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Next generation sequencing in a family with autosomal recessive Kahrizi syndrome (OMIM 612713) reveals a homozygous frameshift mutation in *SRD5A3*

Kimia Kahrizi¹, Cougar Hao Hu², Masoud Garshasbi², Seyedeh Sedigheh Abedini¹, Shirin Ghadami¹, Roxana Kariminejad¹, Reinhard Ullmann², Wei Chen², H.-Hilger Ropers², Andreas W. Kuss², Hossein Najmabadi¹, Andreas Tzschach²

¹Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

²Max Planck Institute for Molecular Genetics, Berlin, Germany

Correspondence:

Andreas Tzschach MD

Max Planck Institute for Molecular Genetics

Department Human Molecular Genetics

Innestr. 73

14195 Berlin

Germany

E-mail: tzschach@molgen.mpg.de

Tel: +49 30 8413 1416

FAX: +49 30 8413 1383

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Abstract

As part of a large-scale, systematic effort to unravel the molecular causes of autosomal recessive mental retardation, we have previously described a novel syndrome consisting of mental retardation, coloboma, cataract and kyphosis (Kahrizi syndrome, OMIM 612713) and mapped the underlying gene to a 10.4 Mb interval near the centromere on chromosome 4. By combining array-based exon enrichment and next generation sequencing, we have now identified a homozygous frameshift mutation (c.203dupC; p.Phe69LeufsX2) in the gene for steroid 5 α -reductase type 3 (*SRD5A3*) as the disease-causing change in this interval. Recent evidence indicates that this enzyme is required for the conversion of polyprenol to dolichol, a step that is essential for N-linked protein glycosylation. Independently, another group has recently observed *SRD5A3* mutations in several families with a type 1 congenital disorder of glycosylation (CDG type Ix, OMIM 212067), mental retardation, cerebellar ataxia and eye disorders. Our results show that Kahrizi syndrome and this CDG Ix subtype are allelic disorders, and they illustrate the potential of next-generation sequencing strategies for the elucidation of single gene defects.

Introduction

We have recently reported on a remotely consanguineous Iranian family of three affected siblings who suffered from a novel mental retardation syndrome characterised by severe mental retardation, cataract, coloboma and kyphosis (Kahrizi syndrome, OMIM 612713).¹ Linkage analysis in this family revealed a gene locus in the pericentromeric region of chromosome 4.

Here, we report on the results of mutation screening in the genes of this interval by next-generation sequencing, and on the identification of a frameshift mutation in the steroid 5 α -reductase type 3 gene *SRD5A3*. In combination with other recent observations, this finding indicates that the clinical spectrum of Kahrizi syndrome is wide, and that mutations in the *SRD5A3* gene lead to defective protein glycosylation, even though this feature may be missed upon routine testing.

Methods

Homozygosity mapping in this family had revealed a single linkage 10.4 Mb interval in the pericentromeric region of chromosome 4 flanked by markers rs728293 (chr4: 47052440, HG18) and rs1105434 (chr4: 57488508, HG18).¹

Library preparation, DNA enrichment and Illumina/Solexa Sequencing

For enrichment of the coding regions of all RefSeq genes and flanking intronic sequences of this interval, complementary oligonucleotide baits were designed for all 602 exons including 30 bp extensions at either end, using Agilent's eArray version 6.4. On the 244k sequence capture array, each of these oligonucleotides was represented four times. Repetitive sequences were not excluded. After fragmentation

of the sample of genomic DNA of the index patient (II:4),¹ DNA fragments were repaired to generate blunt ends by T4 polymerase and Klenow DNA polymerase, and phosphorylated with T4 polynucleotide kinase. We measured the DNA concentration with a Nanodrop 7500 spectrophotometer, and a 1 µl aliquot was diluted to 10 nM. After adding a single 'A' base to the 3' end of the DNA fragments using Klenow exo (3' to 5' exo minus), we ligated Solexa adaptors (with a 'T' overhang) with the DNA fragments using DNA ligase. Ligated products (size range 150–200 bp) were gel purified on 2 % agarose, followed by 18 cycles of PCR-amplification.

The hybridization of 20 µg of library DNA and the subsequent sequence capture was performed according to the manufacturers' recommendations (Agilent, Santa Clara, CA). Adaptor-ligated DNA was hybridized to the surface of one lane of a flow cell, and DNA clusters were generated using the Illumina/Solexa cluster station, followed by 76 cycles of sequencing on the Illumina/Solexa 1G analyzer.

Data analysis

Data analysis was performed as described previously.²

RT-Analysis

To check for *SRD5A3* expression, total RNA was extracted from Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines (LCLs) of the index patient and two controls by using the TRIzol reagent (Invitrogen), and cDNA was generated by using the SuperScript III Reverse Transcriptase (Invitrogen) together with random hexamer primers. This cDNA was used to perform PCRs with a series of primer combinations

for two adjacent exon sequences and together encompassing the entire gene (primer sequences are available upon request).

Results

Illumina/Solexa sequencing

In total, 25 million single-end 76 nucleotide (nt) reads were generated, which allowed sufficient coverage of approximately 94 % of the target sequence. 167 single-nucleotide variants (SNVs) matched variants from the reference sequence, 13 of which were not annotated in the dbSNP database. These changes were either silent changes in the coding regions or changes in non-coding regions. A total of 31 indels were detected, 22 of which were novel, and all but one (in exon 1 of *SRD5A3*) were located in non-coding regions. The results of next-generation sequencing analysis of this interval are summarized in Table 1.

Mutation in SRD5A3

The only remaining variant in coding regions of this interval was the duplication of one cytosine in exon 1 of *SRD5A3*. According to NM_024592.3, the position of this change in the protein is c.203dupC (p.Phe69LeufsX2). The genomic position of this duplication is chr4:55907464dupC (HG18).

This mutation was subsequently confirmed by conventional Sanger sequencing in the index patient and his two affected siblings (II:5 and II:6 according to Fig 2a in Kahrizi et al, 2009)¹ (Figure 1a). The parents and two healthy siblings (II:1 and II:3) were heterozygous carriers of this duplication. We failed to detect this

mutation in 366 control chromosomes from healthy Iranian and German control individuals.

RT-PCR

RT-PCR using RNA extracted from a lymphoblastoid cell line of the index patient showed missing or reduced expression of the five *SRD5A3* exons (Figure 1b).

Discussion

In a consanguineous family of three affected siblings who were suffering from a complex congenital disorder, homozygosity mapping had revealed a linkage interval on chromosome 4, and mutation screening of the coding regions in this interval by exon enrichment and Illumina/Solexa sequencing revealed a frameshift mutation in *SRD5A3*.

Mutations in *SRD5A3* have been identified independently of this study in other patients and families with partially overlapping clinical features and congenital defects of glycosylation.³⁻⁴ *SRD5A3* encodes steroid 5 α -reductase type 3, an enzyme which is required for the conversion of polyprenol to dolichol, the lipid anchor for N-glycosylation in the endoplasmatic reticulum.⁴ *SRD5A3* is highly expressed in fetal brain.³

Cantagrel et al (2010)³ and Morava et al (personal communication)⁴ identified a total of 7 different *SRD5A3* mutations in 9 families. Most of these cases were ascertained by abnormal transferrin isoelectric focussing patterns suggesting a type I congenital defect of glycosylation (“CDG type Ix”, OMIM 212067).⁵ In the family

reported here, no clearly abnormal transferrin mobility had been detected in two independent routine tests for CDG screening. Interestingly, in the family reported by Al-Gazali et al (2008),⁶ now also known to carry an *SRD5A3* mutation,³ repeated CDG testing had failed to detect any abnormality, too. Thus, in patients with clinical features suggestive of Kahrizi syndrome, *SRD5A3* mutations should be ruled out even if CDG testing is negative.

Apart from severe mental retardation which was observed in all patients with *SRD5A3* mutations, the presence of additional clinical features seems to vary considerably, not only between different families, but also within families. Cerebellar ataxia was present in most patients, but malformation of the cerebellum or the cerebellar vermis were absent in the original family with Kahrizi syndrome and only detectable in some patients by MRI investigation. Ophthalmic problems (cataract, iris coloboma, optic nerve hypoplasia) were frequently observed. Cataracts were only present in two of the patients of the Cantagrel/Morava cohort.³ In the family reported here, all three siblings developed cataracts at the age of about 17 years. Since the oldest patient in the Cantagrel/Morava cohort was only 12 years old, it may be expected that other patients will also develop cataracts as they grow older.

The syndrome reported here has some phenotypic overlap with Peter's plus syndrome (OMIM 261540), an autosomal recessive disorder which is caused by mutations in the gene encoding B3GALTL, a putative glycosyltransferase.⁷ Also in patients with Peter's plus syndrome, routine CDG testing by isoelectric focussing of transferrin (IFT) failed to reveal any abnormalities. This example, and the family reported here, may serve as a reminder that a normal IFT result does not always

exclude glycosylation defects, and - based on the clinical phenotype - additional molecular genetic tests may be necessary.

In summary, the detection of an *SRD5A3* mutation in this family shows that the recently described Kahrizi syndrome (OMIM 612713) and a specific CDG type Ix subtype (OMIM 212067) are allelic disorders. This study also demonstrates the effectiveness of combining exon enrichment and next-generation sequencing technology for detecting causative gene defects.

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Conflict of interest

The authors declare no conflict of interest.

Legends to table and figure

Table 1 Summary of the results of next-generation sequencing after exon enrichment of the interval on chromosome 4

Figure 1 a) Graphical representation of the linkage interval in the pericentromeric region of chromosome 4 (red box), the position of *SRD5A3*, the position of the mutation and partial chromatogram of exon 1 of the index patient. The asterisk denotes the duplication of one cytosine (c.203dupC;p.Phe69LeufsX2). b) Results of the RT-PCR using RNA derived from a lymphoblastoid cell line of the index patient showing absence (ex 1-4) or reduced (ex 4-5) expression of *SRD5A3* transcripts. *HUWE* was used as an internal control.

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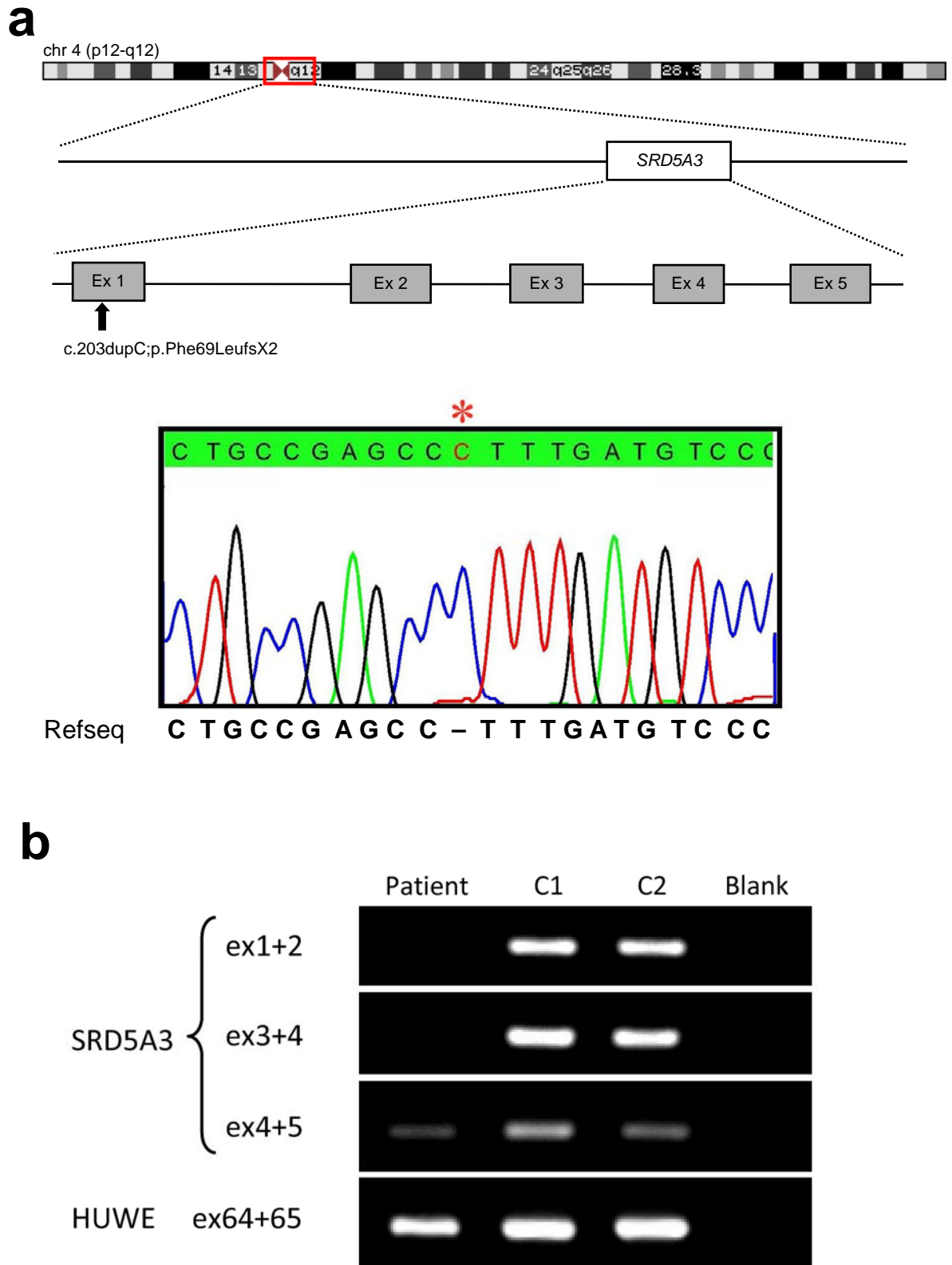


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Table 1 Summary of the results of next-generation sequencing after exon enrichment of the interval on chromosome 4

| Parameter | Result |
|---|--|
| Total length of the enriched region | 171,642 bp |
| Number of annotated (refseq) genes within the enriched region | 46 |
| Number of exons within the enriched region | 580 |
| Number of raw sequencing reads | 25,216,503 × 76 bp |
| Percentage of sequencing reads uniquely mapped on the genome | 43.75% |
| Percentage of sequencing reads uniquely mapped within the enriched region | 8.2% |
| Median sequencing depth within the enriched region | 574 (redundant) or 129 (non-redundant) |
| Percentage of enriched region sufficiently covered (non-redundant coverage >=4) | 99.986% |
| Total number of SNVs detected | 167 |
| Total number of indels detected | 31 |
| Total number of novel SNVs detected* | 13 |
| Total number of novel indels detected* | 22 |
| Number of novel synonymous changes detected | 34 |
| Number of novel non-synonymous changes detected | 1 |

Table 1 Summary of the results of next-generation sequencing after exon enrichment of the interval on chromosome 4

* not annotated in dbSNP (version 130)

Note: All variants mentioned in the list are located in the linkage interval (chr4:
47,052,440-57,488,508)