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 maternally or X linked trait. 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-syndromic, progressive, and severe-profound impairment is associated with mutations in a majority of DFNB loci. 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.

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. All other dominant missense alleles of TECTA are associated with stable, non-progressive hearing loss. The only known recessive allele of TECTA is a splice site mutation resulting in a frameshift, causing severe-profound deafness linked to DFNB21.

α-tectorin is one of the major glycoproteins of the tectorial membrane, the acellular matrix overlying the cochlear neuroepithelium. α-tectorin has predicted structural domains with similarity to protein modules important for cross linking with other proteins. 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. In contrast, missense substitutions in the zonadhesin domain of TECTA cause mild to moderate hearing loss primarily affecting high frequencies.

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 genetic diagnosis.

### Table 1  TECTA mutations and associated phenotype

<table>
<thead>
<tr>
<th>Mutant allele</th>
<th>Location domain</th>
<th>Hearing loss</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td>DFNA8/A12</td>
<td>Exon 10</td>
<td>Postlingual</td>
<td>Mild to severe, progressive</td>
</tr>
<tr>
<td>Cys1619Ser</td>
<td>Exon 14</td>
<td>Variable</td>
<td>Mild to moderate-severe, progressive</td>
</tr>
<tr>
<td>Cys1619Ser</td>
<td>Exon 17</td>
<td>Prelingual</td>
<td>Mild to moderate, stable</td>
</tr>
<tr>
<td>Cys1637Gly</td>
<td>Exon 18</td>
<td>Prelingual</td>
<td>Mild to moderate, stable</td>
</tr>
<tr>
<td>Cys1637Gly</td>
<td>Exon 20</td>
<td>Prelingual</td>
<td>Moderate to moderate-severe, stable</td>
</tr>
<tr>
<td>DFNB21</td>
<td>Exon 5</td>
<td>Prelingual</td>
<td>Moderate-severe</td>
</tr>
<tr>
<td>IVS9+1G→A</td>
<td>Intron 9</td>
<td>Prelingual</td>
<td>Severe-profound</td>
</tr>
<tr>
<td>6037delG</td>
<td>Exon 20</td>
<td>Prelingual</td>
<td>Moderate-severe</td>
</tr>
</tbody>
</table>

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.
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 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 performed 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 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 (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 6037delG.
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 non-sense mediated decay mechanism. Alternatively, the truncated mutant α-tectorin polypeptide may be non-functional. The lack of a phenotype in 649insC and 6037delG heterozygous

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**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).
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<sup>−/−</sup> mice have moderate-severe hearing loss. The only inner ear abnormality in these Tecta<sup>−/−</sup> 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. 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. 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. Furthermore, in the presence of wild type TECTA, the missense mutant protein may be secreted as shown for pathogenic mutations in another ZP domain.

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References


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