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Original Article

Heterozygous variants in transmembrane channel-like 1 gene cause autosomal recessive nonsyndromic hearing loss

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Abstract

Autosomal recessive non-syndromic hearing loss (ARNSHL) can cause severe or very severe pre-speech hearing loss. Transmembrane channel-like 1 (TMC1) gene is the sixth deafness gene discovered, but the precise extent of its protein structure and function is unknown. First, history collection, audiology examination and imaging examination were performed on the proband and his family members. Peripheral blood of proband and family members was collected, genomic DNA was extracted, exon high-throughput sequencing technology was used to detect the deafness gene mutation of the proband, and Sanger sequencing was performed to verify the TMC1 gene of the proband's parents. The proband was born with hearing impairment, normal tympanic function, inability to induce acoustic reflex in both ears (acoustic reflex threshold is 100 dBHL), and severe sensorineural deafness. One of his sisters has severe sensorineural hearing loss, and neither his parents nor his other sister is hearing impaired. High-throughput sequencing of the proband identified mutations at c.741+3_741+6delAAGT (splicing) and c.884C>T (p.A295V) of the TMC1 gene, two of which were heterozygous mutations. Sanger sequencing confirmed that the c.884C > T mutation was inherited from the mother, while the c.741+3_741+6delAAGT mutation was derived from the father. Prediction of amino acid function suggested that both mutations were pathogenic mutations. In conclusion, we found a new pathogenic complex heterozygous mutation of the TMC1 gene, which enriched the mutation spectrum of the TMC1 gene and provided a basis for genetic counseling and prenatal diagnosis of ARNSHL.

Keywords: Amino acid function, Autosomal recessive non-syndromic hearing loss, Exon high-throughput sequencing technology, Gene mutation, Transmembrane channel-like 1.

1. Introduction

About 50% of patients with deafness are caused by genetic factors [1]. Hereditary deafness is a typical monogenic genetic disease with high specificity. Hearing loss is mainly divided into syndromic and non-syndromic hearing loss. Non-syndromic hearing loss can be subdivided into autosomal recessive non-syndromic hearing loss (ARNSHL, 77%), autosomal dominant non-syndromic hearing loss (ADNSHL, 22%), sex chromosome-related hearing loss, y chromosome-related hearing loss and mitochondrial hearing loss [2].

Non-syndromic hearing loss (NSHL), which accounts for about 70% of inherited hearing loss, has strong genetic heterogeneity. Total nonsyndromic hearing loss genes: 138.Autosomal dominant nonsyndromic hearing loss genes: 63. Autosomal recessive nonsyndromic hearing loss genes: 82. Sex-linked nonsyndromic hearing loss genes: 7 (http://hereditaryhearingloss.org/). Among them, GJB2, SLC26A4 and mitochondrial 12S rRNA gene mutations are COL11A2, GJB2, GJB6, MYO6, MYO7A, TBC1D24, TECTA and TMC1 can cause ARNSHL and ADNSHL.

Transmembrane channel-like 1 (TMC1) gene is the sixth deafness gene discovered, which belongs to autosomal recessive inheritance. It is located on chromosome 9q21.13 and contains 24 exons. Coding of transmembrane protein is composed of 760 amino acids, but the precision of TMC1 protein structure and function is not yet clear [3].

In this study, whole exome sequencing (WES), Sanger sequencing and Sanger verification were used to detect the genetic causes of a non-syndromic deafness family with rare hot spot mutations. For non-clinical hot-spot mutation, molecular diagnosis of deafness patients to provide more genetic data reference, for this kind of family genetic counseling for theoretical basis.

2. Materials and methods

2.1. Data collection and clinical evaluation

In the study, the family members were in The Second Hospital, Hebei Medical University Otolaryngology Head

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and Neck Surgery Clinical Evaluation. This study was approved by the ethics committee. Written informed consent was obtained from all study participants and from parents of participants younger than 18 years of age. All participants were informed that all collected data would be used for scientific research only and not for any commercial purposes. The evaluation consisted of a complete history interview and a complete physical examination, including otoscopy, to rule out hearing loss due to infection, trauma or other factors.

2.2. Audiological examination and imaging examination

Middle ear function was assessed by tympanic pressure method, and outer hair cell function was assessed by distortion generation otoacoustic emission (DPOAE). Pure-tone audiometry (PTA) was calculated as the mean of the patient's hearing thresholds at 500, 1000, 2000 and 4000 Hz. The degree of hearing loss was defined as mild (26-40 dB HL), moderate (41-55 dB HL), moderately severe (56-70 dB HL), severe (71-90 dB HL), and extremely severe (> 90 dB HL). The hearing thresholds reported in this study were the average pure-tone thresholds on each side. Tandem gait and Romberg test were performed for vestibular function examination. Computed tomography (CT) of temporal bone scan, in the evaluation of the middle ear and inner ear anatomy available subjects.

2.3. High-throughput sequencing and bioinformatic analysis

DNA extraction and the common deafness gene hotspot mutation screening: follow the principle of informed consent, to extract the proband and family members of peripheral blood 1 mL peripheral blood. Using Beijing MyGenostics medical examination center to screen for common deafness genes. It covers 751 loci of 147 genes in deep intron region reported by HGMD and increases the probe coverage density of 29 genes (such as: PCDH15, COL4A6, FGF3, STRC, EYA1 and other gene encryption probe designs, among which GJB2, SLC26A4, POU3F4 gene full-length design), and detection of deafness related genes in mitochondrial circular DNA.

High-throughput sequencing and analysis of data: are propositus genealogy human all exons of high-throughput sequencing. After quality control analysis, the sequencing data were compared and filtered in ClinVar database according to ACMG guidelines, and known pathogenic loci and suspicious variants with population mutation frequency less than 0.005 were found. Bioinformatics analysis was performed using REVEL software to identify suspected pathogenic mutations.

The other family members were subjected to Sanger sequencing, and the parents of the family were verified by Sanger sequencing. The proband was screened for candidate pathogenic mutations by high-throughput sequencing, and the other members of each family were subjected to the same site detection to determine whether the pathogenic mutation was consistent with family separation. In addition, according to the American College of Medical Genetics and Genomics (ACMG) guidelines, the detected candidate pathogenic mutations were divided into five pathogenic grades, including pathogenic, suspected pathogenic, of undetermined significance, suspected benign and benign [4, 5].

3. Results

3.1. Clinical description

The proband is a boy, aged 1 month and 22 days, he had hearing impairment at birth. Tympanometry showed an A-shaped tympanogram, indicating normal tympanic function and the acoustic reflex could not be evoked in both ears (the acoustic reflex threshold was 100 dBHL); ABR prompted ears severe sensorineural deafness. Otoscopy and CT examination of temporal bone showed no obvious abnormality. His two elder sisters, an 11-year-old who is hearing impairment, pure tone hearing threshold determination shows profound sensorineural hearing loss. Otoscopy and CT examination of temporal bone showed no obvious abnormality. Both patients delivered at term after normal delivery, and neither parent had a history of persistent noise or ototoxic drug exposure or of severe infections during pregnancy. Clinical and equipment assessment found no evidence of syndrome characteristics, such as cardiovascular disease, diabetes, vision problems or neurological diseases. Both the brother and sister appeared to have normal intelligence. Neither of their parents nor the proband's other sister had hearing impairment (Table 1, Fig. 1).

3.2. Screening for common genetic screening and mitochondria

The following common deafness gene mutations were not detected in the proband and his family members: including the GJB2 gene (c.35delG, c.176_191del16, c.235delC, c.299-300delAT), SLC26A4 gene (c.919-2A>G, c.1174A>T, c.1226G>A, c.1229C>T, c.1707+5G>A, c.1975G>C, c.2027T>A, c.2168A>G), mitochondrial genes12S rRNA (m.1494C>T, m.1555A>G) and GJB3 gene (c.538C>T) (Table 2).

3.3. High throughput sequencing results

High-throughput sequencing of the proband revealed TMC1 gene c.741+3_741+6delAAGT (splicing) and c.884C>T (p.A295V) locus mutation, two loci are heterozygous mutation. Department members at home generation sequencing phenotype for hearing impairment sister also found in TMC1 genes in c.741+3_741+ 6delAAGT (splicing) and c.884C>T (p.A295V) was found in the sister with normal phenotype. Heterozygous mutation of T locus. The father carried c.741+3_741+6delAAGT mutation, while the mother did not. c.884C>T (p.A295V). His father had no mutation at T locus, but his mother had heterozygous mutation at T locus (Fig. 2, Tables 3, 4).

Combined with the mutation analysis results, protein alignment showed conservation of residues TMC1 across five species. SWISS-model software was used to predict

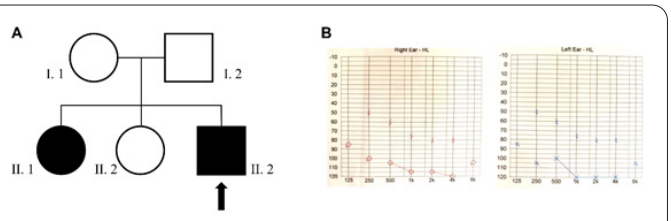

Fig. 1. Clinical description. (A) Pedigrees of the families with ARNSHL-affected subjects are denoted in black. Arrow indicates the proband. (B) Audiogram of affected subjects showed hearing loss ranged from severe to profound.

Table1. Clinical characteristics of patients.

	I.1	I.2	II.1	II.2	II.3
Current age	36	38	11	9	1month22day
Sex	F	M	F	F	M
Genotype	c.884C>T (p.A295V) heterozygote	c.741+3_741+6delAAGT (splicing) heterozygote	c.884C>T (p.A295V) heterozygote and c.741+3_741+6delAAGT (splicing) heterozygote	c.884C>T (p.A295V) heterozygote	c.884C>T (p.A295V) heterozygote and c.741+3_741+6delAAGT (splicing) heterozygote
Newborn screening	NA	NA heterozygote	Abnormal	NA	Abnormal
ABR (dB)	NA	NA	l.dx: no response/l.sin: 90 (4 kHz) and click 85	NA	No response
ASSR	NA	NA	--	NA	(right)80-80-80-80dB HL (left) 95-90-90-90dB HL
Age at diagnosis	--	--	From birth	--	From birth
Severity	--	--	Profound	--	Profound
Progression	--	--	No	--	No
Balance problems	No	No	No	No	No

Table 2. Overview of all TMC1 mutations identified to date.

Nucleotide position (cDNA)		Type of variant	Exon (E) /Intron (I)	Onset of HL	Severity of HL	Domain
-258A>C	–	Regulatory	E3	–	Severe to profound	–
-259C>T	–	Regulatory	E3	–	Severe to profound	–
16+1G>T	Splice disruption	Splicing	I5	Prelingual	Severe to profound	–
64+2T>A	Splice disruption	Splicing	I6	Congenital/Prelingual	Profound	–
100C>T	p.R34X	Nonsense	E7	Prelingual	Severe to profound	N-terminus
150delT	p.N50KfsX26	Frameshift	E7	Congenital	Profound	N-terminus
-195_16del	27 Kb deletion	Deletion	E5	Prelingual	Severe to profound	–
236+1G>A	p.E83X	Nonsense	I7	Congenital	Severe to profound	–
237-6T>G	Splice disruption	Splicing	I7	Prelingual	Severe to profound	–
256G>T	p.Glu86X	Nonsense	E8	Prelingual	Profound	N-terminus
295delA	p.K99KfsX4	Frameshift	E8	Prelingual	Severe to profound	N-terminus
362+18A>G	p.Glu122Tyrfs*10	Frameshift	I8	Congenital	Severe to profound	–
362+3A>G	Splice disruption	Splicing	E8	Prelingual	Severe to profound	–
453+2T>C	Splice disruption	Splicing	-	Prelingual	Severe to profound	–
530T>C	p.(lle177Thr)	Missense	E10	Prelingual	Profound	–
536-8T>A	Splice disruption	Splicing	I10	Prelingual	Severe to profound	–
536-8T>A	Splice disruption	Splicing	I10	Prelingual	Severe to profound	–
c.758C>T	p.Ser253Phe	Missense	E8	Prelingual	Severe	–
767delT	p.F255FfsX14	Frameshift	E13	Congenital	Severe to profound	T1-T2
776A>G	p.T259C	Missense	E13	Prelingual	Profound	T1-T2
776+1G>A	Splice disruption	Splicing	E13	Prelingual	Profound	–
797T>C	p.I266T	Missense	E13	Prelingual	Severe to profound	–
821C>T	p.P274L	Missense	E13	Prelingual	Profound	T2
830A>G	p.Y277C	Missense	E13	Prelingual	Severe to profound	T2
884+1G>A	Splice disruption	Splicing	E13	Prelingual	Severe to profound	–
1083_1087del	p.R362PfsX6	Frameshift	E15	Prelingual	Profound	T2-T3
1114G>A	p.V372M	Missense	E15	Prelingual	Severe to profound	T3
1165C>T	p.R389X	Nonsense	E15	Congenital	Profound	T3-T4
1166G>A	p.R389Q	Missense	E15	Congenital	Severe to profound	T3-T4
1209G>C	p.W403C	Missense	E15	Prelingual	Severe to profound	T3-T4
1253T>A	p.M418K	Missense	E16	Prelingual	Severe to profound	T4
1283C>A	p.Ala428Asp	Missense	E16	Prelingual	Severe to profound	T4
1330G>A	p.G444R	Missense	E16	Congenital/Prelingual	Profound	T4

1333C>T	p.R445C	Missense	E16	Congenital/Prelingual	Severe to profound	T4
1334G>A	p.R445H	Missense	E16	Prelingual	Profound	T4
c.1404+1G > T	Splice disruption	Splicing	E16	Prelingual	Moderate to severe	-
1534C>T	p.R512X	Nonsense	E17	Prelingual	Severe to profound	T4-T5
1541C>T	p.P514L	Missense	E17	Prelingual	Severe to profound	T4-T5
1543T>C	p.C515R	Missense	E17	Prelingual	Severe to profound	T4-T5
c.1566+1G>A	Splice disruption	Splicing	–	Prelingual	Severe to profound	–
1586_1587del	-	Frameshift	E18	–	Severe to profound	–
1589_1590del	p.S530X	Nonsense	E18	–	Profound	–
1703A>G	p.Y568C	Missense	E19	–	Profound	–
1714G>A	p.D572N	Missense	E19	Prelingual	Severe to profound	–
1763+3A>G	p.W588WfsX81	Frameshift	I19	Post-lingual	Profound	–
1764G>A	p.W588X	Nonsense	E20	Congenital	Profound	T4-T5
1810C>T	p.R604X	Nonsense	E20	Congenital	Severe to profound	T4-T5
1810C>G	p.R604G	Missense	E20	Prelingual	Severe	T4
1960A>G	p.M654V	Missense	E20	Prelingual	Severe to profound	T5
1979C>T	p.P660L	Missense	E20	Congenital	Profound	T5-T6
2004T>G	p.S668R	Missense	E21	Prelingual	Severe to profound	T5-T6
2030T>C	p.I677T	Missense	E21	Congenital/Prelingual	Profound	T5-T6
2035G>A	p.E679K	Missense	E21	Prelingual	Severe to profound	T5-T6
2260+2T>A	Splice disruption	Splicing	I23	Prelingual	Severe to profound	–
1696_2283del	Genomic deletion	Deletion	–	Congenital/Prelingual	Profound	–

Table 3. High-throughput sequencing and analysis results of the proband.

Gene	Nucleotide	Location of chromosomes	Exon of the transcript	Zygosity	Normal human frequency (PM2)	ACMG Analysis of pathogenicity	Disease/Phenotype (Mode of inheritance)	Sources of variation
TMC1	c.741+3_741+6delAAGT (splicing)	chr9:7536 9802-7536 9806	NM_138691;exon12	het	0.0001	Uncertain	Autosomal recessive hearing loss type 7 (AR)	Father
TMC1	c.884C>T (p.A295V)	chr9:7538 7471	NM_138691;exon13	het	0.0001087	Uncertain	Autosomal recessive hearing loss type 7 (AR)	Mother

Table 4. TMC1 c.884C>T variation Prediction information.

Nucleotide	Amino Acid	Prediction information				
		REVEL	SIFT	PolyPhen_2	MutationTaster	GERP+
c.884C>T	p.A295V	Benign	Damaging	Benign	Damaging	Damaging

the protein sequence, and the results showed that the alanine at position 295 was changed to valine, but the hydrogen bond was not changed. It is speculated that TMC1 cannot function normally due to local amino acid changes (Fig. 3, Fig. 4). The mutation at this site leads to a change in the amino acid encoded by this site, leading to a change in the protein encoded by TMC1, which is presumed to be harmful.

4. Discussion

Mutations in TMC1 have been found in deafness families in many countries around the world, and have been shown to be associated with ARNSHL [6, 7]. However, these mutations are rarely found in the Chinese population (Table 2). Cases of autosomal recessive characteristic clinical manifestations. Prelingual severe to profound deafness. TMC1 gene (MIM 606706) is located in the dyeing body 9 q21. 13 area, a total of 24 exons, can encode 760 amino acid composition of the membrane channel protein 1, is part of the inner ear hair cells mechanical transduction channel, also is one of the important participate in inner ear hair cells growth and form to maintain protein [8, 9]. Therefore, TMC1 allele mutation can lead to the loss of normal physiological function of TMC1 protein, and then lead to abnormal development of inner ear hair cells, abnormal morphology and mechanical transduction process, and finally loss of normal inner ear function,

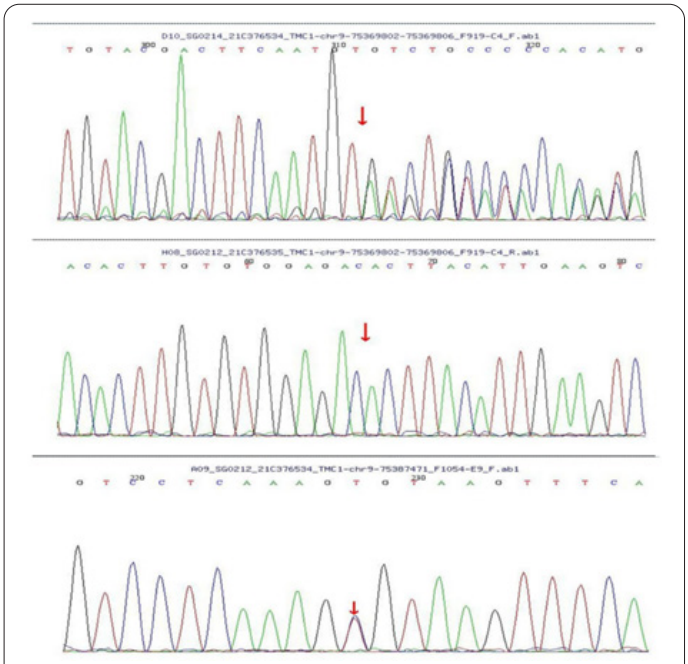

Fig. 2. Sanger sequencing analysis of TMC1 in family showing the compound heterozygous mutations (c.741+3_741+6delAAGT and c.884C>T) co-segregated with the phenotype.

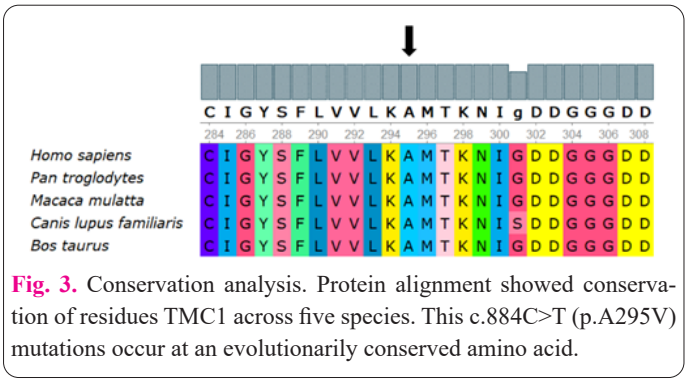

Fig. 3. Conservation analysis. Protein alignment showed conservation of residues TMC1 across five species. This c.884C>T (p.A295V) mutations occur at an evolutionarily conserved amino acid.

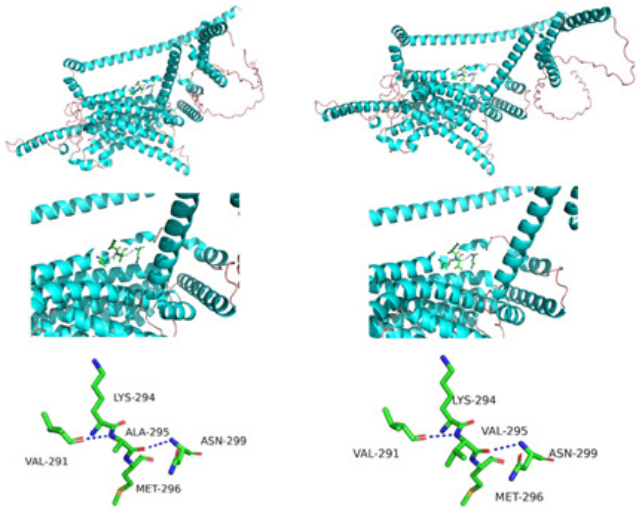

Fig. 4. Effects of mutations predicted by SWISS-model protein on TMC1 structure (c.884C>T). The change of alanine to valine at position 295 does not change the hydrogen bond (blue color). Protein 3D structure prediction map (local) (left wild type, right mutant).

and deafness [7]. Area TMC1 gene mutations have been reported in several countries, including in countries such as Iran, Turkey, Pakistan, and North America is relatively common. The two heterozygous mutations of TMC1 gene in the proband and the family members with hearing impairment involved in this study came from the father and mother with normal clinical phenotype, respectively, which was consistent with the deafness pattern of autosomal recessive inheritance of TMC1 gene.

This study found TMC1 gene c.741+3_741+ 6delAAGT and gene c.884C>T (p.A295V) were detected in the proband. Both of them were heterozygous mutations. Propositus after inductive sound nerve deafness, it has to do with literature reports TMC1 genetic mutations can lead to congenital or preverbal severely so severely deaf clinical phenotype. Combined with the ABR examination results, click stimuli presented at 100 dBnHL in both ears failed to induce well-differentiated wave profiles and regular latencies, suggesting that mechanosensory hair cells in the inner ear organ do not produce or send effective acoustic information to the brain. In his department two TMC1 alleles in patients with heterozygous mutations cause coding protein change, lead to TMC1 protein loss of normal physiological function, and then make the abnormal development of the inner ear hair cells, morphology and mechanical process exception can change, eventually lose normal function of the inner ear, thus appear deafness [10].

In this study, the probands and his two sisters have c.884C>T (p.A295V) heterozygous mutations inTMC1. This mutation by Sanger validation analysis confirmed no mutation was found in the father of the proband, while heterozygous mutation was found in the mother. According to the ACMG guidelines, the mutation was preliminarily determined to be of Uncertain clinical significance. PM2+BP4 showed that the frequency of PM2 in the normal population database was 0.0001087, which was a low-frequency variation. SIFT, MutationTaster and GERP+ protein function predicted that all mutations were harmful. The prediction result of BP4 bioinformatics protein function comprehensive prediction software REVEL

was benign. PolyPhen forecast analysis for benign. Literature database to retrieve the correlation of the site reported ClinVar database without the dot pathogenic analysis results.

The mutation of TMC1 site in the proband caused the change of the amino acid encoded by the site [11-14]. Transmembrane transporters are necessary for mammalian hair cells to convert mechanical stimuli into electrical signals and are involved in auditory transmission guided by inner ear hair cells. Studies on the mouse model of TMC1 have shown that TMC1 may be involved in the most basic auditory process of hair cell transduction, mainly affecting the permeability of cell membrane and regulating intracellular signals, and is essential for hair cell maturation. The mutation of TMC1 changes the encoded protein, which causes K+ reflux disorder, affects the swallowing function of inner hair cells, and eventually leads to auditory system dysfunction, which is characterized by sensorineural hearing loss [15-17].

Family first disease and phenotype of hearing impairment is elder sister, there is also another hybrid mutations for TMC1 genes in c.741+3+6delaagt_741. This site caused a frameshift mutation due to base deletion. Bioinformatics analysis using PolyPhen-2 and SIFT software showed that the mutation was a suspected pathogenic site (PM2). Based on ACMG guidelines, the variant c.741+3_741+6delAAGT (splicing) was considered to be of Uncertain clinical significance. PM2: The frequency of PM2 in the normal population database was 0.0001, which was a low-frequency variation. No association of this locus was reported in the literature database and no pathogenicity analysis was performed in the ClinVar database. By Sanger validation analysis tips, the father of the proband carried heterozygous mutation at this site, while the mother had no mutation.

According to the phenotypic characteristics of the family, two members of the family with hearing impairment had two TCM1 gene mutations, c.741+3_741+6delAAGT and c.884C>T, while the other one with normal phenotype only had a heterozygous mutation of c.741+3_741+6delAAGT mutation was not found. At the same time, the parents and grandparents of the family were normal phenotypically. Combined with the characteristics of autosomal recessive inheritance, the mutation of two loci of TMC1 at the same time caused the change of protein structure, which caused the disease of the family members and congenital severe to profound hearing impairment. The locus c.800G>A between the two loci is a known pathogenic variant reported in India [18].

5. Conclusion

Based on all of the above results and the clinical features of the family, we conclude that c.741+6+3_741 delegate and c.884C>T, double variant of interaction may be the cause of the hearing loss of the family. This is only a new suspected therapeutic mutation reported in the Chinese population. Further confirmation of this mutation as a pathogenic mutation will contribute to the clinical understanding of HL caused by this gene mutation.

Conflict of Interests

The authors declare no competing interests.

Consent for publications

The author read and approved the final manuscript for publication.

Ethics approval and consent to participate

We have received approval from the Ethics Committee of The Second Hospital of Hebei Medical University.

Informed Consent

We have received informed consent from the Ethics Committee of The Second Hospital of Hebei Medical University.

Availability of data and material

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors' contributions

ZQ contributed to the study conception and design. Examination, data collection and analysis were performed by ZL, WY and SP. The first draft of the manuscript was written by ZL and all authors commented on previous versions of the manuscript.

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