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CATSPER2, a human autosomal nonsyndromic male infertility gene

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In the course of positional cloning of the Congenital Dyserythropoietic Anemia type I (CDAI) [MIM 224120] gene on 15q15.1–15.3, we examined a family of French origin, in which the proband suffered from asthenoteratozoospermia and nonsyndromic deafness in addition to CDAI. Two of his brothers had a similar phenotype. All three siblings were homozygous carriers of the *CDAI* mutation as well as of a distally located ~70 kb deletion of the proximal copy of a 106 kb tandem repeat on chromosome 15q15. These repeats encode four genes whose distal copies may be considered pseudogenes. Lack of functional stereocilin and CATSPER2 (a voltage-gate cation channel expressed specifically in spermatozoa) may explain the observed deafness and male infertility phenotypes. To the best of our knowledge, the involvement of CATSPER2 in asthenoteratozoospermia is the first description of a human autosomal gene defect associated with nonsyndromic male infertility.

European Journal of Human Genetics (2003) 11, 497–502. doi:10.1038/sj.ejhg.5200991

Keywords: CATSPER2; Male-infertility; Tandem duplication; Contiguous gene syndromes; 15q15; Genomic deletion

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Received 26 July 2002; revised 4 February 2003; accepted 12 February 2003

Introduction

Several loci have been implicated in syndromic male infertility with oligospermia or azoospermia, such as the receptor genes for the follicle-stimulating hormone^{1,2} and X-linked androgen.^{3–5} Despite an estimated prevalence of ~0.3%, only few genes or loci are known to be involved in human asthenospermia or asthenoteratozoospermia. These include the '9+0' syndrome, in which the central pair of

sperm cell microtubules is missing, leading to complete sperm immotility,⁶ and primary ciliary dyskinesia (PCD; MIM 242650), a group of heterogeneous disorders of unknown origin, usually inherited as autosomal recessive traits. The PCD phenotype is characterized by axonemal abnormalities of respiratory cilia and sperm tails, leading to bronchiectasis, sinusitis, sometimes associated with situs invertus (Kartagener syndrome), and male infertility with immobile spermatozoa.⁷ DNAH11, encoding for axonemal heavy-chain dynein type 11, was the first PCD gene identified.^{8,9} It was recently demonstrated that DNAH11 mutations account for situs inversus totalis but probably only for a minority of PCD cases. No gene involved in pure asthenospermia or asthenoteratozoospermia has been identified so far, although associations with mtDNA haplogroups¹⁰ and an autosomal dominant gene¹¹ were reported. Two genes were recently shown to be involved in mouse sperm motility, CATSPER and CATSPER2.^{12,13} The CATSPER2 human homolog was localized to chromosome 15q, but no mutation affecting male infertility has been described in humans so far.

In the course of positional cloning of the Congenital Dyserythropoietic Anemia type I (CDAI) gene [MIM 224120],¹⁴ which was previously localized to chromosome 15q15.1–15.3,¹⁵ we examined a family of French origin, in which the clinical and hematological features of the propositus (II-2, Figure 1a) and of his two affected brothers were compatible with CDAI (Table 1). However, unlike other CDAI patients, the brothers suffered also from sensorineural deafness, and a severe reduction in the percentage of motile spermatozoa in their ejaculates (Table 1). As a result of the perfect cosegregation of these additional features with their CDAI status, these patients were meticulously examined for the possible involvement of chromosome 15 in their condition.¹⁵ Detailed molecular analysis of this family's DNA samples revealed a ~70 kb deletion in chromosome 15q15, removing part of the CATSPER2 gene, and suggesting the importance of this gene for sperm motility in humans.

Materials and methods

Patients

The propositus (II-2, Figure 1), a 56-year-old male from a nonconsanguineous family, from a small country town in southwest France, was found to be deaf in infancy. Later he proved to be sterile, and at the age of 53, presenting with mild jaundice and high serum ferritin, he was diagnosed, based on bone marrow electron microscopy, as having CDAI. Similar phenotypic findings were noted in his two affected brothers (Figure 1, Table 1). A detailed otorhinolaryngologic investigation (including tympanometric studies, a CT-scan of the temporal bones, brain stem-evoked potentials, videonystagmography, rotary/vestibular and caloric tests) revealed a moderate symmetrical sensorineur-

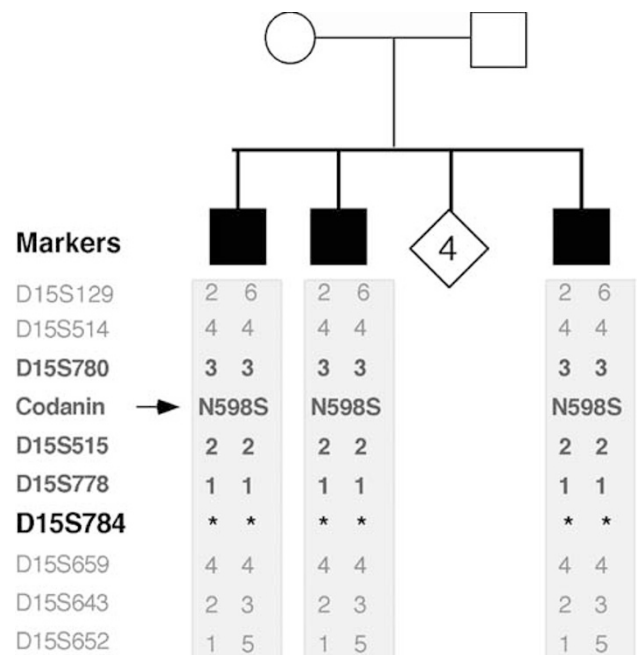


Figure 1 Pedigree of family with three affected male siblings suffering from CDAI, sensorineural deafness and lack of motile sperm cells, and their chromosome 15q15 haplotypes. The location of the N598S codanin I mutation is indicated by an arrow.¹⁴ As D15S784 could not be amplified, it is marked in larger characters, while markers within the defined Bedouin CDAI linkage interval are in bold.

al hearing loss (40 dB) involving all frequencies (patients II-2, II-4). Semen analysis showed a normal volume and sperm concentration. The motility and morphology of the spermatozoa were, however, abnormal, indicative of asthenoteratozoospermia. The percentage of motile cells at 1 and 2 h postejaculation was 3 and 1%, respectively. The majority (91%) of the sperm cells had short, coiled flagella. A similar clinical phenotype was observed in the third brother, although no semen analysis or audiographic tests were performed.

Molecular analysis

The genomic clones (AC011330, AC019011 and AC018512) used to validate the duplication were obtained from Multimegabase Sequencing Center, University of Washington, USA. Genotyping was performed according to standard procedures. Small copy-specific islands (mostly Alu-inserts) allowing discrimination between the proximal and distal copies of the repeat were identified by sequence alignments. To delineate the extent of the deletion, PCR reactions were performed with repeat-unit-specific primers as shown in Figure 2c. Sequence analysis of the CATSPER2 gene and pseudogene were performed by *in silico* alignment, sequence variants being subsequently validated and mapped on short- or long-range PCR fragments generated

Table 1 Main clinical and laboratory features of the three affected brothers

Patient	II-1	II-2	II-4
Age (years)	58	56	54
Hemoglobin (g/dl)	13.9	12.3	13.1
MCV fL (Normal value 80–90 fl)	99	98	97
Peripheral blood smear	Basophilic stippling and schizocytes	Basophilic stippling and schizocytes	Basophilic stippling and schizocytes
Serum ferritin (ng/ml) (normal value 25–250 ng/mL)	1550	1399	2100
Bone marrow aspiration			
Light microscopy	Intercellular chromatin bridges	Intercellular chromatin bridges	Intercellular chromatin bridges
Electron microscopy	ND	Spongy heterochromatin Enlarged nuclear pores	ND
Deafness	+	+	+
Auditory testing	ND	Sensorineural deafness	Sensorineural deafness
Infertility	+	+	+
Spermogram	ND	Asthenoteratospermia	Asthenoteratospermia

ND, not done, MCV, mean corpuscular volume.

on DNA from control individuals. Primer pairs were designed using Oligo primer software. All PCR reactions were performed according to standard procedures, and the products were subsequently sequenced using Big-Dyes dye terminators kit on an ABI 3700 sequencer (Perkin-Elmer/Applied biosystems). Sequence comparisons were performed using a STADEN package¹⁶ and Sequencer software (gene codes cooperation: www.genecodes.com).

Full-length KIAA0377 transcripts were amplified, using long-range PCR of human cDNA libraries (Clontech) and of mRNAs from erythroid precursors grown in a two-phase liquid culture¹⁷ as previously described.¹⁸ The PCR products were cloned into a pCR2.1-TOPO vector to separate and sequence alternatively spliced variants expressed in the same tissue.

Web-based databases and sequence analysis

Genomic browsers: Santa Cruz, <http://genome.ucsc.edu/goldenPath/hgTracks.html>;

Celera Human Genome Database (CHGD), <http://cds.celera.com/>;

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim>.

Homology searches. KIAA0377 orthologs (*H. sapiens* KIAA0377: XP_012386, *M. Musculus* KIAA0377: AF50258, *H. sapiens* KIAA0433: NP_056031, *D. melanogaster*: AAF50947, *C. elegans*: T25770, *A. thaliana*: T51452, *S. pombe*: T41050, and *S. cerevisiae*: NP_013514) were identified upon a PSI-BLAST search of KIAA0377 against the NCBI nonredundant database.¹⁹ The KIAA0377-translated protein was analyzed by the following computer programs: EXPASY Proteomic tools containing a Profile scan and PROSITE software²⁰ (<http://www.expasy.ch/>), BLOCKS²¹ (<http://bioinformatics.weizmann.ac.il/blocks/>) and YPD-The Yeast Proteome Database – (<http://www.incyte.com/>

<http://www.incyte.com/sequence/proteome/databases/YPD.shtml>) for KIAA0377 yeast ortholog function.

Results

Although no consanguinity was reported, the affected brothers were homozygous for the same N598S CDAI mutation¹⁴ as well as for a battery of chromosome 15q markers (Figure 1a), which is suggestive of an unsuspected consanguineous antecedent. Marker D15S784 failed, however, to be amplified with several sets of primers, suggesting the existence of a possible deletion in these patients, which could account for their unique syndromic phenotype.

Analysis of control chromosome and identification of an ~106 kb tandem duplication

A detailed analysis of control chromosomes led to the identification, distal to the location of the CDAI linkage interval, of an unsuspected ~106 kb tandem duplication, involving four genes (Figure 2a): (a) The anonymous gene KIAA0377, encoding a 1408 amino-acid-long predicted protein of unidentified function; (b) creatine mitochondrial kinase 1 (CKMT1), which is crucial for energy metabolism, particularly in tissues with high-energy requirements; (c) a transcript expressed almost exclusively in the inner ear, encoding stereocilin (SRTC)²² and (d) CATSPER2, a sperm-specific voltage-gated cation channel.¹³ Detailed *in silico* analysis revealed that although each pair of coding regions shows >98.0% identity, three of the distal genes are likely to be pseudogenes (Figure 2a). The distal copy of KIAA0377 contains only the first 24 of 31 exons, that of SRTC has a stop codon in exon,²² and that of CATSPER2 differs at numerous positions in the transcript, including

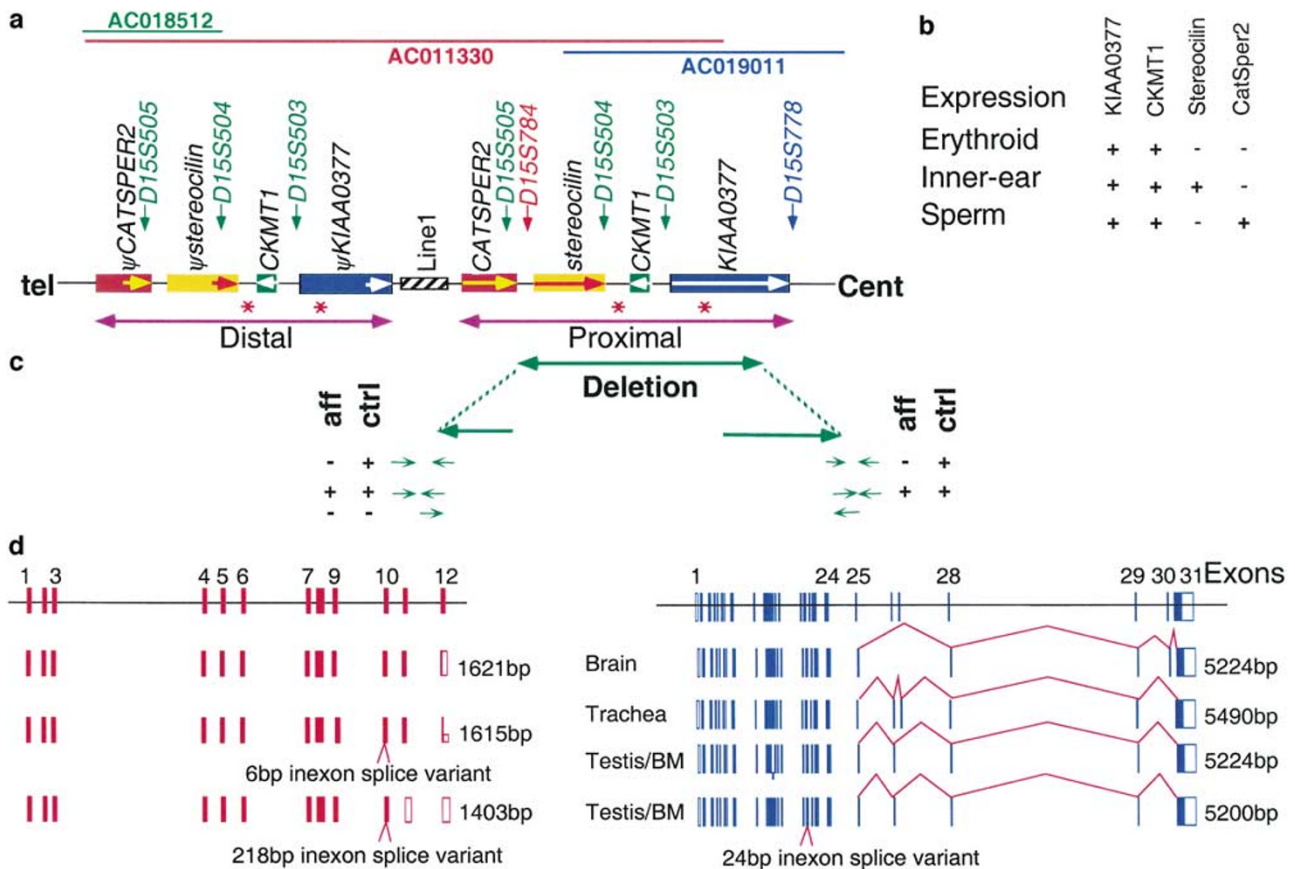


Figure 2 Expanded view of a duplicated genomic region on 15q15 (telomere – left, centromere – right). (a) The blue, green and red lines represent different genomic clones (and accession numbers) according to which the duplication was identified and validated experimentally. Microsatellite marker positions are indicated by arrows on the genomic map. Duplicated markers are in green, unique markers in blue and D15S784 in red. Genes are shown as colored boxes with arrows indicating gene polarity. The unique line repeat separating the tandem duplications is indicated by a striped box. Pseudogenes are marked by a ψ symbol. The purple arrow points to the proximal and distal duplication elements. (b) Expression patterns of KIAA0377 and CKMT1 (based on experimental and *in silico* data) and of stereocilin and CATSPER2 (based on *in silico* data). (c) Primers used to define the deletion boundaries are shown as sets of green arrows. The upper pair amplified only DNA from control individuals; the pair on the second line yielded the expected PCR fragments from both controls and affected brothers; the last set of primers, designed to span the deletion, failed to yield the bridging fragment. (d) Genomic structure of the proximal and distal CATSPER2 (red) and KIAA0377 (blue) genes. Coding and noncoding exons are depicted as filled and open boxes. Alternatively spliced transcripts of CATSPER2 (AF411817, AF411818, and AF411819¹³) and KIAA0377 (AF502586, AF502587, AF502588, AF502589) are indicated, with their corresponding lengths to their right. Five single nucleotide polymorphisms (SNPs) were found in KIAA0377, including the two that cause amino-acid substitutions: a G356A (exon 3) and A4022G transition (exon 31) resulting, respectively, in an Arg77Gln and Glu1305Arg substitution.

base substitutions, gaps and a 1 bp deletion, creating a premature translation termination. CKMT1 is the only gene for which neither the distal nor the proximal copy shows any disruption of coding capacity (the distal copy differs in its cDNA by only two synonymous base substitutions: G1233A and C1263T). The D15S784 marker, which fails to be amplified in these patients, is located within a unique microregion separating the proximal SRTC and CATSPER2 loci. In addition, about 10kb of a unique sequence containing a 6 kb line repeat (L1) separates the large duplicated segments (Figure 2a).

The affected patients carry a ~70 kb deletion of the proximal copy of the repeat

In comparison with genomic DNA from healthy individuals which yielded both the proximal and distal copies of the Alu inserts, genomic DNA from the affected sibs yielded amplicons only of the Alu inserts present in the distal copy, thereby confirming the presence of a substantial deletion (Figure 2c). Figure 2c also shows the distal and proximal borders of the genomic deletion map, respectively, 1 kb centromeric to D15S505 and 2 kb centromeric to exon 24 of the KIAA0377 gene. None of the

tested primer sets allowed amplification of the region bridging the deletion.

The affected siblings proved, based on local sequence divergence, to be homozygous carriers of a ~70 kb deletion of the proximal copy of the repeat unit, encompassing D15S784 and the copy-specific Alu inserts, as well as the first 24 exons of KIAA0377, the entire coding sequences of both CKMT1 and SRTC, and the last two exons of CATSPER2, removing 225 bp of the corresponding mRNA. D15S505, which is within the 10th intron of CATSPER2 and maps 5123 bp distal to D15S784, is not deleted (Figure 2a and c).

The deletion and the observed phenotype

To determine to what extent this deletion could account for the observed deafness and sterile phenotypes encountered in the patients, and which of the encoded genes might underlie these pathologies, we examined each of these genes.

A similarity search revealed several proteins highly homologous to the KIAA0377 translated product. Although they all contain a single histidine acid phosphatase motif (data not shown), none has a known function, except for the yeast ortholog *Asp1/Vip1*, which is involved in the movement of actin patches.²³ Next we, investigated the expression pattern of KIAA0377. RT-PCR amplification showed that it is expressed ubiquitously, yielding a complex pattern of alternatively spliced variants, the full-length mRNAs being between 5400 and 5200 bp long (Figure 2d). Hence, neither KIAA0377 nor the ubiquitous CKMT1 – involved in energy transduction in tissues with large, fluctuating energy demands, of which one intact copy is still present – appear *a priori* to be candidates for the etiology of the observed phenotypes. The lack of their proximal copies seems to have no visible phenotypic effect.

As for the remaining two genes, STRC and CATSPER2, the specificity of their expression pattern is compatible with the hypothesis that the observed deafness and infertility phenotypes are because of their respective inactivation (Figure 2b). Immunohistological labeling previously demonstrated that SRTC is expressed only in the inner ear, with intense staining along the hair bundle.²² Stereocilin is associated with stereocilia, the stiff microvilli forming the mechanoreception structure of sound stimulation. Furthermore, gene inactivating mutations in two DFNB16-linked families suggest that loss of stereocilin is involved in their deafness.²² Hence, the three brothers, having also lost the intact copy of the SRTC gene, belong to the same clinical and genetic deafness entity. CATSPER2 mRNA, however, is expressed during the meiotic stages of spermatogenesis, and all three of the cloned human testis cDNAs are transcribed from the proximal CATSPER2 gene,¹³ which is truncated in these patients (Figure 2c). CATSPER2 is thus a strong candidate for the observed infertility phenotype.

Discussion

Recent estimates suggest that as much as 5–10% of the human genome might be duplicated. These estimates of the number of duplicated segments suggest that rearrangements involving these sequences may play a prominent role in the etiology of a significant fraction of genetic disorders.^{24,25} Indeed, nonallelic homologous recombination (NAHR) between duplicated sequences may result in microdeletions, microduplications or inversions of genomic segments, causing Mendelian disorders, contiguous gene syndromes and whole-arm chromosome aberrations, depending on the size of the genomic segment involved. Of the 169 regions identified thus far as segmental duplications (and constituting roughly 1/10th of the genome (298 Mb)), 24 were found to be associated with genomic disorders.²⁴ In the present study, we identified a 106 kb tandem duplication and a large genomic deletion that may underlie the observed deafness and male infertility phenotypes. Thus, these patients carry besides the CDAI mutation, a deletion inactivating four genes. CATSPER2, partially removed by the deletion, appears to be the best candidate for the etiology of the observed male infertility.

The CATSPER2 protein resembles the voltage-gated potassium channel.¹³ This ion channel is present on the sperm flagellum, suggesting its role in the regulation of sperm motility. CATSPER2 also strongly resembles another sperm-specific putative cation channel, namely CATSPER,^{12,13} whose targeted disruption results in male sterility in otherwise normal mice with reduced sperm motility and lack of cyclic AMP-induced Ca²⁺ influx.¹² Both proteins possess a single, six-transmembrane-spanning repeat whose pore region and overall homology are close to a single domain of the much larger four-repeat Ca-selective ion channel. In both proteins, the pore-forming residue is located between the fifth and sixth transmembrane segments. The deletion found in our patients removes the last 225 bp of CATSPER2 mRNA. This fragment corresponds to the protein segment that mediates the actual channel pore complex,¹³ suggesting that the natural pore complex of CATSPER2 is not formed in these patients, thereby affecting sperm motility, a functional assignment compatible with the observed phenotype, that is, asthenozoospermia.

In view of their post-meiosis colocalization within the sperm flagellum, it has been suggested that these two proteins of the CATSPER family interact functionally and are part of a heterotetrameric Ca channel.^{13,26} Although coimmunoprecipitation failed to demonstrate any homo- or heterotetramer formation,¹³ it would be of interest to measure the impact that the loss of one CATSPER family member has on the subcellular localization of the other. Finally, considering the decreased sperm motility and male infertility phenotype of the CATSPER mouse knockout model, these data warrant the systematic screening of

CATSPER, which maps to 11q13.1, as an additional potential candidate in the generation of this particular phenotype.¹³ Both CATSPER and CATSPER2 may also be candidate genes for autosomal dominant asthenoteratozoospermia.¹¹

To sum up, the high structural and functional homology of CATSPER2 with its paralog, CATSPER, and the fact that inactivation of the latter leads to loss of sperm motility in mice, and the phenotype observed in our patients, indicate that disruption of CATSPER2 might underlie highly reduced sperm motility in man. Last but not least, to the best of our knowledge, the implication of CATSPER2 in asthenoteratozoospermia is the first description of an autosomal gene associated with nonsyndromic male infertility in humans.

Acknowledgements

We are indebted to Professor E Fibach from the Hematology Department, Hadassah Medical Center, Jerusalem, Israel, for culturing erythroid precursors from peripheral blood. This work was supported by a grant from the Israel Ministry of Health (HT), and by the J Maus and G Ceasemann-Maus, Chair in Pediatric Hematology (RZ). It is also supported by the Crown Human Genome Center, an Israel Ministry of Science grant to the National Laboratory for Genome Infrastructure, the Alfred Krupp Foundation, the German-Israeli Foundation for Scientific Research and Development, and The Weizmann Institute Glasberg, Levy, Nathan Brunschwig and Levine funds (DL). Doron Lancet holds the Ralph and Lois Silver Chair in Human Genomics. JSB is a recipient of the Hermann-Mayer chair.

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