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Targeted next-generation sequencing of deaf patients from Southwestern China

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Abstract

Background: Targeted next-generation sequencing is an efficient tool to identify pathogenic mutations of hereditary deafness. The molecular pathology of deaf patients in southwestern China is not fully understood.

Methods: In this study, targeted next-generation sequencing of 127 deafness genes was performed on 84 deaf patients. They were not caused by common mutations of GJB2 gene, including c.35delG, c.109 G>A, c.167delT, c.176_191del16, c.235delC and c.299_300delAT.

Results: In the cohorts of 84 deaf patients, we did not find any candidate pathogenic variants in 14 deaf patients (16.7%, 14/84). In other 70 deaf patients (83.3%, 70/84), candidate pathogenic variants were identified in 34 genes. Of these 70 deaf patients, the percentage of "Solved" and "Unsolved" patients was 51.43% (36/70) and 48.57% (34/70), respectively. The most common causative genes were SLC26A4 (12.9%, 9/70), MT-RNR1 (11.4%, 8/70), and MYO7A (2.9%, 2/70) in deaf patients. In "Unsolved" patients, possible pathogenic variants were most found in SLC26A4 (8.9%, 3/34), MYO7A (5.9%, 2/34), OTOF (5.9%, 2/34), and PDZD7 (5.9%, 2/34) genes. Interesting, several novel recessive pathogenic variants were identified, like SLC26A4 c.290T>G, SLC26A4 c.599A>G, PDZD7c.490 C>T, etc.

Conclusion: In addition to common deafness genes, like GJB2, SLC26A4, and MT-RNR1 genes, other deafness genes (MYO7A, OTOF, PDZD7, etc.) were identified in deaf patients from southwestern China. Therefore, the spectrum of deafness genes in this area should be further studied.

KEYWORDS
hereditary deafness, mutations, targeted next-generation sequencing

1 | INTRODUCTION

Deafness (or hearing loss) is a disease characterized by partial or total inability to hear. It can be inherited (Duman & Tekin, 2012; Meena & Ayub, 2017; da Silva & Duarte, 1995). Most of the heritable deafness is inherited by an autosomal recessive pattern. Autosomal dominant, X-linked, mitochondrial, and digenic inheritance were also observed in heritable deafness. More than 100 genes have been identified in heritable deafness. Targeted next-generation sequencing

enabled us to efficiently screen mutations in several deafness genes compared to PCR-based Sanger sequencing (Lin et al., 2012; Yang et al., 2013). It evolutionarily improves the clinical diagnosis rate of heritable deafness (Shearer & Smith, 2012; Vona et al., 2014). Several studies showed that it is an efficient tool to identify causative genes in deaf patients (Miyagawa et al., 2013, 2015). Different cultures, ethnicities, and living environments lead to various genetic backgrounds in the world (Donovan, 1984). Yunnan province, a region in southwestern China, has a higher attitude compared to other plain areas of China. The populations in this region might have their unique features, and the molecular epidemiology of deaf patients here is still unclear now.

In the present study, a panel containing 127 deafness genes was generated. These genes were associated with non-syndromic, syndromic, and mitochondrial deafness. Eighty-four deaf patients were subjected to 127 deafness genes sequencing. We aimed to preliminarily analyze the molecular epidemiology of deaf patients from southwestern China.

2 | MATERIALS AND METHODS

2.1 | Statement of ethics, patients, and DNA preparation

Peripheral blood samples of deaf patients were collected in First People's Hospital of Yunnan Province from 2016 to 2019. The study was approved by the Ethics Committee of First People's Hospital of Yunnan Province (Affiliated Hospital of Kunming University of Science and Technology). Each patient in this study signed informed consent. By E.Z.N.A.® Blood DNA Kit (cat. no. D3392-02; Omega Biotek, Inc.), genomic DNA was extracted from the peripheral blood leukocytes.

2.2 | Targeted sequence capture and high-throughput sequencing

One hundred and twenty-seven deafness genes (Table S1) were sequenced and captured in this study. The experimental procedures were referred to our previously published article (Li et al., 2016; Li et al., 2019). Briefly, probe capture panels (Roche NimbleGen Inc.) were designed for 127 deafness genes, and total size for targeted regions (exons, splicing sites, and immediate flanking intron sequences of 100 bp) was 619167 bp. DNA was fragmented, and the fragmented DNA was end-repaired and ligated to the adapter oligonucleotides. After that, PCR was used to make a library preenrichment amplification. The qualified libraries were used for the capture of fragments of 127 genes. The capture of 127 genes was performed according to the NimbleGen capture

protocol. The captured fragments were further sequenced by HiSeq 2500 Analyzers (Illumina).

2.3 | Data filtering, read mapping, variant detection, and analysis pipeline

Data filtering, read mapping, and variant detection were based on our previous descriptions (Li et al., 2016). Briefly, following the Illumina Pipeline (version 1.3.4), primary data were achieved after image analyses, error estimation, and base calling. Data were further filtered, and clean reads were kept. Clean reads were aligned to the UCSC hg19 reference genome by the BWA (Burrows-Wheeler Aligner) Multi-Vision software package. SOAPsnp software (Li et al., 2009) and GATK Indel Genotyper (http://www.broadinstitute.org/gsa/wiki/index.php/, The Genome Analysis Toolkit) were used to detect SNPs (single-nucleotide polymorphisms) and indels (insertion-deletions).

Missense, indel, nonsense and splice-site variants in each patient were selected out and screened for quality. The frequency of these variants was further referred to 1000 Genomes public variant databases, dbSNP, and ExAC databases. The known disease-causing mutations were determined by the HGMD database (Stenson et al., 2014).

2.4 | Molecular modeling

Amino acids sequences of candidate causative genes were referred to the GenBank database. 3D protein structure of candidate pathogenic genes was modeled by RaptorX web server (Wang et al., 2016). PyMOL was used to view 3D structure of proteins which was constructed by RaptorX. Structure of mutated and wild-type protein was compared.

3 | RESULTS

Total of 84 deaf patients participated in this study, including 70 simplex probands and 14 multiplex probands. They are clinically diagnosed with deafness. Environmental causes were excluded in these patients. Most of them were congenital profound deafness (>95 dB). Of these patients, two patients were diagnosed with Charge syndrome and Usher syndrome 1B, respectively.

Patients participated in this study were previously screened by common mutations in GJB2 gene, including c.35delG, c.109 G>A, c.167delT, c.176_191del16, c.235delC and c.299_300delAT. Only the negative patients of GJB2 common mutations were subjected to 127 deafness genes sequencing. The lists of genes are shown in Table S1. The targeted region was 619167 bp. Sequencing data showed

high quality. The average sequencing depth was approximately 200 X. Several variants were detected in each patient, including SNPs and indels. In further data analysis, variants with minor allele frequency (MAF)>0.01 were filtered out. Subsequently, nonsynonymous and splicing site variants were selected out for the next data analysis. Splicing site variants located in 100 bp upstream and 100 bp downstream of the exon–intron or intron–exon boundary. At last, candidate pathogenic variants were identified by combining analysis of genotype and clinical phenotype of each deaf patient. According to the ACMG guideline, candidate variants were defined as "pathogenic," "likely pathogenic," "variants of unknown significance (VUS)," "likely benign," and "benign."

In total 84 deaf patients, we did not find any pathogenic variants in 14 patients (16%). In other 74 patients (84% of total patients), we found candidate pathogenic variants which

were directly or possibly associated with phenotypes of patients, as shown in Figure 1a. Both monogenic and digenic deafness were considered during analysis. Candidate pathogenic variants could be classified as three inheritance modes: autosomal dominant, autosomal recessive, maternally inherited (mitochondrial). Digenic inheritance of deafness was not observed in our deafness patients. Subsequently, based on the descriptions by Yan et al., (2016), we classified deaf patients harboring candidate variants as "Solved" and "Unsolved" groups, respectively. "Solved" patients were caused by "pathogenic" or "likely pathogenic" variants, while "Unsolved" patients harbored at least one "VUS" variant. The percentage of "Solved" and "Unsolved" deaf patients was 51.43% (36/70) and 48.57% (34/70), respectively. In multiplex families, the solved rate in the patients' group of "Solved" and "Unsolved" were 36.11% (13/36) and 20.59%
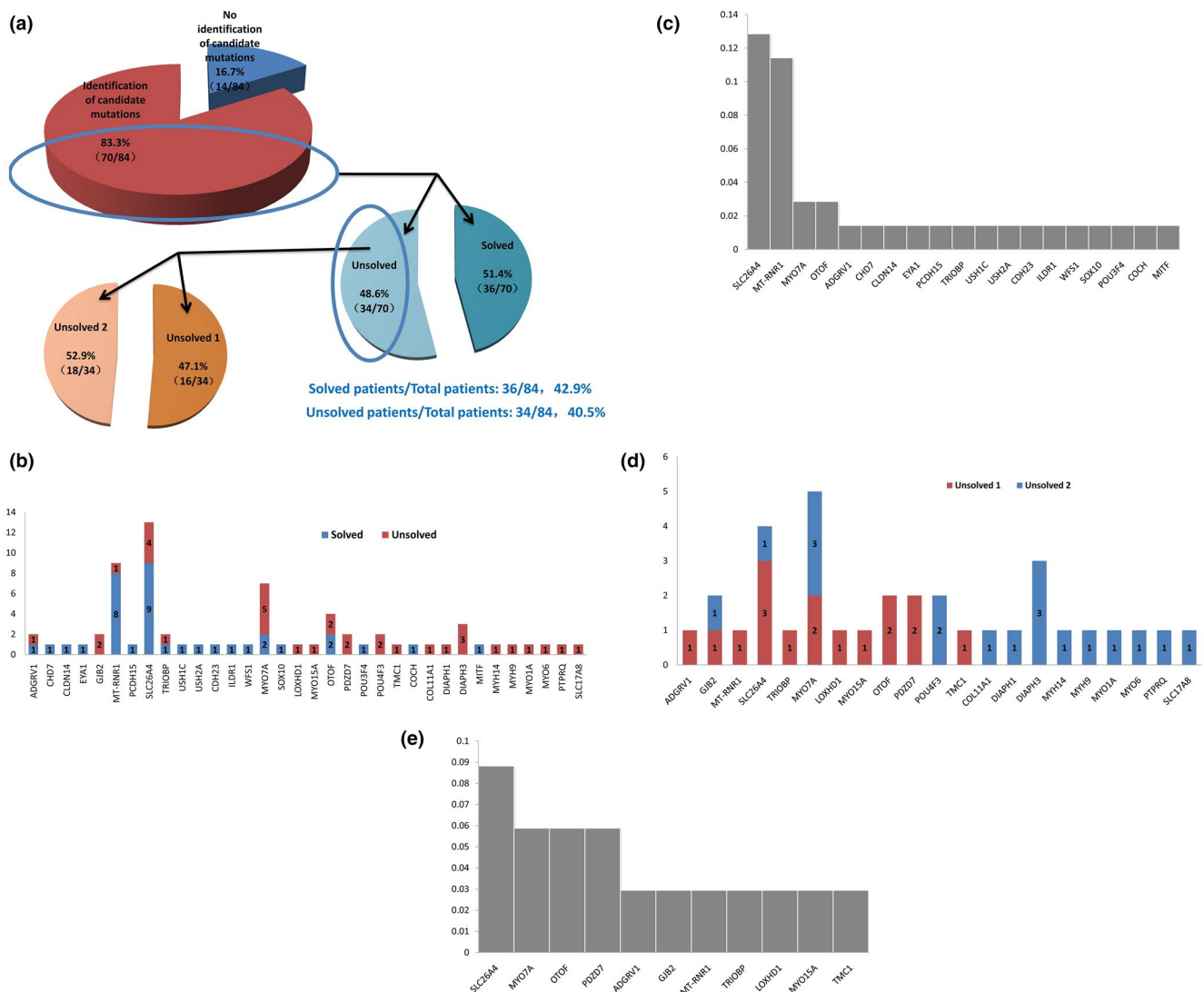

FIGURE 1 (a) Percentage of patients in "Solved" and "Unsolved" groups. (b and c). Candidate causative genes in "Solved" and "Unsolved" groups. The number of patients was annotated in each gene group. (d and e) Candidate causative genes in "Unsolved1" and "Unsolved2" groups. The number of patients was annotated in each gene group.

(7/34), respectively. "Unsolved" deaf patients were divided into two groups: "Unsolved 1" and "Unsolved 2." Deaf patients in "Unsovled1" group harbored candidate pathogenic variants that could possibly explain their pathology. Candidate pathogenic variants identified in "Unsolved2" patients could not possibly explain their pathology. Some specific candidate pathogenic variants in "Unsolved" patients of multiplex families are highlighted in Table 2. Compared with simplex families, these variants in multiplex families were more likely to be associated with deafness.

In the "Solved" group, most of the causative genes were SLC26A4 and MT-RNR1 (Table 1 and Figure 1b,c). Other causative genes were also identified, like MYO7A, SOX10, CHD7, etc. Nine patients were caused by mutations in the SLC26A4 gene. SLC264 c.919-2A>G was a frequent mutation. Eight patients were caused by the MT-RNR1 gene, all of them were caused by homogeneous m.1555A>G mutation. Two patients were caused by compound heterozygous mutations in the MYO7A gene. Mutations were c.1679A>C, c.6115G>C, c.2183T>C and c.2187 +2_+8 delTGAGCAC. Two patients harbored mutations in OTOF gene. Other patients were caused by each one different gene. There were two patients with syndromic deafness in "Solved" patients. They were Charge syndrome and Usher syndrome 1B, respectively. A patient with Charge syndrome harbored CHD7 c.1714C>T mutation. Compound mutations of MYO7A c.1679 A>C and c.6115 G>C were identified in a patient with Usher syndrome 1B.

In the "Unsolved" group, we found 21 possibly causative genes (Table 2 and Figure 1D,E), including MYO7A, DIAPH3, PDZD7, OTOF, SLC26A4, GPR98, etc. In the dominant inheritance model, we found candidate pathogenic variants in COCH, DIAPH1, DIAPH3, MYH14, MYH9, MYO1A, MYO6, MYO7A, POU4F3, PTPRQ, and SLC17A8 genes. In the recessive inheritance model, candidate pathogenic variants were identified in GJB2, ADGRV1, LOXHD1, MYO15A, OTOF, PDZD7, SLC26A4, TMC1, and TRIOBP genes. In the mitochondrial inheritance model, m.681T>C was identified in the MT-RNR1 gene. Nonsynonymous variants were predicted by bioinformatics tools: SIFT and Polyphen-2. Candidate variants that were predicted to be both tolerated and benign were GJB2 c.471G>A, LOXHD1 c.977A>G, MYH9 c.1897C>T, SLC26A4 c.2110G>A, TMC1 c.373A>C, and TRIOBP c.4442C>T. Four patients were associated with the MYO7A gene. PDZD7 c.490C>T, a recurrent pathogenic variant, was identified in two deaf patients. SLC26A4 c.290T>G and c.599A>G were novel likely pathogenic variants.

To further analyze candidate pathogenic variants classified as "VUS," the 3D protein structure of each mutant were constructed by Raptor X web server, as shown in Figure S1. ADGRV1 p.Leu4546His, LOXHD1 p.Asn326Ser, MYO15A p.Gly1318Ser, MYO7A p.Thr381Met, TMC1 p.Lys125Gln

might result in alteration (increase or disappearance) of hydrogen bonds. PDZD7 p.Arg164Trp, TMC1 p.Glu483Asp, MYO7A p.Arg853His might lead to the adaptation of α-Helix or β-sheet. GJB2 p.Met157Ile, MYO7A p.Pro541Leu, OTOF p.Thr597Met, OTOF p.Arg1583Cys might not have an influence on hydrogen bonds, α-Helix, and β-sheet. We could not analyze some candidate pathogenic variants, like TRIOBP p.Ser1481Phe, since we could not generate the 3D protein structure of its corresponding wild-type protein. Representative 3D protein structure of candidate pathogenic variants in SLC26A4, MYO7A, and PDZD7 genes is shown in Figure 2.

Generally, autosomal dominant, autosomal recessive, and maternally (mitochondrial) inheritance were observed in our cohorts of deaf patients. Candidate pathogenic variants were identified in 34 deafness genes, like SLC26A4, MT-RNR1, MYO7A, OTOF, and PDZD7 genes.

4 | DISCUSSION

In the present study, by high throughput sequencing of 127 deafness genes, we aimed to preliminarily analyze causative genes of Southwestern China in a cohort of 84 deaf patients from Southwestern China. The deafness genes in this area were rarely studied. In addition to GJB2, SLC26A4, and MT-RNR1 genes (common deafness genes), we found candidate pathogenic variants in 34 deafness genes, like MYO7A, PDZD7, and OTOF genes.

In our cohorts of deaf patients, we found 34 genes related to deafness (Tables 1,2). The number of "Solved" patients was 34, while that of "Unsolved" patients was 36. The percentage of "Unsolved" patients in total deaf patients was higher than that of "Solved" patients. "Unsolved" patients harbored "VUS" and "likely benign" variants. Some of these variants had no allele frequency data in 1000 Genome (Clarke et al., 2012) and ExAC databases. It might be caused by genetic background differences. Altitude is closely related to oxygen density. In the present study, patients were all from Yunnan province, China. The average altitude of Yunnan province is higher than the coastal areas of China. It might lead to genetic background differences between people living in coastal areas and Yunnan province. This might be an explanation for the identification of several "VUS" variants in our cohorts of deaf patients. Several studies have shown that people in different regions have different genetic backgrounds, especially for ethnicities (Liu et al., 2002; Manzoli et al., 2016; Miyagawa et al., 2013; Yan et al., 2016). This suggested that allele frequency database of deafness genes in local people should be established. It might be very helpful. In addition, functional studies should be performed on some recurrent candidate pathogenic variants, like PDZD7 c.490 C>T.

TABLE 1 Variants in the "Solved" patients

Gene	N	Transcript	Genotype	Chromosome	SIFT	Polyphen2	ExAC database	Multiplex/simplex family	Zygosity	Solved	ACMG
ADGRV1	1	NM_032119	c.8551G>T	chr5:90001381	—	—	—	Simplex	Het	Solved	Pathogenic
			c.12631C>T	chr5:90073825	—	—	—		Het		Pathogenic
CHD7	1	NM_017780	c.1714C>T	chr8:61693607	—	—	—	Simplex	Het	Solved	Pathogenic
CLDN14	1	NM_001146077	c.202C>T	chr21:37833792	—	—	—	Simplex	Hom	Solved	Pathogenic
POU3F4	1	NM_000307	EX1 DEL	—	—	—	—	Multiplex	Hemi	Solved	Pathogenic
MT-RNR1	1	NC_012920	m.1555A>G					Simplex	Hom	Solved	Pathogenic
	7	NC_012920	m.1555A>G					Multiplex	Hom	Solved	Pathogenic
MYO7A	1	NM_000260	c.1679A>C	chr11:76874023	Damaging	Probably damaging	—	Simplex	Het	Solved	Likely pathogenic
			c.6115G>C	chr11:76922260	Damaging	Probably damaging	—		Het		Likely pathogenic
	1	NM_000260	c.2183T>C	chr11:7688650	Damaging	Probably damaging	—	Simplex	Het	Solved	Likely pathogenic
			c.2187 +2_+8 delTGAGCAC	chr11:7688651 2..768865186	—	—	—		Het		Pathogenic
PCDH15	1	NM_033056	c.1864_1865 insTA	chr10:55892687..55892688	—	—	—	Simplex	Het	Solved	Pathogenic
			c.2220+1G>A	chr10:55826516	—	—	—		Het		Pathogenic
SLC26A4	2	NM_000441	c.919-2A>G	chr7:107323898	—	—	0.000306	Simplex	Hom	Solved	Pathogenic
	1		c.919-2A>G	chr7:107323898	—	—	0.0003058	Multiplex	Het	Solved	Pathogenic
			c.915_916 insG	chr7:1073237966..107323797	—	—	0.00001650		Het		Pathogenic
	1		c.919-2A>G	chr7:107323898	—	—	0.000306	Multiplex	Hom	Solved	Pathogenic
	1		c.2027T>A	chr7:107342495	Damaging	Damaging	—	Simplex	Het	Solved	Pathogenic
			c.2168A>G	chr7:107350577	Probably damaging	Probably damaging	0.0001238		Het		Pathogenic
	1		c.919-2A>G	chr7:107323898	—	—	0.0003058	Multiplex	Het	Solved	Pathogenic
			EX5_6 DEL	—	—	—	—		Het		Pathogenic
	1		c.1991C>T	chr7:107342459	Tolerated	Damaging	—	Multiplex	Het	Solved	Pathogenic
			c.2168A>G	chr7:107350577	Probably damaging	Probably damaging	0.0001238		Het		Pathogenic

(Continues)

TABLE 1 (Continued)

Gene	N	Transcript	Genotype	Chromosome	SIFT	Polyphen2	ExAC database	Multiplex/simplex family	Zygosity	Solved	ACMG
	1		c.1174A>T	chr7:107330593	Damaging	Probably damaging	0.000008242	Simplex	Hom	Solved	Pathogenic
	1		c.2168A>G	chr7:107350577	Probably damaging	Probably damaging	0.0001238	Simplex	Het	Solved	Pathogenic
			EX1_3 DEL	—	—	—	—		Het	Solved	Pathogenic
TRIOBP	1	NM_001039141	c.1342C>T	chr22:38119905	—	—	—	Simplex	Het	Solved	Pathogenic
			c.2074C>T	chr22:38120637	—	—	—		Het	Solved	Pathogenic
USH1C	1	NM_153676	c.388-1G>A	chr11:17548879	—	—	—	Simplex	Hom	Solved	Pathogenic
USH2A	1	NM_206933	c.99_100insT	chr1:216595579..216595580	—	—	—	Simplex	Het	Solved	Pathogenic
			c.11660G>A	chr1:215914768	—	—	—		Het	Solved	Pathogenic
OTOF	1	NM_194248	c.2236C>T	chr2:26700596	Tolerated	Benign	0.00001	Simplex	Hom	Solved	Likely pathogenic
	1	NM_194248	c.4747C>T	chr2:26688592	Damaging	Probably damaging	0.00002471	Multiplex	Hom	Solved	Likely pathogenic
ILDR1	1	NM_175924	c.206C>A	chr3:121725861	Tolerated	Probably damaging	0.00004	Simplex	Hom	Solved	Pathogenic
CDH23	1	NM_022124	c.6442G>A	chr10:73553127	Tolerated	Probably damaging	—	Simplex	Hom	Solved	Likely pathogenic
EYA1	1	NM_000503	EX1_18 DEL	—	—	—	—	Simplex	Het	Solved	Pathogenic
SOX10	1	NM_006941	c.378C>A	chr22:38378414	—	—	—	Simplex	Het	Solved	Likely pathogenic
WFS1	1	NM_006005	c.1356_1371delGCCCTACACGCGCAGG	chr4:6302878..6302893	—	—	—	Simplex	Het	Solved	Likely pathogenic
COCH	1	NM_004086	c.847G>A	chr14:31354713	Tolerated	Probably damaging	—	Simplex	Het	Solved	Likely pathogenic
MITF	1	NM_198159	c.1021C>G	chr3:70008431	Damaging	Probably damaging	—	Simplex	Het	Solved	Likely pathogenic

Abbreviations: ACMG, a technical standard of the American College of Medical Genetics and Genomics; Exac Database, Exome Aggregation Consortium (ExAC) database.

TABLE 2 Variants in the "Unsolved" ("Unsolved 1" and "Unsolved 2") patients

Unsolved

Gene	N	Transcript	Genotype	Chromosome	SIFT	Polyphen2	ExAC database	Multiplex/ simplex family	Zygosity	Unsolved 1/ unsolved 2	ACMG
COL11A1	1	NM_080630	EX1_EX65 DUP	—	—	—	—	Simplex	Het	Unsolved 2	VUS
DIAPH1	1	NM_001079812	c.2983C>T	chr5:140908277	Damaging	—	—	Simplex	Het	Unsolved 2	VUS
DIAPH3	1	NM_001042517	c.2608-3T>C	chr13:60435673	—	—	0.00005802	Simplex	Het	Unsolved 2	VUS
	1	NM_001042517	c.3124C>T	chr13:60384961	Damaging	Probably damaging	—	Multiplex	Het	Unsolved 2	VUS
	1	NM_001042517	c.1100G>A	chr13:60566632	Damaging	Probably damaging	0.00006994	Simplex	Het	Unsolved 2	VUS
PDZD7	2	NM_024895	c.490C>T	chr10:102783245	Damaging	Probably damaging	0.00004959	Simplex	Hom	Unsolved 1	VUS
GJB2	1	NM_004004	c.109G>A	chr13:20763612	—	Probably damaging	0.006587	**Multiplex**	Het	**Unsolved 1**	Pathogenic
			c.471G>A	**chr13:20763250**	Tolerated	Benign	**0.000008261**		**Het**	Unsolved 1	**VUS**
	1	NM_004004	c.235delC	chr13:20763486	—	—	0.0003625	Simplex	Het	Unsolved 2	Pathogenic
ADGRV1	1	NM_032119	c.2241-10A>T	chr5:89938443	—	—	—	Simplex	Het	Unsolved 1	Likely pathogenic
			c.13637T>A	chr5:90079858	Damaging	Possibly damaging	—		Het	Unsolved 1	VUS
LOXHD1	1	NM_144612	c.1468C>T	chr18:44172511	—	—	—	**Multiplex**	Het	**Unsolved 1**	Pathogenic
			c.977A>G	**chr18:44181337**	Tolerated	—	**0.0003579**		**Het**	Unsolved 1	**likely benign**
MT-RNR1	1	NC_012920	m.681T>C	—	—	—	—	Simplex	Hom	Unsolved 1	VUS
MYH14	1	NM_001077186	c.475G>A	chr19:50720941	Damaging	Probably damaging	0.00001657	Simplex	Het	Unsolved 2	VUS
MYH9	1	NM_002473	c.1897C>T	chr22:36702600	Tolerated	Benign	—	Multiplex	Het	Unsolved 2	VUS
MYO15A	1	NM_016239	c.9243_9251delins AAGGGGGG	chr17:18062933..18062941	—	—	—	Simplex	Het	Unsolved 1	Pathogenic
			c.3952G>A	chr17:18030399	Damaging	Probably damaging	0.000008324		Het	Unsolved 2	VUS
MYO1A	1	NM_005379	c.235G>T	chr12:57441501	Tolerated	Probably damaging	0.002871	Simplex	Het	Unsolved 2	Likely benign
MYO6	1	NM_004999	c.2672C>T	chr6:76599787	Damaging	Possibly damaging	0.0001422	Simplex	Het	Unsolved 2	VUS
MYO7A	1	NM_000260	c.1622C>T	chr11:76873966	Damaging	Probably damaging	—	Simplex	Het	Unsolved 2	VUS
	1	NM_000260	c.2558G>A	chr11:76890971	Damaging	Probably damaging	—	Multiplex	Het	Unsolved 1	VUS
	2	NM_000260	c.1142C>T	chr11:76871270	Tolerated	Possibly damaging	0.000235	Simplex	Het	Unsolved 2	VUS
	1	NM_000260	c.541C>T	chr11:76867776	—	—	—	Simplex	Het	Unsolved 1	Likely pathogenic
		NM_000260	c.617G>A	chr11:76867932	Damaging	Probably damaging	0.00014		Het	Unsolved 2	VUS

(Continues)

TABLE 2 (Continued)

Unsolved

Gene	N	Transcript	Genotype	Chromosome	SIFT	Polyphen2	ExAC database	Multiplex/simplex family	Zygosity	Unsolved 1/unsolved 2	ACMG
OTOF	1	NM_194248	c.1790C>T	chr2:26703667	Tolerated	Possibly damaging	0.000008474	Simplex	Het	Unsolved 1	VUS
			c.829G>T	chr2:26717878	Tolerated	Probably damaging	—		Het		VUS
	1	NM_194248	c.1273C>T	chr2:26706449	Damaging	Probably damaging	0.00002	Simplex	Het	Unsolved 1	Pathogenic
			c.3983G>C	chr2:26693501	Damaging	Probably damaging	—		Het		VUS
POU4F3	1	NM_002700	c.593G>A	chr5:145719583	Damaging	Probably damaging	—	Simplex	Het	Unsolved 2	VUS
	1	NM_002700	c.909G>T	chr5:145719899			—	Simplex	Het	Unsolved 2	VUS
PTPRQ	1	NM_001145026	c.1786A>G	chr12:80889054	Damaging	Benign	—	Simplex	Het	Unsolved 2	VUS
SLC17A8	1	NM_139319	c.760T>G	chr12:100795638	Damaging	Probably damaging	—	Simplex	Het	Unsolved 2	VUS
SLC26A4	1	NM_000441	c.919-2A>G	chr7:107323898	—	—	0.0003058		Het	Unsolved 1	Pathogenic
			c.290T>G	**chr7:107303866**	**Damaging**	**Probably damaging**	—	**Multiplex**	**Het**		**VUS**
	1		c.1746 delG	chr7:107341584			—	Simplex	Het	Unsolved 1	Pathogenic
			c.2110G>A	chr7:107350519	Tolerated	Benign	—		Het		VUS
	1		c.2027T>A	chr7:107342495	Damaging	Probably damaging	—	Simplex	Het	Unsolved 1	Pathogenic
			c.599A>G	chr7:107314792	Damaging	Probably damaging	—		Het		VUS
	1		c.919-2A>G	chr7:107323898	—	—	0.000306	Multiplex	Het	Unsolved 2	Pathogenic
TMC1	1	NM_138691	c.373A>C	chr9:75355045	Tolerated	Benign	0.00001654	Simplex	Het	Unsolved 1	VUS
			c.1449A>C	chr9:75407151	Tolerated	Possibly damaging	—		Het		VUS
TRIOBP	1	NM_001039141	c.1283C>G	chr22:38119846	Damaging	Possibly damaging	0.0001739	Simplex	Het	Unsolved 1	Likely benign
			c.4442C>T	chr22:38130785	Tolerated	Benign	0.0001354	—	Het		VUS

Note: Specific candidate pathogenic variants in "Unsolved" patients of multiplex families (in bold).

"Exac Database," Exome Aggregation Consortium (ExAC) database.

Abbreviations: ACMG, a technical standard of the American College of Medical Genetics and Genomics; unsolved1, deafness patients in unsovled1 group. In this group, deafness patients harbored candidate pathogenic variants that could possibly explain their pathology; unsolved2, deafness patients in unsovled2 group. In this group, deafness patients harbored the candidate pathogenic variants that could not explain their pathology; VUS, variant of unknown significance; likely benign, variant of likely benign.
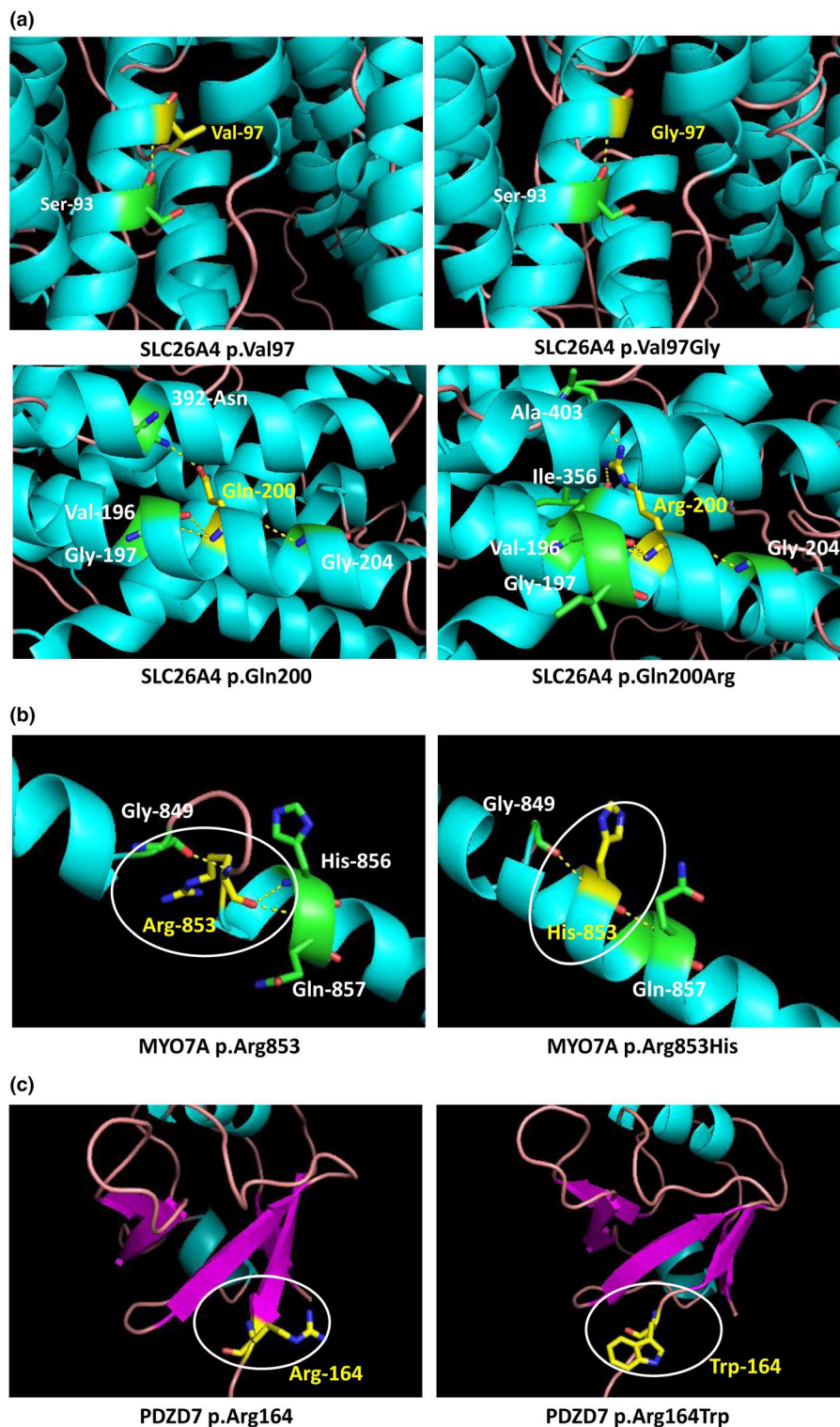
FIGURE 2 Three-dimensional (3D) protein structure prediction and analysis of mutants in genes, like SLC26A4 (a), MYO7A (b), and PDZD7 (c) genes. These genes were shown to contain variants of unknown significance (VUS) in the present study. Hydrogen bonds were predicted by PyMOL software and shown in yellow dotted lines. α-Helix and β-sheet were colored as blue and red, respectively.

GJB2, SLC26A4, and MT-RNR1 genes have been identified in Chinese deaf patients of different regions (Chan & Chang, 2014; Lu et al., 2010; Shen et al., 2011; Tsukada et al., 2015). They are thought to be common deafness genes

in Chinese. The distributions of uncommon deafness genes are still unclear in different populations and regions. In the study of uncommon deafness genes by Yan et al., (2016), MYO15A, USH2A, MYO7A, MYO6, and TRIOBP were

their most identified genes. The results of the present study might have some difference from that of Yan et al., (2016). Although we also identified the MYO7A gene in our cohorts of deaf patients, the frequency of other candidate causative genes in our deaf patients was different from that of Yan et al., (2016), like OTOF, PDZD7, and DIAPH3 genes. The result suggested that the distribution of deafness genes might have its features in deaf patients from Southwestern China. The conclusion needs to be further confirmed, since the sample size was limited in the present study. In addition, candidate pathogenic variants in multiplex families were not tested in affected family members, since we were not able to collect their samples. It was another limitation of the present study.

Heritable deafness is a complicated disease. More than 200 deafness genes have been reported (Angeli et al., 2012; Liu et al., 2006; Petersen, 2002). Here, 127 deafness genes screening was applied to 84 deaf patients from southwestern China. Only half of the deaf patients ("Solved" patients) could be clearly diagnosed. Therefore, 127 deafness genes screening had its limitation in clinical application.

By 127 deafness genes screening, no candidate pathogenic mutations/variants were detected in 14 deaf patients. Whether these patients harbored other pathogenic mutations was still unknown. Whole-exome/genome sequencing has been widely used to identify uncommon deafness genes (Berg et al., 2011; Choi et al., 2009; Ormond et al., 2010; Yang, Muzny, et al., 2013). To further explore molecular pathology of these patients, whole-exome/genome sequencing might be very effective. Our results also indicated the possibility that some deaf patients from southwestern China might be caused by other deafness genes. It should be further explored.

In summary, the present study provided a preliminary overview of the spectrum of mutations in deaf patients from Southwestern China. Thirty-four genes were found to have candidate pathogenic variants in our cohorts of patients. The findings will be helpful in the prevention and molecular diagnosis of deafness.

CONFLICT OF INTEREST
The authors declared no conflicts of interest.

AUTHORS' CONTRIBUTIONS
Study concepts: Yunlong Li and Baosheng Zhu. Literature research: Yunlong Li. Clinical information collection: Jie Su, Jingman Zhang, Jiahong Pei, Dongmei Li, and Yinhong Zhang. Data acquisition: Jingyu Li and Menglang Chen. Data analysis/interpretation: Yunlong Li Jingyu Li, Menglang Chen. Manuscript preparation: Yunlong Li. Manuscript revision/review: Yunlong Li, Jie Su, Jingman Zhang, Jiahong Pei, Dongmei Li, Yinhong Zhang, and Baosheng Zhu. Manuscript final version approval: Yunlong Li and Baosheng Zhu.

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REFERENCES
Angeli, S., Lin, X., & Liu, X. Z. (2012). Genetics of hearing and deafness. *Anatomical Record (Hoboken)*, *295*(11), 1812–1829. https://doi.org/10.1002/ar.22579

Berg, J. S., Khoury, M. J., & Evans, J. P. (2011). Deploying whole genome sequencing in clinical practice and public health: meeting the challenge one bin at a time. *Genetics in Medicine*, *13*(6), 499–504. https://doi.org/10.1097/GIM.0b013e318220aaba

Chan, D. K., & Chang, K. W. (2014). GJB2-associated hearing loss: systematic review of worldwide prevalence, genotype, and auditory phenotype. *Laryngoscope*, *124*(2), E34–E53. https://doi.org/10.1002/lary.24332

Choi, M., Scholl, U. I., Ji, W., Liu, T., Tikhonova, I. R., Zumbo, P., Nayir, A., Bakkaloğlu, A., Özen, S., Sanjad, S., Nelson-Williams, C., Farhi, A., Mane, S., & Lifton, R. P. (2009). Genetic diagnosis by whole exome capture and massively parallel DNA sequencing. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(45), 19096–19101. https://doi.org/10.1073/pnas.0910672106

Clarke, L., Zheng-Bradley, X., Smith, R., Kulesha, E., Xiao, C., Toneva, I., Vaughan, B., Preuss, D., Leinonen, R., Shumway, M., Sherry, S., & Flicek, P. (2012). The 1000 Genomes Project: data management and community access. *Nature Methods*, *9*(5), 459–462. https://doi.org/10.1038/nmeth.1974

da Silva, E. O., & Duarte, A. R. (1995). Genetic deafness: a brief review. *Jornal de Pediatria*, *71*(6), 297–302. https://doi.org/10.2223/jped.796

Donovan, J. L. (1984). Ethnicity and health: a research review. *Social Science and Medicine*, *19*(7), 663–670. https://doi.org/10.1016/0277-9536(84)90237-5

Duman, D., & Tekin, M. (2012). Autosomal recessive nonsyndromic deafness genes: a review. *Frontiers in Bioscience*, *17*, 2213–2236. https://doi.org/10.2741/4046

Li, R., Li, Y., Fang, X., Yang, H., Wang, J., Kristiansen, K., & Wang, J. (2009). SNP detection for massively parallel whole-genome resequencing. *Genome Research*, *19*(6), 1124–1132. https://doi.org/10.1101/gr.088013.108

Li, Y., Su, J., Ding, C., Yu, F., & Zhu, B. (2019). Identification of four novel mutations in MYO7A gene and their association with nonsyndromic deafness and Usher Syndrome 1B. *International Journal of Pediatric Otorhinolaryngology*, *120*, 166–172. https://doi.org/10.1016/j.ijporl.2019.02.021

Li, Y., Zhu, B., Su, J., Yin, Y., & Yu, F. (2016). Identification of SLC26A4 mutations p. L582LfsX4, p.I188T and p.E704K in a Chinese family with large vestibular aqueduct syndrome (LVAS). *International Journal of Pediatric Otorhinolaryngology*, *91*, 1–5. https://doi.org/10.1016/j.ijporl.2016.08.026

Lin, X., Tang, W., Ahmad, S., Lu, J., Colby, C. C., Zhu, J., & Yu, Q. (2012). Applications of targeted gene capture and next-generation sequencing technologies in studies of human deafness and other genetic disabilities. *Hearing Research*, *288*(1–2), 67–76. https://doi.org/10.1016/j.heares.2012.01.004

Liu, X., Ouyang, X., & Yan, D. (2006). The Genetic Deafness in Chinese Population. *Journal of Otology*, *1*(1), 1–10.

Liu, X., Xia, X., Ke, X., Ouyang, X., Du, L. I., Liu, Y. U., Angeli, S., Telischi, F., Nance, W., Balkany, T., & Xu, L. I. (2002). The prevalence of connexin 26 (GJB2) mutations in the Chinese population. *Human Genetics*, *111*(4–5), 394–397. https://doi.org/10.1007/s00439-002-0811-6

Lu, J., Li, Z., Zhu, Y. I., Yang, A., Li, R., Zheng, J., Cai, Q., Peng, G., Zheng, W., Tang, X., Chen, B., Chen, J., Liao, Z., Yang, L. I., Li, Y., You, J., Ding, Y. U., Yu, H., Wang, J., … Guan, M.-X. (2010). Mitochondrial 12S rRNA variants in 1642 Han Chinese pediatric subjects with aminoglycoside-induced and nonsyndromic hearing loss. *Mitochondrion*, *10*(4), 380–390. https://doi.org/10.1016/j.mito.2010.01.007

Manzoli, G. N., Bademci, G., Acosta, A. X., Felix, T. M., Cengiz, F. B., Foster, J. 2nd, & Tekin, M. (2016). Targeted resequencing of deafness genes reveals a founder MYO15A variant in Northeastern Brazil. *Annals of Human Genetics*, *80*(6), 327–331. https://doi.org/10.1111/ahg.12177

Meena, R., & Ayub, M. (2017). Genetics of human hereditary hearing impairment. *Journal of Ayub Medical College, Abbottabad*, *29*(4), 671–676.

Miyagawa, M., Naito, T., Nishio, S. Y., Kamatani, N., & Usami, S. (2013). Targeted exon sequencing successfully discovers rare causative genes and clarifies the molecular epidemiology of Japanese deafness patients. *PLoS One*, *8*(8), e71381. https://doi.org/10.1371/journal.pone.0071381

Miyagawa, M., Nishio, S. Y., Ikeda, T., Fukushima, K., & Usami, S. (2013). Massively parallel DNA sequencing successfully identifies new causative mutations in deafness genes in patients with cochlear implantation and EAS. *PLoS One*, *8*(10), e75793. https://doi.org/10.1371/journal.pone.0075793

Miyagawa, M., Nishio, S. Y., Kumakawa, K., & Usami, S. (2015). Massively parallel DNA sequencing successfully identified seven families with deafness-associated MYO6 mutations: the mutational spectrum and clinical characteristics. *The Annals of Otology, Rhinology, and Laryngology*, *124*(Suppl 1), 148S–157S. https://doi.org/10.1177/0003489415575055

Ormond, K. E., Wheeler, M. T., Hudgins, L., Klein, T. E., Butte, A. J., Altman, R. B., Ashley, E. A., & Greely, H. T. (2010). Challenges in the clinical application of whole-genome sequencing. *Lancet*, *375*(9727), 1749–1751. https://doi.org/10.1016/S0140-6736(10)60599-5

Petersen, M. B. (2002). Non-syndromic autosomal-dominant deafness. *Clinical Genetics*, *62*(1), 1–13. https://doi.org/10.1034/j.1399-0004.2002.620101.x

Shearer, A. E., & Smith, R. J. (2012). Genetics: advances in genetic testing for deafness. *Current Opinion in Pediatrics*, *24*(6), 679–686. https://doi.org/10.1097/MOP.0b013e3283588f5e

Shen, Z., Zheng, J., Chen, B., Peng, G., Zhang, T., Gong, S., Zhu, Y. I., Zhang, C., Li, R., Yang, L. I., Zhou, J., Cai, T., Jin, L., Lu, J., & Guan, M.-X. (2011). Frequency and spectrum of mitochondrial 12S rRNA variants in 440 Han Chinese hearing impaired

pediatric subjects from two otology clinics. *Journal of Translational Medicine*, *9*, 4. https://doi.org/10.1186/1479-5876-9-4

Stenson, P. D., Mort, M., Ball, E. V., Shaw, K., Phillips, A., & Cooper, D. N. (2014). The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Human Genetics*, *133*(1), 1–9. https://doi.org/10.1007/s00439-013-1358-4

Tsukada, K., Nishio, S. Y., Hattori, M., & Usami, S. (2015). Ethnic-specific spectrum of GJB2 and SLC26A4 mutations: their origin and a literature review. *The Annals of Otology, Rhinology, and Laryngology*, *124*(Suppl 1), 61S–76S. https://doi.org/10.1177/0003489415575060

Vona, B., Müller, T., Nanda, I., Neuner, C., Hofrichter, M. A. H., Schröder, J., Bartsch, O., Läßig, A., Keilmann, A., Schraven, S., Kraus, F., Shehata-Dieler, W., & Haaf, T. (2014). Targeted next-generation sequencing of deafness genes in hearing-impaired individuals uncovers informative mutations. *Genetics in Medicine*, *16*(12), 945–953. https://doi.org/10.1038/gim.2014.65

Wang, S., Li, W., Liu, S., & Xu, J. (2016). RaptorX-Property: a web server for protein structure property prediction. *Nucleic Acids Research*, *44*(W1), W430–W435. https://doi.org/10.1093/nar/gkw306

Yan, D., Tekin, D., Bademci, G., Foster, J., Cengiz, F. B., Kannan-Sundhari, A., Guo, S., Mittal, R., Zou, B., Grati, M., Kabahuma, R. I., Kameswaran, M., Lasisi, T. J., Adedeji, W. A., Lasisi, A. O., Menendez, I., Herrera, M., Carranza, C., Maroofian, R., … Tekin, M. (2016). Spectrum of DNA variants for non-syndromic deafness in a large cohort from multiple continents. *Human Genetics*, *135*(8), 953–961. https://doi.org/10.1007/s00439-016-1697-z

Yang, T., Wei, X., Chai, Y., Li, L., & Wu, H. (2013). Genetic etiology study of the non-syndromic deafness in Chinese Hans by targeted next-generation sequencing. *Orphanet Journal of Rare Diseases*, *8*, 85. https://doi.org/10.1186/1750-1172-8-85

Yang, Y., Muzny, D. M., Reid, J. G., Bainbridge, M. N., Willis, A., Ward, P. A., Braxton, A., Beuten, J., Xia, F., Niu, Z., Hardison, M., Person, R., Bekheirnia, M. R., Leduc, M. S., Kirby, A., Pham, P., Scull, J., Wang, M., Ding, Y., … Eng, C. M. (2013). Clinical whole-exome sequencing for the diagnosis of mendelian disorders. *New England Journal of Medicine*, *369*(16), 1502–1511. https://doi.org/10.1056/NEJMoa1306555

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