Identification of a Novel Frameshift Mutation in the *TECTA* Gene in an Iranian Family With Autosomal Nonsyndromic Hearing Loss

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Abstract- Hearing loss (HL) is one of the most frequent birth defects, and genetic factors contribute to the pathogenesis of the disorder in about half of the patients. In the present study, we performed whole-exome sequencing (WES) based on Next-generation sequencing (NGS) in an Iranian family with hereditary HL. Then, Sanger sequencing was used to verify the segregation of the variant recognized in affected family members. A novel homozygous frameshift variation, c.649-650insC, in TECTA was found in the family, which might lead to a truncated TECTA protein (p. Asn218Gln fsX31). Our findings propose that the homozygous TECTA-p.N218QfsX31 mutation is the pathogenic variant for ARNSHL. To the best of our knowledge, this mutation has not been described in patients with the HL phenotype and so far has not to be reported in any of the mutation databases. Our data expand the spectrum of mutations in the TECTA gene in nonsyndromic hearing loss.

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Introduction

Hearing impairment is considered the most common sensorineural disorder in humans and affecting more than 5% of the world population [http://www.who.int/]. Almost 50% of the recognized impairments are caused by genetic factors. Hereditary hearing impairment is divided into two groups, syndromic and nonsyndromic, the latter being highly heterogeneous (1-3). In the majority of patients, the exclusive effect on the auditory sense without causing other identifiable features; these are referred to as "nonsyndromic hearing loss" (NSHL) and represent 70% of all inherited patients. The heredity of nonsyndromic hearing impairments has recessive (ARNSHL) and dominant (ADNSHL) forms of inheritance. It is expected that 80% of genetic modes of hearing impairments are AR and the remaining 20% is AD (3).

So far, numerous genes (more than 150 genes) are estimated because of the hearing impairment, which accounts for the heterogeneity of hearing loss; the fact that necessitates adopting more efficient approaches for routine genetic testing and comprehensive genetic analysis. With the advent of the whole exome sequencing (WES) technique based on next-generation sequencing (NGS) platforms, heterogeneous inherited disorders have become available for the discovery of the causative genes and diagnosis (4). NGS allows for targeted enrichment and sequencing of nearly all exons of protein-coding genes. The approach is a rapid, accurate, and cost-effective method to recognize causative mutations in affected people with single-gene mutations.

Hereditary Hearing Loss (HHL) is of special importance in societies with a high rate of consanguinity that reveals the way for a rare pathogenic mutation to appear, and Iran is no exception with approximately 40% consanguinity. Hearing Loss (HL) ranks as the second most common disability among the Iranian population, and the rate of consanguineous marriage in

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the Iranian HL population is expected to reach up to 65%.

According to this evidence, we aimed to recognize defective genes in an Iranian family with NSHL by NGS. In this study, we report one novel variant in the TECTA gene with a pathogenic effect which may explain the NSHL phenotype in this Iranian family.

Case Report

An Iranian family with unknown hereditary hearing loss was enrolled by using the pedigree information of the patient from the Ahwaz (Southwest Iran). Clinical examination of the affected member in the family performed by an otolaryngologist and a geneticist included a thorough physical evaluation and otoscopy. The subject's developmental history and clinical evaluations did not show any syndromic manifesting and hearing loss was the only complaint; diagnosis of sensorineural HL (SNHL) was made according to pure tone audiometry (PTA), both air and bone conduction at frequencies ranging from 250 to 8000 Hz. Computerized tomography (CT) scan of the temporal bone was obtained in the affected member in the family to look for inner ear anomalies. Written informed consent was obtained from all participants, and the study was, according to the guidelines of the Ethics Committee of Iran's Ministry of Health and Medical Education.

Genomic DNA was isolated from blood leukocyte samples by using standard salting-out methods and the concentration of DNA samples was determined using a NanoDrop 1000 spectrophotometer. Then, stored at -20º C or amplified immediately.

Next, the DNA was used to produce libraries and capture sequencing. A customized Human capture array was designed to capture all coding regions and the intron/exon boundaries of the target genes involved in the pathogenesis of NSHL followed by the Next Generation Sequencing (NGS) method (Macrogen, Seoul, South Korea).

Sequence alignment of the sequence reads was carried out by using the reference human genome build hg19 from UCSC and were annotated through datasets and tools. Previously identified frequent variants (frequency> 1%) and synonymous substitutions were filtered out by use of public databases including the 1000 Genome Project (http://www.1000genomes. org), dbSNP, and EXAc Browser. Potential disease-causing variants were evaluated by using in silico pathogenicity prediction tools such as PolyPhen2 (5), SIFT (6), as well as Mutation Taster predictions (7) and Multiple Alignment tool.

To confirm the true positive of the novel variants recognized by NGS, Sanger sequencing was carried out in the patient (II-2) and family members. The specific primers were used for the amplification of a target gene site according to the reference sequences of the human genome from GenBank in NCBI that previously reported (8-9). PCR products were directly sequenced on the automated genetic analyzer (ABI 3100; Applied Biosystems). Moreover, Sanger sequencing was subsequently carried out to validate segregation in the family for the candidate variant.

The DNA sequence analysis of the genes involved in the pathogenesis of NSHL showed one novel mutation in the TECTA gene cosegregating in the affected members of the family. The mutation is a novel homozygous frameshift mutation, c.649-650insC, in exon 6 of the TECTA gene (c.649-650ins C) and results in a frameshift mutation with substitution of asparagine to glutamine at codon 218, which might lead to a truncated TECTA protein (p. Asn218Glnfs*X31).

The proband (II:2; Figure 1A) in the family, patient with TECTA gene mutation c.649-650insC, (Figure 1B) was a 30-years-old male who had one affected brother, and the hearing impairment in affected members of the family was noticed at school age. The inheritance mode and audiological examination of the proband were bilateral ARNSHL.

DNA sequence analysis of the other genes failed to identify any disease-causing sequence variants in the family. The region of the novel mutation was amplified, and then the PCR product was sequenced. So, the identified novel mutation was verified by using Sanger sequencing. The alignment of TECTA from diverse species was shown in Figure 1E. This result revealed that the mutated asparagine residue was evolutionarily conserved, demonstrating that this residue is important for proper protein function. Also, we confirmed that the unaffected parents and his affected brother (II-3) had the TECTA c.649-650ins C frameshift mutation in the heterozygous and homozygous states, respectively. The genotypes of sibs were also not homozygous for this mutation.

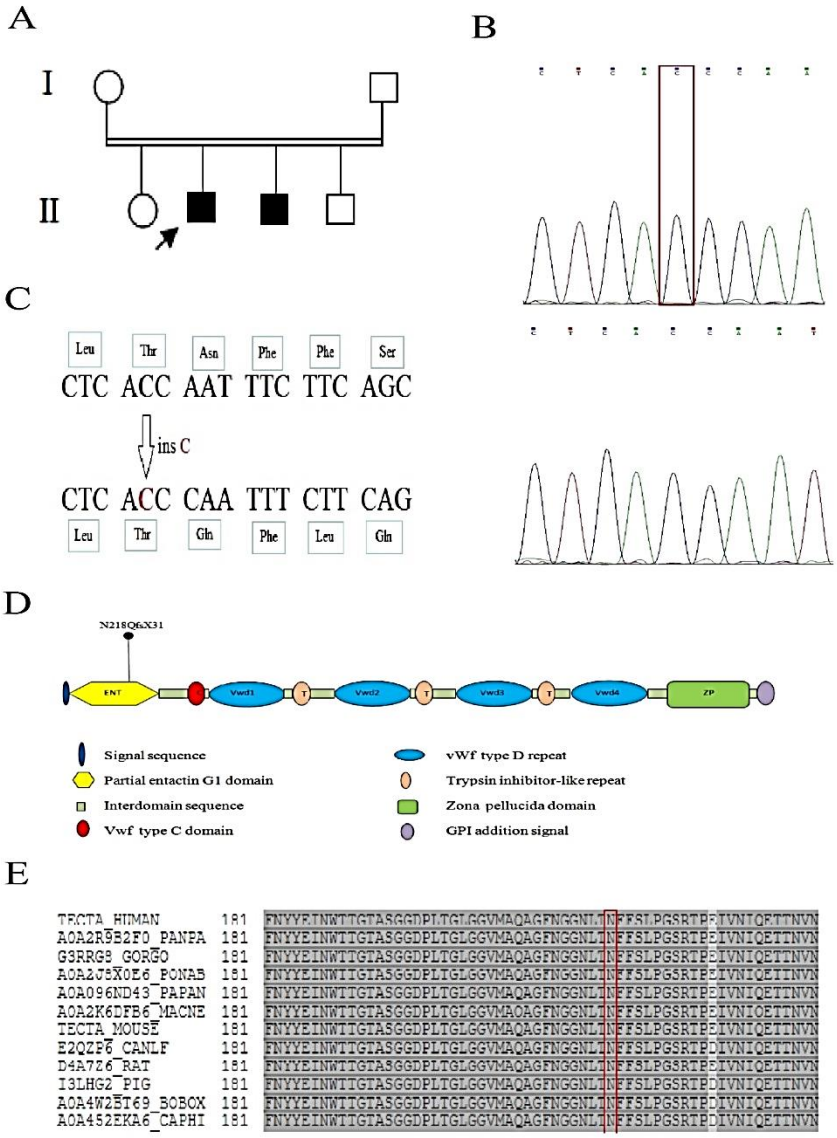

Figure 1. (A) The pedigree of family for the c.649-650 insC mutation. The black symbols indicate affected individuals. (B) Partial DNA sequences of the c.649-650 insC mutation in TECTA gene in the patients and a normal member. (C) The amino acid changes caused by the changes in the DNA sequence. (D) Domain structure of TECTA. The mutation lies within the N-terminal region, in the ENT domain. (E) Alignment of the a-tectorin and homologous sequences in the N-terminal ENT interdomain. The arrow marks the position of the c.649-650 insC mutation (p. Asn218GlnfsX31). The ENT domain is highly conserved in different species

Discussion

In the present study on an Iranian family with deafness, NGS showed one novel mutation in the TECTA gene to be the underlying causes of the impairment in the family.

The proband (II:2, shown with an arrow in Figure 1A) in the family was a 30-year-old male, and his brother was also affected (Figure 1A). Clinical history and audiological evaluation of the affected patients were indicative of progressive, bilateral ARNSHL. This family is affected by a novel frameshift mutation in exon 6 of the TECTA gene (c. 649-650insC, p.Asn218GlnfsX31) in the ENT-like domain of the protein, which is predicted to create a shift in the reading frame and introduce a stop codon at position 1012. The p.Asn218GlnfsX31 mutation was implicated as a pathogenic mutation causing hearing loss. The mutant amino acids are located in the highly conserved ENT-like the domain of TECTA, which includes a domain in

residues 98-252. Overall, truncating mutation is predicted to partially or fully disrupt C-terminal domain-mediated protein-protein interaction as well as its transcriptional regulation function. Also, this mutation may lead to a diminish in the expression level of full-length TECTA mRNA in affected individuals because the mutant mRNA may be degraded by a nonsense-mediated decay mechanism.

Hearing loss caused by TECTA mutations is inherited in two modes of autosomal recessive and autosomal dominant. THE human TECTA gene (MIM 602574) has been situated at the 11q22-q24 chromosome and contains 23 exons, and provides a protein of 2155 amino acids (10-13). Studies have revealed that the TECTA gene is highly conserved in humans, mice, and zebrafish (14). Alpha-tectorin protein in humans is encoded by the TECTA gene and is the most important non-collagen component of the tectorial membrane (TM) of the inner ear (The tectorial membrane is an apical extracellular structure of the cochlea and lies over the organ of Corti) (15). Sound waves cause the movement of TM related cells (16). Also, alpha tectorin is a large modular glycoprotein that contains some protein-protein interaction domains: the entactin domain (ENT), the zonadhesin region (ZA) containing two partial and three full von Willebrand factor (vWF) type D repeats, an N-terminal entactin G1-like domain, three trypsin inhibitor-like cysteine-rich domains, and a C-terminal zonapellucida (ZP) domain (10,15,17).

Interestingly, the truncated mutations, including nonsense, frameshift, and splicing, are responsible for ARNSHL, while all of the missense mutations in the TECTA gene cause ADNSHL (18-20). Thus, any mutation in the TECTA gene that inactivates the gene products is correlated with ARNSHL. Autosomal recessive mutations in TECTA result in a moderate to severe deafness and show an audiogram pattern in a flat or U shape at all frequencies. Although all missense mutations in the TECTA gene cause ADHL, conditional on the involved domain harboring the mutation, clinical features are diverse (19). Also, any defect in TM leads to a decline in the quality of sound waves transferred to stereociliary fibers of hair cells and finally causes hearing loss (21).

The first direct evidence of the contribution of TECTA mutations of hearing loss was provided by a study of a Lebanese family with Severe-to-Profound prelingual deafness. This mutation has been situated at the donor splice site in intron 9 and leads to a stop codon at 972 positions rendering a truncated protein (22). So far, more than 60 mutations have been described in this gene, which is inherited in either dominant or recessive modes that cause NSHL.

This mutation has previously not been reported in mutation databases. This is the first report of mutations of the TECTA gene in patients affected by NSHL. The following evidence proves that this mutation can lead to NSHL: 1-Next generation sequencing only identified this mutation to be the main cause of NSHL in the patient. 2- As can be seen in Fig. 1A, direct Sanger sequencing proved the mutation in the proband and affected members of the family, and based on recognized heterozygote mutations in their parents, the pattern of inheritance must be an autosomal recessive for TECTA. 3- Bioinformatics software such as SIFT, PolyPhen, Mutation Taster software are predicted that this variant will be damaging and disease-causing (For N218Qfs X31). 4. Also, a frameshift mutation in exon 6 of TECTA gene (c. 649-650insC, p.Asn218GlnfsX31) in the ENT domain of the protein (Figure 1D), which is predicted to create a shift in the reading frame and introduce a stop codon at position 1012, can create major problem in the TECTA protein. 5- On the other hand, because this modify was not present in the healthy members, it cannot be ruled out that it affects the phenotype of these patients. Thus, these mutations in the TECTA gene are pathogenic in our patients with NSHL.

In conclusion, we have successfully applied NGS in a family for mutation screening within hearing loss related genes and identified one novel frameshift mutation in the TECTA gene that segregated with the ARNSHL in the Iranian family, thereby confirming previous reports that mutation in TECTA is associated with nonsyndromic hearing loss.

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