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Novel *OTOA* mutations cause autosomal recessive non-syndromic hearing impairment in Pakistani families

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To the Editor:

Mutations in the *OTOA* gene (MIM 607038) have been reported as a cause of autosomal recessive non-syndromic hearing impairment (ARNSHI) in the Palestinian population (1, 2). Here we describe two novel *OTOA* mutations that were discovered in three consanguineous Pakistani families segregating ARNSHI. The study was approved by the Institutional Review Boards of Quaid-I-Azam University and the Baylor College of Medicine and Affiliated Hospitals. Informed consent was obtained from the family members who participated in the study. Based upon medical history the hearing impairment (HI) is prelingual in onset, and possible environmental causes of HI such as perinatal, ototoxic, traumatic and infectious factors were excluded. No evidence of syndromic or vestibular disease was found after physical examination that included balance and gait testing. The family members underwent air conduction audiometric testing and the results of individuals V-1 of family 4223, V-2 of 4309, and IV-1 of 4526 showed bilateral severe-to-profound HI (Fig. 1a).

DNA samples were obtained from hearing impaired and unaffected individuals of the three families. A genome scan was subsequently performed at the Center for Inherited Disease Research (CIDR) using the Human Linkage panel that contains ~6000 SNP (single-nucleotide polymorphism) markers. Linkage and haplotype analyses were performed with

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Electronic Database Information

The following URLs were accessed for data in this article:

Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

Superlink-SNP-Online, http://cbl-hap.cs.technion.ac.il/superlink-snp/main.php

UCSC Genome Browser, http://genome.ucsc.edu

ClustalW2 web server, http://www.ebi.ac.uk/Tools/msa/clustalw2/

Supporting Information

The following Supporting information is available for this article:

Table S1. LOD scores for families 4223, 4309 and 4526 within the 16p region which includes the *OTOA**.

Additional Supporting information may be found in the online version of this article.

Superlink-SNP-Online. Maximum multipoint LOD scores of4.31, 2.36 and 2.05 were observed in families 4223, 4309 and 4526, respectively (Table S1, supporting information) in the *OTOA* gene region where the homozygous haplotypes were found to segregate with ARNSHI (Fig. 1b–d). Thus, DNA samples of two HI and one unaffected individuals from each family were sequenced for the *OTOA* gene. After the identification of mutations, DNA samples from additional family members and 335 unrelated Pakistani controls were sequenced. To determine the pathogenicity of identified mutations, bioinformatics analyses were performed using PolyPhen-2 (3) and MutationTaster(4). Evolutionary conservation of DNA sequence was estimated using the GERP ++(Genomic Evolutionary Rate Profiling) score in the UCSC genome browser, while non-human OTOA proteins were aligned with ClustalW2 web-server to determine the conservation of protein residues. A missense substitution, c.1352G>A(p.Gly451Asp), observed in family 4223 resulted in a pathogenic variant as predicted by PolyPhen-2 and MutationTaster (Table 1). The c.1352G is evolutionarily constrained with a GERP score 5.56 (Table 1) as the protein residue p.Gly451 is highly conserved in otoancorin proteins of other species. Another variant, c.1879C>T (p.Pro627Ser), which segregates with ARNSHI in families 4309 and 4526, is also predicted to be pathogenic (Table 1). The amino acid substitution at the highly conserved proline residue in otoancorin proteins of 11 non-human species may result in a pathogenic change. Neither novel mutation was detected in 670 control chromosomes. Families 4309 and 4526 also segregated an additional missense substitution, c.1141C>G (p.Gln381Glu). The variant was found in 6 out of 400 Pakistani control chromosomes, and the amino acid change is predicted to be benign by PolyPhen-2 (Table 1). Although the variant is not reported in public databases, it is most likely a polymorphism considering an allele frequency of 1.5% in Pakistani controls.

This is the first report of *OTOA* mutations outside of the Palestinian population. Although the severity of HI was not specified for all of the Palestinian individuals, it was reported that the c.1025A>T caused bilateral severe HI (1), while the HI observed for Pakistani individuals with either the c.1325G>A or c.1879C>T variants caused severe-to-profound HI affecting all frequencies (Fig. 1a). The exact structure of otoancorin still remains unknown, although it has been predicted that otoancorin has an ARM-type superhelical structure with small helical segments interspersed with non-helical sections (5). InterProScan (6) also predicts a signal peptide of 22 residues at the N-terminus and a glycosylphosphatidylinositol (GPI) anchor at the C-terminal domain. The GPI anchor serves to attach the apical surface of inner ear epithelial cells to overlying acellular gel extensions from the tectorial membrane through binding of the GPI anchor to glycoproteins of the tectorial membrane (2). Based on the secondary structure prediction server Jpred (7), the Gly451 residue is located within a buried α-helical segment, while the Pro627 residue is within an exposed coil region as a domain linker (8). ARM-type repeats, such as those predicted to occur in otoancorin, have the outer helix broken into two smaller helices with a bend in the middle, and a Gly or a Pro residue is usually found at this bend (5). Thus both Gly451 and Pro627 may contribute to the formation of the superhelical structure of otoancorin, and mutations at these residues might result in an inability of otoancorin to stabilize the tectorial membrane on top of the sensory hair cells, which is essential for inner ear mechanotransduction.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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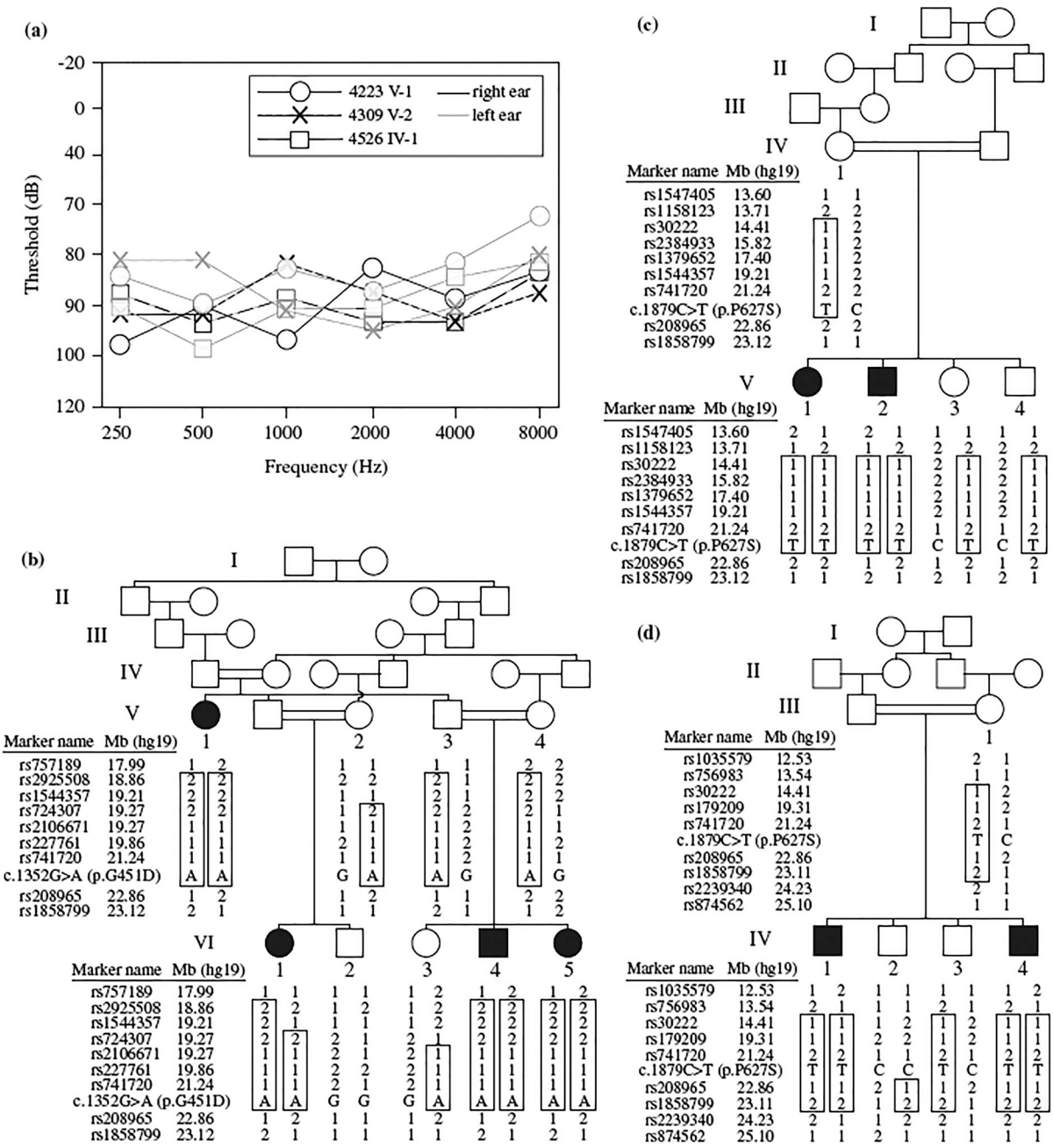

Fig. 1.

(a) Air-conduction thresholds for selected individuals from families 4223, 4309, and 4526 with the homozygous *OTOA* mutations. Right ear hearing threshold is shown with black, left ear with gray. All affected individuals' audiograms show bilateral severe-to-profound hearing loss across all frequencies tested. **(b–d)** Pedigree drawings of three ARNSHI families with *OTOA* mutations. Filled symbols represent hearing-impaired individuals, clear symbols denote hearing individuals. The haplotype segregating with ARNSHI is shown in a box and includes genotypes for SNP markers within the region and variant for the *OTOA*

mutation. **(b)** Family 4223 segregates the novel mutation c.1352G>A (p.G451D), **(c)** families 4309 and **(d)** 4526 segregate the novel mutation c.1879C>T (p.P627S).

Table 1.

Identified mutations and previously reported causal variants

Nucleotide change	Amino acid substitution	Exon number[a]	Family ID	Maximum LOD score	Alleles in controls	GERP++ score	PolyPhen-2 (human v
c.1025A>T[b]	p.D342V	12	BR	3.49	0/400	5.30	Probably damagi
c.1320+2T>C[b]	-	-	A1	3.56	0/400	5.21	-
c.1352G>A[c]	**p.G451D**	13	4223	4.31	0/670	5.56	Probably damagi
c.1141C>G	p.Q381E	12	4309/4526	2.36/2.05	6/400	5.21	Benign
c.1879C>T[c]	**p.P627S**	17	4309/4526	2.36/2.05	0/670	4.77	Probably damagi

[a]The exon number is based on the *OTOA* isoform 1 (NM_144672.3).

[b]These mutations were previously identified in Palestinian families (1, 2).

[c]Mutations in bold type indicate newly identified functional variants from this study.