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# Analysis of *TMIE* gene mutations including the first large deletion of exon 1 with autosomal recessive non-syndromic deafness

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## Abstract

**Background:** Transmembrane inner ear (TMIE) protein is an essential component of the mechanotransduction complex. In collaboration with other components, TMIE aids the maintenance and function of the sensory hair cells. Autosomal recessive deafness-6 (DFNB6) is caused by mutated *TMIE*, a gene in the high genetic heterogeneity spectrum of deafness. Hearing loss has a significant impact on the global economy and the quality of life of affected persons, their families, and society. Here, three unrelated families with *TMIE* variants are presented. All three cases were found while studying the genetic causes of an Iranian cohort of subjects with cochlear implants.

**Methods:** Whole exome sequencing was performed to find possible genetic etiology in probands of families after a comprehensive medical evaluation for hearing loss. Co-segregation analysis in probands and other family members was performed by Sanger sequencing. The variants were interpreted per the American College of Medical Genetics and Genomics guidelines.

**Results:** Three different variants associated with *TMIE* were confirmed as reasons for autosomal recessive non-syndromic deafness. The first novel ~ 10-kb deletion surrounding exon 1 of *TMIE* along with two previously reported variants co-segregated with families including a frameshift variant c.122\_125dup (p.Pro43fs) and a missense variant c.250 C>T; p.(Arg84Trp) in exons 2, and 3, respectively.

**Conclusion:** This study increases the mutational spectrum of the *TMIE* gene and highlights the importance of the large deletion of this gene as a reason for hearing loss. Moreover, an efficient and simple multiplex PCR assay was developed to determine the exact breakpoints of the *TMIE* deletion.

**Keywords:** Hearing loss, DFNB6, *TMIE*, Whole-exome sequencing, CNV

## Introduction

Hearing loss (HL) is the most prevalent sensory impairment, affecting one in every 500 newborns [1, 2]. Currently, HL affects more than 1.5 billion of the world's population, about one in five people, and is expected to affect more than 2.45 billion people in 2050 [3, 4]. HL significantly impacts the global economy and quality of life of affected persons, their families, and society [5–7].

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HL is a highly heterogeneous disorder that results from a wide range of environmental factors, including ototoxic drugs, infections, neonatal complications, and more than 150 auditory development-related genes [8–11]. In fact, genes are responsible for more than 60% of prelingual HL [12]. HL can be classified into different categories based on various factors, including type (conductive, sensorineural, mixed, and central auditory dysfunction), onset (prelingual and postlingual), severity (mild, moderate, moderately severe, severe, and profound), and whether it is isolated (non-syndromic) or associated with other clinical issues (syndromic) [13].

In the last two decades, new generations of sequencing have been applied, and a wide range of HL-related mutations have been uncovered [14]. These factors have provided valuable information about the genotype–phenotype correlation to improve the effectiveness of genetic counseling and prognoses for the disease [2, 15]. Eighty percent of hearing impairment are caused by genes with autosomal recessive inheritance; autosomal dominant, X-linked, and mitochondrial inheritance are less common. The diagnostic rate of hearing impairment-related genes depends heavily on family history, phenotype, onset time, patient ethnicity, and symmetry in HL [2].

To date, 78 genes are known to cause autosomal recessive non-syndromic HL (<https://hereditaryhearingloss.org>). The transmembrane inner ear (*TMIE*) gene participates in the mechanotransduction machinery of hair cells. Loss-of-function mutations in the evolutionarily conserved protein *TMIE* were causally associated with autosomal recessive deafness-6 (DFNB6; OMIM: 600,971) in human and animal studies [16–19]. DFNB6 is categorized as a type of severe-to-profound congenital or prelingual HL [16]. *TMIE* (OMIM: 607,237), with four exons, is located on the 3p21 chromosomal region. *TMIE* produces a protein with two transmembrane domains joined by an extracellular loop, plus N and C intracellular terminus, which play a role in the hearing process by aiding the maintenance, maturation, and development of the inner ear's sensory hair cells [17, 20, 21].

In the present investigation of the genetic background of an Iranian cohort with cochlear implants, we describe three unrelated families with autosomal recessive non-syndromic HL who exhibited mutations in the *TMIE* gene using whole-exome sequencing (WES). Moreover, we introduced a reliable and simple PCR method for finding the boundaries of the novel deletion of the *TMIE* gene.

## Materials and methods

### Ethics statement

This study was approved by the Ethics Committee of the Iran University of Medical Sciences (Tehran, Iran)

according to national law and the World Medical Association Code of Ethical Principles for Medical Research Involving Human Subjects. (Approval number: IR.IUMS.REC.1400.862). Written consent was obtained from all participants according to the Declaration of Helsinki.

### Study participants and clinical evaluations

Three consanguineous, unrelated Iranian families with siblings affected with HL were recruited (Fig. 1). The proband of the first family (IV.1) was a 17-year-old female; her 8-year-old brother also had prelingual HL (Fig. 1A). The proband of the second family (IV.1) was a 6-year-old female (Fig. 1B), and the proband of the third family (VI.1) was a 17-year-old female. The third family had only two children, both of whom had prelingual HL (Fig. 1C). The consanguineous parents and healthy siblings of families had normal auditory and verbal functions. Clinical evaluations, including family history and physical examinations for hearing, as well as tests for the presence of associated symptoms in syndromes and exposure to environmental factors, were performed in Hazrat Rasoul Akram Hospital, Tehran, Iran [22].

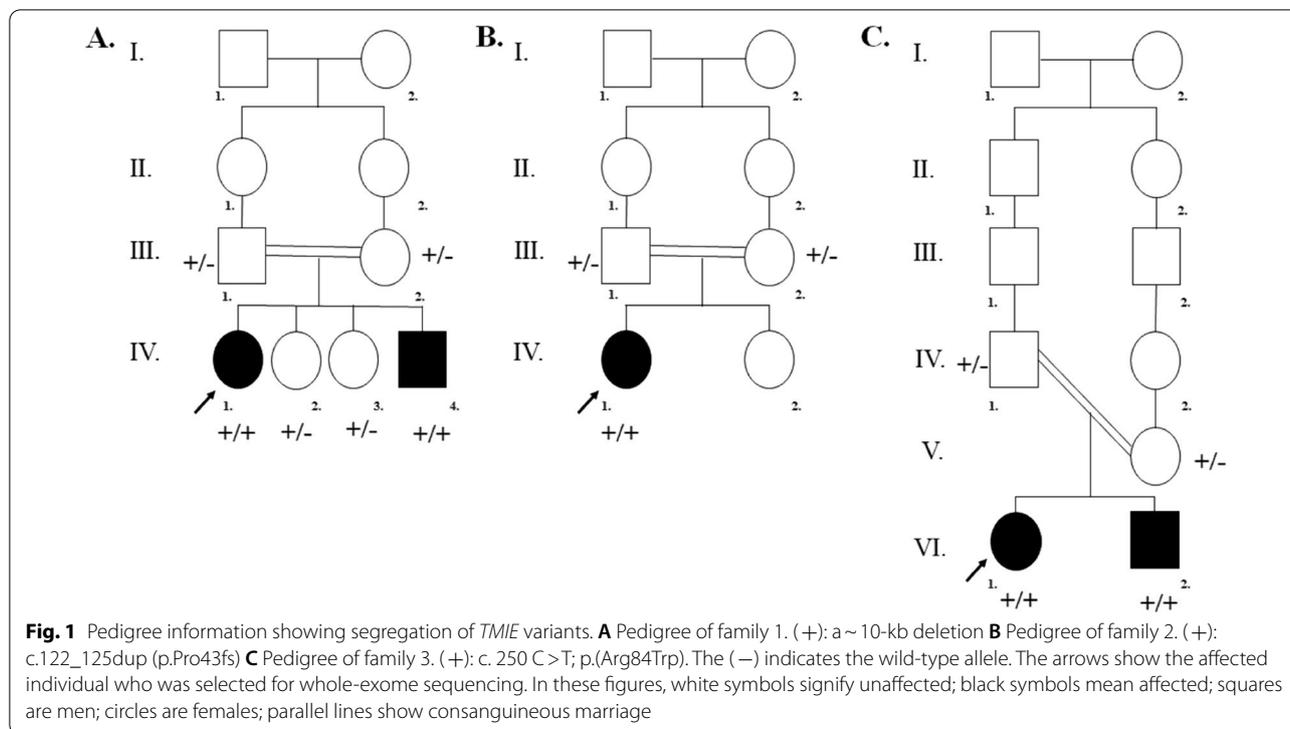
Individuals IV.1, IV.4 (family 1), IV.1 (family 2), and IV.1, VI.1, VI.2 (family3) (Fig. 1) underwent complete ear, nose and throat examination. Routine pure tone audiometry was performed on IV.1 from family 3 according to current standards. Air and bone conduction thresholds were measured at 250–8000 and 250–4000 Hz, respectively [23]. Audiological examinations, including auditory brainstem response, pure tone otoacoustic emission, and auditory steady-state response, were carried out to determine hearing thresholds for individuals IV.1, IV.4 (family 1), IV.1 (family 2), and VI.1, VI.2 (family3).

Blood samples (5 mL) were collected from the contributors, and genomic DNA was extracted as previously described by Falah et al. [9].

### Whole-exome sequencing

WES was performed based on previous work [15]. In short, WES enrichment was performed on the probands' genomic DNA using a Twist Human Core Exome Kit (Twist Bioscience, CA, USA) on an Illumina NovaSeq system (Illumina, San Diego, CA, USA) provided by CeGaT GmbH Company (Tübingen, Germany). The reads were aligned to the GRCh38/hg38 human reference genome sequence. The analytical sensitivity and specificity for detecting point mutations, micro-insertion, deletion, and duplication (within 20 bp) was > 95%.

The stepwise data analysis method was performed to find the causative variants. The first step was to remove variants with a minor allele frequency of > 1% in public databases such as gnomAD [24], 1000 Genome [25], dbSNP [26], and Iranome [27]. In the second step, all



non-coding regions other than the 20 bp flanking regions and synonymous variants were excluded. The results of variants were predicted using bioinformatics tools, including MutationTaster [28], Polyphen2 [29], SIFT [30], Provean [31], and Combined Annotation Dependent Depletion [32]. Human Splicing Finder v.3.1 was applied to find the role of variants in the splicing process [33]. The Berkeley Drosophila Genome Project and NetGene2 were used to analyze the effects of the variant on mRNA splicing [34, 35]. The remaining variants were ranked in order of priority according to patient phenotypes. (e.g., hearing symptoms) using Human Gene Mutation Database (HGMD) [36], ClinVar [37], Deafness Variation Database (DVD) [8], and human phenotype ontology [38]. ACMG/AMP (American College of Genetics and Genomics/Association for Molecular Pathology) guidelines were used for variant interpretation in the context of HL [39].

#### Variant validation

The identified variants were verified by direct Sanger sequencing in patients, and co-segregation analysis of candidate variants was performed using the samples obtained from parents and selected healthy relatives. Primers for the region of the candidate variants were designed using Primer3 (Table 1) [40]. PCRs were performed in a total volume of 25  $\mu$ l, including 12  $\mu$ l 2  $\times$  Taq DNA Polymerase Master Mix RED (Ampliqon, Odense,

Denmark), 1  $\mu$ l of each primer described in (Table 1), 2  $\mu$ l of genomic DNA, and 9  $\mu$ l of ddH<sub>2</sub>O. PCRs were performed with the initial denaturation at 95  $^{\circ}$ C for 4.0 min, followed by 30 cycles at 95  $^{\circ}$ C for 45 s, at 58  $^{\circ}$ C for 45 s, at 72  $^{\circ}$ C for 45 s, and a final extension at 72  $^{\circ}$ C for 7.0 min.

In family 1, the primers (Table 1) were used to find the exact breakpoints of the *TMIE* deletion according to the following method. In the first stage, PCRs were performed with two pairs of upstream primers 1 and 2 and one pair of downstream primer 3 of the deleted exon of the *TMIE* gene. In the next step, in multiplex PCR, all four primers (F2, R3, and the pair of primers 1) were used in a single reaction tube. The PCR products were visualized using ethidium bromide staining and 2% agarose gel electrophoresis. The purified PCR product of affected IV.1 was directly sequenced in forward directions using a PRISM 3100 sequencer (Applied Biosystems, Foster City, CA, USA). Sequencing results were analyzed by Codon code aligner software version 6.0.2 (CodonCode Corp).

In families 2 and 3, the PCR products with primers (F4, R4) and (F5, R5) were used for direct Sanger sequencing (Table 1).

## Results

### Clinical presentation

Clinical examinations and audiology evaluations showed that the three unrelated families (Fig. 1; Table 2) had non-syndromic severe-to-profound HL with an autosomal

**Table 1** Primer sequences and products size of the *TMIE* gene

Family	Exon number	Primer name	Primer sequence (5'–3')	Product size (bp)
1	Upstream Exon 1	F1	5'-AGAATCGAATTGGAAGGCAC-3'	184
		R1	5'-TTAAGGCGAACATCCTGAAAG-3'	
	Upstream Exon 1	F2	5'-CTGTGAGCGGGGTATCTTAC-3'	326
		R2	5'-CGCAACTCCAGAGCAAC-3'	
	Downstream Exon 1	F3	5'-CAGCCACAGATCCTCTG-3'	225
		R3	5'-TAAATAAGGACAGACACGAGAAGTC-3'	
	Multiplex Exon 1	F1	5'-AGAATCGAATTGGAAGGCAC-3'	184
		R1	5'-TTAAGGCGAACATCCTGAAAG-3'	
		R3	5'-TAAATAAGGACAGACACGAGAAGTC-3'	
2	Exon 2	F4	5-CTCCCACTTCAAGTACCTGGCTC-3	616
		R4	5-CACGTCCTCGTCCCAGTCC-3	
3	Exon 3	F5	5-CCATTCCTGGGTCTCTGAA-3	288
		R5	5-AGCAGAGGAACAGGGTGAC-3	

recessive inheritance pattern. Imaging investigations and computed tomography scans did not reveal any abnormalities in the anatomical structures of the middle and inner parts of the patients' ears. The cochlear implant surgery was previously performed for all affected persons (Table 2). After cochlear implantation, the auditory-verbal therapy was provided twice every week for 1 year. The result of categories of auditory performance-II (CAP-II) and speech intelligibility rating (SIR) scores were evaluated after the first year of implantation (Table 2, Additional file 1) [41, 42].

### Molecular findings

In family 1, the WES results indicated a ~10-kb deletion region in proband IV.1, spanning across exon 1 of the *TMIE* gene (NM\_147196.3), likely causing abnormal gene translation or nonsense-mediated mRNA decay. The deletion was not reported in the DGV [43], gnomAD, ClinVar, and UCSC [44]. The allele frequency was also not reported in the local database, Iranome (in same-ethnically individuals).

A frameshift variant in the *TMIE* gene—NM\_147196.3: c.122\_125dup (p.Pro43fs)—was identified that co-segregated with the phenotype in family 2 (Fig. 2A; Table 2). This variant was reported as pathogenic in ClinVar (VCV000984396.1), HGMD, and DVD databases. The variant was classified as “pathogenic” according to ACMG/AMP guidelines (Criteria: PVS1, PM2, and PP5) [39]. Furthermore, a missense variant in the *TMIE* gene—NM\_147196.3: c.250 C>T; p.(Arg84Trp)—was identified that co-segregated with the phenotype in family 3 (Fig. 2B; Table 2). This variant was reported as pathogenic/likely pathogenic in ClinVar (VCV000003391.15),

HGMD, and DVD databases. The variant was classified as “likely pathogenic” according to ACMG/AMP guidelines (Criteria: PM1, PM2, PM5, PP3, and PP5) [39].

### Validation of a large deletion in *TMIE*

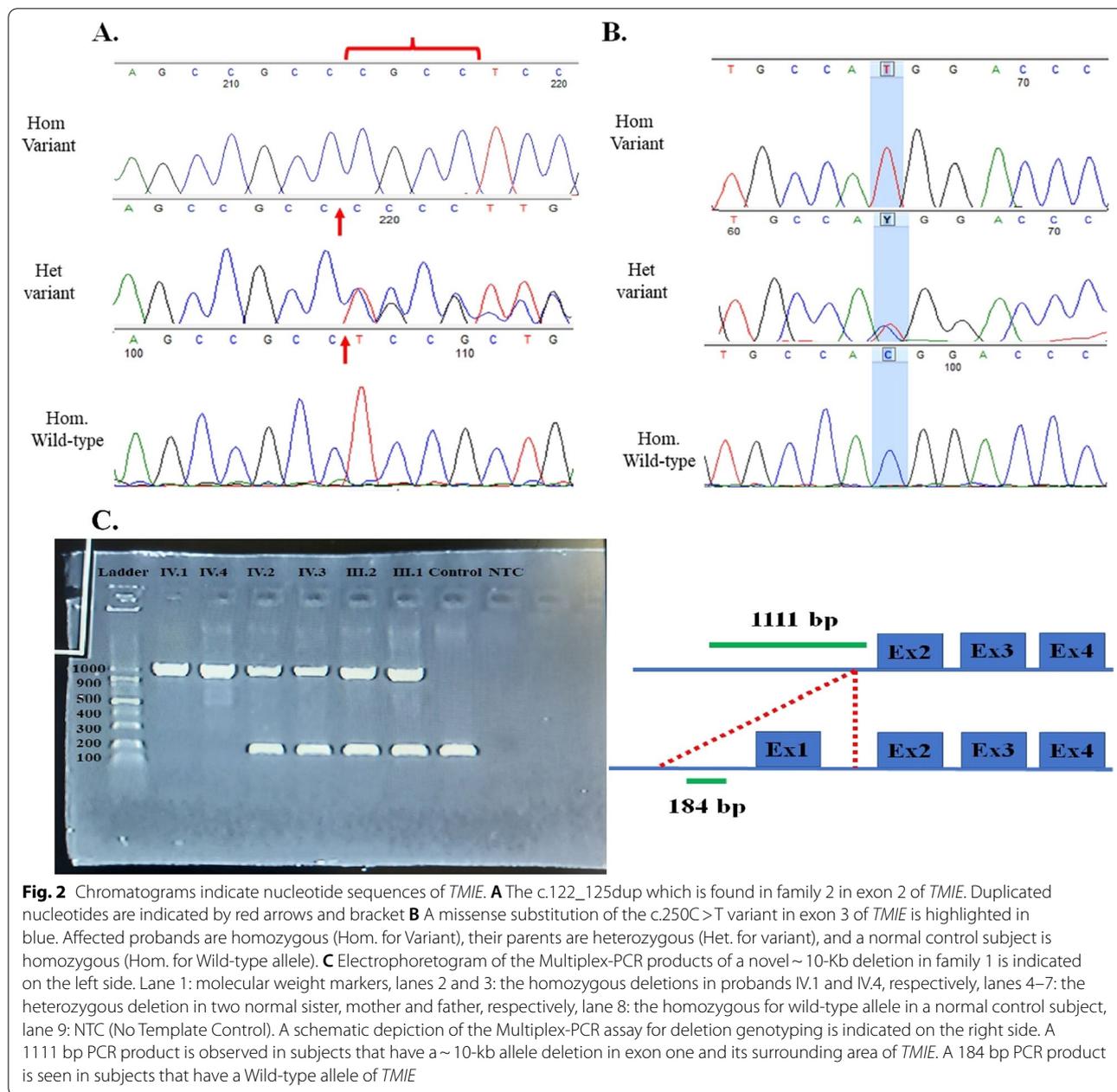
The product of the first PCR using a pair of F1 and R1 primers upstream of exon 1 had a length of 184 bp. This product was observed in all family members but not in patients, which indicated a lack of this area in patients (Figs. 2C and 3B). The second PCR product had a length of 326 bp and the primer pairs F2 and R2. This product was observed in the upstream area of exon 1 in all family members (Fig. 3B). The third PCR product had a length of 225 bp with primers F3 and R3; this product was also observed in all family members (Fig. 3B), thus indicating the presence of these upstream and downstream parts in all individuals. Finally, a multiplex PCR was performed to find the exact deletion point following an easy, accurate, and cost-effective method using primers F1, R1, F2, and R3. This process led to two products with 184 and 1111 bp. The PCR electrophoretogram of the deleted region is shown in (Fig. 2C). Affected family members (IV.1 and IV.4) showed a 1111-bp band, indicating a ~10-kb homozygous deletion. A normal individual without deletion in this region indicated a band with a size of 184 bp. Heterozygous individuals for deletion were seen with two bands (1111 bp and 184 bp) (Figs. 2C and 3B). Direct Sanger sequencing of purified PCR products of an affected individual (IV.1) indicated a novel homozygous deletion of 9283 bp in NC\_000003.12: g. 46,694,176-46703459del (Fig. 3). This was classified as a “pathogenic” variant according to the ACMG/AMP

**Table 2** Summary of genotype and clinical characteristics of three unrelated Iranian families

Family	Pedigree	Variation		Protein	Type	Status	Age at test	Age of onset	Age at cochlear implant	Type of HL	CAP-II	SIR	Gender	
		Chromosome	Nucleotide											
Family 1	IV.1	Chr3:46,694,176-46,703,459	g.46694176-46703459del	-	Deletion	Hom	17y	Prelingual	5y	Profound	7/9	4/5	Female	
		Chr3:46,694,176-46,703,459	g.46694176-46703459del	-	Deletion	Hom	8y	Prelingual	5y	Profound	6/9	3/5	Male	
		Chr3:46,694,176-46,703,459	g.46694176-46703459del	-	Deletion	Het	12y	-	-	-	Normal	-	-	Female
		Chr3:46,694,176-46,703,459	g.46694176-46703459del	-	Deletion	Het	11y	-	-	-	Normal	-	-	Female
III.1	III.1	Chr3:46,694,176-46,703,459	g.46694176-46703459del	-	Deletion	Het	40y	-	-	Normal	-	-	Male	
		Chr3:46,694,176-46,703,459	g.46694176-46703459del	-	Deletion	Het	36y	-	-	Normal	-	-	Female	
Family 2	IV.1	Chr3:46,705,816-46,705,817	c.122_125dup	p.Pro43Alaf1er73	Frameshift	Hom	6y	Prelingual	10 m	Severe to profound	7/9	5/5	Female	
		Chr3:46,705,816-46,705,817	c.122_125dup	p.Pro43Alaf1er73	Frameshift	Het	37y	-	-	Normal	-	-	Male	
		Chr3:46,705,816-46,705,817	c.122_125dup	p.Pro43Alaf1er73	Frameshift	Het	30y	-	-	-	Normal	-	-	Female
Family 3	VI.1	Chr3:46,709,164	c.250 C>T	p.(Arg84Trp)	Missense	Hom	17y	Prelingual	10y	Profound	6/9	4/5	Female	
		Chr3:46,709,164	c.250 C>T	p.(Arg84Trp)	Missense	Hom	7y	Prelingual	10 m	Profound	7/9	5/5	Male	
	V.1	Chr3:46,709,164	c.250 C>T	p.(Arg84Trp)	Missense	Het	38y	-	-	Normal	-	-	Female	
		Chr3:46,709,164	c.250 C>T	p.(Arg84Trp)	Missense	Het	36y	-	-	-	Normal	-	-	Male

The annotation was applied according to the Homo sapiens genome assembly GRCh38 (hg 38)

Hom homozygote, Het heterozygote, Y year, M month, CAP II categories of auditory performance, SIR speech intelligibility rating



**Fig. 2** Chromatograms indicate nucleotide sequences of *TMIE*. **A** The c.122\_125dup which is found in family 2 in exon 2 of *TMIE*. Duplicated nucleotides are indicated by red arrows and bracket **B** A missense substitution of the c.250C>T variant in exon 3 of *TMIE* is highlighted in blue. Affected probands are homozygous (Hom. for Variant), their parents are heterozygous (Het. for variant), and a normal control subject is homozygous (Hom. for Wild-type allele). **C** Electrophoretogram of the Multiplex-PCR products of a novel ~10-Kb deletion in family 1 is indicated on the left side. Lane 1: molecular weight markers, lanes 2 and 3: the homozygous deletions in probands IV.1 and IV.4, respectively, lanes 4–7: the heterozygous deletion in two normal sister, mother and father, respectively, lane 8: the homozygous for wild-type allele in a normal control subject, lane 9: NTC (No Template Control). A schematic depiction of the Multiplex-PCR assay for deletion genotyping is indicated on the right side. A 1111 bp PCR product is observed in subjects that have a ~10-kb allele deletion in exon one and its surrounding area of *TMIE*. A 184 bp PCR product is seen in subjects that have a Wild-type allele of *TMIE*

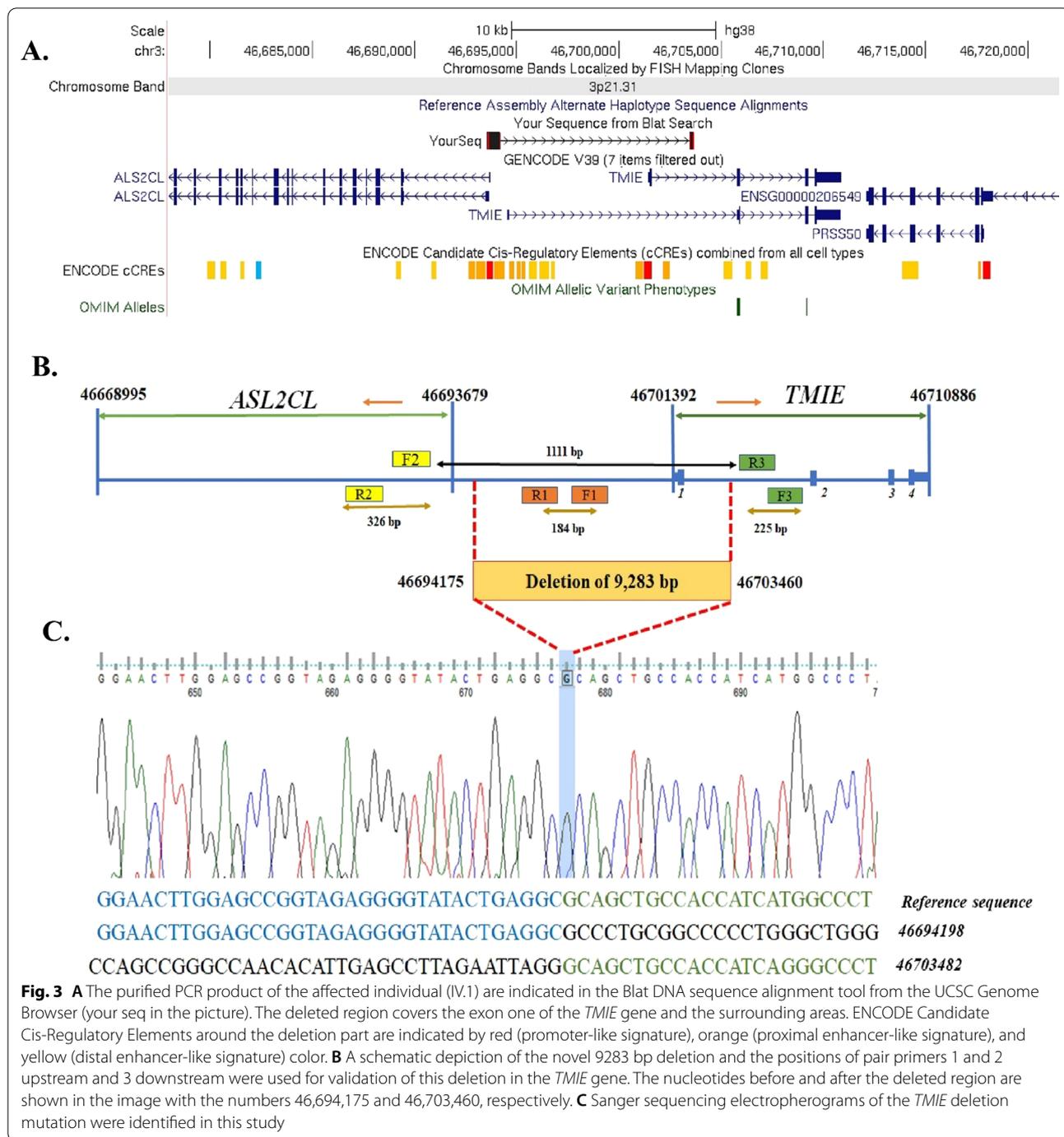
guidelines for variant interpretation regarding HL (Criteria: PVS1, PM1, and PM2) [39].

### Discussion

The loss of function of *TMIE* is responsible for autosomal recessive non-syndromic HL (DFNB6). Previous studies have indicated the role of *TMIE* in the maintenance, maturation, and development of the sensory hair cells in the inner ear (reviewed in [20]). *TMIE* is considered one of the components of the mechanotransduction machinery on the surface of the stereocilia of hair cells [45, 46]. The

mechanotransduction complex converts the mechanical stimuli of sound, gravity, and head accelerations into electrical signals. The transmission of these signals into the central nervous system is essential to the perception of sound [47].

Here, we reported three unrelated non-syndromic HL families due to pathogenic mutations in *TMIE* (Fig. 1). Initially, according to the ACMG guidelines for screening for genes related to HL [39], *GJB2* mutation testing was performed; the results were normal for three probands [12, 48, 49]. In the next step, WES was performed on



the probands of three families. One novel ~10-kb deletion in family 1, a frameshift duplication (c.122\_125dup) in family 2, and a missense (c.250 C>T) in family 3 were detected. These variants co-segregated with HL in families (Table 2; Fig. 2).

A homozygous deletion 9283 bp in NC\_000003.12: g.46,694,176-46703459del was reported for the first time

in this document (Figs. 2C and 3). This deletion removes exon 1 of the *TMIE* gene and its surrounding intronic region, where the cis-regulatory elements are located (Fig. 3A). Therefore, this deletion can prevent transcription initiation or initiation from cryptic sites. As described in our previous review of the *TMIE* gene, no significant deletions to this gene have been associated

with HL [20]. However, the total deletion of the *TMIE* gene has been previously reported in spinner [17] and circling mice [50, 51], which serve as animal models of humans regarding DFNB6. Both models are caused by a spontaneous 40-kb deletion on chromosome 9, which is analogous to human chromosome 3p21. The most important manifestations observed in these mice include HL, typical head tossing, circling, and hyperactivity.

The homozygous c.122\_125dup variant inserts a 4-bp (CGCC) in exon 2, which is predicted to result in a frameshift, substituting 72 incorrect amino acids followed by a premature stop codon [16]. In the wild type of the *TMIE* protein, proline 43 is located on the extracellular loop between two transmembrane domains. This mutation was reported for the first time in an Indian family with a consanguineous marriage and three affected children [16].

The homozygous c.250C>T substitution was identified in exon 3 of the *TMIE* gene. This variant causes an arginine (Arg (R)) substitution to tryptophan (Trp (W)) at codon 84, which is located in a highly conserved residue within the intracellular carboxy-terminus of the *TMIE* protein. The emerging evidence demonstrates the importance of the carboxyl terminus of *TMIE* for its proper functioning. This domain participates in *TMIE* binding to PIP2 (phosphatidylinositol 4,5-bisphosphate) and TMC1 (Transmembrane channel-like 1), and correct collaboration of these components is essential for the proper functioning of the mechanotransduction channel [45, 46, 52]. The first report on p.R84W involved a family with a consanguineous marriage from southern India [16, 53]. Sirmaci et al. reported frequencies of 10.3% and 2.4% in Southeastern Anatolia and Turkey, respectively, while a haplotype analysis indicated that the mutation was due to a 'Founder Effect' that first occurred approximately 1250 years ago [54].

Moreover, in agreement with the previous report, no inner ear abnormality was observed in the affected members of this study [54].

An effective treatment for hearing impairment is still based on hearing amplification and cochlear implantation [55], even though no commonly used procedure can restore normal hearing to patients. A better understanding of the molecular mechanisms that cause hearing impairment can provide new perspectives for treatment [56, 57]. Early studies on the genetic causes of HL led to the introduction of high heterogeneity of single nucleotide variants as a responsible factor in this field. Later, the role of copy number variations (CNVs) as a common reason for HL was indicated using comprehensive genetic testing platforms [58, 59]. So far, CNVs have been reported in 29 non-syndromic HL-related genes,

with *STRC*, *OTOA*, and *GJB2/GJB6* being the most well-known genes with deafness-causing CNVs [59]. To the best of our knowledge, no CNVs have been reported in *TMIE*. The present study expands the mutations varieties in the *TMIE* gene by introducing the first ~10-kb deletion. A noteworthy limitation of this study is that we could not perform a functional analysis of this variant in vitro or in vivo. *TMIE* is considered a critical protein in the mechanotransduction complex, which participates in functional mechanotransduction channel [45, 46, 52] and hair-bundle morphogenesis [21]. Further experimentation is needed to better understand the underlying cellular pathway of *TMIE* in developing HL. Such research could improve the quality of clinical diagnoses, medical management, genetic counseling, and prevention strategies for patients and their relatives.

## Conclusion

We have elucidated the role of the *TMIE* gene as a genetic cause of HL in three Iranian patients. We have also indicated the usefulness of WES, along with multiplex PCR, for identifying a novel ~10-kb causative deletion in *TMIE*. This study expands the mutational spectrum of the *TMIE* gene and indicates the importance of studying the connection between large deletions in this gene in HL.

## Abbreviations

*TMIE*: Transmembrane inner ear; DFNB6: Autosomal recessive deafness-6; HL: Hearing loss; WES: Whole-exome sequencing; HGMD: Human gene mutation database; DVD: Deafness variation database; ACMG: American College of Medical Genetics; AMP: Association for molecular pathology; CREs: Cis-regulatory elements; Arg: Arginine; Trp: Tryptophan; PIP2: Phosphatidylinositol 4,5-bisphosphate; TMC1: Transmembrane channel-like 1; CNVs: Copy number variations.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12920-022-01287-9>.

**Additional file 1** A) Categories of Auditory Performance-II (CAP-II) criteria. B) Speech intelligibility rating (SIR) categories.

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## Author contributions

SR, performed experiments, data analysis and interpretation, manuscript preparation; MF, enrolled the patient and contributed to clinical diagnoses and report writing, proved manuscript final version; HE, enrolled the patient and contributed to clinical diagnoses, revising the manuscript; SM, Study design, enrolled the patient and contributed to clinical diagnoses and report writing, data analysis and interpretation, revising the manuscript; MF, Study design, enrolled the patient and contributed to clinical diagnoses, data analysis and interpretation, manuscript preparation, proved manuscript final version. All authors have read and approved the final manuscript.

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### Availability of data and materials

The whole-exome sequencing datasets used during this manuscript are available from the corresponding author on reasonable request. The novel variant was submitted in ClinVar database (accession number: SCV002098951).

### Declarations

#### Ethics approval and consent to participate

The study was conducted in accordance to the guidelines of the Declaration of Helsinki, and approved by Iran University of Medical Sciences (Tehran, Iran). (Approval number: IR.IUMS.REC.1400.862). Informed consent was obtained from all subjects and/or their legal guardian(s).

#### Consent for publication

Written informed consent for publication of clinical details and clinical images was obtained from the legal guardians.

#### Competing interests

The authors declare no competing interest.

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