Mutations in the human α-tectorin gene cause autosomal dominant non-syndromic hearing impairment

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The tectorial membrane is an extracellular matrix of the inner ear that contacts the stereocilia bundles of specialized sensory hair cells. Sound induces movement of these hair cells relative to the tectorial membrane, deflects the stereocilia, and leads to fluctuations in hair-cell membrane potential, transducing sound into electrical signals. α-tectorin is one of the major non-collagenous components of the tectorial membrane3-5. Recently, the gene encoding mouse α-tectorin (Tecta) was mapped to a region of mouse chromosome 19, which shows evolutionary conservation with human chromosome 11q (ref. 3), where linkage was found in two families, one Belgian (DFNA12; ref. 4) and the other, Austrian (DFNA8; unpublished data), with autosomal dominant non-syndromic hearing impairment. We determined the complete sequence and the intron-exon structure of the human TECTA gene. In both families, mutation analysis revealed nonsense mutations which replace conserved amino-acid residues within the zona pellucida domain of TECTA. These findings indicate that mutations in TECTA are responsible for hearing impairment in these families, and implicate a new type of protein in the pathogenesis of hearing impairment.

As mouse Tecta is only expressed in the inner ear2, and human TECTA maps to the genetic linkage interval for both DFNA8 and DFNA12 (ref. 3), we considered TECTA a candidate gene for the non-syndromic hearing impairment in both families3. We therefore determined the sequence and the intron-exon structure of TECTA by genomic sequencing. Human genomic TECTA sequence was aligned with the mouse Tecta cDNA sequence. In regions where the homology between the mouse cDNA and the human genomic sequence diverged, the presence of splice-site consensus sequences were evaluated, and consensus values (CV) were calculated8. We found a total of 23 exons, ranging in size from 65 to 602 bp.

The composite DNA sequence, comprising all exons of TECTA, defines a single open reading frame of 6465 bp, displaying 88% identity to mouse Tecta. TECTA encodes a protein of 2155 amino acids, with 95% identity to mouse α-tectorin (Fig. 1). Nearly all structural features of TECTA are conserved between man and mouse (Fig. 1). Alpha-tectorin has an amineterminal hydrophobic signal sequence for translocation across

![Fig. 1 Amino-acid sequence alignment of human (H) and mouse (Mm) α-tectorin. Identical amino-acid sequences are indicated with •. Positions showing no homology to any known protein sequence are darkly shaded. The first 219 amino acids show homology with the G1 domain of entactin. Thirty-nine amino acids separate this domain from a 1528-amino-acid domain (amino acids 259-1786) which is homologous with zona pellucida. The third conserved region (amino acids 1805 to 2057) is the zona pellucida domain. The N- and COOH-terminal hydrophobic sequences are boxed. Two stars (••) indicate the cleavage site of the signal peptide. One star (*) indicates the most likely acceptor of the glycosylphosphatidylinositol anchor. The tetrabasic putative endoprotease cleavage site is underlined.](image)

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the membrane and a carbonyl-terminal hydrophobic region characteristic of precursors for glycosylphosphatidylinositol-linked membrane-bound proteins. The protein is probably released from the membrane by proteolysis cleavage at a conserved tetra-basic sequence upstream of the predicted acceptor for the glycosylphosphatidylinositol anchor (residue 2091; Fig. 1; ref. 2). α-tectorin is further processed into three polyepitides: a module containing a region homologous to the G1 domain of entactin, a module similar to zonadhesin, and a module consisting of a zona pellucida domain. These three polyepitides are crosslinked to each other by disulfide bridges and interact with β-tectorin to form the non-collagenous matrix of the tectal membrane.

In the mouse, RT-PCR of Tecta cDNA amplified two splice variants, the larger variant encoding a protein containing five amino acids (RPLAP; ref. 2) not found in the smaller variant. The exact nature of the alternative splicing, however, could not be determined. In the human TECTA genomic sequence, exon 15 was found to contain two possible 5′ splice sites 15 bp apart, with CV values of 0.76 and 0.90, respectively (Fig. 2). Use of one of the splice sites would lead to the insertion of an RPLAP peptide in the same position as in the mouse. These data suggest that in man as well as in mouse, both splice sites are used, giving rise to two isoforms with and without the RPLAP peptide (Fig. 2).

We found no gross rearrangements in TECTA by Southern blot of DNA from either family studied. We then conducted exon-by-exon scanning using Single Stranded Conformation Polymorphism (SSCP) analysis. Mutation analysis of TECTA in the Austrian family revealed an A→G missense mutation at n 5876 (Table 1, Fig. 3). This mutation replaces the tyrosine at residue 1870 with a cysteine (Y1870C). The Y1870C mutation segregated in 8 affected family members and was not found in any of the 6 unaffected family members, 50 Belgian controls or 50 Austrian controls living in the same region as the DFNA8 family.

Mutation analysis of all 23 exons of TECTA in the Austrian DFNA12 family revealed two mutations in exon 17. The first mutation, C→T at nucleotide position 5725, results in phenylalanine replacing leucine residue 1820 (L1820F; Table 1, Fig. 3). The second mutation, G→A at n 5738, leads to a substitution of aspartic acid for glycine at residue 1824 (G1824D; Table 1, Fig. 3). These two mutations are only 12 base pairs apart (Fig. 3). Eighteen affected members of the DFNA12 family had both mutations, but neither was present in any of the 40 unaffected family members or 100 controls. It is possible that one mutation is a rare polymorphism, while the other one is the disease-causing mutation. Alternatively, they might have a synergistic effect, neither being capable of producing disease by itself.

To investigate the evolutionary conservation of the amino acids changed by the missense mutations found in the DFNA8/DFNA12 families, we searched the GenBank database for protein sequences homologous to the zona pellucida domain of TECTA (residues 1805–2057) using BLASTX. Alignment of the 10 genes with the highest homology in the BLAST results showed that all three amino acids changed by the missense mutations were evolutionarily conserved (Fig. 4; refs 2,9–16).

The mutations in the zona pellucida domain of α-tectorin may have dominant-negative phenotypes that disrupt the interactions between the different tectorin polyepitides, and as a consequence, disrupt the structure of the tectal membrane. A deficient tectal membrane is expected to lead to inefficient transmission of sound to the mechanosensitive stereociliary bundles of the hair cells, resulting in hearing impairment. It is also possible that a mutation causes mRNA instability or the degradation of α-tectorin, reducing the amount of this protein in the tectal membrane.

The hearing impairment in both families is prelingual in onset. The fact that the human tectal membrane is formed between the twelfth and nineteenth week of embryonic development is consistent with a defect of the tectal membrane in these families. Furthermore, α- and β-tectorin are only expressed transiently during cochlear development in the mouse. CT scans of the temporal bones of affected members of the DFNA8 family and magnetic resonance imaging of the inner ear of DFNA12 patients (unpublished results) did not reveal any gross structural abnormalities. These in vivo imaging methods are inadequate to visualize the structure of the tectal membrane. Definitive proof of the disease-causing nature of the α-tectorin mutations must, therefore, come from further experiments investigating the effects of the missense mutations in α-tectorin on the structure and the function of the tectal membrane.

Table 1 • TECTA mutations in DFNA12/DFNA8 families

<table>
<thead>
<tr>
<th>Exon</th>
<th>Domain</th>
<th>DNA</th>
<th>Protein</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>ZP</td>
<td>5725C→T</td>
<td>L1820F</td>
<td>Belgian (DFNA12)</td>
</tr>
<tr>
<td>17</td>
<td>ZP</td>
<td>5738G→A</td>
<td>G1824D</td>
<td>Belgian (DFNA12)</td>
</tr>
<tr>
<td>18</td>
<td>ZP</td>
<td>5876A→G</td>
<td>Y1870C</td>
<td>Austrian (DFNA8)</td>
</tr>
</tbody>
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ZH, zona pellucida domain

Fig. 2 Alternative splicing of TECTA. Introns sequences are given in lower-case letters; exon sequences are given in upper-case letters and are boxed. Splice-site consensus sequences with their CV value are indicated above the DNA sequence. The acceptor splice site gives rise to 15 extra nucleotides in the mRNA, leading to 5 extra amino acids (RPLAP). The protein lacks the RPLAP amino acids when the second acceptor splice site is used.

Fig. 3 DNA sequences with TECTA mutations in DFNA8/DFNA12 families. Electropherograms for the regions immediately surrounding the DFNA8/DFNA12 mutations are shown. For each family, an affected patient and a control person is depicted. Arrows indicate the positions of the mutations.
Methods

DFNA12 and DFNA8 families. The Belgian DFNA12 family has already been described clinically and genetically. All 8 affected members of the Austrian DFNA8 family showed a moderate-to-severe hearing deficit (60-80 dB) involving all frequencies. The hearing impairment was bilateral and the patients reported no change in hearing over time. Linkage results have localized the gene responsible for DFNA8 on the long arm of chromosome 11. In the same region as that for DFNA12. A detailed description of the DFNA8 family will be published elsewhere.

Identification of cosmids clones. Cosmids containing TECTA DNA sequences were identified by screening an arrayed chromosome-11-specific cosmid library with the mouse Tecta DNA sequence as a probe. Five positive clones were obtained from the Resource Centre of the German Human Genome Project, and grown overnight in LB medium containing 25 µg/ml kanamycin. Cosmid DNA samples were digested with restriction enzymes, electrophoresed through a 0.8% agarose gel and transferred to a Hybond N+ membrane (Amersham) using standard procedures. The membranes were consecutively hybridized with four overlapping Tecta CDNA fragments.

Shotgun cloning of cosmids clones. DNA from four selected chromosome-specific cosmids clones (ICRF107FI332D, ICRF107FI352D, ICRF107FI371D1) was isolated from fragments of 400 bp to 1.5 kb, blunt-ended and shotgun-cloned into a plasmid. From each cosmids, 768 plasmid subclones were picked in two 384-well microtiter plates. Replicas were made on Hybond N+ membranes (Amersham) using a 384-pin replicator (Genetix). The membranes were hybridized with four overlapping fragments of the mouse Tecta CDNA as probes, and positive plasmid clones were sequenced.

Mutation analysis. All 23 exons were amplified by PCR using primers flanking the different exons. Primers sequence available from author on request) if the corresponding PCR products were smaller than 200 bp. Otherwise, additional primers were designed to generate several overlapping fragments comprising the exon. SSCP analysis was carried out using 0.5×MDE gels (FMC) as described. DNA was sequenced on an ABI 377 automated DNA sequencer (Perkin Elmer), using Thermosequence (Amersham) and Big Dye (Perkin Elmer) dye terminator cycle sequencing kits. Rapid mutation screening was carried out by restriction enzyme digestion of PCR-amplified exons followed by pycromassile gel electrophoresis.

Genbank accession numbers. TECTA genomic sequences, AF055114 to AF055136.

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