

# Gene mutation analysis and genetic counseling for patients with non-syndromic hearing loss in Linyi region

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**Abstract.** Through gene mutation analysis of patients with non-syndromic hearing loss (NSHL) correct genetic counseling for patients with NSHL and their family members were provided. A total of 116 patients suffering from NSHL were selected, and Sanger sequencing was applied to analyze 31 mutation sites in four deafness genes [gap junction  $\beta$ -2 (*GJB2*), solute carrier family 26, member 4 (*SLC26A4*), *GJB3* and mitochondria 12S ribosomal ribonucleic acid (*12SrRNA*)]. Based on detection results, for the families with reproductive needs, amniotic fluid was extracted from pregnant women during proper gestational weeks to identify fetal genotypes and predict hearing state. Among 116 patients with NSHL, 51 patients carrying definite pathogenic mutation were found, including 35 patients with *GJB2* mutations, 14 patients with *SLC26A4* gene mutations and 2 patients with mitochondrial deoxyribonucleic acid *12SrRNA* (mtDNA *12SrRNA*) mutations. No *GJB3* gene mutation site was detected. In addition, prenatal diagnosis to 17 pregnant women who had given birth to babies with deafness was performed, and results suggested that genotypes of 6 fetuses were consistent with those of probands, genotypes of 8 fetuses were consistent with those of their parents, and no mutation was found in the other 3 fetuses. Gene mutation analysis of patients with NSHL can identify the etiology and provide appropriate genetic counseling and birth guiding for patients with NSHL and their family members. In addition, prenatal diagnosis to the families who plan to give birth again can avoid the natality of fetuses with hearing loss.

## Introduction

Hearing loss is one of the most common birth defects in China, and the incidence rate in newborns is 1/1,000-3/1,000 (1). Survey data from the World Health Organization (<http://www.who.int>)

have indicated that among 360 million individuals with hearing loss, there are 32 million children. There are many factors related with hearing loss, and genetic factors, environmental factors or interactions between the two factors can cause deafness (2). It is reported that 50% of deafness is associated with genetic factors, of which 70% are classified into non-syndromic hearing loss (NSHL), and approximately 80% of NSHL are caused by autosomal recessive inheritance (3,4). Previous molecular etiology studies have shown that four NSHL genes including gap junction  $\beta$ -2 (*GJB2*, OMIM: 121011), *GJB3* (OMIM: 603324), solute carrier family 26, member 4 (*SLC26A4*, OMIM: 605646) and mitochondrial deoxyribonucleic acid *12SrRNA* (mtDNA *12SrRNA*, OMIM: 561000) are most common (5-9). Therefore, mutation screening of the four genes in people provides an effective method for the diagnosis of hearing loss. At the same time, prenatal diagnosis to the families with inherited hearing loss or the risk of deafness before the birth of other babies can avoid the birth of baby with hearing loss.

In this study, the above pathogenic genes in 116 patients with hearing loss were directly sequenced by polymerase chain reaction (PCR) amplified products, the causes of hearing loss in family members were identified, and the technical support of prenatal diagnosis was conducted, so as to guide families with hearing loss delivering descents with normal hearing.

## Patients and methods

**Subjects.** Following the principle of informed consent, 116 NSHL patients who were aged from 3 months to 39 years and had genetic counseling in the Prenatal Diagnosis Center of Women and Children's Health Care Hospital of Linyi (Linyi, China) from January 2015 to June 2017 were collected. Hearing loss was >70 dB. Detailed medical history of deaf patients was collected, and patients with systemic diseases, dysnoesia, syndromic hearing loss, history of meningitis, otitis media or ear trauma were excluded. The study was approved by the Ethics Committee of Women and Children's Health Care Hospital of Linyi (Linyi, China). Signed written informed consents were obtained from the patients or the guardians.

## Methods

**DNA extraction.** A total of 2 ml venous blood from patients was collected by the ethylenediaminetetraacetic acid-Na (EDTA-Na) anti-coagulation tube. For pregnant women who

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would receive prenatal diagnosis, 10 ml amniotic fluid was extracted via amniocentesis during 17-20 weeks of gestation. According to the manufacturer's instructions, DNA was extracted by the kit (Tiangen Biotech Co., Ltd., Beijing, China), a part of which was subjected to quantitative and purity tests by an ultraviolet spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and preserved at -20°C.

**PCR amplification and sequencing analysis.** PCR amplification reaction, PCR products purification and sequencing analysis of target DNA fragment were performed. Sequencing reaction was conducted by Shanghai Genome Pilot Institutes of Genomics and Human Health (Shanghai, China) with the use of an American ABI 373XL automated DNA sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Software including Chromas, BioEdit and SeqMan were used to make comparison and analysis of sequencing data with National Center for Biotechnology Information (NCBI) standard sequence and Cambridge Reference Sequence (CRS).

## Results

In this study, 31 mutation sites in the four common genes in Chinese were detected in 116 patients with hearing loss, and 51 patients were diagnosed with definite pathogenic mutations with a positive rate of 43.96%. The remaining 65 patients had no definite pathogenic mutations. The sequencing results are shown in Table I. A total of 35 patients were diagnosed with definite *GJB2* mutation-induced deafness, accounting for 30.17% (35/116) of all subjects. Among them, homozygous mutations were identified in 21 patients, and compound heterozygous mutations were found in 14 patients. Definite *GJB2* gene mutation-induced hearing loss was identified in 14 patients, of which the prevalence rate was 12.07% among all subjects. Among them, homozygous mutations were identified in 7 patients, and compound heterozygous mutations were found in 7 patients. A total of 2 (2/116, 1.72%) patients were found carrying mtDNA *12SrRNA* 1555A>G mutations, and they had administration history of aminoglycoside. No *GJB3* gene mutation site was detected. All in all, a total of six *GJB2* mutation sites (Fig. 1) and five *SLC26A4* mutation sites were identified (Fig. 2).

Among 116 patients with hearing loss, 17 were identified with mutations in the *GJB2* or *SLC26A4* gene. Subsequently, their parents received tests of genes associated with hearing loss, which indicated that they were carriers. Sequencing results are shown in Table II. All of the 17 families had the need for further fertility. During their next pregnancy, prenatal diagnosis was performed. The results suggested that the genotypes of 6 fetuses were consistent with those of the probands, so the 6 families decided to terminate the pregnancy. No mutation was found in 3 fetuses. The genotypes of the other 5 children were consistent with those of their parents. Follow-up results showed that their fetuses had normal hearing after birth.

## Discussion

Due to genetic heterogeneity and phenotypic diversity shown in patients with hearing loss, providing proper genetic counseling for patients is still a great challenge clinically. Up to

Table I. Results of genetic test of 51 patients with hearing loss.

Gene	Mutation site	No. of patients (case)	Total no. (case)	Proportion (%)			
<i>GJB2</i>	235delC/235delC	18	35	30.17			
	235delC/299-300delAT	7					
	235delC/176-191del16bp	1					
	235delC/511-512insAACG	1					
	235delC/30-35insG	1					
	299-300delAT/299-300delAT	2					
	109G>A/109G>A	1					
	109G>A/176-191del16bp	1					
	109G>A/299-300delAT	2					
	176-191del16bp/511-512insAACG	1					
	<i>SLC26A4</i>	IVS7-2A>G/IVS7-2A>G			7	14	12.07
		IVS7-2A>G/1226G>A			2		
IVS7-2A>G/2168A>G		3					
IVS7-2A>G/1614+1G>A		1					
IVS7-2A>G/2009T>C		1					
<i>12SrRNA</i>	1555A>G	2	2	1.72			
Total			51	43.96			

*GJB2*, gap junction  $\beta$ -2; *SLC26A4*, solute carrier family 26, member 4; *12SrRNA*, 12S ribosomal ribonucleic acid.

now, hundreds of genes causing hereditary hearing loss have been identified. Most of NSHL are induced by mutations in the four genes including *GJB2*, *SLC26A4*, mtDNA *12SrRNA* and *GJB3* (5-9). The findings provide theoretic basis for the implementing of prenatal diagnosis of deafness genes. In this study, Sanger sequencing was employed to analyze the 31 mutation sites in these four genes in 116 NSHL patients.

In this study, 35 (30.17%) patients carrying *GJB2* mutations were detected, among which, 21 patients had *GJB2* genetic homozygous mutations, and 14 had *GJB2* compound heterozygous mutations. *GJB2* 235delC had the highest percentage, followed by 299\_300delAT. Epidemiological studies of *GJB2* mutations in deaf patients in different countries have revealed that 46% of Hungarian patients with NSHL were caused by *GJB2* mutations, 24.3% of patients with NSHL among Americans were induced by *GJB2* mutations, and 12.2-33% of patients with NSHL among Chinese were due to *GJB2* mutations (10). In this study, it was found that 30.17% of patients with NSHL were caused by *GJB2* mutations, which suggested that the *GJB2* gene mutation plays an important role in hereditary NSHL. The types of *GJB2* mutation are different in different regions or races. Studies of Yu *et al.* (11) and Dai *et al.* (12) have suggested that the mutation types of the most common *GJB2* in the Chinese people are 235delC, 299\_300delAT and 176\_191del16bp. In this study, 235delC accounted for the highest percentage, followed by 299\_300delAT and 109G>A. A study by Zhang *et al.* (13) found that the mutation types of *GJB2* in patients with HSHL are consistent in Linyi.

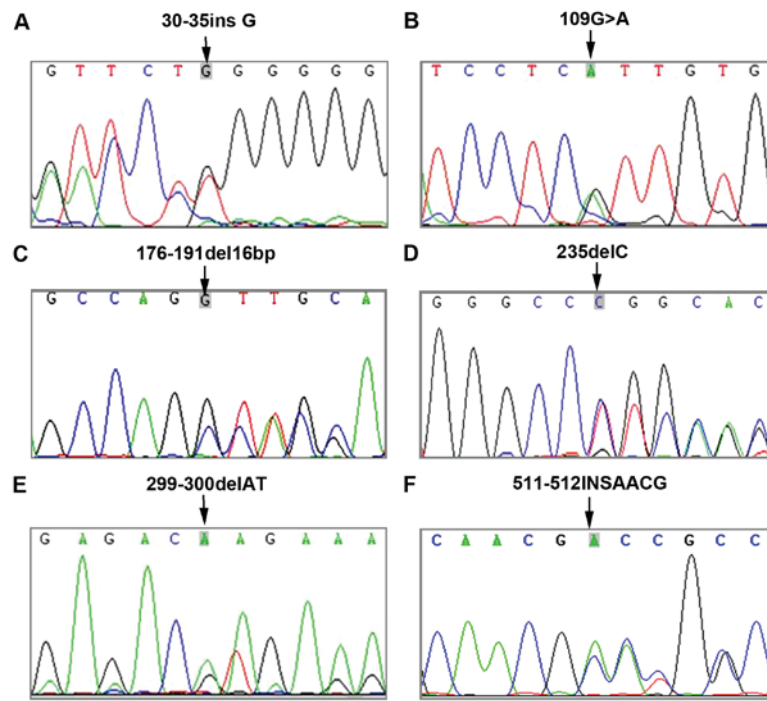


Figure 1. *GJB2* mutation sites and types. (A) 30-35ins G, (B) 109G>A, (C) 176-191del16bp, (D) 235delC, (E) 299-300delAT and (F) 511-512insAACG heterozygous mutations. Nucleotide mutation sites are indicated by arrows. *GJB2*, gap junction  $\beta$ -2.

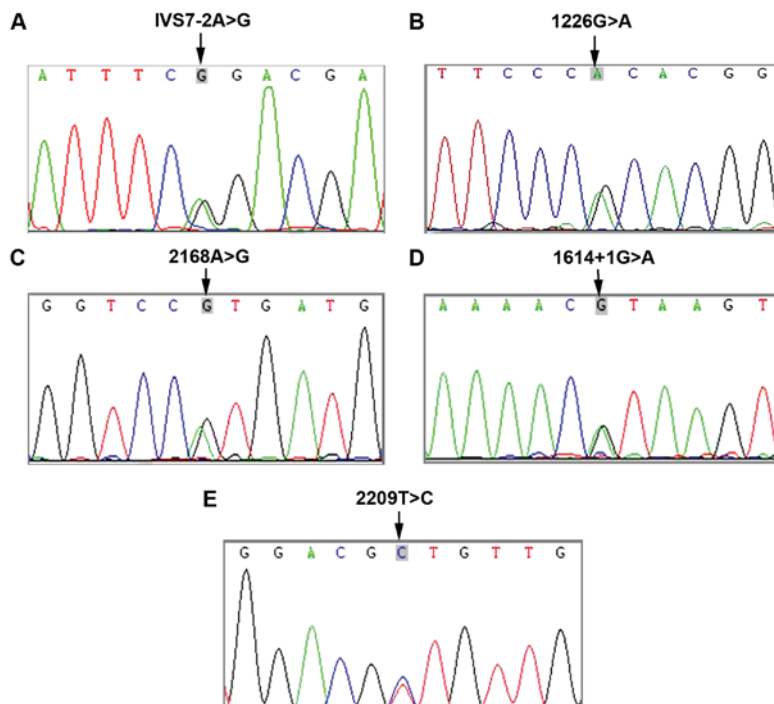


Figure 2. *SLC26A4* mutation sites and types. (A) IVS7-2A>G, (B) 1226G>A, (C) 2168A>G, (D) 1614+1G>A and (E) 2009T>C heterozygous mutations. Nucleotide mutation sites are indicated by arrows. *SLC26A4*, solute carrier family 26, member 4.

*SLC26A4* gene mutations will cause large vestibular aqueduct syndrome, of which the mutation frequency ranks second in patients with NSHL, next to *GJB2* gene (14). Chai *et al* (15) reported that the incidence rate of *SLC26A4* gene mutations in Chinese patients with NSHL is 11.2%, and in this study it was 12.07%. A study on patients with NSHL in China by Yuan *et al* (16) showed that the most common *SLC26A4*

mutations in China are IVS7-2A>G, 2168A>G and 1174A>T. In this study, it was found that the most common mutation in *SLC26A4* was IVS7-2A>G, which is consistent with the findings of Yuan *et al* (16).

In this study, 2 patients were found carrying the mtDNA *12SrRNA* 1555A>G mutations, which accounted for 1.72%. Carrying mtDNA *12SrRNA* mutations can lead to the

Table II. Sequencing results of the family members of the 17 hearing loss patients with prenatal diagnosis.

No. of case	Proband	Father	Mother	Fetus	Follow-up results
1 <sup>b</sup>	IVS7-2A>G/2009T>C	2009T>C/WT	IVS7-2A>G/WT	WT/WT	Normal hearing
2 <sup>a</sup>	299-300delAT/299-300delAT	299-300delAT/WT	299-300delAT/WT	299-300delAT/299-300delAT	Induced labor
3 <sup>a</sup>	235delC/235delC	235delC/WT	235delC/WT	235delC/WT	Normal hearing
4 <sup>a</sup>	235delC/235delC	235delC/WT	235delC/WT	235delC/235delC	Induced labor
5 <sup>a</sup>	235delC/235delC	235delC/WT	235delC/WT	235delC/235delC	Induced labor
6 <sup>a</sup>	235delC/235delC	235delC/WT	235delC/WT	WT/WT	Normal hearing
7 <sup>a</sup>	35insG/235delC	235delC/WT	35insG/WT	235delC/WT	Normal hearing
8 <sup>a</sup>	235delC/235delC	235delC/WT	235delC/WT	235delC/WT	Normal hearing
9 <sup>a</sup>	235delC/235delC	235delC/WT	235delC/WT	235delC/WT	Normal hearing
10 <sup>b</sup>	IVS7-2A>G/IVS7-2A>G	IVS7-2A>G/WT	IVS7-2A>G/WT	IVS7-2A>G/IVS7-2A>G	Induced labor
11 <sup>b</sup>	IVS7-2A>G/1226G>A	IVS7-2A>G/WT	1226G>A/WT	1226G>A/WT	Normal hearing
12 <sup>a</sup>	235delC/511-512insAACG	511-512insAACG/WT	235delC/WT	235delC/WT	Induced labor
13 <sup>a</sup>	235delC/235delC	235delC/WT	235delC/WT	235delC/WT	Induced labor
14 <sup>a</sup>	235delC/235delC	235delC/WT	235delC/WT	WT/WT	Normal hearing
15 <sup>b</sup>	IVS7-2A>G/IVS7-2A>G	IVS7-2A>G/WT	IVS7-2A>G/WT	IVS7-2A>G/IVS7-2A>G	Induced labor
16 <sup>a</sup>	235delC/235delC	235delC/WT	235delC/WT	235delC/235delC	Induced labor
17 <sup>b</sup>	IVS7-2A>G/IVS7-2A>G	IVS7-2A>G/WT	IVS7-2A>G/WT	IVS7-2A>G/WT	Normal hearing

<sup>a</sup>For *GJB2* gene, <sup>b</sup>for *SLC26A4* gene. *GJB2*, gap junction  $\beta$ -2; *SLC26A4*, solute carrier family 26, member 4; WT, wild-type.

occurrence of aminoglycoside drug-induced deafness (17). However, the proportion of patients with drug-induced deafness is small due to the strict application requirements of aminoglycosides in clinical practice. In this study, no carrier of the definite *GJB3* mutation site was discovered, which might be resulted from the small sample size. Therefore, further verification is needed.

In this study, prenatal diagnosis to 17 families with hearing loss induced by definite pathogenic mutation suggested that all their parents were carriers. Moreover, genetic analysis and genetic counseling showed that genotypes of 6 fetuses were in line with those of probands, so the 6 families decided to terminate the pregnancy. No mutation was found in 3 fetuses, and the genotypes of the other 8 fetuses were consistent with their parents. Follow-up results showed that the 11 fetuses had normal hearing after birth.

The deafness genes including *GJB2*, *GJB3* and *SLC26A4* are mainly autosomal recessively inherited, while *12SrRNA* is maternally inherited (18,19). Detection of deafness couples and parents of patients with mutations in *GJB2*, *SLC26A4* and *12SrRNA* genes were performed to determine the genotypes and estimate recurrence risk on the basis of genotypes. If the pathogenic genes carried by parents are located on different genes, their descendants are at a lower risk of illness and do not need prenatal diagnosis. If the parents are carriers of the same gene mutation (*GJB2* or *SLC26A4*), their offspring have a 25% risk of deafness. Prenatal diagnosis is recommended for a further birth. If mothers carry *12SrRNA* mutations, for their siblings, mother as well as offspring, the administration of aminoglycosides should be banned for life, which can effectively prevent the occurrence of deafness.

To sum up, application of gene sequencing is very useful to the genetic testing for patients with NSHL. In addition, this method is simple in operation and can be applied in clinical practice, providing accurate genetic counseling clinically.

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#### Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

#### Authors' contributions

HL drafted the manuscript. HL and JQ were mainly devoted to DNA extraction. JZ and YH were responsible for PCR. All authors read and approved the final manuscript.

#### Ethics approval and consent to participate

The study was approved by the Ethics Committee of Women and Children's Health Care Hospital of Linyi (Linyi, China). Signed written informed consents were obtained from the patients or the guardians.



**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

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