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Comparative analysis of allele frequencies of 15 deafness gene variants between hearing-loss and normal populations in Henan, China☆

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ABSTRACT

Background: Hearing loss is found in more than 5 % of cases worldwide. Hearing loss is divided into three types: Sensorineural hearing loss, Combined hearing loss and Conductive hearing loss. Among them, no less than 50 % of pediatric cases of sensorineural hearing loss are genetic. In Henan, China, there are no statistics on the allele frequency of deafness gene variants.
Methods: We divided 2178 subjects enrolled at the Third Affiliated Hospital of Zhengzhou College from January 2019 to March 2021 into a hearing loss group and a normal control group. We performed array and pathogenicity classification for screening the 15 deafness gene variants, calculated and compared the allele frequency of the deafness gene variants, and then compared the hearing loss diagnosis rate between the hearing loss group and the normal control group.
Results: We found that in the hearing loss group, the overall allele frequency of all detected variants was 16.6 %. Comparative analysis showed that the allele frequencies of *GJB2* c.235delC variant, *GJB3* c.538C > T variant and *SLC26A4* c.919-2A > G variant were significantly higher than those of the East Asian population average in the gnomAD database. At the same time, our study confirmed that *GJB3* c.538C > T variant may not be the disease-causing variant of hearing loss.
Conclusions: These results support genetic counseling and rational prediction of risk for deafness.

1. Introduction

Hearing loss (HL) has emerged as a global health concern, significantly impacting individuals' quality of life [1,2]. According to the World Health Organization statistics for 2021, HL affects no less than 5 % of the world's population, including 34 million children [3]. It is estimated that by 2050 over 700 million people – or one in every ten people will have disabling hearing loss (PRL: https://www.who.int/news-room/fact-sheets/detail/deafness-and-hearing-loss). HL is divided into three types: Sensorineural hearing loss,

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Combined hearing loss and Conductive hearing loss [3]. Sensorineural hearing impairment (SNHL) involves a pathological condition of the inner ear [4]. Among them, no less than 50 % of sensorineural hearing loss in children is due to genetic factors [5].

In recent decades, 27.8 million cases of hearing loss have been reported in China, accounting for 33.5 % of the total cases [6]. Overall, approximately 80 % of NSHL cases are autosomal recessive (AR), autosomal dominant (AD) inheritance accounts for 20 %, and no more than 2 % are caused by mitochondrial or X-linked inheritance patterns [7]. The *GJB2* c.235delC and *SLC26A4* c.919-2A > G variants (also written as IVS7-2A > G) have the highest carrier rates in the Chinese population [8,9].

Currently, newborns are tested for *GJB2* c.35delG variant, c.176del16 variant, c.235delC variant, and c.299delAT variant; *SLC26A4* c.919-2A > G variant, c.2168A > G variant, c.1174A > T variant, c.1226G > A variant, c.1707+5G > A variant, c.1975G > C variant, c.2027T > A variant, and c.1229C > T variant; *GJB3* c.538C > T variant; mtDNA 12S rRNA m.1555A > G variant and m.1494C > T variant to determine variant carrier rate [10–15]. However, all of these variant carrier statistics are based on case numbers and do not include allele frequency statistics (AF). Because of the limited population, not enough cases have been reported to date to assess allele frequency (AF) of deafness gene variants in populations with hearing loss.

The aim of our study is to calculate the allele frequencies of these 15 deafness gene variants in the hearing-loss population in Henan, China, and to investigate the similarities and differences of these allele frequencies between the hearing-loss population and the normal population to establish a unique allele frequency spectrum of deafness gene variants in Henan, China. At the same time, we will also compare the genetic diagnosis rate and carrier rate between the hearing loss population and the normal population to evaluate the diagnostic effect of bio-gene chip array technology for hearing loss.

2. Materials and methods

2.1. Subjects

2178 subjects were enrolled at the Third Affiliated Hospital of Zhengzhou University from January 2019 to March 2021. All subjects that were diagnosed using the American College of Medical Genetics and Genomics guideline for the clinical evaluation and etiologic diagnosis of hearing loss were divided into hearing loss group (HL) and normal control group (NC group) according to the presence or absence of hearing loss phenotype. The severity of the patients is similar and the phenotypes are different, but they are consistent with the detected genes The phenotypes caused by the mutations are consistent. Extracted keywords for hearing loss phenotypes included autism spectrum disorder, expressive language disorder, major vestibular syndrome, cerumen embolism, deafness, ear trauma, ear itching, sensorineural deafness, dysarthria, hearing impairment, neurological deafness, hearing impairment, sudden deafness, otitis externa, speech impairment, and speech delay. Patients and controls were all from unrelated families. In addition, a complete medical history was obtained from all subjects, and they provided written informed consent before the study, while physicians and genetic counsellors were responsible for interpreting the test results and then suggesting possible interventions for variant carriers. It should be noted that this study was approved by the Institutional Review Board of the Third Affiliated Hospital of Zhengzhou University.

2.2. Sample collections and genomic DNA extraction

All subjects had 3 ml of peripheral blood drawn and collected in EDTA tubes containing anticoagulant. DNA was extracted using the QIAGEN 69504 Blood and Tissue DNA Extraction Kit.

2.3. Array for the variants screening

Due to the cost-effectiveness, quick turn-around time, and accessibility, Bio-Gene Chip Array Kit (No.:300,068) from CapitalBio Technology was used to detect deafness gene variants.4 genes (*GJB2*, *GJB3*, *SLC26A4*, and mtDNA 12S rRNA) were tested simultaneously using DNA microarray technology, including c.35delG variant, c.176del16 variant, c.235delC variant, c.299delAT variant, c.919-2A > G variant, c.2168A > G variant, c.1174A > T variant, c.1226G > A variant, c.1707+5G > A variant, c.1975G > C variant, c.2027T > A variant, c.1229C > T variant, c.538C > T variant, m.1555A > G variant, and m.1494C > T variant. The selection of 15 variants in 4 genes was based on previous studies that showed that these variants are the most common causes of nonsyndromic HL. A study of 58,397 Chinese newborns screened for 20 common pathogenic variants in the four genes identified a genetic carrier rate of 5.52 % and detected 20.59 % (7/34) of newborns with deafness-causing genotypes (*GJB2* or *SLC26A4*) who passed hearing screening [9]. The 15 variants with higher frequency among these 20 variants were selected for our study. The amplicons of the 15 variants of four genes were amplified by multiplex PCR. The PCR reaction mixture was then hybridized with specific labeled probes immobilized on the microarray chip. Finally, the chip signals were scanned and imaged using the LuxScan microarray scanner and detection system.

2.4. Pathogenicity classification of deafness gene variants

The 13 variants in 3 nuclear genes for deafness were classified into five categories: pathogenic, likely pathogenic, uncertain significance (VUS), likely benign, and benign in accordance with the expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss [16]. 2 variants in mitochondrial genes were classified into the same five categories by referencing the ClinGen Mitochondrial Disease Nuclear and Mitochondrial Expert Panel specifications to the ACMG/AMP Variant Interpretation Guidelines version1 (PRL:https://clinicalgenome.org/site/assets/files/4953/clingen_mito_disease_acmg_specifications_v1-1.pdf).

2.5. Calculation and comparison of deafness gene variants' allele frequency in hearing loss group and normal control group

Allele frequencies of all variants of the deafness gene were calculated. The allele frequencies (AF) of each gene variant were compared between the hearing loss group and the normal control group. The East Asian AF, listed in the Genome Aggregation Database (gnomAD), was used as a reference. The calculation formula for the allele frequency of the deafness gene variants was as follows:

AF=(2n1+n2)/2 N.

AF: allele frequency; N:total number of individuals; n1: number of homozygotes; n2: number of heterozygotes.

2.6. Statistical analysis

Statistical analysis was performed using SPSS Statistics 22.0 statistical software. The χ2-test was performed to compare the genetic test diagnosis between the HL group and the NC group ($P < 0.05$ was statistically significant). GraphPad Prism 5.0 software was used to generate line plots and compare allele frequencies of deafness gene variants.

3. Results

3.1. Subjects

689 subjects were included in HL group. 1489 subjects were included in NC group. In the HL group, 335 males and 240 females were included. In the NC group, there were 788 males and 596 females. The age distribution of subjects in both groups is shown in Table 1. The specific descriptions of the pathogenic genes, their heterozygosity, and the genetic patterns of the identified hearing loss patients, carriers, and individuals without detected pathogenic gene variants in this study can be found in Supplementary Table 1, Supplementary Table 2 and Supplementary Table 3.

3.2. Pathogenicity classification of deafness gene variants

Classification of the pathogenicity of the 15 variants of the deafness gene detected with the Bio-Gene Chip array revealed that the variants m.1555A > G and m.1494C > T were classified as "likely pathogenic"; the variant c.538C > T was classified as "VUS" (Table 2). With the exception of the 3 variants mentioned above, the remaining 12 variants were all classified as "pathogenic" (Table 2).

P: pathogenic; LP: likely pathogenic; NA: not applicable; ACMG criterias:PVS1: Null variant (nonsense, frameshift, canonical ± 1 or 2 splice sites, initiation codon, single or multi-exon deletion) in a gene where loss of function (LOF)is a known mechanism of disease; PM3: For recessive disorders, detected in trans with a pathogenic variant; PS4: The prevalence of the variant in affected individuals is significantly increased compared to the prevalence in controls; PM2: Absent from controls (or at extremely low frequency if recessive); PS3: Well-established in vitro or in vivo functional studies supportive of a damaging effect on the gene or gene product; BS1: Allele frequency is greater than expected for disorder; BA1: Allele frequency is above 5 % in Exome Sequencing Project, 1000 Genomes,or ExAC; BS2: Allele frequency is greater than expected for disorder; PP1: Co-segregation with disease in multiple affected family members in a gene

definitively known to cause the disease.

3.3. Comparison of deafness gene variants' allele frequency in hearing loss group and normal control group

The calculation results and comparison of allele frequencies of deafness gene variants in the HL group and the NC group were shown in Table 3 and Fig. 1. Of these, in the NC group, the AF of most gene variants was comparable to the East Asian AF provided by the gnomAD database, including the *GJB2* c.176del16 variant, c.35delG variant, and c.299delAT variant; *SLC26A4* c.2168A > G variant, c.1174A > T variant, c.1226G > A variant, c.1229C > T variant, c.1975G > C variant, c.2027T > A variant, and c.1707+5G > A variant; mtDNA 12S rRNA m.1494C > T variant and m.1555A > G variant. In contrast, the AF of *GJB2* c.235delC variant; *GJB3* c.538C > T variant; *SLC26A4* c.919-2A > G variant was significantly larger than the East Asian AF provided by gnomAD database (Table .3 and Fig. 1). In the HL group, the AF of some gene variants was significantly higher than the NC group, including *GJB2* c.176del16 variant, c.235delC variant and c.299delAT variant; *SLC26A4* c.2168A > G variant, c.919-2A > G variant, c.1226G > A variant, c.1229C > T variant and c.1975G > C variant (Table .3).

Table 1
Age characteristics of subjects included in this study.

Age	Hearing loss group (n = 689)			Normal control group (n = 1489)		
	Total, *n*	Male, *n*	Female, *n*	Total, *n*	Male, *n*	Female, *n*
0–1 year	575	335	240	1384	788	596
1–5 years	47	16	31	44	20	24
Above 5 years	67	37	30	61	26	35

n:number.

Table 2
Pathogenicity classification of 15 deafness gene variants.

Gene	Nuclei acid change	Amino acid change	Variant type	Classification	ACMG criteria (s)
GJB2	c.176del16	p.Gly59fs	Frameshift	P	PVS1+PM3_VeryStrong + PS4_Moderate + PM2_Supporting + PS3_Supporting
	c.235delC	p.Leu79fs	Frameshift	P	PVS1+PM3_VeryStrong + PS4+PM3_Moderate + PM2_Supporting + BS1
	c.35delG	p.Gly12fs	Frameshift	P	PVS1+PM3_VeryStrong + PS4+BA1
	c.299delAT	p.His100fs	Frameshift	P	PVS1+PM3_VeryStrong + PS4_Moderate + PM2_Supporting + PS3_Supporting
GJB3	c.538C > T	p.Arg180Ter	Nonsense	VUS	BS2+PS4_Supporting + PS3_Moderate + PM2_Supporting
SLC26A4	c.2168A > G	p.His723Arg	Missense	P	PM3_VeryStrong + PP3_Strong + PS1+PS3_Moderate + PM5+PM2_Supporting + PP2
	c.919-2A > G	p.?	Splice	P	PVS1+PM3_VeryStrong + PP1_Strong + BS1
	c.1174A > T	p.Asn392Tyr	Missense	P	PM3_VeryStrong + PP3_Strong + PS3_Supporting + PM2_Supporting
	c.1226G > A	p.Arg409His	Missense	P	PM3_VeryStrong + PS4+PP3_Strong + PP1_Strong + PM2_Supporting
	c.1229C > T	p.Thr410Met	Missense	P	PM3_VeryStrong + PP1_Strong + PP3_Strong + PS3_Supporting + PM2_Supporting + PP4
	c.1975G > C	p.Val659Leu	Missense	P	PM3_Strong + PS4+PM2_Supporting + PP2+PS3_Supporting
	c.2027T > A	p.Leu676Gln	Missense	P	PM3_Strong + PS4_Moderate + PM5+PM2_Supporting + PP2+PP3
	c.1707+5G > A	p.?	Intron	P	PM3_VeryStrong + PS4_Supporting + PM2_Supporting + PP3
mtDNA 12S rRNA	m.1494C > T	NA	NA	LP	PS4+PP1+PS3_Supporting
	m.1555A > G	NA	NA	LP	PS4+PP1_Moderate + PS3_Supporting

3.4. Comparison of genetic testing diagnosis between hearing loss group and normal control group

Table 4 shows that the difference in diagnosis rate and carrier rate of deafness gene variants between the group with hearing loss and the normal control group was statistically significant ($\chi^2 = 206.303$; P = 0.000). Fig. 2 shows that in the hearing loss group, the rates of diagnosed patients and carriers were higher than in the normal control group; the rate of individuals in whom no gene variant was detected was lower than in the normal control group.

4. Discussion

Non-syndromic hereditary hearing loss exhibits extreme genetic heterogeneity. To date, no fewer than 6000 disease-causing variants have been identified in no fewer than 110 genes [17]. Studies in different regions of China have shown that variant rates for deafness tests in newborns in Beijing, Wuhan, South China, and Northwest China are 4.508 %, 4.51 %, 2.976 %, and 4.04 %, respectively [13,18–20]. In our study, subjects were divided into the HL group and the NC group. In the NC group, the total allele frequency of all detected variants was 3.4 % (Table 3), which was the average carrier rate in different regions of China. In the HL group, the total allele frequency of all detected variants was 16.6 % (Table 3), which was much higher than that in the NC group. It is worth noting that compared with the East Asian allele frequencies listed in the gnomAD database, the allele frequencies in our study were significantly increased for the *GJB2* c.235delC variant, the *SLC26A4* c.919-2A > G variant, and the *GJB3* c.538C > T variant (Fig. 1), suggesting that the carrier rate of these variants was underestimated in Henan, China. Therefore, for each region, it is necessary to formulate allele frequencies that are appropriate for each geographic feature.

The severity of hearing loss varies depending on the type of genes and variants. In most populations, severe to profound autosomal recessive non-syndromic HL is most commonly caused by pathogenic variants in the *GJB2* gene [21]. Among the *GJB2* gene variants associated with autosomal recessive non-syndromic HL, the most common variants are c.35delG [22] (2%–4% for persons of Nordic descent) and c.235delC [23] ((carry in Japanese 1%–2%).Our study showed that in Henan, China, the allele frequency of *GJB2* c.235delC variant was the highest, 7.6 % in the HL group and 0.7 % in the NC group (Table .3), which was consistent with previous genetic studies of the national population HL [6]. The *SLC26A4* gene is associated with autosomal recessive Pendred syndrome/enlarged vestibular aqueduct [24]. Among them, c.919-2A > G variant and c.2168A > G variant are the most common pathogenic variants in Chinese, Japanese and Korean populations [25–27]. Our study showed that the two variants were also the most frequently detected *SLC26A4* variants in Henan, China (Table 3), which is consistent with previous studies [6].

Variants in the *GJB3* gene were originally shown to be the disease-causing gene for autosomal dominant nonsyndromic deafness in the Chinese population, and c.547G > A and c.538C > T were the two most common variants of the *GJB3* gene [28]. However, with the accumulation of a large number of cases, the pathogenicity of the *GJB3* c.538C > T variant became controversial. Some studies [29] showed that 0.40 % of patients but only 0.24 % of normal individuals had the *GJB3* c.538C > T variant, suggesting that detection of the *GJB3* c.538C > T variant in the context of autosomal dominant nonsyndromic deafness was equivocal. Our study showed that the allele frequency of *GJB3* c.538C > T variant was 0.29 % in the HL group and 0.30 % in the NC group (Table .3), and the difference in allele frequency between these two groups was not statistically significant. A query of the ClinGen database revealed that curation of the *GJB3* gene with erythrokeratoderma variabilis was rated as "strong," but curation of the *GJB3* gene with nonsyndromic genetic hearing

Table 3
Calculation of deafness gene variants' allele frequency in hearing loss group and normal control group.

Nuclear gene	Site and nucleotide change	Amino acid change	Hearing loss group (n = 689)			Normal control group (n = 1489)			East Asian AF in gnomAD
			Heterozygote	Homozygotes	Allele frequency	Heterozygote	Homozygotes	Allele frequency	
GJB2	c.176del16	p.Gly59fs	4	0	0.00290276	2	0	0.00067159	0.0001631
	c.235delC	p.Leu79fs	63	21	0.07619739	33	0	0.01108126	0.006515
	c.35delG	p.Gly12fs	1	0	0.00072569	0	0	0	0
	c.299delAT	p.His100fs	25	0	0.01814224	6	0	0.00201478	0.0009023
GJB3	c.538C > T	p.Arg180Ter	4	0	0.00290276	9	0	0.00302216	0.001103
SLC26A4	c.2168A > G	p.His723Arg	11	1	0.00943396	6	0	0.00201478	0.001604
	c.919-2A > G	p.?	36	8	0.03773585	30	0	0.01007388	0.005064
	c.1174A > T	p.Asn392Tyr	1	0	0.00072569	2	0	0.00067159	0.00005438
	c.1226G > A	p.Arg409His	4	0	0.00290276	1	0	0.0003358	0
	c.1229C > T	p.Thr410Met	3	0	0.00217707	1	0	0.0003358	0.0003511
	c.1975G > C	p.Val659Leu	11	0	0.00798258	2	0	0.00067159	0.0002006
	c.2027T > A	p.Leu676Gln	2	0	0.00145138	2	0	0.00067159	0
	c.1707+5G > A	p.?	2	0	0.00145138	3	0	0.00100739	0.0001092
Mitochondrial gene	Site and nucleotide change					Hetero plasmic AC (heteroplasmy level:0.10–0.95)	Homoplasmic AC (heteroplasmy level≥0.95)	Hetero plasmic AF	
mtDNA 12S rRNA	m.1494C > T		NA	NA	NA	1	0	0.0003358	0
	m.1555A > G		NA	NA	NA	4	0	0.00134318	0.0006748
Total			167	30	0.16618287	102	0	0.03425118	

AF: allele frequency; NA: not applicable.

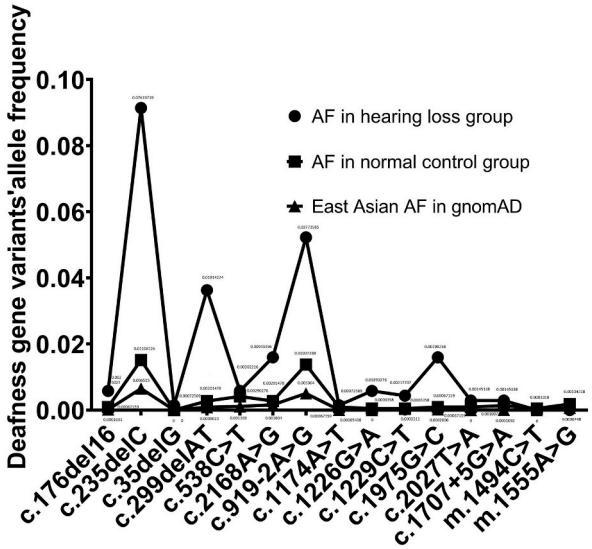

Fig. 1. Comparison of deafness gene variants' allele frequency between hearing loss group and normal control group AF: allele frequency; N:total number of individuals; n1: number of homozygotes; n2: number of heterozygotes; AF = (2n1+n2) /2 N; Hearing loss group (n = 689); Normal control group (n = 1489).

Table 4
Comparison of genetic testing diagnosis between Hearing loss group and Normal control group (($P < 0.05$ was statistically significant).

Group(s)	Total	Diagnosed patients	Carriers	Not detected out	χ^2	*P*
Hearing loss	689	62 (9.0 %)	106 (15.4 %)	521 (75.6 %)	206.303	0.000
Normal control	1489	0 (0)	98 ((6.6 %)	1391 (93.4 %)		

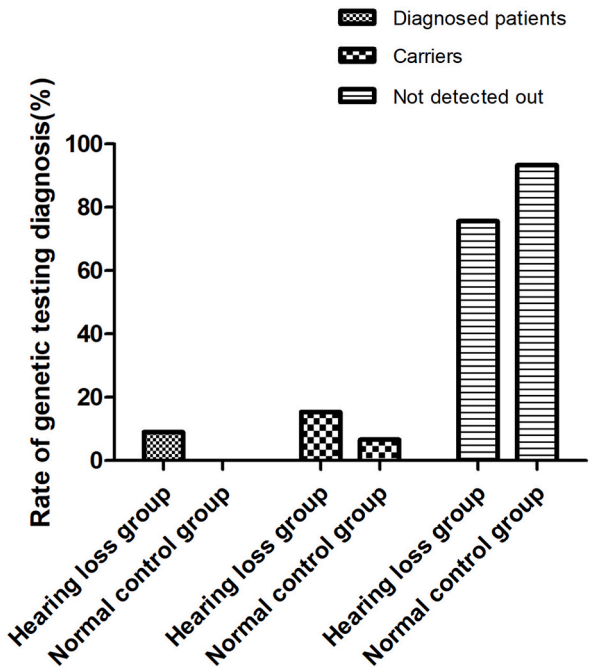

Fig. 2. Rate of genetic testing diagnosis between hearing loss group and normal control group ($\chi^2 = 206.303$; P = 0.000); Hearing loss group (n = 689); Normal control group (n = 1489).

loss was rated as "controversial." This suggests that the association between erythrokeratodermia variabilis and the *GJB3* gene is more definite and the *GJB3* gene variant for nonsyndromic genetic hearing loss is uncertain. Pathogenicity classification of c.538C > T of the *GJB3* gene according to ACMG guidelines [16] suggested that the c.538C > T variant was classified as "uncertain significance (VUS)" because of its high carrier rate in the normal population (BS2) (Table .2). At the same time, according to the ACGS guidelines (https://www.acgs.uk.com/quality/best-practice-guidelines/), this variant can be subdivided into "ice cold VUS", which is a degree close to "likely benign".All this evidence suggests that the *GJB3* c.538C > T variant may not contribute to hearing loss. This finding helps in genetic counseling and rational prediction of the risk for deafness caused by the *GJB3* c.538C > T variant. In addition, Fig. 1 showed that both m.1555A > G and m.1494C > T were present in the normal control group and not in the group with hearing loss, which allowed subjects to make an early prediction about the possibility of drug-induced deafness.

When the diagnosis rate of genetic tests was compared between the HL group and the NC group, there was a statistically significant difference in the diagnosis rate of genetic tests between the 2 groups ($\chi^2 = 206.303$; P = 0.000) (Table .4). This suggests that current bio-gene chip array technology is suitable for performing hearing loss testing on a large scale while providing diagnostic aids to underserved patients. However, as shown in Fig. 2, the detection rate of carriers in the HL group was significantly higher than in the NC group, suggesting that some carriers may be due to the presence of other pathogenic variant alleles in trans not included in these 15 deafness gene variants, leading to missed detection. In addition, there are still a substantial number of patients in the HL group in whom the 15 deafness gene variants were not detected (Fig. 2), which does not rule out the possibility that other pathogenic deafness gene variants cause the subjects' hearing loss.

5. Conclusions

In summary, our study showed that in the hearing loss population of Henan, China, the total allele frequency of all detected variants was 16.6 %. Comparative analysis showed that the allele frequencies of *GJB2* c.235delC variant, *GJB3* c.538C > T variant, and *SLC26A4* c.919-2A > G variant in Henan, China, were significantly higher than those of the East Asian population average in the gnomAD database. At the same time, our study demonstrated that the GJB3 c.538C > T variant is not the disease-causing variant of hearing loss, providing support for genetic counseling and rational prediction of risk for deafness. However, due to limitations in sample size and the number of variants tested, calculations of allele frequencies may be biased and there is a possibility of missing other genes and other variants. For subjects with typical hearing loss phenotypes but no related gene mutations have been detected, we will conduct further tests such as whole exome sequencing on these individuals.

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Ethics statement

This study was reviewed and approved by the Institutional Review Board of the Third Affiliated Hospital of Zhengzhou University, with the approval number: 2021-019-01.The submission has obtained informed consent from all participants.

Data availability statement

Data will be made available on request.

Supplementary content related to this article has been published online at [https://www.editorialmanager.com/heliyon/download.aspx?id=3403494&guid=6c145b9e-914a-4395-9995-a6b22cd7bc09&scheme=1], [https://www.editorialmanager.com/heliyon/download.aspx?id=3403495&guid=fb8de3e5-04b6-4121-b0a3-824f7f5fe60e&scheme=1] and [https://www.editorialmanager.com/heliyon/download.aspx?id=3403496&guid=c186a526-e397-4da7-964c-1c830afdba16&scheme=1]

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests.

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Abbreviations

Hearing loss HL
Autosomal recessive: AR

Autosomal dominant AD
Sensorineural hearing impairment: SNHL
Pathogenic P
Likely pathogenic LP
Uncertain significance VUS
Likely benign LB
Benign B
Allele frequency AF
Normal control NC

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e21185.

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