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A Deletion Mutation in *SANS* Results in Atypical Usher Syndrome

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Keywords

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Usher syndrome is an autosomal, recessively inherited disorder involving progressive retinitis pigmentosa and hearing loss with or without vestibular dysfunction. Usher type I (*USH1*) is the severest form (1). It involves profound deafness, vestibular areflexia and onset of retinitis pigmentosa in early childhood (1). To date seven genetic loci for *USH1* have been mapped (*USH1B-USH1H*) and five of the genes have been identified. *USH1G* (MIM #606943) is associated with mutations in *SANS* (2). The encoded 461 amino acid protein, SANS, is predicted to have three ankyrin-like domains, a PDZ binding motif and a Sterile alpha motif (SAM) domain (2). *SANS* is expressed in many tissues including the inner ear and retina (2,3). Jackson shaker (*js*) mice have a mutation in *Sans*. They exhibit disorganized stereocilia and are profoundly deaf, but do not exhibit a retinal phenotype (3).

USH1G appears to be a rare cause of *USH1* as only five mutations in *SANS* have been reported to cause Usher syndrome in four families (2,4,5) (Table 1). All mutations, except one, cause classic symptoms of *USH1*. A mutation in exon 2 substituting p.V458D in SANS is associated with atypical Usher syndrome. It is therefore hypothesized that missense mutations in *SANS* may result in hypomorphic alleles and cause a less severe phenotype as compared to frameshift mutations (4).

We report a consanguineous family with four affected individuals with moderate to severe hearing loss, mild retinitis pigmentosa and normal vestibular function, a phenotype which resembles an USH2 diagnosis (1). Family HLRB12 (Fig. 1a) was recruited from Sheikhupura, Pakistan with Institutional Review Board approval at University of the Punjab, and written informed consent was obtained from all participants. Three affected children were subjected to audiometric examinations at the age of 12, 14 and 21 years, in ambient noise conditions. Clinical examination revealed moderate to severe hearing loss (Fig. 1b). Vestibular function was evaluated by Romberg and tandem gait tests. Additionally, inquiries were made about whether affected children felt insecure while walking in darkness or had motion sickness. There was no delay in independent ambulation and none of the affected individuals had difficulty with balance suggesting vestibular function was normal. Additionally, none of the patients reported problems with eyesight including night vision. However, funduscopy revealed mild symptoms of retinitis pigmentosa in three of the older

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affected individuals examined at 5, 13, 15, and 22 years respectively. No bone spicules were observed in the retinal epithelium (Fig. 1d). The optic discs were pale as compared to those in normal individuals. Electroretinography could not be performed. Optical testing revealed a mild loss of near sight vision, which was not noted by the patients.

Genotyping with fluorescently labeled markers for linkage analyses excluded all Usher syndrome loci except *USH1G*. Markers D17S1807 and D17S1301 lie close to *SANS* and showed homozygosity by descent in all affected individuals of family HLRB12 (Fig. 1a). Using a disease allele frequency of 0.001 and coding the phenotype as a fully penetrant autosomal recessive disorder, maximum two-point LOD scores of 4.2 and 3.9 were obtained at recombination fraction $\theta = 0$ with the markers D17S1807 and D17S1301 respectively. We PCR amplified and sequenced the three exons and flanking intronic regions of *SANS*. In the DNA of affected individuals of family HLRB12 we identified a homozygous 15bp deletion (c.163_164+13del15) involving nucleotides in the first exon and intron of *SANS* (Fig. 1c, e). We did not detect this mutation in 200 chromosomes from ethnically matched controls assayed by Tetra primers ARMS PCR (6).

In order to identify the effect of c.163_164+13del15 on the *SANS* transcript, we obtained RNA from whole blood and generated cDNA. However we found that *SANS* is not expressed sufficiently in blood samples (data not shown). Usually mutations that delete donor splice sites in first exons result in retention of the following introns or use of a cryptic splice site within the affected exons or introns (7,8) . Indeed, *in silico* analysis for cryptic splice sites in wild type and mutant genomic sequences of *SANS* with GeneSplicer (http://cbcb.umd.edu/software/GeneSplicer/gene_spl.shtml) predicted retention of the first intron of in the RNA resulting from mutant, but not from the normal, *SANS* genomic sequence (data not shown). The retention of the first intron will introduce a frameshift and a premature stop codon in the *SANS* open reading frame. The presence of premature stop codons are known to mark some mRNAs for nonsense mediated decay (9) and it is possible that no mutant mRNA will be produced in patients with the deletion mutation. However, if the mRNA escapes this surveillance mechanism, the frameshift will result in a truncated nonfunctional protein of 58 amino acids.

Our work indicates that both missense and deletion mutations in *SANS* can result in atypical Usher syndrome. Thus the location or type of mutation does not predict the severity of the disorder and the phenotypic course can be modified by unknown genetic or epigenetic factors. Furthermore, some patients with no mutations in genes which cause USH2 may have mutations in *SANS*.

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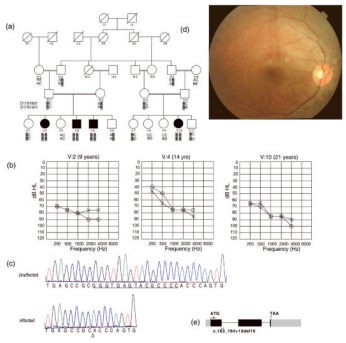

Figure 1.

(a) Pedigree of family HLRB12. Haplotypes for two closest markers to *SANS* are shown for the genotyped members. Alleles of the two markers were homozygous for all affected individuals. The ancestral chromosome with the *SANS* mutation is shaded in gray (b) Audiograms of three affected individuals of family HLRB12 indicate moderate to severe hearing loss. Age at the time of audiometry is indicated on tops of the audiograms. “o” indicates air conduction for right ear, while “x” indicates air conduction for left ear (c) A fifteen nucleotide deletion was observed in *SANS* at the first exon-intron junction in DNA of affected individuals. “Δ” indicates the start of deletion. Deleted bases are underlined in the trace from a control. (d) A representative funduscopic image from a patient with atypical Usher syndrome in family HLRB12. No bone spicules were observed (e) Diagrammatic depiction of *SANS*. Black boxes represent translated exons while gray boxes denote untranslated parts of the exons. The two introns are represented by horizontal lines. The position of the deletion of 15 nucleotides identified in family HLRB12 is shown by a bracket under the depicted exon 1 and the following intron. The arrow and the asterisk denote the start and stop codons in *SANS*, respectively.

Table 1

List of all mutations in *SANS*

List of all known mutations identified in *SANS*.

Country	Exon	Mutation[a]	Consequence	Hearing Loss	Reference
Germany	1	c.143 T>C*	p.L48P	Profound	2
	2	c.186_187delCA	Frameshift		
Tunisia	2	c.393insG	Frameshift	Profound	2
Jordan	2	c.832_851del20**	Frameshift	Profound	2
U.S.A	1	c.113 G>A	p.W38X	Profound	5
Turkey	2	c.1373 A>T	p.D458V	Variable (moderate to profound)	4
Pakistan	1	c.163_164+13del15	Frameshift	Moderate to severe	This report

[a]Numbers with respect to the open reading frame of *SANS*, corresponding to cDNA sequence from GenBank (AK091243) with the first nucleotide in the initiation codon designated as "+1".

*Originally reported as c.142 C>T.

**Originally reported as 829_848del20.