

Genetic screening of common genetic deafness in 60,391 women of childbearing age and intervention of birth defects

Linyuan Niu, Liqin Liu, Jinjun Tian, Wei Chen, Chunxiao Zhang, Xinqiang Lan

Weihai Second Municipal Hospital Affiliated to Qingdao University, Weihai Maternal and Child Health Hospital, Weihai, China

Submitted: 11 October 2021; **Accepted:** 22 January 2022

Online publication: 3 February 2022

Arch Med Sci 2024; 20 (1): 113–123

DOI: <https://doi.org/10.5114/aoms/146024>

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Corresponding author:

Xinqiang Lan

Weihai Second Municipal

Hospital

Affiliated to Qingdao

University

264200, Weihai, China

E-mail: xinqiangl@126.com

Abstract

Introduction: At least 60% of cases of severe hearing loss result from genetic factors. In this study genetic screening was carried out for common genetic deafness in women of childbearing age to prevent deafness and birth defects via providing genetic counseling and follow-up services for high-risk families.

Material and methods: In total 60,391 pre-pregnancy/early-gestation women who received treatment in second-level or above hospitals in Weihai from February 2017 to December 2019 were selected. Venous or peripheral blood was collected to make dried blood slices on filter paper to extract genomic DNA, and high-throughput sequencing was applied to detect 20 variant sites in 4 common deafness genes (*GJB2*, *GJB3*, *SLC26A4* and mitochondrial *12S rRNA*) in the Chinese population. The spouses of women with deafness gene variants were sequenced.

Results: In total 3,761 carriers with deafness gene variants were detected in 60,391 women of childbearing age, with a carrier rate of 6.2%. Among them, 1,739 women (2.88%) only carried *GJB2* pathogenic variants. The carrying rate of c.235delC in *GJB2* pathogenic variants was the highest at 2.08%. 1,553 women (2.58%) only carried *SLC26A4* pathogenic variants. The carrying rate of c.919-2A>G in *SLC26A4* pathogenic variants was the highest at 1.63%. 300 women (0.5%) only carried *GJB3* variants, and 125 women (0.2%) carried the mitochondrial drug-sensitive gene variant.

Conclusions: This screening model will greatly reduce the birth rate of children with hearing disabilities and is an effective way to prevent newborn deafness. In addition, genetic screening provided the related knowledge of hereditary deafness, especially strengthening genetic counseling and the clinical decision making from the genetic screening.

Key words: hereditary deafness genes, women of childbearing age, genetic screening, genetic counseling.

Introduction

Hearing loss is a universal auditory dysfunction. Severe hearing loss strongly impacts human daily communication and social activities. Based on the latest WHO data in 2019, there are about 466 million hearing-impaired people worldwide, accounting for 5% of the world's total population [1]. The Second National Sample Survey on Disabilities in 2006 revealed that there were 27.8 million people with hearing disabilities in China, of whom 20.04 million were simply people with hearing disabili-

ties, accounting for 24.2% of the total [2]. Neonatal deafness incidence is 1–3‰, that is, about 30,000 deaf children are born every year. The China Birth Defects Prevention Report (2012) pointed out that hearing disabilities have become the second largest birth defect in China. Therefore, preventing hearing disabilities and intervening in birth defects are essential aspects to improve the quality of the Chinese population. Studies have shown that at least 60% of cases of severe hearing loss result from genetic factors [3]. Hereditary hearing loss mainly involves four types: autosomal recessive inheritance (approximately 80%), autosomal dominant inheritance (approximately 15–20%), mitochondrial inheritance (approximately 1%) and sex-linked inheritance (approximately 1%) [4]. Based on a molecular epidemiological survey of deafness in China, *GJB2*, *GJB3*, *SLC26A4* and mitochondrial *12S rRNA* are the most universal deafness genes in Chinese [5, 6]. The hereditary hearing loss resulting from *GJB2* and *SLC26A4* follows autosomal recessive inheritance. Carriers of mitochondrial *12S rRNA* variants are sensitive to aminoglycoside drugs, belonging to maternal inheritance, whose deafness rate in Chinese with hearing loss is up to 34% [5]. However, survey data reveal that among the normal population in China, about 6% are carriers of common deafness gene variants [7, 8], and most deaf newborns each year are born to families without a family history of deafness. It can be seen that although deafness gene screening and intervention for people with hearing loss can prevent birth of deaf children by deaf parents to a certain extent, it cannot effectively prevent normal parents from giving birth to children with hereditary hearing loss. Therefore, screening of common deafness genes in the normal population before the marriage check or pregnancy and early gestation, early detection of couples with pathogenic variants in the same gene and corresponding intervention measures are effective means to prevent hearing disabilities.

In this study, we conducted common deafness gene screening in 60,391 women of childbearing age in Weihai, genetic counseling in high-risk families and corresponding intervention measures, which can effectively prevent these families from giving birth to children with hearing loss, thus reducing the incidence in the region and providing genetic counseling and clinical decision making from the genetic screening.

Material and methods

Research subjects

In total 60,391 pre-pregnancy/early-pregnancy women who received treatment in second-level or above hospitals in Weihai between February 2017

and December 2019 were selected, ranging in age from 18 to 47, and gestational age less than 15 + 6 weeks. Among them, there were in total 53 women with different degrees of hearing loss, and 17 with family history of hearing disabilities. This study was approved by the Medical Ethics Committee of Weihai Maternal and Child Health Hospital, and all research subjects signed informed consent forms.

All patients completed the survey by answering questions and filling in the “deafness questionnaire survey form.” The details were as follows: (a) basic information: name, gender, date of birth, nationality, marital status, contact information, etc.; (b) morbidity status: age of onset, presence of other associated symptoms (vertigo, tinnitus, etc.), related medical history (infectious diseases, etc.), history of use of aminoglycoside antibiotics, long-term noise exposure, otitis media or traumatic history of the ear, etc.; (c) the development condition of language, whether wearing a hearing aid or cochlea, etc.; and (d) whether other systemic diseases co-existed (eyes, bones, intellectual disability, etc.). Ear examination and audiological evaluations including pure-tone audiometry, immittance testing, auditory brainstem response, and auditory steady-state response were performed for all hearing impaired patients. Physical and neurological examinations were carried out with special attention to renal and ophthalmologic differences to exclude those with syndromic deafness (Figure 1).

DNA extraction

Venous or peripheral blood of our subjects was collected to prepare dry blood slices on filter papers (Whatman 903), each with at least 2 blood spots. An automatic punching machine was used to take a slice of dried blood sample to corresponding 96-well plates complemented with reagents, and it was sealed with aluminium film. The plates were placed on a heater to boil, and cooled for later use. QIAamp DNA Blood Mini Kit (Qiagen, Germany) was used to extract genomic DNA from each blood sample following the manufacturer’s instructions, and a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) was used to evaluate the quantity and quality of extracted DNA.

Library construction

Twenty common variant sites of *GJB2*, *SLC26A4*, and *GJB3*, as well as *mtDNA*, were detected by using a deafness diagnostic screening panel (CapitalBio Genomics Co., Ltd., China). Library construction, quality control, and sequencing template preparation were performed according to the instructions of a Blood DNA LQ kit. The PCR method was used to amplify 20 variant sites in *GJB2*, *GJB3*,

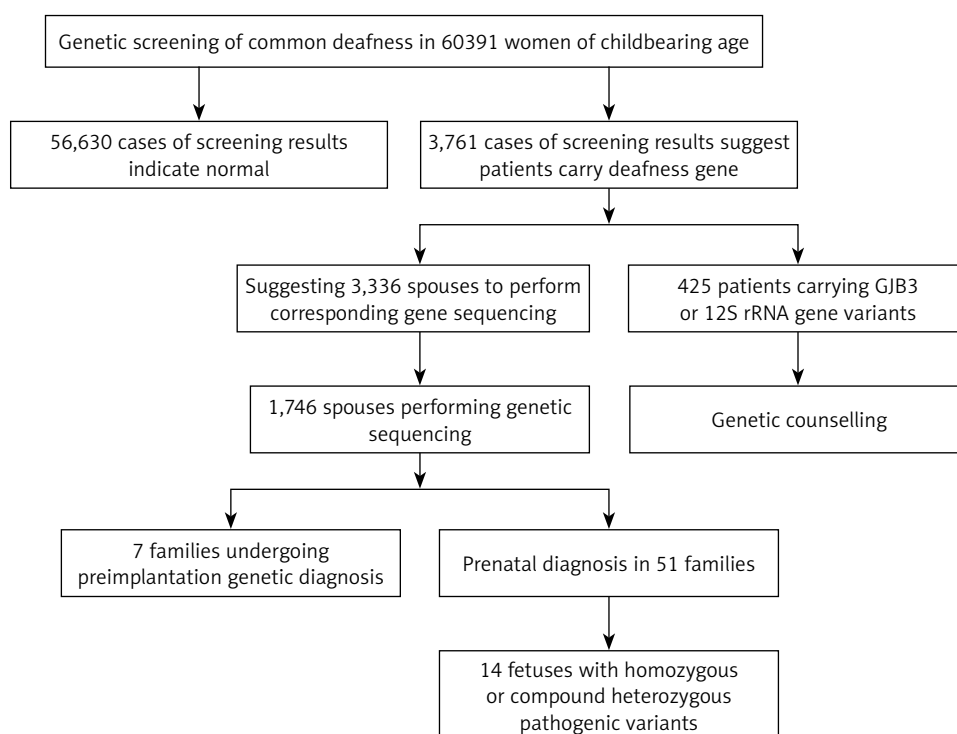


Figure 1. Research procedures

SLC26A4 and mitochondrial *12S rRNA* genes, then sequencing labels were added and purified to complete library construction.

Deafness gene detection

Via the high-throughput sequencing method and gene sequencer BGISEQ-500 to detect deafness genes, 20 pathogenic sites in 4 genes were detected, including in *GJB2* c.35delG, c.167delT, c.176_191del16, c.235delC and c.299_300delAT, *GJB3* gene c.538C>T and c.547G>A, in *SLC26A4* c.281C>T, c.589G>A, c.919-2A>G, c.1174A>T, c.1226G>A, c.1229C>T, c.1707+5G>A, c.1975G>C, c.2027T>A, c.2162C>T and c.2168A>G, and in mitochondrial *12S rRNA* m.1494C>T and m.1555A>G.

For women who have been screened for the pathogenic sites in deafness genes, it is recommended that their spouses undergo corresponding gene sequencing, providing medication guidance for women with drug-sensitive gene variants. For couples who carry pathogenic variants in the same gene, genetic counseling was provided and corresponding intervention measures were taken. Postpartum neonatal hearing screening and follow-up for women with deafness gene mutations were done.

Results

Deafness gene screening results of 60,391 women

Among 60,391 pre-pregnancy/early-pregnancy women, in total 3,761 women carried common

deafness gene variants, with a total carrier rate of 6.23% (3,761/60,391) (Table I), 1724 (2.85%) carried only one *GJB2* variant, 1,534 (2.54%) only carried one *SLC26A4* variant, 300 (0.5%) only carried one *GJB3* variant, and 125 (0.2%) carried the drug-sensitive gene variant. There were 44 women (0.07%) carrying two deafness gene variants, of whom 38 carried both *GJB2* and *SLC26A4* variants, accounting for 0.06% of the total screened population, 3 carried both *GJB2* and *GJB3* variants, and 3 carried *SLC26A4* and *GJB3* variants at the same time. Additionally, there were 53 women with clinical manifestations of varying degrees of hearing loss, of whom 15 (0.02%) carried 2 pathogenic variants in *GJB2* at the same time, 19 (0.03%) carried 2 pathogenic variants in *SLC26A4* at the same time, 3 carried gene variants for drug-sensitive deafness, 4 only carried 1 variant in *GJB2* or *SLC26A4*, and 12 did not carry common variants.

GJB2. In this study, 1,780 women carried the *GJB2* variant, with a carrying rate of 2.92% (1,780/60,391). Among them, the carrying rate of c.235delC was the highest at 2.08%; c.299_300delAT was the second with 0.73%; the carrying rate of c.35delG was the lowest at 0.005% (Table II).

SLC26A4. In total 1,594 women carried the *SLC26A4* variant, with a carrying rate of 2.62% (1,594/60,391). Among them, the carrying rate of c.919-2A>G was the highest at 1.63%, followed by c.2168A>G, with a carrying rate of 0.42% (Table III).

Table I. Common deafness gene screening results of 60,391 cases

Variant types	Cases	Carrying rate (%) (n = 3761)	Carrying rate (%) (n = 60391)
<i>12S rRNA</i> 1555A>G homogeneous variant	97	2.58	0.16
<i>12S rRNA</i> 1555A>G heterogeneous variant	21	0.56	0.03
<i>12S rRNA</i> 1494C>T homogeneous variant	7	0.19	0.01
<i>GJB2</i> homozygous variant	9	0.24	0.01
<i>GJB2</i> compound heterozygous variant	6	0.16	0.01
<i>SLC26A4</i> homozygous variant	5	0.13	0.01
<i>SLC26A4</i> compound heterozygous variant	14	0.37	0.02
Total 1	159	4.23	0.26
<i>GJB2</i> heterozygous variant	1724	45.84	2.85
<i>SLC26A4</i> heterozygous variant	1534	40.79	2.54
<i>GJB3</i> compound heterozygous variant	300	7.98	0.50
Combined heterozygous variants in <i>GJB2</i> and <i>SLC26A4</i>	38	1.01	0.06
Combined heterozygous variants in <i>GJB2</i> and <i>GJB3</i>	3	0.08	0.005
Combined heterozygous variants in <i>SLC26A4</i> and <i>GJB3</i>	3	0.08	0.005
Total 2	3602	95.77	5.96
Total (1 + 2)	3761	100.00	6.23

The statistical results do not include polymorphic sites in the gene.

Table II. Statistics of *GJB2* variant types

Variant types	Variant sites	Cases	Carrying rate (%) (n = 60391)
Homozygous variant	c.235delC	6	0.01
	c.299_300delAT	3	0.005
Heterozygous variant	c.176_191del16	65	0.11
	c.235delC	1257	2.06
	c.299_300delAT	440	0.72
	c.35delG	3	0.005
Compound heterozygous variant	c.176_191del16/c.235delC	1	0.002
	c.235delC/c.299_300delAT	5	0.01
Total		1780	2.92

This statistical data contain 41 cases that carry both *GJB2* and other deafness gene variants.

Deafness gene sequencing results of spouses

Based on the female test findings, we recommend that the husbands of 3,336 women with *GJB2* or *SLC26A4* variants undergo corresponding gene sequencing. In total 1,746 women's spouses agreed to be tested. The detection rate was 52.3% (1746/3336) and 139 men carried *GJB2* or *SLC26A4* variants, with a carrying rate of 7.96% (139/1,746). Among them, 1,146 men were sequenced for *GJB2*; 77 were carrying known pathogenic variants, and 5 carrying suspected pathogenic variants or variants of unknown clinical significance. In total 600 men were sequenced for *SLC26A4*; 31 were carrying known pathogenic variants, and 26 carrying suspected

variants or variants of unknown clinical significance. From the results, the known pathogenic variants were mainly c.109G>A (2.18%), c.235delC (1.54%), c.299_300delAT (0.52%) and c.919-2A> in *SLC26A4* and G (0.98%) in *GJB2*. At the same time, in *SLC26A4* suspected pathogenic variants and variants of unknown clinical significance accounted for a relatively high proportion of 1.49% (Table IV).

Results of fetus receiving prenatal diagnosis

Based on the clinical manifestations and test results of couples, it was finally determined that 99 couples carried known or suspected pathogenic variants in *GJB2* or *SLC26A4*, and their offspring

Table III. Statistics of *SLC26A4* variant types

Variant types	Variant sites	Cases	Carrying rate (%) (n = 60391)
Homozygous variant	c.1229C>T	1	0.00
	c.919-2A>G	4	0.01
Heterozygous variant	c.1174A>T	114	0.19
	c.1226G>A	46	0.08
	c.1229C>T	75	0.12
	c.1975G>C	136	0.23
	c.2027T>A	31	0.05
	c.2168A>G	248	0.41
	c.281C>T	5	0.01
	c.589G>A	27	0.04
	c.1707+5G>A	16	0.03
	c.919-2A>G	877	1.45
Compound heterozygous variant	c.1174A>T/c.1226G>A	1	0.002
	c.919-2A>G/c.1174A>T	2	0.003
	c.919-2A>G/c.1226G>A	2	0.003
	c.919-2A>G/c.1229C>T	1	0.002
	c.919-2A>G/c.1975G>C	2	0.003
	c.919-2A>G/c.2027T>A	2	0.003
	c.919-2A>G/c.2168A>G	4	0.01
Total		1594	2.62

These statistical data include 41 cases that carry both *SLC26A4* and other deafness gene variants.

have a 25% risk of disease, which is in line with the prenatal indications for diagnosis. But among these 99 couples, 38 husbands carried the *GJB2* c.109G>A variant. At present, although the *GJB2* c.109G>A variant is considered to be a pathogenic variant, the hearing phenotype resulting from it is quite different: hearing can be normal or weakened, and it also can be manifested at birth or later, so if the offspring is diagnosed as a *GJB2* c.109G>A homozygous variant or compound heterozygous variant before birth, their hearing phenotype after birth cannot be predicted. Finally, after genetic counseling and full informed consent, in total 51 couples underwent prenatal diagnosis, including 6 with the *GJB2* c.109G>A variant (Table V), and 7 chose to undergo pre-implantation genetic diagnosis. The results of prenatal diagnosis showed that the offspring of 14 couples inherited deafness gene variants other than *GJB2* c.109G>A from their parents. It is most likely that they are hereditary deafness patients, accounting for 27.5%. The offspring of 2 couples were a *GJB2* c.235delC/c.109G>A compound heterozygous variant and a c.109G>A/c.299_300delAT compound heterozygous variant. The offspring of 23 couples may carry only one deaf gene variant or they did not inherit variants of their parents, and the predicted risk of the fetus with hereditary deafness is extremely low.

Among 125 women carrying drug-sensitive gene variants, 97 carried *12S rRNA* 1555A>G homogeneous variants, 21 carried *12S rRNA* 1555A>G heterogeneous variants, and 7 carried *12S rRNA* 1494C>T homogeneous variants (Table I), respectively, accounting for 0.16%, 0.03% and 0.01% of the total people screened. The follow-up of women and their maternal family members revealed 3 women with hearing disability and 1 uncle with hearing disability. Because drug-susceptibility gene mutations follow the maternal inheritance method, we issued medication guide cards to 125 carriers of drug-susceptibility gene mutations, informing them and their maternal family members that they should absolutely avoid using aminoglycoside drugs throughout their lives in case of deafness.

Follow-up results of the offspring's hearing

Our work conducted follow-up of the offspring's hearing of 3,761 female carriers; 21 carriers' children were finally diagnosed with hearing disabilities.

Among them, 18 children were tested for deafness genes; 12 carried two variants in *GJB2* or *SLC26A4*, and they were clearly children with congenital hereditary hearing loss; 6 did not carry or only carried one variant in *GJB2* or *SLC26A4* (Table VI).

Table IV. Deafness gene variant of 1,746 spouses

Detected genes	Variant types	Cases	Carrying rate (%) (n = 1746)
<i>GJB2</i>	c.109G>A heterozygous variant	38	2.18
	c.235delC heterozygous variant	24	1.37
	c.235delC homozygous variant	3	0.17
	c.299_300delAT heterozygous variant	9	0.52
	c.35delG heterozygous variant	1	0.06
	c.9G>A heterozygous variant	1	0.06
	c.187G>T heterozygous variant	1	0.06
	Others	5	0.29
<i>SLC26A4</i>	c.919-2A>G heterozygous variant	16	0.92
	c.919-2A>G homozygous variant	1	0.06
	c.2168A>G heterozygous variant	3	0.17
	c.1226G>A heterozygous variant	2	0.11
	c.1229C>T heterozygous variant	1	0.06
	c.1975G>C heterozygous variant	1	0.06
	c.1263+1G>A heterozygous variant	1	0.06
	c.563T>C heterozygous variant	1	0.06
	c.415+2T>C heterozygous variant	1	0.06
	c.919-2A>G/c.1174A>T compound heterozygous variant	1	0.06
	c.919-2A>G/c.2168A>G compound heterozygous variant	1	0.06
	c.919-2A>G/c.1829C>A compound heterozygous variant	1	0.06
	c.754T>C/c.2168A>G compound heterozygous variant	1	0.06
	Others	26	1.49
Total		139	7.96

Discussion

The *GJB2* variant is the first cause of hereditary nonsyndromic hearing loss (NSHL) among Chinese. At present, there are more than 100 gene variants in *GJB2* that are known to be pathogenic (<https://hereditaryhearingloss.org>), and these variants have obvious regional and ethnic differences [9]. There are also differences in common *GJB2* variant sites in Chinese from different regions [10]. *SLC26A4* is the second most universal variant gene in NSHL patients, and patients of different races also have different gene variant profiles [11–13]. In China, c.919-2A>G is the most common variant [14]. Based on previous studies, in our hospital the main pathogenic variant of NSHL patients in the Jiaodong area is *GJB2* c.235delC, followed by *SLC26A4* c.919-2A>G [15]. Among 60,391 women of childbearing age in this study, 1,739 only carried the *GJB2* variant, with a carrying rate of 2.88%; 1,553 women only carried the *SLC26A4* variant, with a carrying rate of 2.58%. The carrying rates of *GJB2* and *SLC26A4* variants among Chinese were 3% and 2–3%, respectively, which are basically the same [7, 8]. Among them, *GJB2* c.235delC has the highest carrying rate, ac-

counting for 2.08%, followed by *SLC26A4* c.919-2A>G, with a carrying rate of 1.63%, which further confirms that common deafness gene variants in Jiaodong are mainly *GJB2* c.235delC and *SLC26A4* c.919-2A>G.

GJB2 encodes a gap junction protein (connexin 26 protein – Cx26) [16]. The most common pathogenic variant in *GJB2* among Chinese is c.235delC, if which lack its base will result in frameshift variants in the coding region of *GJB2*, leading to premature termination of translation and the formation of non-functional Cx26, which results in sensorineural hearing loss [17]. The *GJB2* c.235delC homozygous variant is mostly manifested as congenital severe-extremely severe hearing loss, and very few cases are delayed moderate post-lingual hearing loss [18]. Chang *et al.* [19] conducted audiometric phenotype statistics on 100 NSHL patients with the *GJB2* c.235delC homozygous variant, finding that patients with deafness caused by the *GJB2* c.235delC homozygous variant showed diversity in both ear hearing phenotypes and the degree of hearing loss is mainly extremely severe, severe and moderately severe, whereas mild is the least common. In this study,

Table V. Prenatal diagnosis results of 51 couples

Detected genes	No.	Female's deafness gene test result	Male's deafness gene test result	Prenatal diagnosis result of fetus
<i>GJB2</i>	1	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC heterozygous variant
	2	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC heterozygous variant
	3	c.235delC heterozygous variant	c.109G>A heterozygous variant	c.235delC heterozygous variant
	4	c.235delC heterozygous variant	c.109G>A heterozygous variant	Normal
	5	c.235delC heterozygous variant	c.299_300delAT heterozygous variant	c.235delC/c.299_300delAT compound heterozygous variant
	6	c.235delC heterozygous variant	c.109G>A heterozygous variant	c.235delC/ c.109G>A compound heterozygous variant
	7	c.235delC heterozygous variant	c.35delG heterozygous variant	c.235delC heterozygous variant
	8	c.235delC heterozygous variant	c.35delG heterozygous variant	c.235delC heterozygous variant
	9	c.235delC heterozygous variant	c.558_605Dup	Normal
	10	c.235delC heterozygous variant	c.109G>A heterozygous variant	Normal
	11	c.235delC heterozygous variant	c.299_300delAT heterozygous variant	c.235delC heterozygous variant
	12	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC heterozygous variant
	13	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC heterozygous variant
	14	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC heterozygous variant
	15	c.235delC heterozygous variant	c.235delC heterozygous variant	Normal
	16	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC homozygous variant
	17	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC homozygous variant
	18	c.235delC heterozygous variant	c.299_300delAT heterozygous variant	c.299_300delAT heterozygous variant
	19	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC homozygous variant
	20	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC homozygous variant
	21	c.235delC heterozygous variant	c.235delC heterozygous variant	Normal
	22	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC heterozygous variant
	23	c.235delC heterozygous variant	c.235delC heterozygous variant	c.235delC heterozygous variant
	24	c.235delC heterozygous variant	c.299_300delAT heterozygous variant	c.235delC heterozygous variant
	25	c.299_300delAT heterozygous variant	c.299_300delAT heterozygous variant	c.299_300delAT homozygous variant
	26	c.299_300delAT heterozygous variant	c.235del heterozygous variant	c.299_300delAT heterozygous variant

Table V. Cont

Detected genes	No.	Female's deafness gene test result	Male's deafness gene test result	Prenatal diagnosis result of fetus
	27	c.299_300delAT heterozygous variant	c.235del heterozygous variant	Normal
	28	c.299_300delAT heterozygous variant	c.235del heterozygous variant	Normal
	29	c.299_300delAT heterozygous variant	c.109G>A heterozygous variant	c.109G>A/c.299_300delAT compound heterozygous variant
	30	c.299_300delAT heterozygous variant	c.109G>A heterozygous variant	c.109G>A heterozygous variant
	31	c.299_300delAT heterozygous variant	c.187G>T heterozygous variant	c.187G>T heterozygous variant
	32	c.299_300delAT heterozygous variant	c.299_300delAT heterozygous variant	c.299_300delAT homozygous variant
	33	c.299_300delAT heterozygous variant	c.230G>A heterozygous variant	c.299_300delAT heterozygous variant
	34	c.299_300delAT heterozygous variant	c.235del heterozygous variant	c.235delC/c.299-300delAT compound heterozygous variant
	35	c.299_300delAT heterozygous variant	c.235del heterozygous variant	c.299_300delAT/c.235delC compound heterozygous variant
	36	c.299_300delAT heterozygous variant	c.235del heterozygous variant	c.299_300delAT/c.235delC compound heterozygous variant
SLC26A4	37	c.1174A>T heterozygous variant	c.487G>C heterozygous variant	c.487G>C heterozygous variant
	38	c.1226G>A heterozygous variant	c.919-2A>G heterozygous variant	Normal
	39	c.919-2A>G heterozygous variant	c.919-2A>G/ c.1829C>A compound heterozygous variant	c.919-2A>G heterozygous variant
	40	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant	Normal
	41	c.919-2A>G heterozygous variant	c.1975G>C heterozygous variant	c.919-2A>G heterozygous variant
	42	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant	Normal
	43	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant	Normal
	44	c.919-2A>G heterozygous variant	c.2168A>G heterozygous variant	Normal
	45	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant
	46	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant	Normal
	47	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant	c.919-2A>G homozygous variant
	48	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant
	49	c.589G>A heterozygous variant	c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant
	50	c.1975G>C heterozygous variant	c.1226G>A heterozygous variant	c.1975G>C/ c.1226G>A compound heterozygous variant
	51	c.1975G>C heterozygous variant	c.919-2A>G heterozygous variant	Normal

Table VI. Confirmed children with hearing and deafness gene test results

No.	Children's hearing test results		Deafness genes	Children's deafness gene test results	Mother's deafness gene test results	Father's corresponding deafness gene sequencing results
	Left ear	Right ear				
1	60dBnHL	70dBnHL	<i>GJB2</i>	c.299_300delAT Heterozygous variation	c.299_300delAT heterozygous variation	Not done
2	No	No		c.235delC/ c.299_300delAT compound heterozygous variation	c.235delC heterozygous variation	c.299_300delAT heterozygous variant
3	No	No		c.235delC/ c.299_300delAT compound heterozygous variation	c.235delC heterozygous variation	c.299_300delAT heterozygous variant
4	60dBnHL	60dBnHL		c.235delC homozygous compound	c.235delC heterozygous variant	Not done
5	30dBnHL	60dBnHL		c.109G>A / c.299_300delAT heterozygous variation	c.299_300delAT heterozygous variant	c.109G>A heterozygous variant
6	No	30dBnHL		c.235delC heterozygous variant	c.235delC heterozygous variant	c.608T>C heterozygous variant
7	No	No		c.9G>A/c.235delC compound heterozygous variant	c.235delC heterozygous variant	c.9G>A heterozygous variant
8	No	No		c.299_300delAT homozygous compound	c.299_300delAT heterozygous variant	c.299_300delAT heterozygous variant
9	90dBnHL	90dBnHL		c.235delC/ c.299_300delAT compound heterozygous variant	c.299_300delAT heterozygous variant	c.235delC heterozygous variant
10	No	No		c.235delC homozygous compound	c.235delC heterozygous variant	Not done
11	100dBnHL	80dBnHL	<i>SLC26A4</i>	c.919-2A>G/ c.2168A>G compound heterozygous variant	c.919-2A>G/ c.2168A>G compound heterozygous variant	c.919-2A>G heterozygous variant
12	No	No		c.919-2A>G homozygous compound	c.919-2A>G heterozygous variant	Not done
13	80dBnHL	70dBnHL		c.919-2A>G homozygous compound	c.919-2A>G heterozygous variant	Not done
14	No	30dBnHL		c.919-2A>G heterozygous variant	c.919-2A>G heterozygous variant	Not done
15	No	90dBnHL		c.919-2A>G homozygous compound	c.919-2A>G heterozygous variant	Not done
16	70dBnHL	30dBnHL		c.1975G>C heterozygous variant	c.1975G>C heterozygous variant	Normal
17	30dBnHL	No	-----	Normal	c.235delC heterozygous variant	Not done
18	60dBnHL	60dBnHL		c.235delC heterozygous variant	c.235delC heterozygous variant	Not done
19	50dBnHL	60dBnHL		Not done	c.235delC heterozygous variant	Not done
20	80dBnHL	70dBnHL		Not done	c.235delC heterozygous variant	Not done
21	70dBnHL	80dBnHL		Not done	c.919-2A>G heterozygous variant	Not done

6 women were detected as *GJB2* c.235delC homozygous variants, and their hearing phenotypes were also heterogeneous, including 4 women with congenital extremely severe hearing loss in both ears, 1 with moderate hearing loss, and 1 with mild hearing loss. This has accumulated original data for us to further exploit the mechanism of different hearing phenotypes of patients carrying the same gene variants, thereby providing a possible basis for treating genes in NSHL patients.

In 2013, Pu *et al.* [20] proposed a tertiary prevention strategy for hearing disabilities of birth defects based on genetic screening and diagnosis: First, via drug-induced deafness susceptibility screening, fertility guidance for deaf couples, early-pregnancy general screening, pre-love guidance for young deaf people to achieve primary prevention; second, through prenatal diagnosis and intervention to achieve the secondary prevention; third, through neonatal gene screening to achieve tertiary prevention. At present, the joint screening of neonatal hearing and deafness genes is being widely promoted in China and has produced certain findings. A number of studies [21, 22] have shown that hearing combined with deafness gene screening is an effective means for early detection of late-onset hereditary hearing loss and drug-sensitive individuals. It provides opportunities for early treatment and lingual rehabilitation for children, with important clinical significance.

In this study, 60,391 women of childbearing age were screened for common deafness genes. With the actual detection rate of spouses being 52.3%, 99 high-risk families were finally identified. After genetic counseling, 7 families opted for preimplantation genetic diagnosis, and 51 families for prenatal diagnosis. The findings of prenatal diagnosis showed that 14 fetuses were most likely to have hearing loss, and the diagnosis rate was 27.5% (14/51). It can be seen that carrying out common deafness genetic screening among women of childbearing age and providing follow-up services such as genetic counseling for high-risk families is essential for reducing birth of children with hearing disabilities.

In our practice of screening common deafness genes in women of childbearing age in this region, we have summarized the following experiences:

1. Common deafness gene screening is highly accepted among women of childbearing age in this region. On average, more than 90% of pre-pregnancy/early-pregnancy women are voluntarily tested each year. In addition to publicity and education, government departments also played an important role in including the testing project in people's livelihood to implement free testing. It can be seen that the government departments provide strong support for preventing

and controlling birth defects in terms of policies and funds, which effectively promotes the prevention and control of birth defects in the region.

2. The rate of further testing for the spouses of female carriers is low, 52.3%, which is far lower than that of females, especially in *SLC26A4* sequencing, only 37.6% (600/1594). After analysis, the first reason is that most families believe that the hearing of both parents is normal, with no family history of hearing disabilities, and that the probability that both couples carry the same type of deaf gene variant is very low. Second, the cost for spouses to receive corresponding gene sequencing is relatively high and needs to be borne by themselves. If these families were highly compliant with our recommendation at that time, through genetic counseling and prenatal diagnosis, we could at least predict the hearing status of the offspring in 47.6% (10/21) of families and provide them with the opportunity to choose. Therefore, how to improve the general people's correct understanding of hereditary deafness is a problem that we urgently need to solve.
3. Be cautious about genetic counseling of controversial pathogenic variants. The detection rate of the *GJB2* c.109G>A variant is relatively high in both normal people and patients with hearing disabilities. Existing research statistics show that c.109G>A is a pathogenic variant, but the hearing phenotypes caused by it are quite different: hearing can be normal or weakened, and the degree of hearing loss can range from mild to severe. This study finally determined that in families where both couples carry known pathogenic variants in the *GJB2* gene, 38 husbands are carriers of c.109G>A. With full knowledge, 6 families chose prenatal diagnosis, and finally 2 families had fetuses with the c.109G>A compound heterozygous variant (1 case was the c.109G>A/c.299_300delAT compound heterozygous variant; 1 case was the c.235delC/c.109G>A compound heterozygous variant). We followed up the hearing of their offspring, and both families indicated that their offspring had passed the newborn hearing screening. Because the later clinical manifestations of this group of people are unpredictable, we recommend that the two families undergo regular hearing monitoring to accurately assess their hearing level.
4. Although hearing impairment is not a serious disability or mortal disease, it will seriously affect the quality of life and inflict a heavy economic and psychological burden on families. In this study, prenatal diagnosis was applied to predict that 14 fetuses were likely to have hearing loss. We learned from follow-up that

12 of them chose to terminate the pregnancy and suggested that these families should give priority to preimplantation genetics if they have reproductive needs, with diagnosis to prevent the occurrence of hereditary deafness. Therefore, with continuously advancing society and generally improved awareness, realizing primary prevention of birth defects of hearing disabilities, that is, to carry out genetic screening for hereditary deafness before pregnancy or even before marriage, and actively adopt corresponding intervention measures, will more effectively avoid various economic and social ethical issues.

In conclusion, screening for common deafness genes in women of childbearing age and providing follow-up consultation and diagnosis are effective means to prevent birth deafness. Such a screening mode is available to achieve early diagnosis and intervention of hereditary deafness, providing families with an opportunity to make informed choices. It can also warn those carrying drug-sensitive deafness genes to avoid using ototoxic drugs in case of hearing loss. But it is still necessary to further promote the publicity and education of deafness gene screening, popularizing the related knowledge of hereditary deafness, especially to strengthen genetic counseling for high-risk families, so that they have a clearer understanding of hereditary deafness, are more willing to receive deafness gene testing, and further understand the importance of preimplantation genetic diagnosis or prenatal diagnosis to prevent hereditary deafness. Additionally, the government's strong support for preventing and controlling birth defects will also encourage more people to receive testing to a certain extent.

Conflict of interest

The authors declare no conflict of interest.

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