Phenotypic Expansion of Congenital Disorder of Glycosylation Due to *SRD5A3* Null Mutation

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Abstract We present a boy, admitted at 4 months, with facial dysmorphism, hypertrichosis, loose skin, bilateral inguinal hernia, severe hypotonia, psychomotor disability, seizures with hypsarrhythmia (West syndrome), hepatosplenomegaly, increased serum transaminases, iris coloboma, glaucoma, corneal clouding and bilateral dilated lateral ventricles, and extra-axial post-cerebellar space. Serum transferrin isoelectrofocusing (IEF) showed a type 1 pattern. Whole-exome genotyping showed a previously reported homozygous nonsense mutation c.320G>A; p.Trp107X in *SRD5A3*. Epilepsy and glaucoma have been

reported only once in the 19 described *SRD5A3*-congenital glycosylation defect patients, and corneal clouding not at all.

Introduction

SRD5A3-CDG is one of about 80 known congenital disorders of glycosylation (CDG). It is a defect in the penultimate step of the synthesis of dolicholphosphate, the carrier of the glycan intermediates in the assembly of GlcNAc₂Man₉Glc₃ in the endoplasmic reticulum. It affects protein N-glycosylation, as well as the synthesis of mannose-linked glycans, C-mannosylation, and glycosphingolipid anchor synthesis. *SRD5A3*-CDG is characterized by neurological and ophthalmological findings such as nystagmus, visual impairment, microphthalmia, cataract, coloboma (iris, chorioretinal), optic disk hypoplasia, and optic nerve hypoplasia/atrophy (Assmann et al. 2001; Prietsch et al. 2002; Al-Gazali et al. 2008; Kahrizi et al. 2009, 2011; Morava et al. 2009, 2010; Cantagrel et al. 2010; Gründahl et al. 2012; Kara et al. 2014; Kasapkara et al. 2012).

Corneal clouding has not been reported in this disorder and epilepsy as well as glaucoma only once. We report a patient with corneal clouding, epilepsy, and glaucoma.

Clinical Report

This 4-month-old boy is the fourth child of first-cousin parents. Following an uncomplicated pregnancy, the patient was born with normal birth weight, height, and head

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Fig. 1 The patient showed a coarse face with hairy forehead, depressed nasal bridge, proptotic eyes, low-set ears, smooth and long philtrum, and thin lips at 4 months (a) and at 31 months (b). Cranial

MRI study at the age of 13 months showed bilateral dilated lateral ventricles and extra-axial post-cerebellar space in coronal T2 (c) and sagittal T1 (d)

circumference. His weight, height, and occipitofrontal circumference (OFC) were 5,600 g (−1.2 SD), 66 cm (+1.3 SD), and 41 cm (−0.2 SD), respectively, at 4 months of age. He was hypotonic and showed facial dysmorphism [hairy forehead, depressed nasal bridge, large fontanelle (6 × 8 cm), low-set ears, smooth philtrum, and thin lips], loose skin, hypermobility of elbows and fingers, hepatosplenomegaly, bilateral inguinal hernia, and hypertrichosis (Fig. 1a, b). Eye examination revealed iris coloboma, glaucoma, nystagmus, and corneal clouding. He had severe feeding difficulty. Serum transaminase (ALT 1,151 U/L, reference values 0–40 U/L; AST 144 U/L, reference values 0–40 U/L) and creatine kinase (291 U/L; reference value <170 U/L) levels were elevated. On abdominal ultrasonog-

raphy there was a dilatation of the right pelvicalyceal system and ureter. Echocardiography revealed a patent foramen ovale. Osteoporosis was observed on skeletal X-ray. He underwent surgical repair for bilateral inguinal hernia at 8 months of age, and bilateral vitreo-trabeculectomy was performed at 9 months of age. Severe gastroesophageal reflux disease (GERD) was diagnosed at 2 years of age.

Infantile spasms were noticed at the age of 7 months. Electroencephalography showed hypsarrhythmia. West syndrome was diagnosed and treated with IM ACTH. Levetiracetam was added to this treatment. His seizures were under control by levetiracetam for 1 year, and EEG showed some improvement. Brain MRI at 1 year old

Fig. 2 Molecular and computational studies of the *SRD5A3* variant. (a) Protein structure of *SRD5A3* and location of the mutation in our patient. Our mutation affects the protein domain before steroid dehydrogenase domain. (b) Conservation alignment indicating that the tryptophan residue at position 107 is conserved across all

vertebrates. (c) Pedigree of the family with Sanger sequencing results showing homozygosity of the c.320G>A; p.W107X variant in the proband and heterozygosity in both parents. Unaffected siblings were not available for segregation

revealed bilateral dilated lateral ventricles and extra-axial post-cerebellar space (Fig. 1c, d).

Weight, height, and head circumference were 10.5 kg (−3.5 SD), 92 cm (−1 SD), and 47 cm (−2.3 SD), respectively, at his last examination at 3 years and 4 months. A dark pigmentation on the dorsum of the feet was observed. Severe hypotonia persisted. He was unable to say single words and to walk. Head control was achieved at 18 months of age and sitting without support at 3 years of age and 4 months. Severe GERD and feeding problems persisted, and he had recurrent respiratory tract infections. His seizures were under control by levetiracetam, and EEG showed improvement in hypsarrhythmia. Serum IGF1 was normal (94.06 ng/ml; reference values 50–143), and antithrombin levels were decreased mildly, %73.5 (reference values %75–125).

Serum transferrin IEF showed which was consistent with type 1 pattern.

Mutation Analysis

Whole-exome sequencing (WES) was performed as described previously (Lupski et al. 2013). Whole-exome sequencing analysis showed a homozygous nonsense mutation c.320G>A; p.Trp107X (chr4:g.56,225,610 G>A [hg19]) in the *SRD5A3* gene that was predicted to terminate protein before the steroid reductase domain (Fig. 2a–c). The mutation was predicted to be deleterious by several bioinformatic tools including Polyphen2, LRT, MutationTaster, and SIFT. The mutation was not found in publicly available (i.e., ARIC, 1,000 genomes, NHLBI Exome Sequencing Project and dbSNP) or internal human genome databases (>4,000 exomes). The affected amino acid was evolutionarily well controlled in all vertebrates except jawless fish and zebra fish (no amino acid counterpart in these two vertebrates). Both parents were heterozygous consistent with Mendelian expectations for an

autosomal recessive trait. Furthermore, the mutation was previously reported in a CDG patient (Cantagrel et al. 2010).

Discussion

The serum transferrin IEF type 1 pattern pointed to a glycan assembly defect. Whole-exome sequencing revealed a homozygous mutation in *SRD5A3*, previously reported in one patient (Cantagrel et al. 2010). Nineteen patients have been reported with *SRD5A3*-CDG, belonging to 13 families. Among them are five adults belonging to two families. They showed various combinations of mainly facial dysmorphisms and neurological, ophthalmological, and cutaneous manifestations (Assmann et al. 2001; Prietsch et al. 2002; Al-Gazali et al. 2008; Kahrizi et al.

2009, 2011; Morava et al. 2009, 2010; Cantagrel et al. 2010; Gründahl et al. 2012; Kara et al. 2014; Kasapkara et al. 2012). Other clinical symptoms, present in a few patients, were feeding problems, failure to thrive, microcytic anemia, cardiac malformations/hypertrophy, hepatosplenomegaly, and cryptorchidism. Besides the type 1 serum transferrin IEF, laboratory investigations showed microcytic anemia, mildly increased serum transaminases, decreased antithrombin, protein C, protein S, and low IGF1 and IGFBP3. We compared the clinical features of the patient presented here with those reported 14 children with mutations in *SRD5A3* gene in Table 1.

The patient presented here showed the full clinical spectrum of this neuro-ophthalmo-cutaneous syndrome, caused by a known mutation. In addition, he had bilateral corneal clouding. Moreover, two of his symptoms have been reported only once in *SRD5A3*, namely, glaucoma and

Table 1 Comparison of the clinical features of our patient to those reported in patients with mutations in *SRD5A3*

	Present patient	Al-Gazali et al. (2008)	Morava et al. (2009, 2010)	Kasapkara et al. (2012)	Assmann et al. (2001)	Prietsch et al. (2002)	Gründahl et al. (2012)
			Polish (3)				
Ethnic background	Turkish	Baluchi (4)	Turkish (3)	Turkish	Turkish	Turkish	Pakistani
<i>Facial dysmorphic features</i>							
Brachycephaly	—	4/4	NA	NA	NA	NA	NA
Hairy forehead	+	4/4	NA	NA	NA	NA	NA
Arched eyebrows	+	4/4	NA	NA	NA	NA	NA
Hypertelorism	+	4/4	NA	NA	NA	NA	NA
Depressed nasal bridge	+	4/4	NA	NA	NA	NA	NA
Short upturned nose	+	4/4	NA	NA	NA	NA	NA
Smooth philtrum	+	4/4	NA	NA	NA	NA	NA
Large mouth	+	4/4	NA	NA	NA	NA	NA
Thin upper lip or thick lips	+	4/4	NA	+	NA	NA	NA
Abnormally formed, low-set ears	+	4/4	NA	+	NA	NA	NA
<i>Neurological findings</i>							
Developmental delay	+	4/4	5/6	+	+	+	+
Hypotonia	+	4/4	5/6	+	+	+	—
Ataxia/stereotypic movements	—	—	2/6	—	+	+	—
Spasticity	—	—	1/6	—	+	—	+
Midline brain malformation	—	3/4	—	—	—	—	—
Frontal polymicrogyria	—	2/4	—	—	—	—	—
Pituitary gland hypoplasia	—	3/4	—	—	—	—	—
Lateral ventricle dilatation	+	1/4	—	+	—	—	—
Global/cerebellar vermis hypoplasia	—	2/4	4/6	+	—	+	—
Extra-axial post-cerebellar space	+	1/4	—	+	—	—	—
Seizures	+	—	1/6	—	—	—	—

(continued)

Table 1 (continued)

	Present patient	Al-Gazali et al. (2008)	Morava et al. (2009, 2010)	Kasapkara et al. (2012)	Assmann et al. (2001)	Prietsch et al. (2002)	Gründahl et al. (2012)
			Polish (3)				
Ethnic background	Turkish	Baluchi (4)	Turkish (3)	Turkish	Turkish	Turkish	Pakistani
<i>Ophthalmological abnormalities</i>							
Microphthalmia	—	1/4	1/6	—	—	—	—
Nystagmus	+	—	5/6	—	+	+	+
Strabismus	+	—	—	+	+	+	NA
Cataract	—	—	2/6	—	—	—	NA
Coloboma	+	4/4	4/6	+	—	—	NA
Optic disk/nerve hypoplasia	—	2/4	3/6	+	+	+	NA
Glaucoma	+	—	1/6	—	—	—	NA
Visual loss	+	—	5/6	—	+	+	NA
Corneal clouding	+	—	—	—	—	—	NA
<i>Cutaneous manifestations</i>							
Erythroderma	—	1/4	—	+	NA	NA	—
Dry skin	—	4/4	3/6	+	NA	NA	—
Atopic dermatitis	+	2/4	—	—	NA	NA	—
Ichthyosis	—	3/4	—	+	NA	NA	—
Dark pigmentation	+	3/4	4/6	—	NA	NA	—
Thick palms and soles	—	3/4	—	—	NA	NA	—
Loose skin	+	3/4	—	—	NA	NA	—
Hypertrichosis	+	4/4	—	—	NA	NA	—
<i>Other clinical symptoms</i>							
Large fontanelle	+	—	—	—	—	—	—
Inguinal hernia	+	—	—	—	—	—	—
Joint hypermobility	+	—	—	—	—	—	—
Hepatosplenomegaly	+	—	—	—	—	—	—
Dilated cardiomyopathy	—	4/4	—	+	—	—	—
Feeding difficulty	+	1/4	2/6	+	—	—	—
Microcytic anemia	+	2/4	4/6	—	+	—	—
Increased transaminases	+	1/4	6/6	NA	+	—	—
Decreased antithrombin and protein C/S	+	4/4	6/6	NA	—	—	—
Low IGF1 and IGFBP3	+	3/4	NA	NA	—	—	—

NA not assessed

epilepsy. The reported epilepsy was absence seizures (Morava et al. 2010), while in the present patient it was West syndrome and localization-related epilepsy that responded well to levetiracetam. In conclusion, the present patient broadens the phenotype of SRD5A3-CDG.

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Take-Home Message

New features such as epilepsy, glaucoma, and corneal clouding were described in the patient with CDG1q.

Details of the Contributions of Individual Authors

BT was the leading physician who diagnosed the condition and to the writing of the manuscript.

DP: sequencing the sample and writing the manuscript.

AÖ, ÇZ, and CY were the physicians who followed up the patient during his admission to the pediatric ophthalmology, neurology, and metabolism departments.

JS: sequencing the sample.

RAG: sequencing the sample and organizing the project.

DMM: sequencing the sample.

JRL: sequencing the sample, organizing the project, and writing the manuscript.

JJ was revising the manuscript critically for important intellectual content and analysis of serum transferrin IEF level.

Conflict of Interest

BT, DP, ÇZ, CY, JS, RAG, DMM, JRL, and JJ declare that they have no conflict of interest.

Informed Consent

Informed consent was obtained from the parents of the patient.

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