A Novel Missense Mutation in SRD5A3 Causes Congenital Disorder of Glycosylation Type I (Cerebello-Ocular Syndrome)

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Abstract
A consanguineous Qatari family having an autosomal recessive disorder characterized by severe mental retardation, cerebellar vermis hypoplasia, retinal degeneration, optic nerve atrophy, ataxic gait, and seizures was studied for identification of the offending gene and mutation. Homozygosity mapping identified an 11.4 Mb critical interval at 4q12 to q13.2 that would contain the gene responsible for the disorder. Ten positional candidate genes were screened for pathogenic mutations, but none were identified. Next-generation exome sequencing in one affected individual identified a novel SRD5A3 missense mutation c.T744G/p.F248L, which was subsequently confirmed by Sanger sequencing, suggesting a congenital disorder of glycosylation type I defect. Isoelectric focusing of serum transferrin showed a type I pattern indicative of an N-glycan assembly defect. This is a novel pathogenic mutation and the first SRD5A3 missense mutation as all others are protein-truncating mutations.

Keywords
congenital glycosylation disorder type I, SRD5A3 gene, homozygosity, mapping, next-generation exome sequencing, cerebello-ocular syndrome

Introduction
A consanguineous Qatari family with 2 female children (Figure 1) having an apparently autosomal recessive disorder characterized by severe mental retardation, and brain malformations including cerebellar vermis hypoplasia, retinal degeneration, optic nerve atrophy, ataxic gait, and seizures presented for evaluation. The birth history for both girls was unremarkable.

Clinical Presentation
The older child developed vision problems at 4 months of age. On evaluation, pendular nystagmus was detected. Brain magnetic resonance imaging (MRI) at that time showed hypoplastic cerebellar vermis, cerebral atrophy, delayed myelination, and bilateral optic nerve hypoplasia. Subsequent repetitive eye examinations showed myopia and poor vision in general. She has severe intellectual impairment and global developmental delay. She walked at 4 years of age and toilet trained by the age of 6 year. At 9 years of age, she was admitted with a 2-month history of repeated attacks of what seemed to be seizures.

Electroencephalogram (EEG) was abnormal with slow background activity, and antiseizure medication (Oxcarbazepine) was started. Currently at 12 years of age, she can recognize her family members, speak single words, has what seems to be gelastic seizures, and attends a school for children with disabilities. Dysmorphic features include hypertelorism, a gaping mouth with tented upper lip, and a broad nose (Figure 2).

The younger child developed generalized tonic–clonic seizures as well as gelastic seizures at about 20 months of age. The EEG showed asymmetrical activity with slower activity

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on the left side and poorly developed sleep spindles with slow delta waves bilaterally at the background. In addition, there were high-voltage delta sharp waves mainly in the left side. Brain MRI at 20 months of age showed diffuse prominence of the ventricular system and extra-axial cerebrospinal spaces, indicating some degree of brain atrophy or mild communicating hydrocephalus and a Dandy-Walker malformation variant. Ophthalmology evaluation showed bilateral horizontal nystagmus. Anterior segment was normal, both pupils were equal and reactive to light, and both fundi showed bilateral optic nerve atrophy. Electrophysiologic studies showed diffuse retinal dystrophy and delayed visually evoked potential response. Hearing was normal. Abdominal ultrasound and echocardiography were unremarkable. Currently, at the age of 5 years, her intellectual state, behaviors, and dysmorphism are similar to her sister’s, but she also has generalized hypotonia (Figure 2).

Both patients had a serum sialo-transferrin pattern suggestive of congenital disorder of glycosylation (CDG) type I. Commercial DNA diagnostic testing for the most frequent genes associated with type I glycosylation disorders (PMM2, MPI, ALG6, ALG3, ALG12, ALG8, ALG2, DPAGT1, ALG9, RFT1) did not identify any pathogenic mutations.

Methods

Blood samples from all family members (Figure 1) were obtained after informed parental consent. Genomic DNA was extracted from whole peripheral blood with the Gentra Puregene Blood kit (cat#158422; Qiagen Valencia, CA, USA) according to the manufacturer’s guidelines.

Homozygosity mapping was performed for all family members utilizing the Human Mapping 370K-Cyto12 SNP genotyping array (Illumina San Diego, CA, USA). Data filtration and genotyping were done with GenomeStudio v2011.1. For determination of linkage intervals, data were analyzed by the homozygosity mapper software [http://www.homozygosity mapper.org/].

Figure 1. Pedigree of the family segregating the SRD5A3 c.T744G / p.F248L mutation. Genotypes are indicated as are the relevant chromatograms. The colors blue, red, black, and green represent the bases C, T, G, and A, respectively. “W” indicates a “wild-type” or normal allele. Consanguinity line represents a double first cousin marriage.

Figure 2. Dysmorphic features of individuals II:3 and II:6, showing hypertelorism, a gaping mouth with tented upper lip, and a broad nose.
Targeted resequencing was done by Sanger Big-dye terminator v.3.1 cycle sequencing (Applied Biosystems Foster City, CA, USA) on an ABI-3700 automatic sequencer (Applied Biosystems Foster City, CA, USA) to screen for mutations in candidate genes, perform population frequency studies for variants, and determine cosegregation of variants with the disease phenotype within the family.

Whole exome target enrichment next-generation sequencing (NGS) was performed on ABI SOLiD4 platform (Applied Biosystems Foster City, CA, USA) according to manufacturer’s specifications. DNA library was prepared using the TargetSeq Exome Enrichment system (Applied Biosystems Foster City, CA, USA) as multiplex fragments libraries utilizing both the SOLiD Fragment Library Construction Kits and the SOLiD Fragment Library Barcoding Kit Module 1–16 for the SOLiD 4 System (Applied Biosystems, Foster City, CA, USA). Bead preparation and enriching were done on an EZ Bead Emulsifier, Amplifier, and Enricher utilizing E80 scale (Applied Biosystems, Foster City, CA, USA). Sequencing modality was performed with multiplex fragment paired end.

Bioinformatic analyses of whole exome NGS data were as follows: raw data files (in a proprietary XSQ file format) were analyzed with the LifeScope v2.5.4 software (Life Technologies Carlsbad, CA, USA) running on a dedicated cluster to align the reads produced by the SOLiD to a hg19 whole genome reference sequence, sourced from the University of California, Santa Cruz Genome Informatics Group. The aligned BAM files were validated, duplicate sequences were identified and removed, and incorrectly identified mate pairs were corrected using the Picard v1.87 software. The Genome Analysis Tool Kit v3.0.0 was applied to the “corrected” output files to recalibrate the base quality scores, using machine learning to model any systematic errors in the data, carry out localized realignments around possible insertion/deletion sequences to ensure mapping accuracy, identify viable variants from the sequence reads, and recalibrate the variants to ensure accuracy of the variant calling in a specific variant-type manner. Once a suitable list of variants was produced in this manner, the list was filtered using in-house scripts to confirm variant zygosity and identify those variants that conform to the inheritance model. These variants were annotated using an in-house script in conjunction with ANNOVAR [http://www.openbioinformatics.org/annovar/] to produce an annotated list of variants with the most recent information available on a number of reference Web sites.

Results
Genotyping identified a single 11.4 Mb homozygosity interval at Hsa 4q12–q13.2 (rs7684211/55823638 to rs1877513/67194620) containing 62 genes of which 24 are protein coding. Candidate genes within the region of homozygosity were prioritized by examination of their physiologic roles and possibility of producing the disease phenotype. Sanger sequencing screening of the first 10 positional candidate genes (AASDH, c4orf14, CEP135, EPHA5, HOPX, IGFBP7, KIAA1211, LOC644145, PPAT, and SRP72) did not identify any pathogenic mutations. Thus, whole exome target enrichment sequencing (NGS) was performed on a single affected family member. Only variants localized within the critical interval were further evaluated and validated. One novel missense mutation c.T744G/p.F248L in the SRD5A3 gene was detected suggesting a CDG type IQ. Subsequently, the entire SRD5A3 coding region was screened by Sanger sequencing and the presence of the mutation was confirmed. The mutation affects an absolutely conserved amino acid, and it is predicted as damaging by Polyphen (version 2.2.2) and SIFT (DB 36 Database) in silico protein modeling softwares. In addition, 2 different bioinformatics tools (Alternative Splice Site Predictor [http://wangcomputing.com/assp/] and NetGene2 World Wide Web Server [http://www.cbs.dtu.dk/services/NetGene2/]) were utilized to determine whether the mutation creates a cryptic splice site that might result in differential messenger RNA splicing and formation of an aberrant isoform. Each tool indicated no change in the predicted splice sites between the wild-type and mutant alleles. The mutation cosegregates with the disease phenotype in the family (Figure 1) is not present in any of the publicly available 1000 Genomes (http://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/) and NCBI dbSNP Build 135 (http://www.ncbi.nlm.nih.gov/projects/SNP/) variant databases, and it is absent in 400 ethnically matched control chromosomes.

Discussion
Congenital disorders of glycosylation are a group of disorders caused by the defective synthesis of N-linked oligosaccharides. Because of the important biologic functions of these oligosaccharides for protein stability and cell communication, incorrect synthesis results in multisystem involvement. The CDG syndromes cause serious, sometimes fatal, malfunction of several different organ systems (especially the nervous system, muscles, and intestines) in affected individuals. Frequency of CDG is estimated at between 1 in 50 000 and 1 in 100 000. Type I CDG syndromes have a type I serum sialo-transferrin pattern on isoelectric focusing characterized by increased di- and asialo-transferrin bands. They are mostly due to defects in the N-glycan synthesis in the cytosol or in the endoplasmic reticulum (ER); some may be due to an N-glycan processing defect in the Golgi apparatus. Two pre-ER defects have been well characterized, namely phosphomannomutase deficiency (carbohydrate-deficient glycoconjugate syndromes (CDGS type IA) and phosphomannose isomerase deficiency (CDGS type IB). Evidence has been reported in 1 family for deficient glycosylation of the dolichol-oligosaccharide ER intermediate.

SRD5A3 is encoding a 318-amino acid enzyme that belongs to the steroid 5-α reductase family and polyisoprenyl reductase subfamily. It is essential for the conversion of polyisopren into dolichol, which is required for the synthesis of dolichol-linked monosaccharides and the oligosaccharide precursor used for N-linked glycosylation of proteins. SRD5A3 mutations have been associated with CDG type IQ defects. Cantagrel et al
identified multiple mutations in SRD5A3. In 2 consanguineous Baluchi-Emirati families originally reported by Al-Gazali et al., a homozygous 3-bp deletion and a 10-bp insertion (286delCAAinsTGAGTAAGGC) in exon 2 in SRD5A3 were observed, which resulted in a predicted stop codon at amino acid 96 in transmembrane domain II. The phenotype included coloboma, optic disk atrophy with loss of vision, mental retardation, cerebellar malformations, and coagulation defects. In a consanguineous Turkish family, a homozygous c.G320A/p.W107X resulted in a termination codon in the first intracellular loop. In a Polish family, a compound heterozygosity was characterized by mental retardation, cataracts, coloboma, and kyphosis.

This report describes a novel SRD5A3 missense mutation associated with a typical CDG type IQ identified by homozygosity mapping in a consanguineous Qatari family, positional candidate gene screening, and NGS exome sequencing. The clinical picture in the 2 patients homozygous for the mutation is summarized in Table 1, with comparison to the cases in the literature.

| Patient | Neurologic findings | Ophthalmologic findings |
|---------|---------------------|-------------------------|
| Sex     |                     |                         |
| II:3    | Muscle hypotonia    | Visual impairment       |
| Male    | –                   | –                       |
| II:6    | Motor retardation   | Optic disk hypoplasia   |
| Female  | +                   | –                       |
|         | Intellectual disability | –                      |
|         | +                   | +                       |
|         | Vermis hypoplasia   | Nystagmus               |
|         | +                   | +                       |
|         | Ataxia              | Optic nerve hypoplasia  |
|         | +                   | +                       |
|         | Glaucoma            | Microphthalmia          |
|         | –                   | –                       |
|         | Cataract            | Glaucoma                |
|         | –                   | –                       |
|         | Glaucosa            | –                       |

|          | II:3 | II:6 | Morava et al. |
|----------|-----|-----|---------------|
| Muscle hypotonia | –   | +   | 10/12         |
| Motor retardation | +   | +   | 8/12          |
| Intellectual disability | +   | +   | 12/12         |
| Vermis hypoplasia | +   | –   | 4/11          |
| Ataxia | +   | +   | 10/11         |
| Visual impairment | +   | +   | 11/12         |
| Optic disk hypoplasia | –   | –   | 3/12          |
| Ocular coloboma | –   | –   | 5/12          |
| Nystagmus | +   | +   | 12/12         |
| Optic nerve hypoplasia | +   | +   | 8/11          |
| Microphthalmia | –   | –   | 2/12          |
| Cataract | –   | –   | 2/12          |

All SRD5A3 mutations identified to date are protein-truncating mutations that are either homozygous or compound heterozygous, which are characterized by ocular and neurologic findings. The c.T744G/p.F248L mutation shares its association with severe mental retardation, optic nerve atrophy, and cerebellar malformations with the other SRD5A3 mutations but lacks the other ocular findings such as cataracts, ocular colobomas as well as ichthyosis and heart defects. This is the only SRD5A3 missense mutation reported to date; however, the ocular and neurologic pictures are similar to the more deleterious mutations. The mutation substitutes a phenylalanine with a leucine residue. Phenylalanine is an aromatic, hydrophobic, 6-carbon ring containing amino acid involved in stacking interactions with other aromatic side chains and in interactions with nonprotein ligands that themselves contain aromatic groups via stacking interactions. It is also involved in binding to polyproline-containing peptides, for example, in SH3 or WW domains. On the other hand, leucine is an aliphatic, branched-chain amino acid usually buried in protein hydrophobic cores with a preference for being within alpha helices more so than in beta strands. The leucine side chain is very nonreactive and is rarely directly involved in protein function, although it can play a role in substrate binding and/or recognition of ligands such as lipids. Such a substitution will severely compromise the stacking ability of that part of the protein which will affect not only its secondary structure (folding) but also its interaction with nonprotein ligands that would require stacking due to a lack of the phenylalanine residue. The presence of leucine at the mutation site will cause a completely different folding and different interactions with nonprotein ligands because of its inability to participate in stacking interactions. These indicate severe compromising in protein folding and interactions, which significantly affect function resulting in a clinical picture similar to that of protein truncating mutations.

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