LETTER TO JMG

Distinctive audiometric profile associated with DFNB21 alleles of *TECTA*

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Genetic factors are thought to account for approximately one half of cases of childhood hearing loss, the majority of which is non-syndromic and not associated with other abnormalities. Seventy-seven percent of hereditary, non-syndromic, prelingual deafness is autosomal recessive, 22% is autosomal dominant, and 1% is transmitted as a matrilineal or X linked trait.[1] So far, more than 30 distinct genetic loci (known as DFNB loci) have been mapped for non-syndromic recessive deafness (NSRD). In the absence of syndromic associations to guide genetic diagnosis, the auditory and vestibular features provide the only phenotypic clues to direct molecular diagnostic testing. Unfortunately, the phenotype of NSRD is usually non-specific; prelingual, non-progressive, and severe-profound impairment is associated with mutations in a majority of DFNB loci.[2] In contrast, inherited dominant hearing loss is more phenotypically heterogeneous; it is usually postlingual, progressive, and can be associated with a variety of different audiometric configurations.[2]

Mutations in the gene encoding α-tectorin (*TECTA*) are associated with both dominant and recessive modes of inherited hearing loss, DFNA8/A12 (MIM 601543 and MIM 601842) and DFNB21 (MIM 603629), respectively, and provide a robust model of genotype-phenotype correlation. Missense substitutions in *TECTA* result in dominant hearing loss (table 1). Three of these missense dominant alleles result in substitution of cysteine residues and are associated with progressive hearing loss.[3–5] All other dominant missense alleles of *TECTA* are associated with stable, non-progressive hearing loss.[6 7] The only known recessive allele of *TECTA* is a splice site mutation that causes prelingual, severe-profound deafness linked to DFNB21.[8]

α-tectorin is one of the major glycoproteins of the tectorial membrane, the acellular matrix overlying the cochlear neuroepithelium.[9 10] α-tectorin has predicted structural domains with similarity to protein modules important for cross linking with other proteins.[6 10] One such region is similar to a sperm protein, zonadhesin, while another predicted domain resembles proteins found in the zona pellucida, an extracellular matrix surrounding the oocyte. Missense substitutions in the zona pellucida domain of *TECTA* cause a moderate degree of hearing loss with greater involvement of mid frequencies.[5–7 11] In contrast, missense substitutions in the zonadhesin domain of *TECTA* cause mild to moderate hearing loss primarily affecting high frequencies.[3 4]

Here we report two novel mutations of *TECTA*, predicted to be functional null alleles, which cosegregate with recessive, moderate to severe hearing loss in large consanguineous families. The distinctive phenotype associated with DFNB21 deafness provides a useful clinical marker to facilitate its genetic diagnosis.

Key points

- Mutations of *TECTA* result in dominantly (DFNA8/A12) or recessively (DFNB21) inherited hearing loss linked to markers on chromosome 11q23.
- We describe a distinctive phenotype associated with homozygosity for two novel frameshift mutations (649insC and 6037delG) of *TECTA* cosegregating with hearing loss linked to DFNB21.
- Affected subjects exhibit a severe hearing loss that is more pronounced in the 1000-2000 Hz frequency range resulting in a flat to a shallow "U" shaped audiogram.
- The phenotype associated with DFNB21 deafness provides a useful clinical marker to facilitate genetic diagnosis.

Table 1 *TECTA* mutations and associated phenotype

Mutant allele	Location domain	Hearing loss			References
		Onset	Phenotype	Audiometric profile	
DFNA8/A12					
Cys1057Ser	Exon 10 (zonadhesin)	Postlingual	Mild to severe, progressive	High frequency	4
Cys1619Ser	Exon 14 (zonadhesin)	Variable	Mild to moderate-severe, progressive	High frequency	3
Leu1820Phe, Gly1824Glu	Exon 17 (zona pellucida)	Prelingual	Mild to moderate, stable	Mid frequency	6
Cys1837Gly	Exon 17 (zona pellucida)	Postlingual	Mild to moderate, progressive	Mid frequency	5
Tyr1870Cys	Exon 18 (zona pellucida)	Prelingual	Moderate to moderate-severe, stable	Mid frequency	6
Arg2021His	Exon 20 (zona pellucida)	Prelingual	Moderate, stable	Mid frequency	7
DFNB21					
649insC	Exon 5	Prelingual	Moderate-severe	Flat or mid frequency	Present report
IVS9+1G→A	Intron 9	Prelingual	Severe-profound	Not defined	8
6037delG	Exon 20	Prelingual	Moderate-severe	Flat or mid frequency	Present report

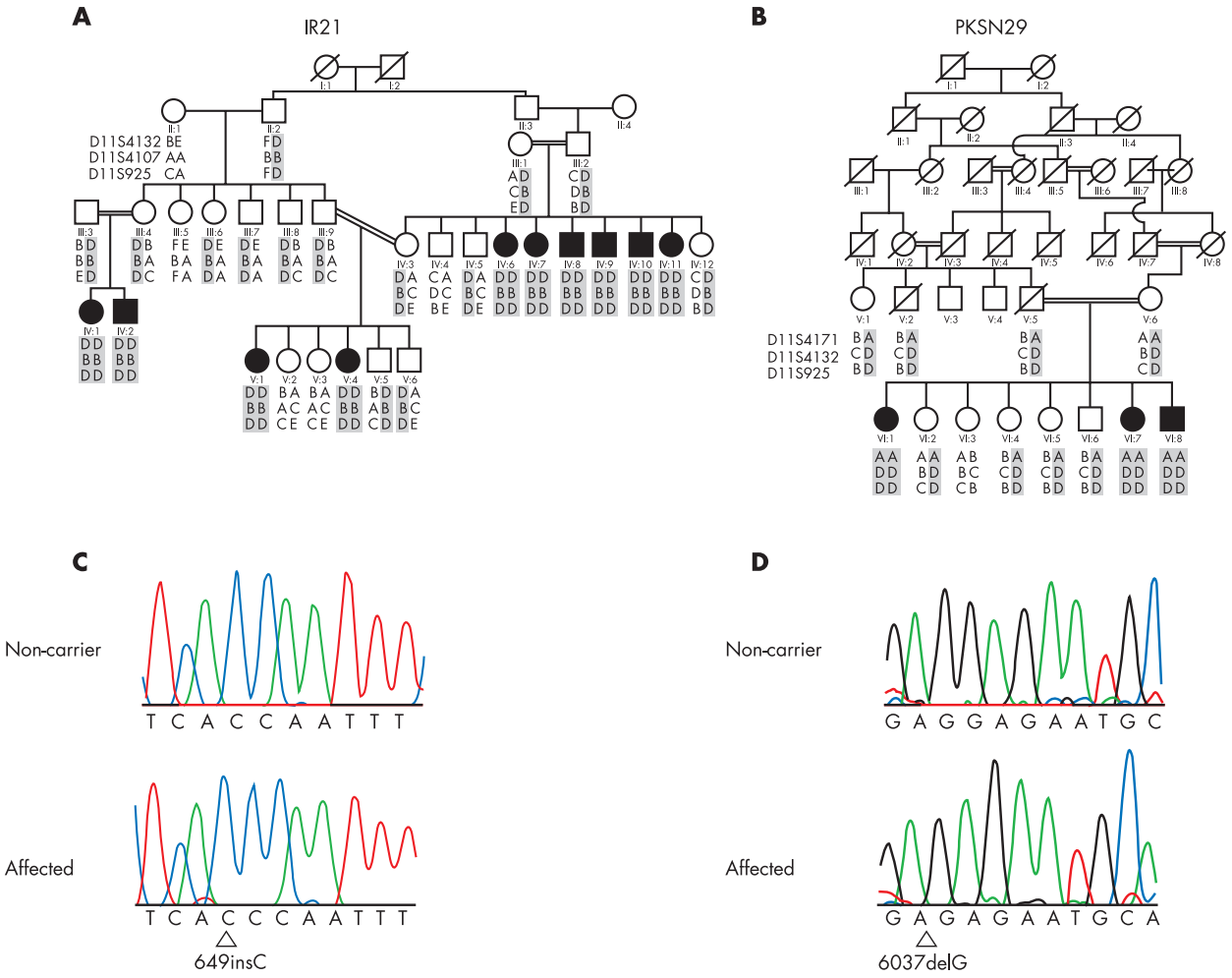

Figure 1 Linkage to DFNB21 and *TECTA* mutations. (A) Family IR21 shows cosegregation of non-syndromic recessive deafness with DFNB21 linked markers on chromosome 11q. Black symbols represent deaf subjects. Grey shaded haplotypes represent the ancestral chromosome harbouring the *TECTA* mutation. (B) Family PKSN29. Deaf subjects VI.1, VI.7, and VI.8 show homozygosity for markers linked to DFNB21. (C) DNA sequence chromatogram from an unaffected homozygous wild type subject V.2 and an affected subject, V.1 in family IR21, homozygous for 649insC. The arrowhead in the affected subject's sequence trace shows the inserted cytosine in exon 5 of *TECTA*. (D) For family PKSN29, a wild type sequence trace of *TECTA* exon 20 from a normal hearing subject VI.3 and deaf subject VI.1 homozygous for 6037delG. The site of deletion of guanine is shown with an arrowhead in the affected subject's chromatogram.

MATERIALS AND METHODS

IRB approval (OH93-DC-016) was obtained for this study from the National Institutes of Health, USA, the Centre of Excellence in Molecular Biology, Lahore, Pakistan, and the National Research Center for Genetic Engineering and Biotechnology, Tehran, Iran. The participating subjects gave written informed consent. Medical histories were obtained for affected subjects in families IR21 and PKSN29. Pure tone air conduction audiometry was performed under quiet ambient environmental conditions. Blood samples were collected from participating subjects of families IR21 and PKSN29 in Iran and Pakistan, respectively. DNA was extracted by a non-organic method[12] followed by removal of proteins using a saturated solution of sodium chloride followed by isopropanol precipitation of DNA.

PCR amplifications of microsatellite repeats linked to the known deafness loci (Hereditary Hearing Loss Homepage, http://www.uia.ac.be/dnalab/hhh) were carried out with fluorescently labelled primers. The resulting PCR products were separated on ABI Prism® 377 polyacrylamide gels. The 23 exons of *TECTA* were PCR amplified with flanking intronic primers[6] from genomic DNA. Primers and dNTPs were removed by incubating 20 µl of the PCR products with 3 units of exonuclease 1 (USB) and 0.3 units of shrimp alkaline phosphatase (Amersham) as recommended by the manufacturers. BigDye™ Terminator cycle sequencing (PE-ABI) products were analysed on an ABI Prism® 377 DNA sequencer.

RESULTS

Families IR21 (fig 1A) and PKSN29 (fig 1B) segregated prelingual, bilateral, moderate to severe sensorineural hearing loss. Anamnestic reports indicated that the hearing impairment was non-progressive. The hearing loss affects all frequencies, resulting in a flat to shallow "U" shaped pattern on the audiograms (fig 2A-F). The heterozygous carriers of *TECTA* frameshift mutations had normal hearing thresholds (fig 2G-H).

Linkage analyses with markers for DFNB21 (*TECTA*) showed shared homozygosity by descent in deaf subjects of families IR21 and PKSN29, respectively (fig 1A, B). The 23 exons of *TECTA* were sequenced for all affected members of the two families. Affected members of family IR21 were homozygous for an insertion of cytosine (649insC) in exon 5 of *TECTA* (fig 1C). In family PKSN29, a deletion (6037delG) in exon 20 was detected in affected subjects (fig 1D). Homozygosity for the mutations cosegregated with the deafness phenotype in both families and no mutations were identified in any other exons of *TECTA*. We did not detect 649insC or

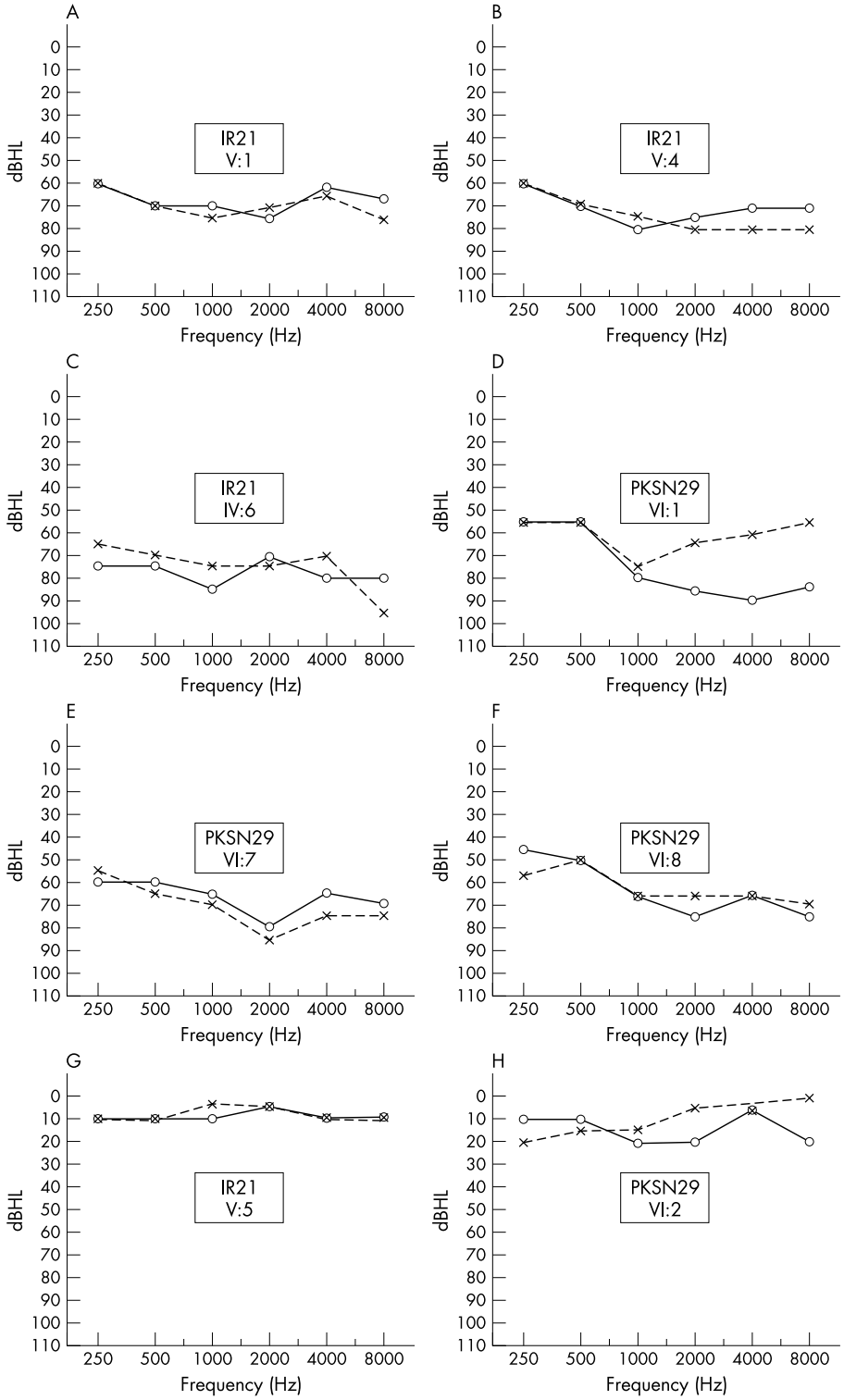

Figure 2 Representative pure tone air conduction audiograms from families IR21 and PKSN29 for deaf subjects and heterozygous carriers of *TECTA* mutations. (A) IR21-V.1 (aged 19). (B) IR21-V.4 (aged 13). (C) IR21-IV.6 (aged 35). (D) PKSN29-VI.1 (aged 28). (E) PKSN29-VI.7 (aged 13). (F) PKSN29-VI.8 (aged 10). The observed thresholds showed a moderate to severe hearing loss that is slightly greater in the middle frequencies. (G) IR21-V.5 (aged 11). (H) PKSN29-VI.2 (aged 20). The two subjects are carriers of *TECTA* mutations and have normal hearing thresholds (O, thresholds for right ear, X, thresholds for left ear).

6037delG in DNA from 72 unrelated Iranian or 180 Pakistani normal hearing subjects.

DISCUSSION

Our results show that homozygosity for functional null alleles of *TECTA* causes moderate to severe, prelingual hearing loss. The frameshifts introduced by 649insC and 6037delG are predicted to result in premature stop codons within exon 5 and exon 20, respectively, and the mutant mRNA may be degraded by a nonsense mediated decay mechanism.[13 14] Alternatively, the truncated mutant α-tectorin polypeptide may be non-functional. The lack of a phenotype in 649insC and 6037delG heterozygous

carriers, in combination with the observation of mutant auditory phenotypes in heterozygotes for missense alleles of *TECTA*, confirms that 649insC and 6037delG are indeed null alleles.

A previously described splice site mutation of *TECTA* cosegregating with DFNB21 hearing loss in a Lebanese family is also predicted to result in a functional null allele.[8] Although the hearing loss in this family was reported to vary from 70 to 110 dB HL, the subjects' ages and audiometric configurations were not described.

The distinctive hearing loss associated with recessive *TECTA* alleles provides a useful clinical marker for genetic diagnosis of DFNB21 deafness. Some of the audiometric configurations show a greater loss in the middle frequencies, but only one (fig 2D) meets the proposed guidelines of the European Working Group on Genetics of Hearing Impairment (http://hear.unife.it/he_infol.htm) for mid frequency hearing loss (⩾15 dB difference between the poorest thresholds in the middle frequencies as compared to those at higher and low frequencies). Mid frequency hearing loss is associated with dominant DFNA13 alleles of *COL11A2*[15] and some DFNA8/A12 alleles of *TECTA* but is currently not associated with any reported DFNB loci.[2]

The cellular basis of deafness associated with recessive *TECTA* alleles may be inferred from a mouse model segregating a recessive, functional null allele of *Tecta*. $Tecta^{\Delta ENT/\Delta ENT}$ mice have moderate-severe hearing loss. The only inner ear abnormality in these $Tecta^{\Delta ENT/\Delta ENT}$ mice is the detachment of the tectorial membrane from the organ of Corti, showing the importance of the tectorial membrane for amplification of auditory stimuli.[16] Homozygosity for recessive, predicted null alleles of α-tectorin in humans might exert similar effects upon the tectorial membrane.

The potential pathogenic mechanism of DFNA8/A12 mutations in the ZP domain of *TECTA* was indirectly addressed in a study of oocytes transfected with a mutant mouse zona pellucida protein, ZP2.[17] The instability of the mutant protein resulted in its reduced secretion, leading the authors to conclude that dominant mutations of zona pellucida domains act via a haploinsufficiency mechanism in causing disease. However, the normal hearing status of heterozygous carriers of recessive *TECTA* alleles indicates that missense DFNA8/A12 alleles of *TECTA* exert their effects via a dominant negative (or gain of function) mechanism.[8] Furthermore, in the presence of wild type *TECTA*, the missense mutant protein may be secreted as shown for pathogenic mutations in another ZP protein, endoglin.[18] We anticipate that continued characterisation of humans and mice segregating mutant alleles of tectorial membrane components will elucidate the structural and functional requirements of the tectorial membrane for auditory function.

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GenBank accession numbers. *TECTA* genomic sequences, AF055114-AF055136. *TECTA*, cDNA NM_005422.

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