RFT1-CDG: Absence of Epilepsy and Deafness in Two Patients with Novel Pathogenic Variants

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Abstract This report is on two novel patients with RFT1-CDG. Their phenotype is characterized by mild psychomotor disability, behavioral problems, ataxia, and mild dysmorphism. Neither of them shows signs of epilepsy, which was observed in all RFT1-CDG patients reported to date ($n = 14$). Also, deafness, which is often associated with this condition, was not observed in our patients. Molecular analysis of RFT1 showed biallelic missense variants including three novel ones: c.827G > A (p.G276D), c.73C > T (p.R25W), and c.208T > C (p.C70R).

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Introduction

RFT1-CDG is an autosomal recessive congenital disorder of glycosylation (CDG), caused by mutations in *RFT1*. It is a defect in the assembly of N-glycans since RFT1 is a putative flippase (Haeuptle et al. 2008) involved in the transfer of Man5GlcNAc2-PP-Dol from the cytoplasmic to the luminal side of the endoplasmic reticulum. To date, 14 RFT1-CDG cases have been reported, and the phenotype is characterized mainly by encephalopathy including epilepsy ($n = 14$) and hearing impairment ($n = 11$), both hallmarks of the disease, as well as variable feeding problems, dysmorphism, and hypotonia (Imtiaz et al. 2000; Haeuptle et al. 2008; Clayton and Grunewald 2009; Haeuptle and Hennot 2009; Jaeken et al. 2009; Vleugels et al. 2009, 2011; Ondruskova et al. 2012; Aeby et al. 2016; Barba et al. 2016; Bastaki et al. 2018; Monies et al. 2017; Pérez-Cerdá et al. 2017).

This report describes two novel patients without epilepsy and deafness and with three novel variants, broadening the clinical and mutational spectrum of RFT1-CDG.

Patients

Patient 1

This Portuguese girl was first evaluated at a metabolic pediatrics consultation at the age of 4 years. She is the first child of healthy, unrelated parents and has two healthy siblings. She was born by vaginal delivery after 40 weeks, with a birth weight of 2,700 g, length of 47.5 cm, and head circumference of 33.5 cm. Apgar scores were 9 and 10, and she had no feeding problems or failure to thrive.

Growth parameters remained on the 50th centile for weight, between the 50th and 75th centiles for length, and on the 90th centile for head circumference. She showed mild psychomotor (first words and walking alone at the age of 24 and 30 months, respectively) and intellectual disability and behavioral problems. At 5 years, she presented a global score of 42 on the Wechsler Intelligence Scale for Children (WISC) III, a tetrapyramidal syndrome, ataxia but no epilepsy [without epileptic pattern on the electroencephalogram (EEG)], and no hearing impairment [normal audiogram and brainstem evoked response audiometry (BERA)]. Brain MRI was normal. At 18 years, her weight was 70 kg (SDS 1.13), height 159 cm (SDS -0.64), and head circumference 56 cm (SDS 1.52). She showed very mild dysmorphism: a prominent forehead and widely spaced nipples. Ultrasonography of the liver, spleen, and kidneys and echocardiography were normal. The fundoscopic ophthalmological investigation was normal. The audiogram and brain MRI were still normal.

Biochemical investigation at 4 years showed a normal blood count and normal levels of blood lactate, serum albumin, cholesterol, transaminases, creatine kinase, lactate dehydrogenase, thyroid-stimulating hormone, and plasma amino acids. Levels of blood coagulation factors V, VII, and XI were normal.

Isoelectric focusing of serum sialotransferrins revealed a type 1 pattern. Phosphomannomutase and phosphomannose isomerase activities were normal in fibroblasts.

Lipid-linked oligosaccharide analysis in patient-derived fibroblasts showed an accumulation of Man5GlcNAc2-PP-Dol. This compound is normally undetectable.

Genetic testing was performed by a capture panel that includes targets for 79 CDG-associated genes.

The mean coverage in the target region was about 600×, and a genotype (homozygous reference, heterozygous or homozygous variant) was called for more than 95% of the targeted bases. Variant annotation and prioritization were done using the Cartagenia Bench tool. Analysis was done for the exonic sequences and 20 bp of the flanking intronic regions. In addition, deep intronic and promoter regions in which variants were known in the HGMD database were also scrutinized. An initial filtering of the variants was done on the basis of population frequency (<5% in at least two population databases).

The CDG panel showed two novel variants in *RFT1*: c.827G > A (p.G276D) and c.208T > C (p.C70R), respectively, paternally and maternally inherited, were confirmed by Sanger sequencing.

Patient 2

This Portuguese man was first observed by a geneticist at the age of 20 years. He was the second child of healthy,

unrelated parents and had a healthy older sister. He was born after a 38 weeks gestation. Birth weight was 3,450 g and length 53 cm. Apgar scores were 9 and 10. There were no feeding problems, nor failure to thrive. He showed mild psychomotor disability (first words and walking alone at the age of 24 and 18 months, respectively), and at 32 years, he presents borderline intellectual disability with supervised independent living (global quotient of 72 on WASI-II scale evaluation (Wechsler Abbreviated Scale of Intelligence), ataxia, but no epilepsy (without epileptic pattern on EEG), and no hearing impairment (normal audiogram). At the age of 33, his weight was 85 kg (SDS 0.93), height 171.5 (SDS 0.75), and head circumference 58 cm (SDS 2.0). He showed mild dysmorphism: synophrys, prominent forehead, widely spaced teeth, second and third toe syndactyly, and widely spaced nipples. Neurological examination showed a bilateral pyramidal syndrome and normal base walking. There was no epilepsy. The otorhinolaryngological examination was normal, and there is still no hearing impairment on the audiogram. The fundoscopic ophthalmological investigation was normal. Liver and renal echography and brain MRI were normal.

Biochemical and technical investigations showed similar results as in patient 1.

Molecular genetic testing using a CDG capture panel as described for patient 1, followed by Sanger sequencing, showed two variants in *RFT1*: c.73C > T (p.R25W), maternally inherited, and c.208T > C (p.C70R), paternally inherited, also present in patient 1.

Discussion

The type 1 serum sialotransferrin pattern in these patients pointed to a defect in the glycan assembly pathway. The result of the dolichol-linked analysis in fibroblasts suggested a defect in *ALG11* or *RFT1*. CDG panel mutation analysis (with 79 genes that, when defective, cause a CDG) showed compound heterozygosity of *RFT1* in both patients. Three novel missense variants were found, c.827G > A, c.73C > T, and c.208T > C, the latter shared by both patients. Arguments in favor of the pathogenicity of these variants are as follows: (1) they were not found in the 1000G (www.1000genomes.org) nor in Exome Variant Server (<http://evs.gs.washington.edu/EVS>) databases; (2) the programs SIFT and POLYPHEN predict a pathogenic effect; and (3) analysis of all human orthologues available in the Ensembl Genome Browser (<http://www.ensembl.org>; release 90) provides evidence of highly conserved residues among all species, pointing to a critical functional role. The fact that both patients share the c.208T > C variant suggests that they may have shared a common ancestor, although there is no evidence of a familial link. Moreover,

Table 1 Clinical and mutational data in reported patients with RFT1-CDG

	Pt 1	Pt 2	Pt 3	Pt 4	Pt 5	Pt 6	Pt 7	Pt 8	Pt 9
Reference	Imtiaz et al. (2000), Hauptle et al. (2008), Hauptle and Henne (2009), Clayton and Grunewald (2009)	Vleugels et al. (2009)	Vleugels et al. (2009)	Vleugels et al. (2009)	Jaeken et al. (2009)	Jaeken et al. (2009)	Ondruskova et al. (2012)	Ondruskova et al. (2012)	Barba et al. (2016)
Gender	Female	Female	Male	Male	Male	Female	Male	Female	Female
Age at publication	Died 4 years 3 months (pulmonary embolus)	Died 8 months	5.5 years	2.2 years	8 months	11 months	21 years	19 years	3.5 years
Onset of symptoms	Birth	NM	NM	NM	NM	Birth	8 months	6 months	NM
FTT	+	+	+	+	NM	NM	–	NM	NM
Feeding problems	+	+	+	+	+	+	–	NM	NM
Dysmorphism	+	+	+	+	–	+	+	+	NM
Short length/stature	+	NM	NM	NM	NM	–	+	+	NM
Microcephaly	NM	+	+	–	–	+	–	–	+
Psychomotor disability	+	+	+	+	+	+	+	+	+
Epilepsy	+	+	+	+	+	+	+	+	+
Deafness	+	+	+	+	+	+	+	–	+
Visual impairment	+	+	+	+	+	+	–	–	NM
Hypotonia	+	+	+	+	+	+	+	+	+
Abnormal brain MRI	+	– ^a	+	–	–	+	–	–	+
Hepatomegaly	+	NM	NM	NM	–	–	+	+	NM
<i>RFT1</i> mutation	Homozygous c.199C>T	Homozygous c.199C>T	Homozygous c.454A>G	Homozygous c.454A>G	Homozygous c.454A>G	c.887T>A/ c.887T>G	c.1222A>G/ c.1325G>A	c.1222A>G/ c.1325G>A	Homozygous c.454A>G
Amino acid change	p.R67C	p.R67C	p.K152E	p.K152E	p.K152E	p.I296K/p. I296R	p.M408V/p. R442Q	p.M408V/p. R442Q	p.K152E

(continued)

Table 1 (continued)

	Pt 10	Pt 11	Pt 12	Pt13	Pt14	Pt 15	Pt 16
Reference	Aeby et al. (2016)	Aeby et al. (2016)	Pérez-Cerdá et al. (2017)	Bastaki et al. (2018)	Monies et al. (2017)	Present paper (patient 1)	Present paper (patient 2)
Gender	Female	Male	Female	Female	NM	Female	Male
Age at publication	Died 1 year (cardioresp. arrest)	Died 2 years (resp. insufficiency)	1.5 years	NM	NM	18 years	20 years
Onset of symptoms	Birth	Birth	NM	NM	NM	NM	NM
FTT	+	NM	NM	NM	NM	—	—
Feeding problems	+	+	NM	NM	NM	—	—
Dysmorphism	NM	NM	NM	NM	NM	+	+
Short length/stature	NM	NM	NM	NM	NM	—	—
Microcephaly	NM	NM	NM	NM	+	—	—
Psychomotor disability	+	+	+	+	+	+	+
Epilepsy	+	+	+	+	+	—	—
Deafness	+	+	+	NM ^b	NM	—	—
Visual impairment	NM	NM	NM	— ^b	NM	—	—
Hypotonia	Hypertonia	Hypertonia	+	+	—	—	NM
Abnormal brain MRI	—	+	+	+	NM	—	—
Hepatomegaly	NM	NM	NM	NM	—	—	—
<i>RFT1</i> mutation	Homozygous c.454A>G	c.1325G>A/ c.110G>T	Not reported	Homozygous c.902A>G	Homozygous c.775 + 1G>C	c.827G>A/ c.208T>C	c.73C>T/ c.208T>C
Amino acid change	p.K152E	p.R442Q/p.R37L	Not reported	p.Y301C	p.G276D/p.C70R	p.R25W/p.C70R	

NM Not mentioned

^a At autopsy, weight, and gyral/sulcal pattern consistent with a degree of cerebral atrophy was reported^b Strabismus

the fact that this variant is not in a CpG mutational hotspot reinforces the possibility of a common origin.

Of note are that the siblings with a mild phenotype reported by Ondruskova et al. (2012) had variants located in the transmembrane domain and that our patient 2 also had a variant in the transmembrane domain (Haeuptle and Hennet 2009).

Table 1 summarizes the clinical findings of the 14 previously reported and the present patients (Imtiaz et al. 2000; Haeuptle et al. 2008; Clayton and Grunewald 2009; Haeuptle and Hennet 2009; Jaeken et al. 2009; Vleugels et al. 2009, 2011; Ondruskova et al. 2012; Aeby et al. 2016; Barba et al. 2016; Bastaki et al. 2018; Monies et al. 2017; Pérez-Cerdá et al. 2017). Common to all 16 patients is a psychomotor disability, which is mostly severe. The majority of patients showed the following symptoms: epilepsy (14/16), sensorineural hearing loss (11/16), hypotonia (11/16), dysmorphism (9/16), and feeding problems (8/16). In a minority of patients, there was failure to thrive, short length, arthrogryposis, ataxia, basal ganglia lesions, behavioral problems, cerebral and/or cerebellar atrophy, hyperreflexia, hypertonia, microcephaly, spastic tetraparesis, visual impairment, white matter abnormalities, convergent strabismus, glaucoma, pale optic nerves, respiratory problems, hepatomegaly, and deep venous thrombosis. The dysmorphism comprised variable features: down slanting palpebral fissures, drooping eyelids, depressed broad nasal bridge, small nose, anteverted nostrils, small mouth, glossoptosis, micrognathia, short neck, inverted nipples, kyphoscoliosis, adducted thumbs, short femora, and valgus feet.

The here reported patients have the mildest reported RFT1-CDG symptoms, suggesting that the novel *RFT1* variants they carry may result in a mild RFT1-CDG phenotype.

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Contributors

Dulce Quelhas – writing and collection of published information, performed initial laboratory screening for CDG.

Jaak Jaeken – collaboration in paper writing and critical review.

Ana Fortuna – clinical evaluation and information collection from patient 2.

Luísa Azevedo – collaboration in writing on genetic information and critical review.

Anabela Bandeira – collected clinical information from patient 1.

Gert Matthijs – molecular screening for CDG genes.

Esmeralda Martins – supervised patient's clinical information and critical review.

Competing Interests

None.

Synopsis

Lack of epilepsy and deafness in two RFT1-CDG patients.

Compliance with Ethics Guidelines

Conflict of Interest

Dulce Quelhas, Jaak Jaeken, Ana Fortuna, Luísa Azevedo, Anabela Bandeira, Gert Matthijs, and Esmeralda Martins declare they have no conflict of interest.

Informed Consent

The authors performed this study in accordance with the Declaration of Helsinki of the World Medical Association. Authors did not request ethical approval from the local ethics committee due to the nature of the study.

Animal Rights

This article does not contain any studies with animal subjects performed by any of the authors.

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