Association of Clinical Features With Mutation of TECTA in a Family With Autosomal Dominant Hearing Loss

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Background: The TECTA gene, which encodes α-tectorin, has recently been cloned. α-Tectorin is a major component of the noncollagenous matrix of the tectorial membrane. Nonsyndromic hearing impairment caused by TECTA mutations has been reported in Austrian, Belgian, Swedish, French, and Lebanese families. The phenotypes and genotypes were different among these families.

Materials and Methods: Our study family displayed autosomal dominant hearing impairment through 3 generations. We sequenced the coding exons of the TECTA gene in 4 affected individuals, and we report the clinical features in a Japanese family with nonsyndromic hearing impairment and a mutation in the TECTA gene.

Results: The 5-frequency average of 250, 500, 1000, 2000, and 4000 Hz in 4 affected individuals was 42.2±3.7 (mean±SD) dB in the right ear and 42.3±4.5 dB in the left ear. The mean age at onset of hearing impairment was 5 years. The progression of hearing impairment was not confirmed for a 15-year period, from the age of 6 to 21 years, in 1 affected member. The 4 patients had a G→A missense mutation at nucleotide 6063 in exon 20. This mutation replaces arginine at residue 2021 with histidine (R2021H).

Conclusions: All 4 affected members showed symmetrical and stable bilateral mild to moderate hearing impairment in the midfrequencies. The mean threshold level of 2000 Hz was the worst among the 5 frequencies. All the affected members had normal vestibular function. The mutation in the TECTA gene, localized in the zona pellucida domain, was detected in all 4 affected individuals. The localization of the mutation in the different modules of the protein may have caused the different clinical features.


There has been tremendous progress in the research of the genetic basis of deafness. It had always been assumed that single-gene defects were responsible for hearing impairment, but many different genes causing deafness, which probably account for more than 50% of the cases of childhood deafness, have recently been reported.1 So far, 70 loci involved in nonsyndromic deafness have also been reported.2 The tectorial membrane is an extracellular gel-like matrix leaf that attaches to the tallest row of the stereociliary bundles of the outer hair cells. The displacement of the tectorial membrane stimulates the outer hair cells, which open the transduction channels and lead to hair cell depolarization. The ultrastructural defects of the tectorial membrane are caused by mutations in 3 different points of genes, namely encoding α-tectorin (TECTA), collagen 11-α2 (COL11A2), and otogelin (Otog), which lead to human hearing impairment.4 The tectorial membrane contains collagenase-sensitive and -inensitive proteins. The major components of the noncollagenous matrix are α-tectorin and β-tectorin, which interact with each other.2 The TECTA gene has recently been cloned and shown to be associated with nonsyndromic hearing impairment.3 It encodes a protein of 2155 amino acids, 95% of which are identical to mouse α-tectorin, which has an aminoterminal hydrophobic signal sequence for translocation across the membrane and a carboxy-terminal hydrophobic region characteristic of precursors for glycosylphosphatidylinositol-linked membrane-bound proteins.7 An alteration in α-tectorin is likely to disrupt the structure of this matrix. DFNA8,8 DFNA12,9 and DFNB2110 loci are mapped on chromosome 11q11 and are all segregating alleles of TECTA. Nonsyndromic hearing impairment caused by TECTA mutations has been reported in Austrian,3 Belgian,3 Swedish,12 French,13

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SUBJECTS AND METHODS

CLINICAL DIAGNOSIS

A family pedigree was constructed at Hamamatsu University Hospital, Hamamatsu City, Japan (Figure 1). Otoscopic and audiometric examinations were performed on all cooperative family members. Blood samples from the family members were obtained after informed consent was granted. Pure-tone audiometry was performed with air conduction at 125, 250, 500, 1000, 2000, 4000, and 8000 Hz and with bone conduction at 250, 500, 1000, 2000, and 4000 Hz. Audiograms were available for 4 members (II:2, III:3, II:2, and I:7) of the pedigree. A speech discrimination test, otoacoustic emissions screening (Capella; GN Otometrics, Tokyo, Japan), a caloric test, and computed tomography were also performed. For 1 member (II:2), we can chart the hearing impairment via audiometric examinations over a long term. His hearing impairment was detected during an examination in primary school when he was 6 years old, and he experienced head trauma while playing a sport at the age of 10 years. Congenital aural fistula of the right ear was confirmed in patient III:3, and her hearing impairment was noticed by her parents when she was 4 years old. Patient II:2 was diagnosed as having hypothyroidism (Hashimoto disease). Patient I:3 had a noise-induced hearing impairment. A hearing aid was used by patients I:4 and I:6. None of the 4 affected family members, all of whom underwent audiometric testing, had complained of tinnitus or vertigo.

MUTATION ANALYSIS

Intronic polymerase chain reaction (PCR) amplification primers flanking each exon were used to detect mutations. Exons 1-20 of TECTA were amplified from genomic DNA samples by PCR. A 5-minute denaturation at 95°C was followed by 35 three-step cycles (95°C for 30 seconds, 55°C for 1 minute, and 72°C for 1 minute), followed by 72°C for 10 minutes, and ending with a holding period at 4°C in a thermal cycler (Perkin-Elmer Corp., Norwalk, Conn.). The PCR products were directly sequenced after removal of unincorporated dinucleotide triphosphates and primers by incubation at 37°C for 30 minutes with 50 to 100-ng PCR product with 0.1 μL of exonuclease I (Amersham Life Science, Cleveland, Ohio) and 1 μL of shrimp alkaline phosphatase (Amersham Life Science). The enzymes were heat inactivated at 80°C for 15 minutes. An aliquot of 6 pmol of either the forward or the reverse primer was used in standard cycle sequencing reactions and run on a sequencer. DNA samples from 96 unrelated Japanese, who had normal hearing, were used as controls.

RESULTS

Audiograms of the 4 affected members are shown in Figure 2. They demonstrated bilateral mild to moderate, symmetrical, and stable sensorineural hearing impairment in the midfrequencies. The mean±SD level of hearing impairment at 250, 500, 1000, 2000, and 4000 Hz was 42.2±3.7 dB (range, 38-47dB) in the right ear and 42.3±4.5 dB (range, 36-47dB) in the left ear. The mean±SD level at each frequency was 22.6±5.5 dB (250 Hz), 32.5±14.4 dB (500 Hz), 51.3±14.3 dB (1000 Hz), 66.3±12.5 dB (2000 Hz), and 35±20.0 dB (4000 Hz) in the right ear and 26.3±7.5 dB (250 Hz), 36.3±8.5 dB (500 Hz), 48.8±7.5 dB (1000 Hz), 57.5±15.5 dB (2000 Hz), and 42.5±17.0 dB (4000 Hz) in the left ear. The history of the progression of hearing impairment charted by audiometry over 15 years (from age 6-21 years) in patient III:2 is shown in Figure 3. The mean level of maximum speech discrimination was 95% at the stimulus level of 70 dB. The responses on the distortion-product otoacoustic emissions and the transient evoked otoacoustic emissions were decreased, which indicated that the current hearing impairment was caused by inner ear dysfunction. All the affected members had normal vestibular function. Abnormality of the inner ear was not found with computed tomography.

The G→A missense mutation at nucleotide 6063 in exon 20 in the TECTA gene was detected in all 4 affected members (Figure 4). This mutation replaces arginine at residue 2021 with histidine (R2021H). All 4 affected members were heterozygous for this mutation. The present mutation was not found in any of the samples from Japanese controls.

COMMENT

A Japanese family with nonsyndromic autosomal dominant hearing loss was investigated. The hearing loss was bilateral and symmetrical, and there was inner ear dysfunction. Stable, moderate, and midfrequency hearing loss was detected on auditory examinations of all 4 affected members at a mean age of 5 years. Histories of delayed speech development and distortion of utterance suggested a prelingual onset of hearing impairment. The
mean ± SD level of hearing impairment was 42.2 ± 3.7 dB (right ear) and 42.3 ± 4.5 dB (left ear). The mean threshold level of 2000 Hz was the worst among the 5 frequencies (250-8000 Hz). We were able to follow the history of hearing impairment with audiometry for 15 years (from age 6-21 years) in 1 affected member. The hearing impairment did not change during that period. The head trauma of 1 affected member did not induce a progression of hearing impairment.

Mutation of the TECTA gene has been identified in Belgian,3 Austrian,3 French,13 Swedish,12 and Lebanese10 families with nonsyndromic hearing loss. The characteristics of nonsyndromic hearing loss in these families were classified into 2 phenotypes. Nonsyndromic autosomal dominant hearing loss was found in the Belgian,3,9,14 Austrian,3,15 French,13 and Swedish,12,16 families in addition to the current family. Nonsyndromic autosomal recessive hearing loss was found in the Lebanese family.10 Mild to severe and progressive hearing loss in the high frequencies was reported in the French and Swedish autosomal dominant families. The onset of hearing impairment was different between the French and Swedish families. Although the hearing impairment in the Swedish family was postlingual, with a mean age at on-

Figure 2. Representation of audiograms for the 4 affected family members (II:2, II:7, III:2, and III:3) at the age of 7 years (III:3), 18 years (III:2), 33 years (II:7), and 42 years (II:2). The mean ± SD level of hearing loss at 5 frequencies was 42.2 ± 3.7 dB (right ear) and 42.3 ± 4.5 dB (left ear), and the maximum level of hearing loss was 2000 Hz (57.5 ± 15.5 dB).
III:3). B, Unaffected normal control. Thr indicates threonine; Cys, cysteine; and Phe, phenylalanine.

The same mutation was found in all 4 affected members (II:2, II:7, III:2, and III:3). B, Unaffected normal control. Thr indicates threonine; Cys, cysteine; and Phe, phenylalanine.

**Figure 4.** Sequence electropherograms. A, Affected member (III:3) with heterozygous G→A missense mutation at nucleotide 6063 in the α-tectorin exon 20 (arrow): an arginine (Arg) to histidine (His) substitution (R2021H). The same mutation was found in all 4 affected members (II:2, II:7, III:2, and III:3). B, Unaffected normal control. Thr indicates threonine; Cys, cysteine; and Phe, phenylalanine.

**Figure 3.** Long-term follow-up on audiometric tests in an affected member (III:2). The history over 15 years, from the age of 6 years to 21 years, of hearing impairment at 7 frequencies and the mean level of hearing loss in the right ear are shown.

There was no history of vertigo or dizziness in the present family or in the previously described families. A caloric test as a vestibular examination also revealed normal function. However, some affected members of the French family were late to start walking, which can be explained by a deficit of α-tectorin in the vestibular organ. α-Tectorin is expressed in the 2-day mouse utricle and saccule.

The TECTA gene is composed of 3 distinct modules: entactin G1 domain, zonadhesin domain (with von Willebrand factor type D repeat), and zona pellucida domain. The mutations found in the Belgian, Austrian, and present families were localized in the zona pellucida domain. A missense mutation at nucleotide 5876 in exon 18 replaced tyrosine at residue 1870 with cysteine (Y1870C) in the Austrian family. The Belgian family demonstrated 2 mutations in exon 17, which replaced leucine at residue 1820 with phenylalanine (L1820F) and aspartic acid at residue 1824 with glycine (G1824D). The present missense mutation at nucleotide 6063 in exon 20 leads to a substitution of arginine for histidine at residue 2021 (R2021H).
due 2021 (R2021H) and has been identified in 4 affected members. The mutation of TECTA in the zona pellucida domain (residues 1805-2057) may disrupt the interactions between the different polypeptides of tectorial membrane, and, as a consequence, improper assembly of the tectorial membrane might cause an inefficient mechanotransduction process. The mutations found in the French and Swedish families were identified in the zonadhesin domain of TECTA. This mutation abolishes the first of the vicinal cysteine present in the D4 von Willebrand factor type D repeat and may cause a change in the cross-linking of the polypeptide. A missense mutation at nucleotide 4857 in exon 14 replaced the cysteine at residue 1619 with serine (C1619S) in the French family. The mutation found in the Lebanese family resulted in the replacement of cysteine with serine at residue 1057 (C1057S) in exon 10. Although these mutations were identified heterozygously in the affected members, the mutation found in the Lebanese family led to the identification of a G→A transition in the donor splice site of intron 9 homozygously in the affected members, skipping exon 9 and resulting in a stop codon at amino acid position 972.

The mutations localized in the zona pellucida domain resulted in the prelingual and stable hearing loss in the midfrequencies in the autosomal dominant family in which the mutation was identified in the zonadhesin domain. The progressive hearing loss in the high frequency was found in the autosomal dominant family in which the mutation was identified in the zonadhesin domain. These findings suggest that the localization of the mutation in the different modules of the protein may result in the different phenotypes.

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