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Mutations in *TMC1* contribute significantly to nonsyndromic autosomal recessive sensorineural hearing loss: A report of five novel mutations

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ABSTRACT

Genome wide homozygosity mapping using Affymetrix 10K arrays revealed the DFNB7/11 locus including the *TMC1* gene in 5 of 35 Turkish families with autosomal recessive nonsyndromic severe to profound congenital or prelingual-onset sensorineural hearing loss (SNHL). Additional 51 families were later screened for co-segregation of the locus with the phenotype using microsatellite markers. *GJB2* and mtDNA A1555G mutations were negative in probands from each family. Mutation analysis was performed in families showing co-segregation of autosomal recessive SNHL with haplotypes at the DFNB7/11 locus. A total of six different mutations in seven families were identified, including novel missense alterations, p.G444R (c.1330G>A), p.R445C (c.1333C>T), and p.I677T (c.2030T>C), one novel splice site mutation IVS6+2 T>A (c.64+2T>A), and a novel large deletion of approximately 31 kb at the 3′ region of the gene including exons 19–24, as well as a previously reported nonsense mutation, p.R34X (c.100C>T). All identified mutations co-segregated with autosomal recessive SNHL in all families and were not found in Turkish hearing controls. These results expand the mutation spectrum of *TMC1* with five novel mutations and provide data for the significant contribution of *TMC1* mutations in hearing loss.

1. Introduction

The prevalence and causes of profound congenital and prelingual hearing loss can vary widely at different times and among populations. Clinically significant hearing loss has been estimated to be present in at least 1.9 per 1000 infants at birth and rises to at least 2.7 per 1000 births by the age of 4 in the USA [1]. Genetic causes are estimated to account for at least 50% of all cases with congenital or prelingual hearing loss; autosomal recessive, dominant, and X-linked genes being responsible for 75–77%, 22%, and 1% of the genetic cases, respectively, in Western populations [2]. It has recently been demonstrated that genetic causes are responsible in 76% of probands with hearing loss in Turkey, 93% of which are inherited with autosomal recessive transmission [3]. At least 70% of individuals with congenital or prelingual-onset severe to profound hearing loss are considered to have no additional findings (nonsydromic hearing loss). Mutations in more than 45 genes have been identified as a cause of dominant or recessive

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nonsyndromic hearing loss (hereditary hearing loss homepage—http://webh01.ua.ac.be/hhh/). However, frequencies of mutations in most of these genes among individuals with hearing loss have remained unknown. Some of these genes, such as *GJB2*, are associated with both dominant and recessive transmission. Mutations in the transmembrane cochlear expressed gene 1 (*TMC1*) were, similarly, found to be associated with both autosomal dominant and autosomal recessive sensorineural hearing loss (ARSNHL) with no additional findings [4].

In this study, we aimed to find the prevalence of biallelic mutations in the *TMC1* gene in a large group of multiplex families with nonsyndromic ARSNHL, after mapping this gene in 5 of 35 families.

2. Materials and methods

2.1. Families

A total of 86 families from Turkey were included in this study. A signed informed consent was obtained from each participant. The study and consent forms were approved by Ankara University Ethics Committee. A standardized history taking and clinical examination were performed on each participant. Laboratory investigations were performed when required. None of the affected individuals was diagnosed with a syndrome in these families. Each family included at least two affected siblings born to consanguineous parents. The number of affected members in the families ranged from 2 to 15.

Pure tone hearing threshold measurements of all the cases were made between 125 and 6000 Hz, and were established according to ISO-1964 standards. Hearing threshold measurements and speech tests were made in the sound proof rooms of Industrial Acoustic Company (IAC) Inc. with Interacoustics AC-40 audiometry. Air conduction hearing thresholds were measured between 125 and 6000 Hz using TDH-39 standard earphones. Bone conduction hearing thresholds were measured between 0.5 and 4 kHz using Oticon 60273 vibrators. The speech reception threshold (SRT) was performed using a three-syllable word list and the speech recognition was performed using a mono-syllable phonetically balanced word list. The speech awareness threshold (SAT) was obtained for patients who were not able to repeat words. The uncomfortable loudness level was also determined. For the Impedancemetric tests, middle ear pressure and acoustic reflex measurements were made, using Interacoustics AT-22 impedancemeter and TDH-39 earphones. Pure tone average (PTA) of air conduction thresholds at 500, 1000, and 2000 Hz were used for classification of the severity of hearing loss—normal hearing: <26 dB HL (Hearing Level), mild hearing loss: 26–40 dB HL, moderate hearing loss: 41–55 dB HL, moderately severe hearing loss: 56–70 dB HL, severe hearing loss: 71–90 dB HL, profound hearing loss >90 dB HL. All probands included in this study had bilateral severe to profound sensorineural hearing loss, congenital or prelingual-onset.

High-resolution temporal bone computerized tomography (CT) scans were performed when available.

At least one affected member from each family was screened and found to be negative for mutations in the noncoding and coding exons of the *GJB2* gene and mtDNA A1555G before inclusion in this study. The coding exon of *GJB2* was sequenced bidirectionally. The noncoding exon was screened using a single strand conformational polymorphism (SSCP) protocol that has been shown to be sensitive for the previously described mutation in this region.

2.2. Locus assignment

At least two affected members of 35 families were genotyped using Affymetrix GeneChip 10K 2.0 Xba Arrays. Genotype data were transferred into a Microsoft Excel sheet and sorted according to genomic localization of the genotyped SNPs. When a homozygous block flanking the *TMC1* gene (located between 74,326,537 and 74,641,087 bps on chromosome 9 – according to Ensembl release 50 – July 2008) was demonstrated in all affected family members who were included, microsatellite markers D9S1837 (74,374,949–74,375,187 bps), D9S1806 (73,391,177–73,391,440 bps), and D9S1876 (74,422,611–74,422,758 bps) were genotyped to demonstrate co-segregation of a haplotype with the phenotype. All affected members of the remaining 51 families were genotyped using the same microsatellite markers. When all affected members showed homozygosity for the typed markers, mutation analysis in *TMC1* was performed.

Two-point LOD scores were calculated using computer program SUPERLINK [5] in the easyLINKAGE program package [6]. Completely penetrant autosomal recessive inheritance with an allele frequency of 0.001 was used during analysis.

2.3. Mutation analysis

Twenty coding exons and intron–exon boundaries of *TMC1* were amplified using polymerase chain reaction (PCR) with appropriate primers (Table 1). All exons were screened using SSCP. PCR products were run on 7% non-denaturing polyacrylamide gels at temperatures calculated based on a previously reported study [7] with addition of forward and reverse primers. When a different band profile was detected, direct DNA sequencing with a Beckman Coulter 2000XL automated sequencer was performed. Detected mutations were screened using SSCP in unrelated Turkish controls from the same geographic origin.

After a large homozygous deletion including exons 19–24 were suspected in family 551, deletion points were narrowed down using a series of PCR studies, which were repeated twice for each region.

Pathogenic nature of a mutation was assessed with two *in silico* analyses: PolyPhen (http://genetics.bwh.harvard.edu/pph/) and ConSeq (http://conseq.tau.ac.il/). PolyPhen considers the evolutionary protection and calculates profile scores for two amino acid variants at the mutation point. ConSeq provides information about the conservation of each amino acid in the protein among orthologs and paralogs.

3. Results

Homozygous SNP haplotypes flanking the *TMC1* gene were demonstrated in five families, out of 35, during initial studies. Microsatellite genotyping confirmed co-segregation in four of these families. Affected members were found to be homozygous for all typed microsatellites in additional seven families. Therefore, 11 families were screened for mutations in the *TMC1* gene. A total of six different homozygous mutations in seven families were identified (Fig. 1; Table 2). These include three previously unreported missense alterations, p.G444R (c.1330G>A), p.R445C (c.1333C>T), and p.I677T (c.2030T>C), one novel splice site mutation IVS6+2 T>A (c.64+2T>A), and a large deletion of approximately 31 kb, starting from intron 18 and ending after 3′ UTR of the *TMC1* gene, as well as a previously reported nonsense mutation, p.R34X (c.100C>T). All identified alterations co-segregated with hearing loss in all available family members and were not found in Turkish hearing controls (Fig. 2; Table 2). *In silico* analyses for three new missense mutations also predicted their pathogenic nature (Table 3).

A large homozygous deletion was identified in all five affected members of family 551. A total of 23 PCR reactions were used to identify the deletion points in family 551. We were able to obtain PCR products in all family members for the first 14 (exons 5–18)