results were obtained.

The patient was also found to bear a de novo heterozygous variant in the *FBN2* gene (c.733C>T; p.Arg245Trp). This gene has been associated with Congenital Contractural Arachnodactyly (MIM #121050) a rare, autosomal dominant connective tissue disorder. This mutation is reported in ClinVar as a "variant of uncertain significance." While the *FBN2* variant does not seem to be responsible for the main clinical characteristics of the patient, with the information available at this time we cannot rule out that this change may have some effect and may be modifying the patient's clinical presentation.

MCAHS3 (or PIGT syndrome) represents an emerging and distinctive entity, which is potentially clinically diagnosable. Given the severity of its clinical features and its reproductive implications, it is important to make the diagnosis of this condition.

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