Reproductive Carrier Screening for Genes Associated with Hereditary Deafness in 9993 Chinese Individuals

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Article

Reproductive Carrier Screening for Genes Associated with Hereditary Deafness in 9993 Chinese Individuals

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Abstract: Preconception or prenatal carrier screening for hereditary deafness is an effective early intervention strategy to reduce the incidence of newborn deafness, but current research is limited. This study aimed to investigate the carrier frequencies of common deafness genes in the Chinese population and to follow up on pregnancy outcomes in high-risk couples. Carrier screening for common deafness genes in the Chinese population, including the GJB2 and SLC26A4 genes, was performed using next-generation sequencing technology. Of the 9,993 subjects screened, the carrier rate was 2.86% for the GJB2 gene and 2.63% for the SLC26A4 gene. Of the six high-risk couples, four made alternative reproductive decisions (three with prenatal diagnosis and one with preimplantation genetic testing), with consequent successful termination of the birth of two affected fetuses. The frequencies of preconception or prenatal carrier screening for the deafness genes GJB2 and SLC26A4 in 9,993 subjects from China in this study were 2.86% and 2.63%, respectively. In addition, four out of six high-risk couples made alternative reproductive decisions, followed by the successful prevention of the birth of two affected fetuses. These findings confirmed the clinical utility of preconception or prenatal carrier screening for hereditary deafness.

Keywords: hereditary deafness; carrier screening; reproductive decision making

1. Introduction

The prevalence of deafness in newborns worldwide is two to three out of every 1,000 births [1,2], with more than 50% of the cases attributed to genetic factors [3]. In China, the neonatal deafness prevalence rate ranges from 1 ‰ to 3.47 ‰ [4]. The consequences of untreated deafness, including hereditary deafness, can have a serious impact on a child's speech, language, education, and social integration [3]. Hereditary deafness is a monogenic disorder that follows the Mendelian inheritance pattern and exhibits significant genetic heterogeneity [4]. It is estimated that 30% of hereditary deafness is syndromic, and 70% is non-syndromic [3,5]. Of the non-syndromic cases, autosomal dominant and X-linked inheritance account for about 15% and 1%, respectively, while autosomal recessive inheritance is responsible for up to 80% [5]. With the emergence of next-generation sequencing technologies and the decline of sequencing costs, knowledge about the genetic etiology of deafness is rapidly increasing [5]. To date, over 100 related genes are associated with non-

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syndromic deafness [6]. In the Chinese population, the two primary causative genes for autosomal recessive non-syndromic deafness are the gap junction beta 2 gene (*GJB2*; OMIM: 121011) and solute carrier family 26 member 4 (*SLC26A4*; OMIM: 605646) [4,7,8]. For intervention and prevention of hereditary deafness, the key is to identify the genetic etiology. Genetic testing can help identify the causative genes, which is essential for accurate genetic counseling, prognosis, and the potential development of possible gene therapy strategies in the future [5,9].

It is worth noting that 95% of newborns with deafness identified by newborn hearing screening had hearing parents [5], suggesting that normal hearing parents who carry the autosomal recessive gene for deafness are at risk of having an affected offspring [10]. Combined newborn hearing and genetic screening has become widespread and is considered tertiary prevention of deafness [2,11], which allows early detection of the molecular etiology and significantly shortens the time to diagnosis and intervention for deafness [11]. Advancing genetic screening for deafness from the newborn to the prenatal period, namely carrier screening of pregnant females or couples at risk, together with prenatal diagnosis such as chorionic villus sampling or amniocentesis, can provide secondary prevention of deafness [2,12]. Compared to prenatal carrier screening, preconception carrier screening is more recommended [12]. Preimplantation genetic testing (PGT) or gamete donation is an option if high-risk couples are detected to be at risk of reproduction [2,12]. Thus, preconception carrier screening can prevent and interrupt the birth of a child with hereditary deafness while avoiding the risk of termination of pregnancy or recurrent abortions [2]. Essentially, it is a form of primary prevention for deafness [2]. A couple who are both confirmed carriers of the same autosomal recessive disorder has a 25% probability of having an affected child with each pregnancy. If the mother carries an X-linked recessive disease, the male offspring of the couple has a 50% probability of being affected [13]. In 2013, the first study of carrier screening for the deafness gene in women of childbearing age showed that eight out of nine couples carrying a mutation in the same deafness gene received the prenatal diagnosis and subsequently prevented the birth of one affected fetus [14]. Accordingly, preconception or prenatal carrier screening for hereditary deafness enables those screened to consider their reproductive risk and promotes additional reproductive options [12,15,16], contributing to reducing the incidence of hereditary deafness in newborns [2,10]. However, studies of preconception or prenatal carrier screening for hereditary deafness are limited, and more investigations are needed.

There is no consensus on which genes for hereditary deafness should be tested for preconception or prenatal carrier screening. Given the high carrier rates of the *GJB2* and *SLC26A4* genes in the Chinese population [17] and following the latest recommendations of the American College of Medical Genetics and Genomics in 2021 [12], *GJB2*-associated autosomal recessive deafness-1A (DFNB1A; OMIM: 220290) and *SLC26A4*-associated autosomal recessive deafness-4 (DFNB4) with enlarged vestibular aqueduct (OMIM: 600791) or Pendred syndrome (PDS; OMIM: 274600) were included in this study for preconception or prenatal carrier screening. This study aimed to perform carrier screening in individual females or couples before or early in pregnancy to determine carrier rates of the *GJB2* and *SLC26A4* genes and their mutations. In addition, we provided genetic counseling to all subjects and followed up on reproductive decision-making, pregnancy outcomes and neonatal health status in high-risk couples.

2. Materials and Methods

2.1. Subjects and study design

A total of 9,993 subjects, including 1,783 couples, were enrolled in this study from Jiangxi Maternal and Child Health Hospital (from March 2020 to July 2023) and Dalian Woman and Children's Medical Center (from August 2020 to July 2023) in China. These subjects attended an expanded carrier screening program (including preconception or prenatal) for 155 monogenic disorders. The study focused on preconception or prenatal carrier screening for hereditary deafness of our interest, consisting of DFNB1A (GJB2 gene) and DFNB4 or PDS (SLC26A4 gene). The criteria for inclusion were: no family history of hereditary deafness without the phenotype

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of deafness; couples of childbearing age preparing for pregnancy or with gestational weeks of less than sixteen; couples seeking a healthy child through assisted reproductive technology; and couples of close consanguinity. Those with a family history of hereditary deafness or gestational age of sixteen weeks or greater were excluded. Genetic counseling prior to testing was provided to all subjects. All subjects signed an informed consent form. Approval for this study was granted by the Institutional Review Board of BGI.

Two strategies were used for carrier screening: sequential and concurrent screening. In sequential screening, females were tested first, and their partners were recalled if they carried at least one mutation in the autosomal recessive deafness gene. In concurrent screening, the couples underwent genetic testing at the same time. After testing, subjects likely to carry the pathogenic deafness gene were provided genetic counseling. Additionally, high-risk couples were given recommendations such as prenatal diagnosis, PGT or gamete donation.

2.2. Selection of hereditary deafness diseases and sequencing

DFNB1A (OMIM: 220290) and DFNB4 with enlarged vestibular aqueduct (OMIM: 600791) or PDS (OMIM: 274600) were selected for preconception or prenatal carrier screening in this study.

The peripheral blood samples of the subjects were collected in 2-5 mL, and genomic DNA was extracted. The target regions (exons and splice site sequences of genes) in genomic DNA were captured using a set of oligonucleotide probes, followed by detection using high-throughput sequencing technology. Bioinformatic analysis of the assay results was used to identify the likely pathogenic or pathogenic variants of the target genes. According to the guidelines of the American College of Medical Genetics and Genomics [18], the screened loci were interpreted and reports were generated.

2.3. Follow up

High-risk couples were followed up on their reproductive decision-making (e.g., prenatal diagnosis and PGT), pregnancy outcomes, and neonatal health conditions.

2.4. Statistical analysis

Numbers (percentage) were reported for categorical variables, and means (standard deviation) for continuous variables. All statistical analyses were performed with R version 4.2.3.

3. Results

3.1. Baseline characteristics of enrolled subjects

Of the 9,993 subjects recruited for this study, 82.2% (n = 8,210) were females, and 17.8% (n = 1,783) were males (i.e., 17,83 couples and 6,427 females). A greater number of 69.1% (n = 6,903) were from Jiangxi Province, and the remaining 30.9% (n = 3,090) were from Liaoning Province. Figure 1 shows the strategy and flowchart for carrier screening in this study.

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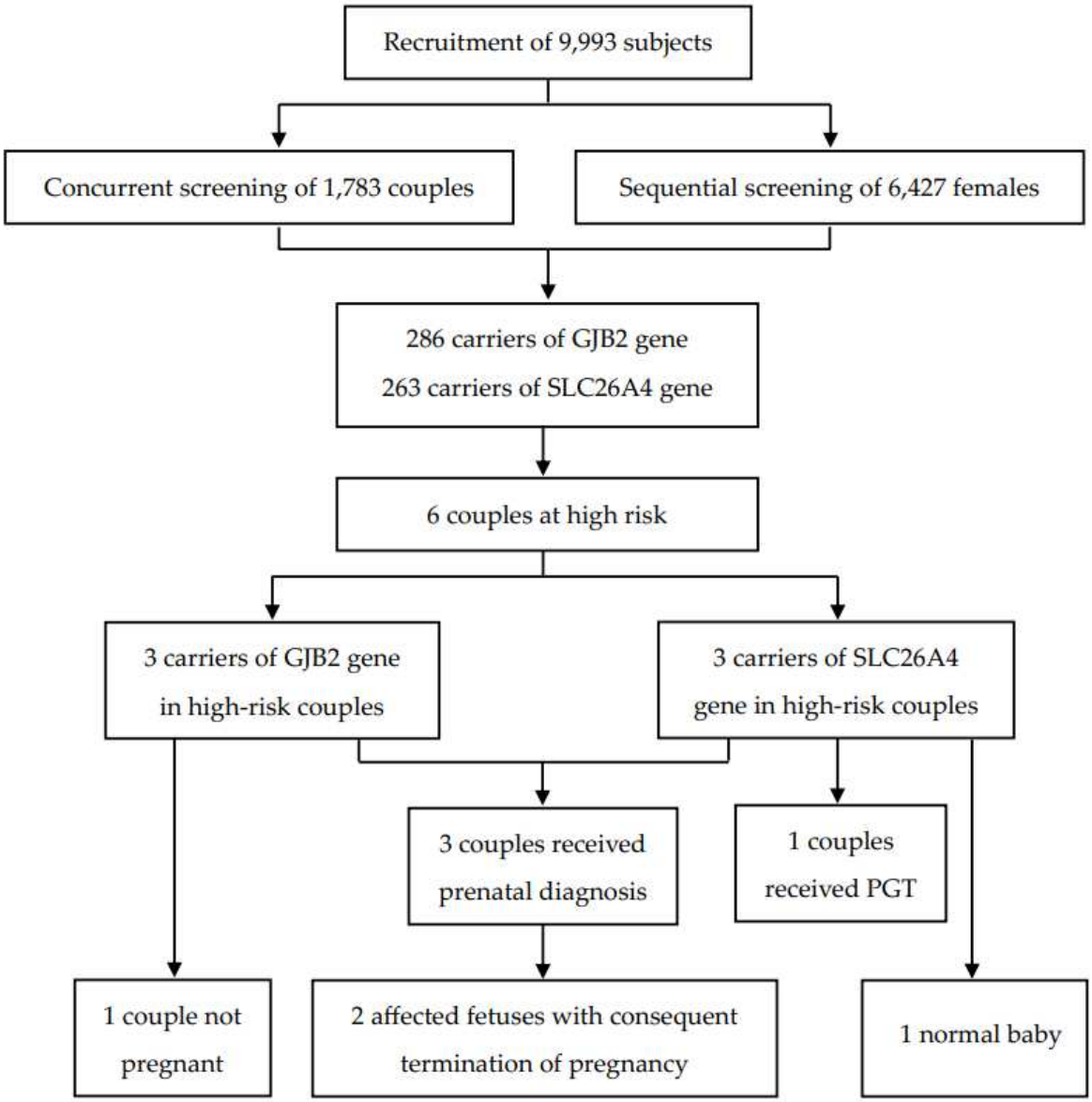

Figure 1. Strategy and flowchart for carrier screening. PGT, preimplantation genetic testing.

3.2. Carrier frequencies of genes and variants associated with deafness

In total, 549 subjects were identified as carriers of deafness-related genes, yielding a carrier frequency of 5.49%. As shown in Table 1, there were 286 carriers of the GJB2 gene with a frequency of 2.86%, and 263 carriers of the SLC26A4 gene with a frequency of 2.63%. Table 2 shows the top three carrier frequencies of variants in the GJB2 and SLC26A4 genes. The top three variants in the GJB2 gene were c.235delC with a frequency of 1.89%, c.299_300del with a frequency of 0.58%, and c.176_191del with a frequency of 0.11%. For the SLC26A4 gene, they were c.919-2A>G with a frequency of 1.08%, c.2168A>G with a frequency of 0.21%, and c.1229C>T with a frequency of 0.20%. In addition, six subjects carried compound heterozygotes with a combination of variants in the GJB2 and SLC26A4 genes, and three subjects carried compound heterozygous variants in the SLC26A4 gene (Tables S1 and S2). The detailed variant spectra of the GJB2 and SLC26A4 genes were shown in Table S1 and Table S2.

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Table 1. Carrier frequencies of deafness genes among 9,993 subjects.

OMIM gene	Gene	OMIM phenotype	Type of hereditary deafness	Count	Carrier frequency (%)
121011	*GJB2*	220290	DFNB1A	286	2.86
605646	*SLC26A4*	600791/274600	DFNB4/PDS	263	2.63
-	-	-	-	549	5.49

DFNB1A, nonsyndromic deafness, autosomal recessive 1A; DFNB4, deafness autosomal recessive 4; PDS, Pendred syndrome.

Table 2. The top three carrier frequencies of variants in the GJB2 and SLC26A4 genes among 9,993 subjects.

Gene	Variant	SNP ID	Count	Carrier frequency (%)
GJB2				
	c.235delC (p.Leu79Cysfs)	rs80338943	189	1.89
	c.299_300del (p.His100fs)	rs111033204	58	0.58
	c.176_191del (p.Gly59fs)	rs750188782	11	0.11
SLC26A4				
	c.919-2A>G	rs111033313	108	1.08
	c.2168A>G (p.His723Arg)	rs121908362	21	0.21
	c.1229C>T (p.Thr410Met)	rs111033220	20	0.20

SNP, single nucleotide polymorphism.

3.3. Couples at high risk and status of follow-up

As shown in Table 3, there were six couples (two pregnant and four not pregnant when recruited) carrying the same GJB2 or SLC26A4 gene. Among the high-risk couples carrying the GJB2 gene, two couples underwent prenatal diagnosis, resulting in the termination of their pregnancy due to the fetus being affected; and one was not pregnant at the time of follow-up. Of the high-risk couples carriers of the SLC26A4 gene, one was pregnant during the follow-up and underwent prenatal diagnosis with the fetus detected as heterozygous for the c.919-2A> mutation; one was performing preimplantation genetic testing to reduce the risk of affected offspring; and the remaining one did not receive a prenatal diagnosis but gave birth to a baby with normal hearing.

Table 3. High risk couples and follow-up status.

Gene	Gender	Variant	Prenatal diagnosis/ outcomes of fetus	Affected fetus	Pregnancy outcome
GJB2					
	Female	c.299_300del (p.His100fs)	Yes/-	Yes	Termination
	Male	c.235delC (p.Leu79Cysfs)			
	Female	c.235delC (p.Leu79Cysfs)	Yes/-	Yes	Termination
	Male	c.235delC (p.Leu79Cysfs)			
	Female	c.176_191del (p.Gly59fs)	No/-	-	Not pregnant
	Male	c.235delC (p.Leu79Cysfs)			
SLC26A4					

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Female	c.2168A>G (p.His723Arg)	Yes/heterozygous variant of c.919-2A>G	No	-
Male	c.919-2A>G			
Female	c.919-2A>G	No/-	Three blastocysts obtained by PGT await for testing	-
Male	c.1595G>T (p.Ser532Ile)			
Female	c.1692dup (p.Cys565fs)	No/-	No	A normal baby
Male	c.1226G>A (p.Arg409His)			

PGT, preimplantation genetic testing.

4. Discussion

In this study, preconception or prenatal carrier screening for the deafness genes GJB2 and SLC26A4 was performed in 9,993 individuals from China, with frequencies of 2.86% and 2.63%, respectively. The most common variant in GJB2 was c.235delC, while in SLC26A4, it was c.919-2A>G. In addition, three out of six high-risk couples underwent prenatal diagnosis with the successful avoidance of the birth of two fetuses with hereditary deafness, and one out of six chose PGT to reduce the risk of offspring being affected. The carrier frequencies of common deafness genes and their mutations in the Chinese population identified in this study might provide data support for future research and clinical genetic counseling. Moreover, this study suggested the importance of preconception or prenatal carrier screening for hereditary deafness to assess reproductive risk and guide reproductive decision-making. Such carrier screening was an effective early intervention strategy to reduce the incidence of neonatal deafness.

The carrier frequency of the GJB2 gene in this study was 2.86%, which was slightly higher than the frequency of 1.66% found in a preconception or prenatal expanded carrier screening study of 10,476 couples (i.e., 20,952 individuals) from southern China [19]. However, a recent study of 3,555,336 neonates from the Chinese Newborn Concurrent Hearing and Genetic Screening cohort reported a similar carrier rate, with an overall *GJB2* carrier rate of 2.53% [17]. Consistent with previous studies in the Chinese population [10,11,17], our study found the top three carrier frequency variants of the *GJB2* gene to be c.235delC (1.89%), c.299_300del (0.58%) and c.176_191del (0.11%). A recent study conducted concurrent hearing and genetic screening for 180,469 newborns in Beijing showed that the frequencies of c.235delC, c.299_300del, and c.176_191del were 1.80%, 0.50%, and 0.12%, respectively [11]. In another study of newborns, the carrier frequencies were 1.95%, 0.48%, and 0.11%, respectively [17]. Variants in the *GJB2* gene are the leading cause of autosomal recessive non-syndromic deafness in the Chinese population [7,8]. *GJB2* encodes the gap junction protein, which is essential for the maintenance of cochlear homeostasis through the recycling of potassium in the inner ear [20,21]. The deafness associated with *GJB2* is sensorineural and varies in severity from mild to profound [5]. Generally, it is present at birth [5].

The second most common cause of autosomal recessive non-syndromic deafness in the Chinese population is the *SLC26A4* gene [7,8]. We found that the frequency of the *SLC26A4* gene in preconception or prenatal carrier screening was 2.63%, which was comparable to the previously reported carrier rate of 2.05% in 3,555,336 newborns in China [17], but higher than the 1.59% in 10,476 couples [19]. Furthermore, the three most common variants of the *SLC26A4* gene were c.919-2A>G with a frequency of 1.08%, c.2168A>G with a frequency of 0.21% and c.1229C>T with a frequency of 0.20%. Similarly, these variants were the top three in frequency among 3,555,336 newborns in China, with frequencies of 1.32%, 0.24%, and 0.12%, respectively [17]. The *SLC26A4* gene encodes the pendrin protein, which is expressed in the inner ear, thyroid and kidney [21,22]. *SLC26A4* is the gene causing DFNB4 and PDS, whose related phenotypes are described as inner ear malformations, hearing impairment, vestibular dysfunction, and thyroid abnormalities [22]. The hearing impairment associated with *SLC26A4* is typically fluctuant or progressive sensorineural [22].

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Carrier screening can provide an opportunity for individuals or couples to know their risk and consider available reproductive options at a preconception or prenatal stage, which demonstrates its clinical utility [12]. If identified before pregnancy, high-risk couples have the options of preimplantation genetic testing, gamete or embryo donation, prenatal diagnosis (chorionic villus sampling or amniocentesis), and adoption [12]. If identified during pregnancy, however, the only option is a prenatal diagnosis [12]. Of the six high-risk couples, except for one who did not become pregnant, four made an alternative reproductive choice (three underwent prenatal diagnosis and one chose PGT), resulting in the successful termination of the birth of two affected fetuses. Therefore, our results supported the clinical utility of preconception or prenatal carrier screening for hereditary deafness.

There were several limitations to the present study. Firstly, the sample was underrepresented because of the inclusion of only subjects from Jiangxi and Liaoning provinces in China. In addition, detailed information on ethnicity was not available for this study. Secondly, although the GJB2 and SLC26A4 genes are the major genetic causes of autosomal recessive non-syndromic deafness in the Chinese population, other related deafness genes that were not screened might have been missed. The discrepancy between the carrier frequencies obtained in this study and those in other studies may be due to variations in the representativeness of the samples (e.g., ethnicity and geographical location) and the coverage of the genes and their variants screened. Therefore, future studies should optimize carrier screening for deafness genes and their variants suitable for different ethnic backgrounds and geographical locations. Finally, complete information on pregnancy outcomes in high-risk couples could not be tracked.

5. Conclusions

In conclusion, this study performed preconception or prenatal carrier screening for the common deafness genes GJB2 and SLC26A4 in 9,993 individuals from China, showing carrier frequencies of 2.86% and 2.63%, respectively. In addition, four out of six high-risk couples made alternative reproductive decisions, followed by the successful prevention of the birth of two affected fetuses. These findings confirmed the clinical utility of preconception or prenatal carrier screening for hereditary deafness. Meanwhile, this study demonstrated the necessity of preconception or prenatal carrier screening for hereditary deafness in the high-risk population, which helped radically reduce the incidence of newborn deafness.

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Institutional Review Board Statement: All procedures conducted in this study involving human participants were in accordance with the ethical standards of the National Research Committee and the Declaration of Helsinki of 1975. Approval for this study was granted by the Institutional Review Board of BGI (No. BGI-S103-T6).

Informed Consent Statement: All subjects signed an informed consent form.

Data Availability Statement: Requests may be addressed to the corresponding authors.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations: PGT, preimplantation genetic testing; DFNB1A, nonsyndromic deafness, autosomal recessive 1A; DFNB4, deafness autosomal recessive 4; PDS, Pendred syndrome; SNP, single nucleotide polymorphism.

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