

# Identification of a novel pathogenic *OTOF* variant causative of nonsyndromic hearing loss with high frequency in the Ashkenazi Jewish population

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**Abstract:** Mutations in the *OTOF* gene have previously been shown to cause nonsyndromic prelingual deafness (DFNB9, OMIM 601071) as well as auditory neuropathy/dys-synchrony. In this study, the *OTOF* NM\_194248.2 c.5332G>T, p.Val1778Phe variant was identified in a large Ashkenazi Jewish family as the causative variant in four siblings with hearing loss. Our analysis reveals a carrier frequency of the *OTOF* c.5332G>T, p.Val1778Phe variant of 1.27% in the Ashkenazi Jewish population, suggesting that this variant may be a significant contributor to nonsyndromic sensorineural hearing loss and should be considered for inclusion in targeted hearing loss panels for this population. Of note, the degree of hearing loss associated with this phenotype ranged from mild to moderately severe, with two of the four siblings not known to have hearing loss until they were genotyped and underwent pure tone audiometry and auditory brainstem response testing. The phenotypic variability along with the auditory neuropathy/dys-synchrony, which allows for the production of otoacoustic emissions, supports that nonsyndromic hearing loss caused by *OTOF* mutations may be much more common in the Ashkenazi Jewish population than currently appreciated due to a lack of diagnosis.

**Keywords:** hearing loss, *OTOF*, c.5332G>T, p.Val1778Phe, Ashkenazi Jewish, otoferlin

## Introduction

Otoferlin is a six C<sub>2</sub> domain transmembrane protein responsible for priming and fusing synaptic vesicles during auditory hair cell exocytosis to allow for sound encoding.<sup>1,2</sup> In the inner hair cell ribbon synapse, otoferlin is the major calcium sensor and binds to membranes as well as syntaxin 1A/SNAP-25 heterodimers in a calcium-dependent manner suggesting a role in regulating SNARE activity.<sup>3,4</sup> It also allows for calcium-dependent exocytosis in immature outer hair cells that act as sound signal amplifiers in the mature cochlea<sup>5</sup> as well as in type 1 vestibular hair cells that play roles in sensing head acceleration and maintaining balance.<sup>6</sup>

Mutations in the *OTOF* gene cause nonsyndromic prelingual deafness (DFNB9, OMIM 601071) in humans<sup>7</sup> and profound hearing loss in *OTOF* knockout mice.<sup>3</sup> Specific phenotypes, including severe, profound, progressive, and temperature-sensitive hearing loss, have been consistently seen for different *OTOF* mutations, which have allowed for genotype-phenotype correlations to be established.<sup>8</sup> *OTOF* mutations have also been associated with auditory neuropathy/dys-synchrony (also referred to as auditory neuropathy spectrum disorder [ANS]), which is characterized by functioning outer hair cells, but damaged auditory nerve and/or inner hair cells leading to disturbed pure tone audiometry, the absence of auditory brainstem responses (ABRs), and the detection of otoacoustic emissions (OAEs).<sup>9-11</sup>

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In this study, the *OTOF* NM\_194248.2 c.5332G>T, p.Val1778Phe variant was identified in homozygous form in a nonconsanguineous Ashkenazi Jewish (AJ) family in siblings with symptoms of hearing loss presenting for genetic evaluation. We identified the carrier frequency of this variant to be 1.27% in the AJ population, suggesting that this variant may be a significant contributor to nonsyndromic sensorineural hearing loss (SNHL) and should be considered for inclusion in targeted hearing loss panels for this population.

## Materials and methods

### Ethics statement

All family members from the index case were enrolled in a Mount Sinai Institutional Review Board (IRB)-approved genetics research study. Written informed consent was obtained from all the family members, and investigations were conducted in accordance with the principles of the Declaration of Helsinki. For the carrier frequency study, all samples were obtained with written patient consent from self-identified Ashkenazi Jews to be used for research purposes. These individuals were a mix of male and female young adults, who were presumed to be healthy. Consent form information included that patient material would be used for clinical testing and that excess material would be de-identified and used for research purposes to characterize single gene disorders in the AJ population. IRB permission was not required for the samples used in the carrier frequency study because all sample identifiers were removed prior to receipt by our lab (45 CFR part 46.101(b)(4)).

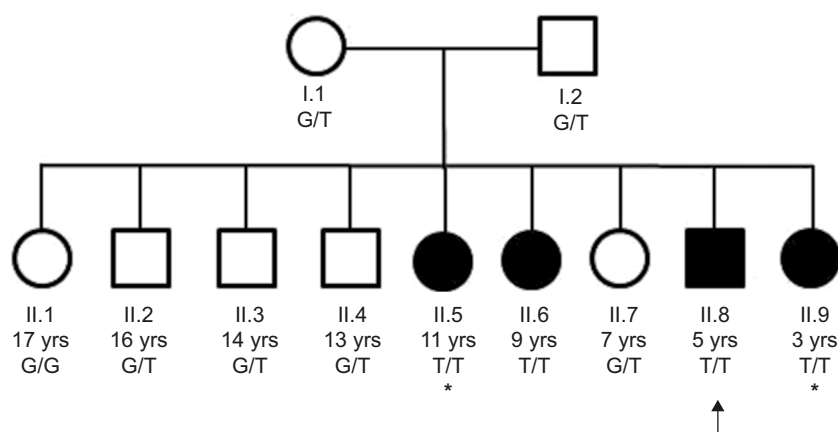
### Human subjects

DNA was isolated from peripheral blood samples obtained from the proband (II.8) as well as his siblings and parents

(Figure 1). The subjects were all of Hungarian and Romanian AJ descent, and the parents denied consanguinity. Of the nine siblings, siblings II.6 and II.8 were reported to have hearing loss at the time of presentation. The proband (II.8) was a male, aged 5 years, who had mild-to-moderate progressive SNHL. The hearing loss was first noted at the age of 3 years due to articulation difficulties, although the patient had failed his hearing test at birth, and follow-up by OAEs indicated hearing within normal limits. He was treated with binaural Oticon Safari hearing aids and uses a frequency-modulated system in school. His hearing exam at the time of genetic testing indicated that he had high-frequency moderately severe SNHL bilaterally, but normal middle ear function, and distortion product otoacoustic emissions (DPOAEs) that were present and robust.<sup>12</sup>

Sibling II.6 was a female, aged 9 years, who had bilateral mild SNHL. She had failed her newborn hearing screen, but follow-up OAE testing was within normal limits. At 4 years, her mother noticed that she was having difficulty hearing, which led to a full audiology evaluation. Sibling II.6 did not require hearing aids or assistive devices. Her hearing exam at the time of genetic testing revealed mild high-frequency hearing loss in the right ear, absence of contralateral acoustic reflexes in the left ear, and ANSD (patient had normal middle ear function, present and robust DPOAEs and present ipsilateral acoustic reflexes bilaterally) (Table 1).

The Harvard OtoGenome testing panel, which sequences 71 genes involved in hereditary hearing loss, was ordered for the proband II.8. After completion of clinical testing for the proband, all the siblings and both the parents were enrolled into our research study for completion of segregation analysis with targeted genotyping. After genetic testing, two additional siblings were evaluated for hearing loss. Audiology testing



**Figure 1** Pedigree.

**Notes:** This pedigree shows the autosomal recessive inheritance of the *OTOF* variant. Males are represented with squares and females are represented with circles. Affected individuals are shaded. Genotypes are shown for each family member, with affected individuals displaying homozygosity for the minor allele. The proband II.8 is indicated by the arrow. The asterisks indicate the two siblings (II.5 and II.9) that were not known to have hearing loss until they were genotyped and underwent hearing evaluations. Of note, the proband's paternal great-grandmother had unilateral early-onset hearing loss of unknown etiology (not shown).

**Abbreviation:** yrs, years.

**Table 1** Audiometry findings

Subject	Sex, Age (years)	Ear	250 Hz	500 Hz	1,000 Hz	2,000 Hz	4,000 Hz	6,000 Hz	8,000 Hz	PTA	SRT	SDS
<b>II.8 (Proband)</b>	Male, 5	AD		25	20	35	40	55	65	32	30	CNT
		AS		20	15	30	40	55	65	23	25	CNT
<b>II.6</b>	Female, 9	AD	15	10	10	15	30	40	40	12	5	92
		AS	5	10	10	10	15	25	20	10	10	100
<b>II.5</b>	Female, 11	AD	5	5	10	10	15	40	45	8	5	88
		AS	5	10	5	10	5	35	45	7	5	92
<b>II.9</b>	Female, 3	AD		15	15	15	35			15		CNT
		AS		15	15	10	25			13		CNT

**Abbreviations:** AD, right ear; AS, left ear; PTA, pure tone average; SRT, speech reception threshold; SDS, speech discrimination score; CNT, could not test.

of sibling II.5 revealed mild-to-moderate SNHL at 6–8 kHz bilaterally with normal hearing at  $\leq 4$  kHz, and robust DPOAEs with the absence of ABR waves 1–5 bilaterally, consistent with ANSD. Similarly, sibling II.9 was found to have normal hearing at 4 kHz and below bilaterally, slight high-frequency SNHL in the left ear and mild high-frequency SNHL in the right ear, and robust bilateral DPOAEs with the absence of ABR waves 1–5 with cochlear microphonic inversion bilaterally, consistent with ANSD (Table 1). In both children, speech and language development were normal and there were no communication impairments; the auditory deficits, which are specific for ANSD, would probably not have been suspected had the testing not been ordered.

## Segregation analysis

PCR and Sanger sequencing were used to perform segregation analysis. Parents and all siblings were genotyped. Standard polymerase chain reaction (PCR) conditions were used at the following settings: hold at 95°C for 5 minutes, 35 cycles at 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 25 seconds, and a final hold at 72°C for 7 minutes along with forward (5'-GTCTCGTCCCAGGAGAACAT-3') and reverse (5'-AGAGGAGGCAGAGGGAAGGT-3') primers. PCR products were separated by agarose gel electrophoresis to ensure proper amplification, which was indicated by a single strong band at the expected amplicon size. The samples were then prepared for sequencing using BigDye and run on a 3730xl DNA Analyzer (Thermo Fisher Scientific, Waltham, MA, USA).

## Carrier frequency study

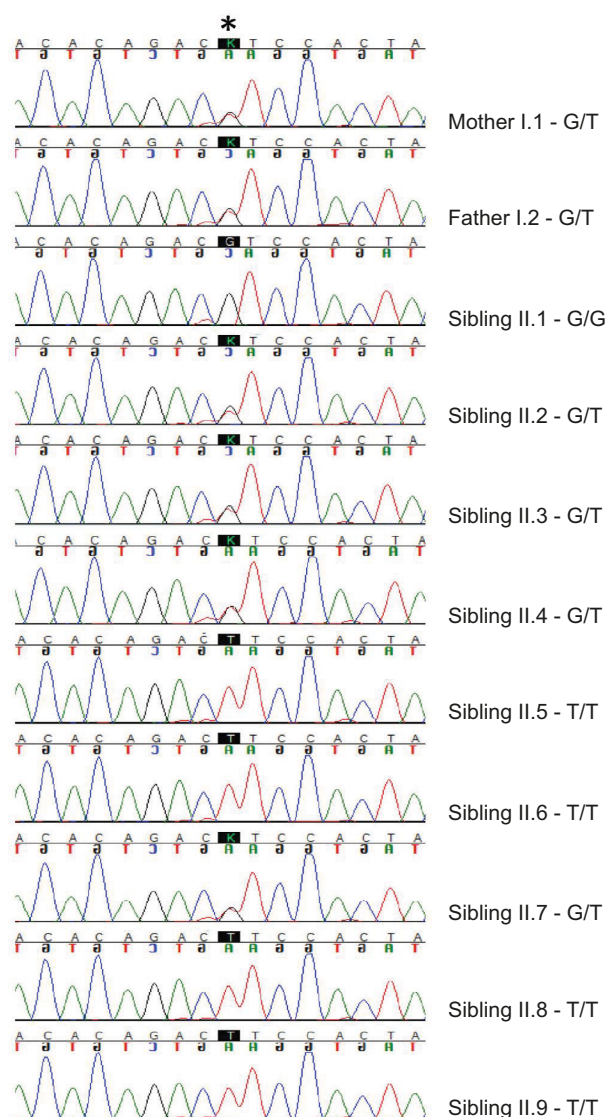
File Builder software (Thermo Fisher Scientific) was used to design the forward (5'-GGCCAGCAGGAGGACAAG-3') and reverse (5'-GTTGCCCTCGCCAGTGA-3') primers as well as VIC (5'-AGGACACAGACGTCCACT-3') and FAM (5'-AGGACACAGACTTCCACT-3') labeled probes. The assay was validated on control samples obtained from the proband and his family, and then the carrier frequency study

was performed on 1,021 samples. The samples were run on the GeneAmp® PCR System 9700 (Thermo Fisher Scientific) at the following PCR conditions: hold at 50°C for 2 minutes and 95°C for 10 minutes, then 40 cycles at 95°C for 15 seconds and 60°C for 1 minute. Allelic discrimination was then performed on the ABI PRISM® 7900 HT Sequence Detection System (Thermo Fisher Scientific), and data were analyzed using TaqMan Genotyper v1.2 software (Thermo Fisher Scientific).

## Results

The OtoGenome testing panel, which screens 71 genes involved in hereditary hearing loss, revealed a homozygous, likely pathogenic missense variant NM\_194248.2 c.5332G>T, p.Val1778Phe in the *OTOF* gene in the proband II.8. This missense change occurred at position chr2:26684765 (on assembly hg19/GRCh37) and is recorded in dbSNP as rs111033330. While *OTOF* mutations are known to cause autosomal recessive nonsyndromic hearing impairment associated with ANSD, the c.5332G>T, p.Val1778Phe variant has not been reported in any manuscripts previously, although this variant has been reported once in ClinVar. Computational analysis of this variant supported that the variant was pathogenic based on biochemical amino acid properties, conservation, Align-GVGD, PolyPhen2 (probably damaging by HumDiv, score 0.971; possibly damaging by HumVar, score 0.681), and SIFT (damaging, score 0.001). Additionally, there was a low allele frequency in ExAC in Europeans (0.0001799 [12/66720]), with no homozygotes reported, and the other ExAC populations reported no carriers (overall allele frequency = 9.885e-05 [12/121390]). Additionally, the valine at amino acid 1778 of *OTOF* shows strong evolutionary conservation. This residue is conserved in rhesus monkey, mouse, dog, chicken, western clawed frog, zebrafish, and lamprey.

The family was then enrolled into an IRB-approved research study and segregation of the variant was assessed. Sanger sequencing confirmed that each parent was an obligate



**Figure 2** Sanger sequencing results.

**Notes:** The Sanger sequencing traces for the proband and his family are depicted in the figure. The asterisk denotes the location of the variant. The black trace indicates the reference G allele, while the red trace indicates the mutant T allele.

carrier of the variant and that sibling II.6 was homozygous for the variant (Figure 2). Interestingly, when the remaining seven siblings were genotyped, two additional siblings (siblings II.5 and II.9) were also found to be homozygous for the pathogenic variant, suggesting that they should also have hearing difficulties. Audiology exams, along with ABR and OAE testing, were subsequently performed for siblings II.5 and II.9. In accordance with her genotype, audiology testing on sibling II.5 revealed mild-to-moderate high-frequency SNHL bilaterally and ANSD. Similarly, sibling II.9 was found to have slight high-frequency SNHL in the left ear and mild high-frequency SNHL in the right ear, as well as a low conductive component and ANSD. In both the siblings, the audiometric findings were significant, specific

for ANSD, and were not suspected clinically as the children had normal speech and language, and no outward signs of a hearing problem.

As the proband was of AJ descent, we performed a carrier frequency study on 1,021 AJ samples, revealing a carrier frequency of 1.27% or ~1 in 79 individuals, based on the presence of 13 heterozygous carriers and 1,008 wild-type samples (Figure 3). All of the samples identified as being heterozygous carriers had their genotypes confirmed through Sanger sequencing, and all the results were concurrent.

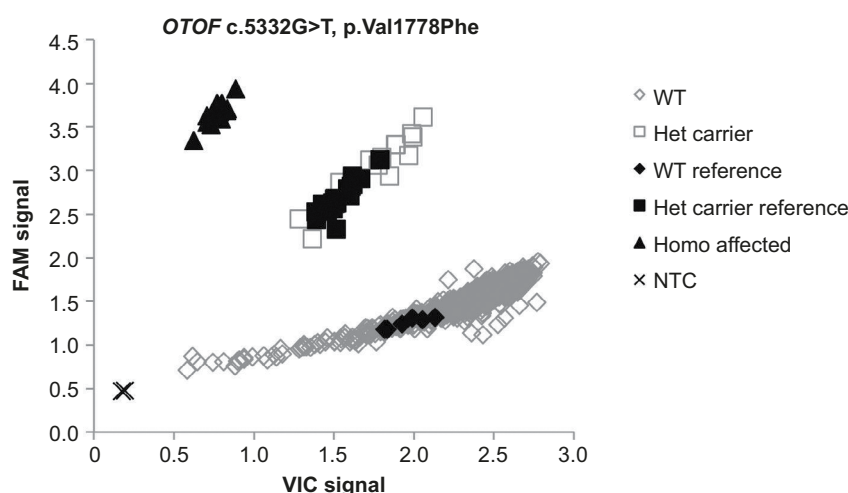
## Discussion

Most isolated childhood hearing loss cases have a genetic etiology. In the AJ population, two frameshift mutations in the gap junction beta 2 (*GJB2*) gene, c.167delT and c.30delG, are primarily responsible for congenital, nonsyndromic autosomal recessive SNHL with carrier frequencies of 4.03% and 0.73%, respectively.<sup>13</sup> The *OTOF* c.5332G>T, p.Val1778Phe variant identified in this study may be another significant contributor to SNHL, and specifically ANSD, in the AJ population with a carrier frequency of 1.27%. This high frequency suggests that this variant is likely a founder mutation, and we recommend adding this variant to targeted hearing loss panels for this population.

Interestingly, a second case of the *OTOF* c.5332G>T, p.Val1778Phe variant has been reported in ClinVar (ClinVar submission # SCV000065265.3). This variant was identified in a pair of AJ siblings with ANSD, who were found to be compound heterozygous for the c.5332G>T, p.Val1778Phe variant and a known pathogenic *OTOF* variant (personal communication with Dr Heidi Rehm, Laboratory for Molecular Medicine, Harvard Medical School), further supporting the potential frequency of this variant in AJ patients with hearing loss. *OTOF* founder mutations have been recognized in other populations and have helped to increase the number of molecular diagnoses made for patients of these ethnicities. These variants include the Spanish c.2485C>T, p.Q829X,<sup>14</sup> Taiwanese c.5098G>C, p.E1700Q,<sup>15</sup> Japanese c.5816G>A, p.R1939Q,<sup>16</sup> and Argentinian c.2905\_2923delinsCTCCGAGCGCA variants.<sup>9</sup> It is important to now associate the c.5332G>T, p.Val1778Phe variant with the AJ population.

Of note, the degree of hearing loss associated with the homozygous *OTOF* c.5332G>T, p.Val1778Phe genotype ranged from mild to moderately severe, with two of the four siblings not reported to have hearing loss until they were genotyped and underwent pure tone audiometry, DPOAE, and ABR testing. The mild phenotype along with ANSD and the presence of OAEs support that nonsyndromic hearing loss caused by *OTOF* mutations may be much more common in





**Figure 3** TaqMan allelic discrimination carrier frequency plot.

**Notes:** The VIC probe is represented by the x-axis and signifies the WT allele, and the FAM probe is represented by the y-axis and signifies the minor allele. Water was used as the NTC.

**Abbreviations:** WT, wild type; NTC, no template control; Het, heterozygous; Homo, homozygous.

the AJ population than currently appreciated and clinically unapparent. Additionally, newborn screening, which is based on OAEs, may fail to identify individuals with this mutation. It is therefore possible that affected individuals may not present for evaluation and/or may not be properly diagnosed and treated unless they receive specific testing.

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## Disclosure

The authors report no conflicts of interest in this work.

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