Two Novel Missense Mutations in the \textit{TECTA} Gene in Korean Families with Autosomal Dominant Nonsyndromic Hearing Loss

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Abstract. The \textit{TECTA} gene, which encodes alpha-tectorin, is known as a causative gene for DFNA8/DFNA12, and DFNB21 hearing loss in humans. In the present study, mutation analysis of the \textit{TECTA} gene was performed in 62 Korean patients with hereditary hearing loss. Two novel nucleotide substitutions, p.V317E and p.T1866M, were identified for the first time in the Korean population. These mutations result in the substitution of amino acids in the zonadhesin (ZA) and the zona pellucida (ZP) domains, and show a genotype-phenotype correlation, which is a characteristic of \textit{TECTA}-related mutations in autosomal dominant nonsyndromic hearing loss. Both mutations are located in highly conserved regions of alpha-tectorin and were not found in 120 unrelated control subjects with normal hearing. Based on this evidence, it is likely that both mutations are the pathogenic ones causing the hearing loss. This study provides useful information for the functional study of hereditary hearing loss caused by tectorial membrane defects.

Keywords: hereditary hearing loss, \textit{TECTA} gene, tectorial membrane, alpha-tectorin

Introduction

The tectorial membrane of the organ of Corti is a sheet of extracellular matrix composed of three different types of collagen and three noncollagenous glycoproteins that are expressed at high levels only in the inner ear [1]. Alpha-tectorin, a major noncollagenous component of the tectorial membrane, has several functional domains: the entactin (ENT) -like domain, four von Willebrand factor-like type D (vWFD) domains in the zonadhesin (ZA) domain, and the zona pellucida (ZP) domain [2-5]. Alpha-tectorin is encoded by the \textit{TECTA} gene, which contains 23 exons and is situated on human chromosome 11q22-24 [3,6-8]. Several studies have shown that mutations in the \textit{TECTA} gene cause autosomal dominant (DFNA8/DFNA12) and autosomal recessive (DFNB21) nonsyndromic hearing loss [7,9] and 18 different mutations have been identified [5,7,9-20]. Interestingly, all of the missense mutations in the \textit{TECTA} gene are responsible for autosomal dominant nonsyndromic hearing loss (ADNSHL), whereas the truncated mutations, including nonsense, frameshift, and splicing, cause autosomal recessive nonsyndromic hearing loss (ARNSHL) [10,15-16]. Unlike autosomal recessive mutations, which show similar phenotypes, autosomal dominant mutations in the \textit{TECTA} gene result in different hearing loss phenotypes depending on the protein domain that is affected [5,7,11,14,19-21].

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In this study, we identified two novel missense mutations in the *TECTA* gene in Korean families with ADNSHL, and we confirmed the association between the mutation positions in the protein domain and their respective phenotypes.

**Materials and Methods**

**Subjects.** A total of 62 subjects with hereditary hearing loss were recruited using the pedigree information of each individual from the Department of Otorhinolaryngology-Head and Neck Surgery, Kyungpook National University Hospital. Physical examinations, including an otolaryngologic examination, were carried out after their previous medical histories were reported by the subjects. According to the inheritance patterns of hearing loss in their pedigrees, the 62 individuals were classified into two groups: 43 individuals with ADNSHL and 19 individuals with ARNSHL. One hundred-twenty unrelated Koreans with normal hearing were used as controls. Written informed consent was obtained from all individuals, and the study was approved by the Institutional Review Board of Kyungpook National University Hospital.

**Audiometric evaluation.** Pure tone audiometry (PTA) was performed in a sound-controlled room at frequencies ranging from 500 to 8000 Hz, according to standard protocols. The level of hearing loss was described as follows based on the mean PTA calculated using the thresholds measured at 500, 1000, 2000, and 3000 Hz: normal hearing, below 20 dB; mild hearing impairment, 21 - 40 dB; moderate hearing impairment, 41 - 70 dB; severe hearing impairment, 71 - 95 dB; and profound hearing impairment > 95 dB [22].

**Mutation analysis.** Genomic DNAs of the 62 subjects and 120 controls were extracted from blood samples using a FlexiGene DNA kit (Qiagen, Hilden, Germany). All 23 exons and intron-exon boundaries of the *TECTA* gene (GenBank ID NM_005422.2) were amplified by polymerase chain reaction (PCR) in the 62 subjects. The quality of the PCR products was monitored by electrophoresis on 2% agarose gels. Shrimp alkaline phosphatase (USB, Cleveland, OH) and exonuclease I (USB) were used to purify PCR products. Sequences of the purified PCR products were obtained from direct sequencing using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Extended products were purified by ethanol precipitation. The 3130xl Genetic Analyzer (Applied Biosystems) was used to resolve the products and the data were analyzed using Sequencing Analysis v5.2 (Applied Biosystems) and Chromas Lite v2.01 (Technelysium, Tewantin, QL, Australia) software. Potential pathogenicity of novel missense mutations was evaluated by SIFT (http://blocks.fhcrc.org/sift/sift.html), PolyPhen (http://genetics.bwh.harvard.edu/pph/), and multiple sequence alignment using Sequence Viewer v6.0.1 (CLC Bio, Aarhus, Denmark).

**Results**

**Mutation analysis of the TECTA gene.** We identified two novel missense mutations of the *TECTA* gene from the DNA samples of the 62 patients with hereditary HL who participated in this study. First, the single nucleotide change of a cytosine for a thymine at nucleotide position 5597 (c.5597 C>T) in exon 18 was detected in family KNUF08. This substitution converts threonine to methionine at amino acid position 1866 (p. T1866M). The affected members in this family (II-2, III-1, III-2, and III-3) were heterozygous for this mutation, but it was not observed in the father (II-1), which is consistent with the affected status in this family (Fig. 1A and 1C). This mutation was not detected in 120 unrelated control subjects and was located in the ZP domain, which is highly conserved across species (Fig. 1D).

Another missense mutation of *TECTA* was a thymine to adenine transversion at nucleotide position 950 (c. 950 T>A) in exon 6, which results in a valine to a glutamic acid substitution at amino acid position 317 (p.V317E) (Fig. 2A). The patient was heterozygous for this mutation and other family members were not available for study. However, the inheritance pattern of hearing loss in this family was autosomal dominant (data not shown) and this variant is located in the highly conserved vWFD0 repeat of the ZA domain (Fig. 2C). No variation was identified in any other exons of *TECTA* in the patients with hereditary HL nor in 120 unrelated control subjects. This evidence supports the inference that p.V317E is a pathogenic mutation rather than a rare polymorphism.

**Audiological features.** Fig. 1 shows the pedigree and the plotted PTAs of affected patients in the KNUF08 family in which the p.T1866M mutation was found. None of the affected subjects had a past history nor evidence of any other cause of hearing loss. The affected subjects in family KNUF08 had suffered from nonprogressive bilateral hearing loss since childhood according to their past medical histories. They all had mild to moderate sensorineural hearing loss. The propositus, a 64-yr-old male with the p.V317E mutation, first visited the hospital due to bilateral hearing loss at the age of 58.
Fig. 1. The p.T1866M mutation detected in family KNUF08. (A) Pedigree of family KNUF08 with the p.T1866M mutation. Filled symbols indicate affected individuals. (B) Pure tone audiograms for left and right ears of 4 affected individuals (II-2, III-1, III-2, and III-3). Numbers in parenthesis refer to age. (C) Partial DNA sequences of the TECTA gene showing the c.5597C>T change in family member (III-1) and in a normal control. (D) Multiple alignments of the alpha-tectorin and homologous sequences in the zona pellucida (ZP) domain partial region. The amino acid sequence of human alpha-tectorin is aligned with sequences of other species. The arrow marks the p.T1866M mutation. The hTECTA = alpha-tectorin of Homo sapiens; mTECTA, alpha-tectorin of Mus musculus; rTECTA, alpha-tectorin of Rattus norvegicus, and gTECTA, alpha-tectorin of Gallus gallus.
Fig. 2. (A) Partial DNA sequences of the TECTA gene showing the c.950T>A change in an affected patient vs a normal individual. The affected individual reveals heterozygosity for valine and glutamic acid at amino acid position 317, and the normal individual is homozygous for valine. (B) Pure tone audiograms of affected individual. The dashed lines and the solid lines show thresholds at the age of 58 and 63 yr, respectively. (C) Multiple alignments of the alpha-rectorin and homologous sequences in the von Willebrand factor-like type D0 (vWF D0) repeat partial region. The arrow marks the position of the p.V317E mutation. The vWF D0 domain shows high conservation in the different species (see Fig. 1).
yr. He showed bilateral mild hearing loss with down-sloping audiogram. The hearing level showed progressive deterioration at some frequencies at the age of 63 yr (Fig. 2B).

Discussion

In the present study, the TECTA gene was screened in 62 Korean patients with hereditary hearing loss. Two novel variants were found, p.V317E and p.T1866M, which were implicated to be pathogenic missense mutations causing hearing loss. Most of the missense mutations that have been identified in alpha-tectorin are located in the ZP domain and a few are located in the ZA domain (Table 1). The novel variation p.V317E in the ZA domain was substitution of valine with an aliphatic hydrophobic side chain to glutamic acid with an acidic side chain. Further, p.T1866M in the ZP domain shows the substitution of the properties of amino acids, threonine to methionine, which are a hydrophilic to a hydrophobic side chain. Thus, it seems likely that the structural variations in the ZP and ZA domains might cause functional problems in alpha-tectorin.

All mutations described to date in TECTA-related ADNSHL have shown significant genotype-phenotype correlation between the mutated domains and the respective hearing loss [5-6,9,19]. According to previous studies, mutations in the ZP and ZA domains are related to mid- and high-frequency hearing loss, respectively (Table 1). The p.V317E in the ZA domain was linked to progressive high frequency hearing loss in the patient’s audiogram and the p.T1866M in the ZP domain showed mild-moderate hearing loss at mid-high frequency in the affected members of the family. These results confirm earlier reports on TECTA-related ADNSHL. Therefore, it appears that the two variations, p.V317E and p.T1866M, identified in this study are pathogenic missense mutations causing hearing loss in two Korean families with ADNSHL. Further studies on the mechanical association between mutation types and inheritance patterns of hearing loss, and between mutation-affected domains and onset frequency are necessary to elucidate the genotype-phenotype correlations in patients with TECTA-related HL.

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Table 1. Missense mutations in the TECTA gene and the associated phenotypes.

<table>
<thead>
<tr>
<th>Family origin</th>
<th>Amino acid change</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Domain</th>
<th>Frequency</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Korean</td>
<td>V317E†</td>
<td>6</td>
<td>c.950T&gt;A</td>
<td>ZA⁺(D0)</td>
<td>High</td>
<td>Mild</td>
<td>This study</td>
</tr>
<tr>
<td>Swedish</td>
<td>C1057S</td>
<td>10</td>
<td>c.3169T&gt;A</td>
<td>ZA (D2)</td>
<td>High</td>
<td>Variable</td>
<td>[5]</td>
</tr>
<tr>
<td>Turkish</td>
<td>C1509G</td>
<td>13</td>
<td>c.4525T&gt;G</td>
<td>ZA (D4)</td>
<td>High</td>
<td>Mild to moderate</td>
<td>[19]</td>
</tr>
<tr>
<td>French</td>
<td>C1619S</td>
<td>14</td>
<td>c.4856G&gt;C</td>
<td>ZA (D4)</td>
<td>High</td>
<td>Mild to moderate</td>
<td>[11]</td>
</tr>
<tr>
<td>Belgian</td>
<td>L1820F</td>
<td>17</td>
<td>c.5458C&gt;T</td>
<td>ZP⁺</td>
<td>Mid</td>
<td>Mild to severe</td>
<td>[7]</td>
</tr>
<tr>
<td>Belgian</td>
<td>G1824D</td>
<td>17</td>
<td>c.5471G&gt;A</td>
<td>ZP</td>
<td>Mid</td>
<td>Mild to severe</td>
<td>[7]</td>
</tr>
<tr>
<td>Spanish</td>
<td>C1837G</td>
<td>17</td>
<td>c.5509T&gt;G</td>
<td>ZP⁺</td>
<td>Mid</td>
<td>Mild to moderate</td>
<td>[21]</td>
</tr>
<tr>
<td>American</td>
<td>C1837R</td>
<td>17</td>
<td>c.5509T&gt;C</td>
<td>ZP⁺</td>
<td>Mid</td>
<td>Mild to moderate</td>
<td>[15]</td>
</tr>
<tr>
<td>Korean</td>
<td>T1866M†</td>
<td>18</td>
<td>c.5597C&gt;T</td>
<td>ZP⁺</td>
<td>Mid</td>
<td>Mild to moderate</td>
<td>This study</td>
</tr>
<tr>
<td>Austrian</td>
<td>Y1870C</td>
<td>18</td>
<td>c.5609A&gt;G</td>
<td>ZP</td>
<td>Mid</td>
<td>Moderate to severe</td>
<td>[7]</td>
</tr>
<tr>
<td>Dutch</td>
<td>R1890C</td>
<td>18</td>
<td>c.5668C&gt;T</td>
<td>ZP</td>
<td>Mid</td>
<td>Mild to moderate</td>
<td>[23]</td>
</tr>
<tr>
<td>Japanese</td>
<td>R2021H</td>
<td>20</td>
<td>c.6062G&gt;A</td>
<td>ZP</td>
<td>Mid</td>
<td>Mild to moderate</td>
<td>[14]</td>
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</tbody>
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†Novel mutations identified in this study.
TECTA gene mutations in familial hearing loss

References


