

ORIGINAL ARTICLE

Report of rare and novel mutations in candidate genes in a cohort of hearing-impaired patients

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Abstract

Background: Many hearing-impaired patients carry mutations in rare or novel genes undetected in regular genetic hot regions/genes screening.

Methods: We collected clinical and genetic data from subjects with hearing loss who visited our department for genetic counseling. Next-generation sequencing was conducted after 154 deafness-related genes were captured using a designed genes panels in 14 unrelated families (37 participants). The results were filtered and assessed with *in silico* tools, in combination with pedigree mapping.

Results: Ten mutations in regular deafness genes (*GJB2*, *SLC26A4*) and uncommon genes (*OTOF*, *MYO7A*, *MYO15A*, and *KARS*) were detected, which constituted 57.2% of yielded rate. In particular, two patients with nonsyndromic deafness carried biallelic *KARS* mutations. In addition, we identified an unreported digenic mutational inheritance in *GRP98/USH2A* genes in a proband with isolated hearing loss. Functional analyses and molecular modeling suggested the damaging consequence of these variants on encoded proteins. According to the variant pathogenicity guidelines, the 17 identified variants in total were classified as “pathogenic” or “likely pathogenic.”

Conclusion: The candidate mutations in deafness genes were suggested to be co-segregated in at least 57.2% of the studied pedigrees. This is the new report of rare/novel mutations causing inherited hearing loss in Chinese.

KEYWORDS

hearing loss, mutations, sensorineural hearing loss

1 | INTRODUCTION

A recent global estimation that hearing loss will affect 2.45 billion people by 2050 (Haile et al., 2021) raises serious concern of an early prevention and appropriate treatment regarding etiologies, in which genetics accounted for 50% of hearing loss (Morton & Nance, 2006). Regional and

ethnic differences between populations exist. Although great effort has been made toward clarifying hereditary origins during the past decades, the genetic and phenotypic heterogeneity of this disease remain to be studied.

A large proportion of hearing-impaired patients carry mutations in rare or novel genes undetected in regular genetic screening (Chen et al., 2014; Doll et al., 2020;

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Yang et al., 2013). Routine Sanger sequencing failed to address this issue due to the enormous DNA sequences in numerous genes. With the advance of genomic capturing and next-generation sequencing (NGS) in many less frequent deafness genes, it is feasible and valuable for public health in identifying genetic etiology for hearing loss.

Our earlier cohort study conferred a genetic etiology in only one-third of patients using Sanger sequencing and pyrosequencing (Chen et al., 2014). Here, we extend this project by adapting an alternative strategy using NGS of captured exons in 154 hearing-related genes in another cohort of Chinese families. Our present results demonstrated that genetic causes clearly contributed to at least 57.2% of the families in this cohort. The increased yielded rate and distinct mutational spectrums due to detection of novel or rare mutations were interesting. This is the new source of data regarding uncommon genetic mutations causing hearing loss in Chinese patients.

2 | MATERIALS AND METHODS

2.1 | Patients

All patients were enrolled from our outpatient department. Family history and medical records were carefully reviewed from all subjects and/or their parents. After signing informed consents for participation, 5 ml of peripheral blood samples from the probands and related family members was collected and subjected to a large-scale targeted deafness-related genes panel (including 154 genes) for NGS.

2.2 | Clinical evaluations

All the participants were completely evaluated by at least one senior otorhinolaryngologist. Potential predisposing events related to hearing loss, such as otitis media with effusion, newborn hyperbilirubinemia, hypoxia, and aminoglycoside use, were precluded. Age-appropriate hearing tests, including pure tone audiometry (PTA), distortion product otoacoustic emission (DPOAE), and auditory brainstem response (ABR), were given. The severity of hearing loss was assorted according to the average estimated PTA as follows: mild (20–40 dB), moderate (41–70 dB), severe (71–95 dB), and profound (>95 dB). Temporal radiological tests help to identify any possible developmental malformation.

2.3 | Targeted deafness genes panel and NGS

We adopt a new designed commercial deafness-related genes panel xGen (Integrated DNA Technologies). Briefly, genomic DNA was extracted from peripheral blood samples (QIAamp DNA Blood Mini Kit), followed by the preparation of DNA library as the procedure. The regions of interest covering exons, exon-intron boundaries in 154 genes was probed and sent for NGS. The raw data were aligned to hg19 with Burrows–Wheeler Aligner (BWA) and Genome Analysis Toolkit (GATK) for variant calling. The variant call format files were analyzed using the ANNOVAR tools containing annotation databases (e.g., the 1000 genome, dbSNP, ClinVar database). Pedigree mapping of the suspected variants was amplified via PCR and sequenced with Sanger sequencing in the probands and their family members.

2.4 | Genetic data interpretation and analysis

The nonsynonymous variants and indels were filtrated based on the criteria: >0.05 frequency in 1000 Genome, dbSNP, and Hapmap and >0.3 in YH database. We used *PolyPhen2*, *SIFT*, *MutationTaster*, *MutationAssessor*, and *FATHMM* to evaluate possible pathogenicity. Conservation alignments among different species were completed with *ClustalX2*. The amino acid sequences are available on NCBI. Genetic interaction was predicted through the STRING network. To evaluate the effect of variant in the splice site and the recognition of donor and acceptor sites, NetGene2 sever was applied. The online HOPE protein prediction tool was utilized to assess the potential effect of the variants on secondary structure. Three-dimensional modeling of human wild-type KARS, MYOVIIA, MYO15A, and the corresponding mutants were performed using SWISS-MODEL (Waterhouse et al., 2018). Finally, all variants were classified according to the American College of Medical Genetics and Genomics/Association for Molecular Pathology (ACMG/AMP) guidelines (Oza et al., 2018; Richards et al., 2015).

3 | RESULTS

This cohort consisted of 14 unrelated Chinese families (37 participants), with 18 hearing-impaired patients and 19 families' members. Detailed information has been listed in Table 1. Briefly, the average age of the patients was

11.7 ± 13.6 years. (1–53 years). Familial history was present in four families, and the rest 10 were sporadic. The hearing tests revealed bilaterally moderate to profound hearing loss. Other possible syndromic symptom was tentatively not found, except one patient in family 11, who presented with delayed gross movement development. The phenotype was classified as nonsyndromic hearing loss in most cases according to the medical record.

The NGS deafness panels yielded at least 17 variants in 12 candidate genes (Table 1). Mutations in common genes *GJB2* (NM_004004.5) and *SLC26A4* (NM_000441.1) (*GJB2* c.235delC, c.299-300delAT, *SLC26A4* c.290T>A, and c.919-2A>G) were detected in three families with five hearing loss subjects, contributing to 21.4% (3/14) of this cohort. Two probands carried homozygous mutation *OTOF* (NM_194248.2) c.5098G>C (14.3%, 2/14), and manifested as auditory neuropathy. Interestingly, we detected a compound heterozygous mutation in a rare gene *KARS* (NM_001130089.2) in family #5, in which the two probands showed single hearing loss without syndromic symptoms. Hearing loss was also resulted from biallelic pathogenic mutations in *MYO7A* (NM_000260.3) and *MYO15A* (NM_016239.3) genes in two families (#2 and #8). In a particular family #9, digenic mutations in *GPR98* (NM_032119)/*USH2A* (NM_206933.2) genes (*GPR98* c.6317C>T/c.10790A>T, *USH2A* c.997T>C) were suspected. In addition, two families (#10 and #11) carried variants unexplainable for the hearing loss in rare genes, for example, *ESPN* (NM_031475.2), *NDUFS3* (NM_004551.2), *NF1* (NM_000267.3), and *TSHZ1* (NM_005786.5). In the rest three cases (#12–#14), NGS failed to identify any suspected variants.

Bioinformatic analysis of the variants are presented in Table 2. Five uncommon and novel mutations identified in *KARS*, *MYO7A*, and *MYO15A* genes are highly conserved among species (Table 2 and Figures 1 and 2). They were predicted to be pathogenic/likely pathogenic through the following mechanisms: (a) To disrupt the mRNA splice site within the exon-intron boundary, leading to mRNA mis-splicing (*MYO7A* c.1003+1G>A). (b) Mutation changes the amino acid sequences (*KARS* c.1212_1220del). (c) Secondary structure alterations were in protein level to disturb the domain and abolish its function (*KARS* c.1570T>A, *MYO15A* c.3926A>T, and *MYO7A* c.472G>C).

In particular, we found a possible digenic inheritance between *GPR98* and *USH2A* genes in family #9. Parental DNA sequences analysis suggested the *in cis* mutations c.6317C>T/c.10790A>T in *GPR98* gene. The pathogenicity of the three variants in this family was also suggested. Detailed clinical and genetic data are shown in Figure 3 and Table 2.

4 | DISCUSSION

In this study, using a large-scale targeted hearing-related genes panel and NGS platform, several candidate mutations were effectively validated in a cohort of families with deafness phenotype. Our results revealed that genetic etiology contributed to at least 57.2% of the studied families, an increased rate higher than the earlier result (32%) (Chen et al., 2014). This new report not only stretched the genetic spectrums in Chinese but also paved way for our further genetic scheme in many other regions and populations.

Coexistence of syndromic and nonsyndromic hearing loss caused by the same gene is not uncommon. For example, *MYO7A* causes either NSHI or Usher syndrome (USH). Early-onset hearing loss and leukoencephalopathy was reported in a patient carrying p.R477H and p.P505S in DNFB89 gene *KARS* (Zhou et al., 2017). We could not ascertain other syndromic symptoms in our cohort at the latest follow-up (e.g., #2, #5, and #9). Possible explanations were as followed. First, the phenotypic manifestation mostly depends on the intrinsic pathogenesis of gene and is largely affected by many miscellaneous factors, for example, ethnic background difference, environment. Second, late onset of syndromic symptoms other than deafness are common (Zong et al., 2016) and the possibility of syndromic hearing loss in this cohort could not be ruled out.

OTOF gene is frequently detected in auditory neuropathy, and otoferlin is mainly expressed under inner hair cells to modulate ribbon synapses function (Yasunaga et al., 2000). Some common alleles in *OTOF* with founder effect were revealed. For example, p.Q829X accounted for 4.4% of hearing loss in Spain (Migliosi et al., 2002), while the Japanese carried a common variant p.R1939Q (56.5%) (Matsunaga et al., 2012). Similar to our findings, the c.5098G>C was mainly shared by Chinese (Chiu et al., 2010). Considering its prevalence and founder effect, the incorporation of c.5098G>C into general genetic screening may gain some benefit in Chinese patients with auditory neuropathy in the future.

The novel variant *MYO7A* c.1003+1G>A is within the intronic region predicted to result in mRNA mis-splicing, a process occurred in many known *MOY7A* mutations (e.g., c.470G>A, c.1342_1343delAG, c.5856G>A, and c.3652G>A) (Aparisi et al., 2013). Another residue p.G158W would force the local backbone into an incorrect conformation and break the unique torsion angle at protein level. Combined with their high conservation and parental analysis, both variants *MOY7A* c.1003+1G>A/c.474G>C were concluded to co-segregate with the phenotype of sensorineural hearing loss. Mutations in *MYO7A* cause different phenotypes from isolated hearing loss to

TABLE 1 Clinical data of studied patients

Family	Gender	Age (years)	Reported onset of deafness	Hearing loss	Inner ear morphology	Genetic results	Intervention
1	Male	3	3 years	Profound	Normal	OTOF c.5098G>C/c.5098G>C	CI
2	Female	2	At birth	Profound	Normal	MYO7A c.472G>C/c.1003+1G>A	CI
3	Male	1	At birth	Profound	EVA	SLC26A4 c.290T>A/c.919-2A>G	CI
4-1	Male	15	At birth	Profound	Normal	GJB2 c.235delC/c.299-300delAT	CI
4-2	Male	13	At birth	Profound	Normal	GJB2 c.235delC/c.299-300delAT	CI
5-1	Male	17	2 years	Profound	Normal	KARS c.1570T>C/c.1212_1220del	CI
5-2	Male	15	2 years	Profound	Normal	KARS c.1570T>C/c.1212_1220del	CI
6-1	Female	11	At birth	Profound	EVA	SLC26A4 c.919-2A>G/c.919-2A>G	CI
6-2	Female	8	At birth	Profound	EVA	SLC26A4 c.919-2A>G/c.919-2A>G	CI
7	Female	3	2 years	Profound	Normal	OTOF c.5098G>C/c.5098G>C	CI
8-1	Male	5	4 years	Profound	Normal	MYO15A c.3926A>T/c.3926A>T	CI
8-2	Female	3	1 year	Profound	Normal	MYO15A c.3926A>T/c.3926A>T	CI
9	Male	5	4 years	Severe	Normal	GPR98 c.6317C>T/c.10790A>T, USH2A c.997T>C	CI
10	Female	53	Unknown	Severe	NA	EPSN c.990+4C>T/+	CI
11	Male	3	3 years	Moderate	Normal	NF1 c.4084C>T/+ , NDUFS3 c.746dupC/+ , TSHZ1 c2394G>A/+	CI
12	Female	2	1 year	Profound	Normal	—	CI
13	Male	38	Unknown	Anacusis (R)/Moderate (L)	Normal	—	CI
14	Male	13	1 year	Profound	Normal	—	CI

Note: GJB2 (NM_004004.5), ESPN (NM_031475.2), GPR98 (NM_032119), KARS (NM_001130089.2), MYO7A (NM_000260.3), MYO15A (NM_016239.3), NDUFS3 (NM_004551.2), NF1 (NM_000267.3), OTOF (NM_194248.2), SLC26A4 (NM_000441.1), TSHZ1 (NM_005786.5) and USH2A (NM_206933.2).

Abbreviations: CI, cochlear implantation; EVA, enlarged vestibular aqueduct; L, left; NA, not available; R, right.

TABLE 2 Bioinformatics data of the suspected variants identified in the probands

Gene	Mutation	AA change	Mutationtaster	Polyphen-2	SIFT	MutationAssessor	FATHMM	NetGene2	ACMG/AMP classification	Ref
<i>OTOF</i>	c.5098G>C	p.E1700Q	Disease causing	Probably damaging	Deleterious	Medium	Damaging	—	Likely Pathogenic	Rs199766465
<i>MYO7A</i>	c.472G>C	p.G158W	Disease causing	Probably damaging	Deleterious	High	Damaging	—	Pathogenic	Novel
	c.1003+1G>A	Splice site change	—	—	—	—	—	missplicing	Likely Pathogenic	Novel
<i>KARS</i>	c.1212_1220del	p.D404-P406del	Disease causing	—	Deleterious	—	—	missplicing	Pathogenic	Novel
	c.1570T>C	p.C524W	Disease causing	Probably damaging	Deleterious	High	Tolerated	—	Likely Pathogenic	Rs776736207
<i>MYO15A</i>	c.3926A>T	p.Q1309L	Disease causing	Probably damaging	Deleterious	High	Damaging	—	Pathogenic	Novel
<i>GPR98</i>	c.6317C>T	p.A2106V	Disease causing	Probably damaging	Neutral	Medium	Tolerated	—	Likely Pathogenic	Rs186999408
	c.10790A>T	p.E3597V	Disease causing	Benign	Neutral	Medium	Tolerated	—	Likely Pathogenic	Novel
<i>USH2A</i>	c.997T>C	p.S333P	Polymorphism	Possible damaging	Neutral	Medium	Tolerated	—	Likely Pathogenic	Xu et al. (2014)

Note: *GPR98* (NM_032119), *KARS* (NM_001130089.2), *MYO7A* (NM_000260.3), *MYO15A* (NM_016239.3), *OTOF* (NM_194248.2), *USH2A* (NM_206933.2). ACMG/AMP: American College of Medical Genetics and Genomics/Association for Molecular Pathology.

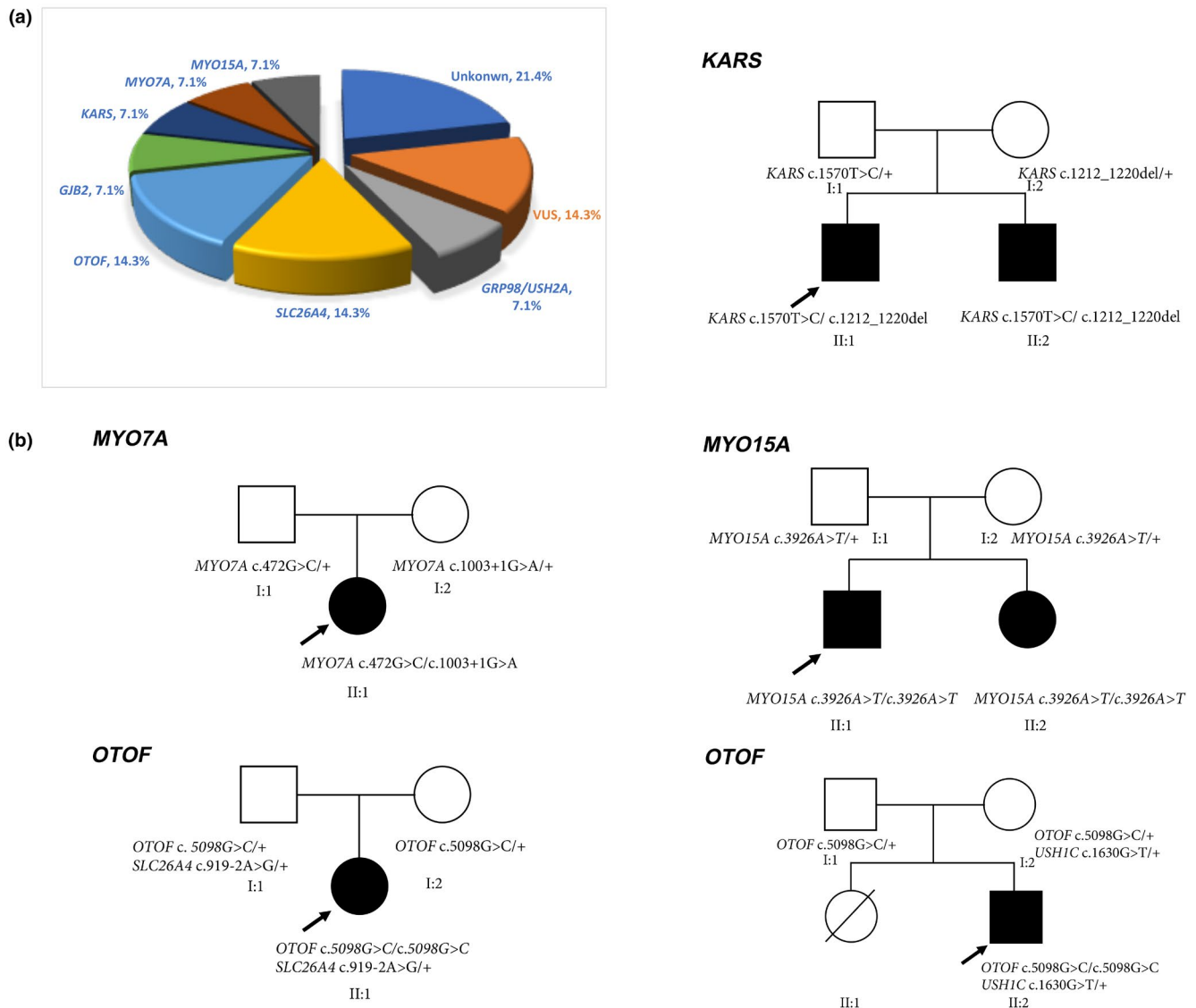


FIGURE 1 (a) The mutational spectrum yielded by the deafness panel. (b) Five pedigrees with clear genetic etiology are presented. +, Wild-type nucleotide; VUS, variant with unknown significance. *GJB2* (NM_004004.5), *GPR98* (NM_032119), *KARS* (NM_001130089.1), *MYO7A* (NM_000260.3), *MYO15A* (NM_016239.3), *OTOF* (NM_194248.2), *SLC26A4* (NM_000441.1), and *USH2A* (NM_206933.2)

USH. There is conflicting evidence of whether recessive inherited hearing loss is an isolated phenotype or part of the USH in *MYO7A* carriers. Delay-onset of visual lesions indicative of USH in nonsyndromic hearing loss frequently occurs (Zina et al., 2001; Zong et al., 2016). Thus, the ophthalmic symptoms in II:1 in family #2 should be closely followed up.

KARS gene encodes a lysyl-transfer ribonucleic acid synthetase to catalyze the aminoacylation of tRNA-Lys in the cytoplasm and mitochondria (Tolkunova et al., 2000). *KARS* protein locates in the spiral ligament, Deiters' cells, the sulcus epithelium, the basilar membrane, and spiral limbus in inner ear (Santos-Cortez et al., 2013). The associated clinical phenotype is heterogeneous. Mutation in *KARS* gene associated with only hearing loss is extremely rare (Santos-Cortez

et al., 2013). Additional syndromic symptoms varied from peripheral nerve disorder disease (McLaughlin et al., 2010), leukoencephalopathy (Zhou et al., 2017), and progressive microcephaly to cognitive impairment (McMillan et al., 2015). The *KARS* mutation c.1212_1220del/c.1570T>C found in this study was predicted to be associated with nonsyndromic hearing loss in family #5. Both patients were finally subjected to cochlear implantation. Other symptoms except hearing loss was absent during peri-implantation regular examinations and at the latest follow-up according to the medical record.

The majority of *MYO15A* variants have been related to congenital or postlingual deafness (Zhang et al., 2019). They are frequently found in consanguineous families in Mideast countries (Doll et al., 2020; Noman et al., 2019;

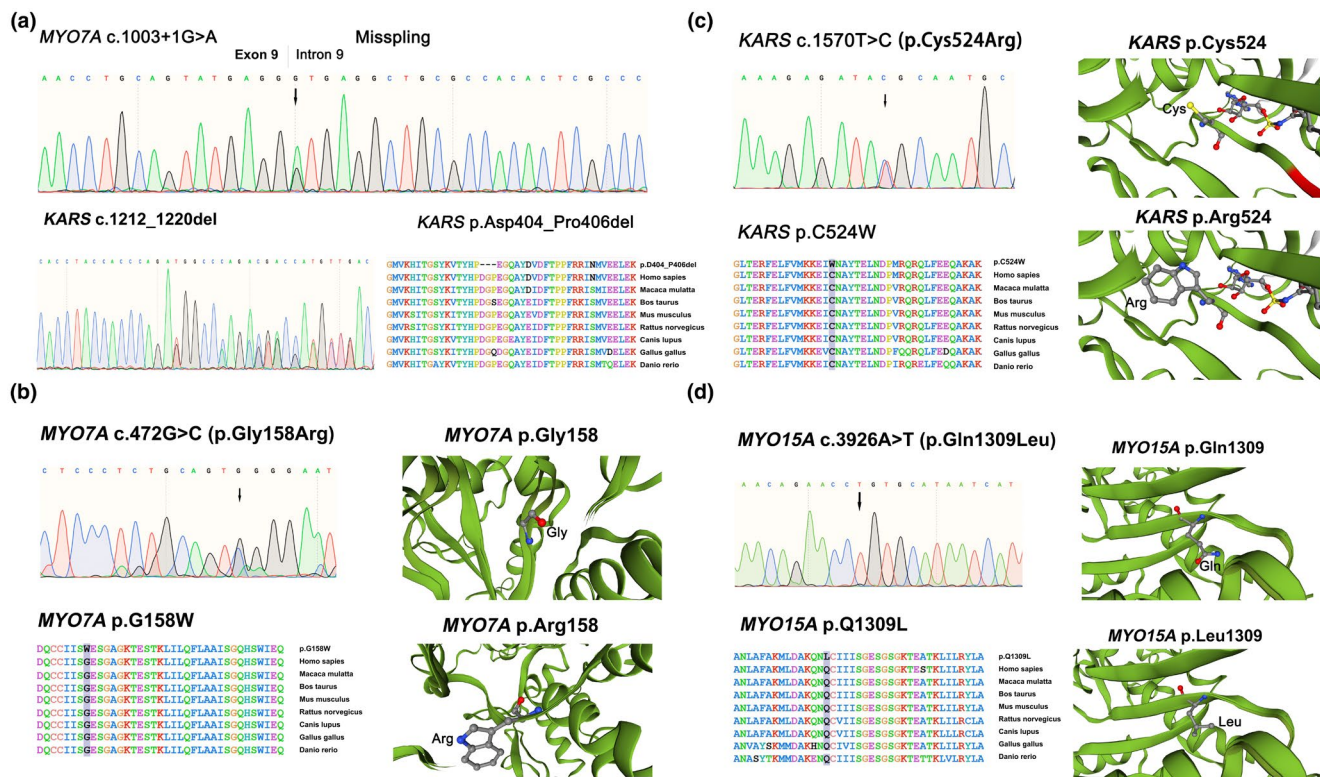


FIGURE 2 The sequences of novel and rare mutations identified in this study are shown. (a). The *MYO7A* c.1003+1G>A disrupts the splice site (GT), and mRNA mis-splicing occurs. The *KARS* frameshift mutations c.1212_1220del caused the alteration of amino acids. (b–d) The DNA sequences, conservation analysis and predicted protein structures of *MYO7A*, *KARS* and *MYO15A*. *KARS* (NM_001130089.2), *MYO7A* (NM_000260.3), *MYO15A* (NM_016239.3)

Shafique et al., 2014). In contrary, genetic impact of *MYO15A* was less reported in China (Wang et al., 2020; Zhang et al., 2019), where nonconsanguineous marriage is predominant. The frequency and spectrum of *MYO15A* mutations in Chinese are largely unknown. The homozygous mutation *MYO15A* c.3926A>T in family #8 adds to the current data. The heterozygous state in the unconsanguineous parents suggested that the *MYO15A* mutation may not be uncommon in Chinese. The genetic load of *MYO15A* in East Asian populations is debated. For example, a relatively low frequency of 0.89% (10/1120) was reported in a study of Japanese deafness cohort (Miyagawa et al., 2015). In another study, 4% (5/125) was found in a Chinese ethnic population (Yang et al., 2013).

In this study, we detected a possible digenic inheritance in two USH-related genes, *GPR98* and *USH2A*. The co-existence of copy number variants or deletions *in trans* with the monoallelic variant in *USH2A* had been ruled out by the deafness genes panel in this study. Pedigree mapping revealed in *cis* configuration of alleles in *GPR98*. The mutants in *GPR98* gene were located *in trans* with a single mutant allele in *USH2A* gene. The mutated residue *GPR98* p.2106V is inferred to disturb the functional domain that is important for the main activity of the protein. Moreover, the replacement of a more hydrophobic residue

by p.E3597V caused the loss of hydrogen bonds and incorrect protein folding (Venselaar et al., 2010). The mutation *USH2A* c.997T>C was previously detected in a patient with retinitis pigmentosa (Xu et al., 2014). Direct interactions between the two genes *USH2A/GPR98* support their involvement in a molecular complex associated with the ankle links in cochlear hair cells (Michalski et al., 2007). The co-expression and protein homology between the two genes is also suggested according to STRING interaction network (Figure 3). Research about the interactive effect between these two genes will be significant.

USH2A, *GPR98*, and *WHRN* genes encode a cell adhesion protein, a G-protein-coupled receptor and a scaffold protein, respectively. The three types of proteins form a protein complex which is expressed in photoreceptors (Yang et al., 2010) and cochlear hair cells (Michalski et al., 2007; Zou et al., 2014). *USH2A*, *GPR98*, and *PDZD7* may interact with each other to function in the ankle links (Zou et al., 2014). Loss of any one of these proteins will reduce expression of the other two proteins (Yang et al., 2010). These findings support the possibility of digenic inheritance of *GPR98/USH2A* in family #9. The development of nonsyndromic hearing loss to USH is not a rare phenomenon, and retinitis pigmentosa usually occurs post

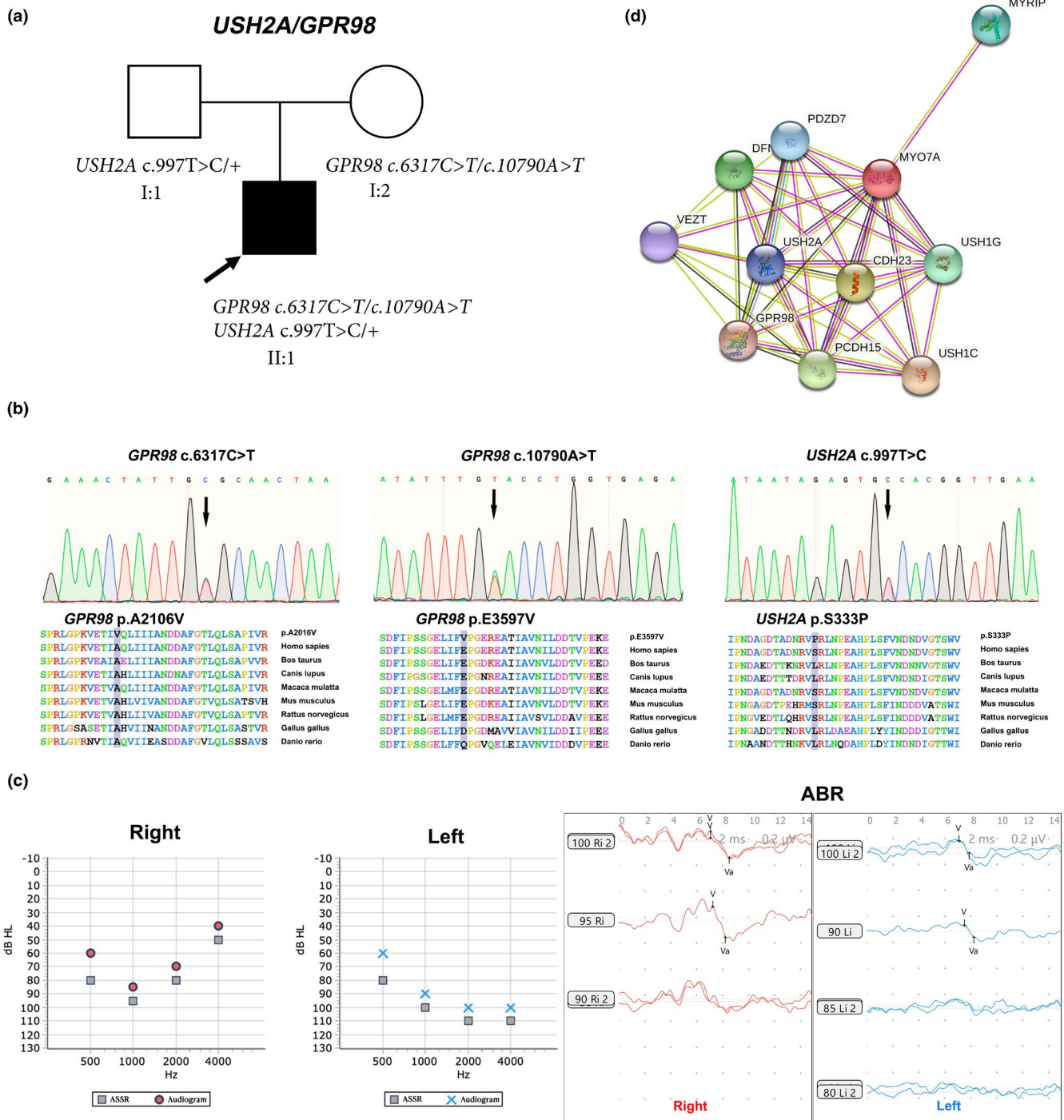


FIGURE 3 A pedigree #9 with suspected double genes inheritance *GPR98/USH2A* is shown (a). The mutated residue is highly conserved (b). (c) Profound hearing loss was detected in this 5-year-old proband, without any detectable ophthalmic symptoms at the latest follow-up. (d) The co-expression between *GPR98* and *USH2A* were predicted by STRING network. *GPR98* (NM_032119), *USH2A* (NM_206933.2)

puberty. Thus, it was reasonable that this proband was only hearing affected. Similarly, Kim et al. (2015) reported digenic mutants in two *USH* genes (*GPR98/PDZD7*) in a 26-month-old child, who showed isolated mild to moderate sensorineural hearing loss. Further investigation of the

underlying mechanism of double *USH* gene mutations in hearing in these cases is advised.

One major limitation of us is the small sample size of this cohort, and our results did not represent a regional mutation spectrum. Moreover, the cellular or animal

study not being conducted here dispute the pathogenicity of identified alleles in this study.

5 | CONCLUSION

The candidate mutations in deafness genes were suggested to be co-segregated in at least 57.2% of the studied pedigrees. This is the new report of rare/novel mutations causing inherited hearing loss in Chinese.

ETHICAL COMPLIANCE

This study was in accordance with the Declaration of Helsinki and approved by the Institutional Review Board at the First Affiliated Hospital, Sun Yat-sen University.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

AUTHOR'S CONTRIBUTIONS

M.L. and Y.L. collected clinical data, participated in genetic studies, data analysis, and drafted the manuscript. B.H. and J.S. collected clinical data and participated in the sequence alignment. K.C. conceived of the study, participated in its design, and coordination and manuscript review. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

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

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