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Reproductive guidance through prenatal diagnosis and genetic counseling for recessive hereditary hearing loss in high-risk families

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ABSTRACT

Objective: To evaluate the accuracy and validity of our protocol for prenatal diagnosis and genetic counseling in high-risk families at a clinic.

Methods: Fifteen unrelated families with recessive nonsyndromic hearing loss (NSHL) in their family history and a positive attitude towards prenatal diagnosis were recruited in the present study. According to genetic information for each family, Sanger sequencing, fluorescence polymerase chain reaction (PCR)-based congenital deafness gene detection kit and multiple PCR-based target gene capture and high-throughput sequencing were used. Genetic counseling was offered to all participating families by genetic counselors and otologists. Prenatal diagnosis was provided to families with detected pathogenic mutations and who were expected to participate in subsequent prenatal diagnosis.

Results: In this study, confirmed pathogenic mutations were detected in eight families, who were defined as high-risk families. These families all participated in prenatal diagnosis with positive attitudes. One novel variant (c.1687dupA) in the *SLC264* gene was detected in a family. Through genetic counseling, the recurrence probability of NSHL in fetuses was 25% in six families, 0% in one family, and 50% in one family. The results of fetal DNA detection showed that one fetal variant was wild type, three were heterozygous mutations in *SLC26A4*, and one was a compound heterozygous mutation in *SLC26A4*. Two variants were heterozygous mutations in *GJB2*, and one was a homozygous mutation in *GJB2*. According to the test results for fetal DNA, prenatal diagnosis found that six fetuses had normal hearing, whereas two fetuses suffered from NSHL. After birth, six infants predicted to have normal hearing passed a newborn hearing screening test and two infants predicted to have NSHL were diagnosed with NSHL and received cochlear implants.

Conclusion: Our protocol for prenatal diagnosis and genetic counseling provides detailed information that can assist couples in high-risk families in preparing for infant arrival and future family planning. For the affected neonates, prenatal diagnosis and genetic counseling achieve an “early screening, early diagnosis, early intervention” strategy.

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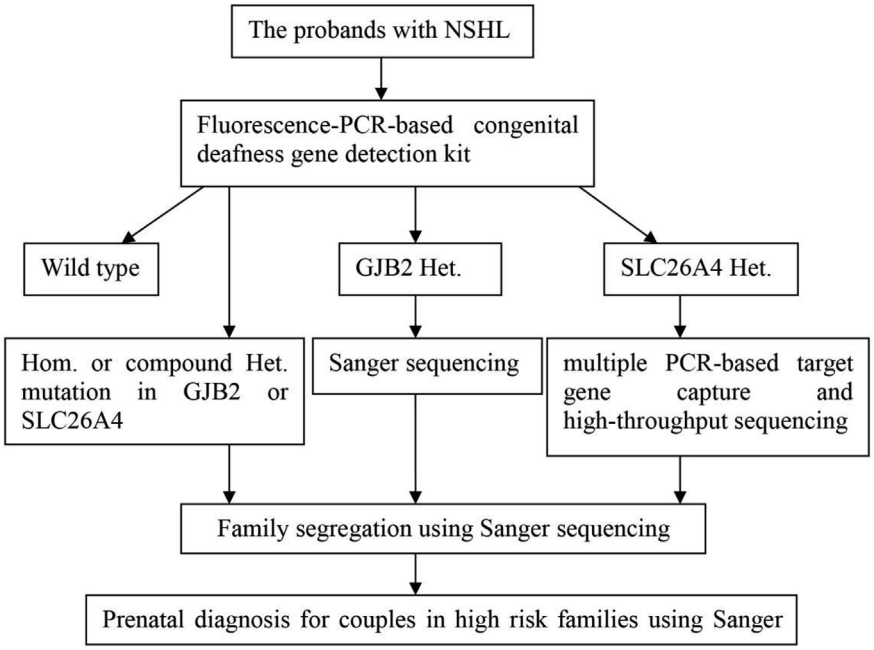

Fig. 1. Workflow diagram of prenatal diagnosis. Hom., homozygous mutation; Het.,heterozygous mutation; compound Het., compound heterozygous mutation.

1. Background

Currently, prenatal diagnosis (PD) is mainly applied to pregnant woman who are at high-risk of pregnancy with single-gene disorders [1]. Hearing loss (HL) is the most common sensorial disease [2], affecting more than 32 million children around the world (http://www.who.int/en/). Approximately 1 in 500 newborns suffers from HL [3]. Statistical evidence shows that 30 000 infants in China are born with congenital nonsyndromic HL (NSHL) each year [4]. The causes of HL include genetic factors, environmental factors, or a synergy of both [5]. For pediatric HL, genetic factors have been deemed as the dominant cause [6], especially for children with a family history of HL. It has been reported that 30% of genetic HL are syndromic HL (SHL) and that the rest (70%) are NSHL [7,8]. HL is the only phenotypic expression observed in NSHL, which is generally caused by mutations in single genes [5]. Therefore, the field of NSHL is ideally positioned to employ the strategies of PD. The overwhelming majority of normal hearing parents with deaf children showed a positive attitude towards genetic screening and PD [9–11]. Additionally, many newly married couples with a family history of HL would be willing to accept PD.

Hereditary HL is an extremely heterogeneous disorder with more than 140 identified loci (http://hereditaryhearingloss.org). Research has shown that the mutation spectrum, prevalence, and expression of various Mendelian diseases is a result of groups with the same ancestry sharing similar genetic risk factors [12]. For example, the c.235delC mutation in *GJB2*(MIM 121011) is most commonly found in eastern Asian populations [13], whereas the c.35delG mutation in *GJB2* accounts for 70% of the variants found in Caucasian populations [14]. Hence, genetic counseling and PD should fully consider differences in the HL gene mutation spectrum across ethnicities. Liu et al. summarized the mutation spectrum of HL genes in the Chinese population and reported that *GJB2*, *SLC26A4* (MIM 605646)and mitochondrial DNA (mtDNA, MIM 561000) were the most frequent HL genes [4]. However, subjects with mutations in mitochondrial DNA pass their mutations on to offspring in a matrilineal inheritance pattern. As a result, we selected pathogenic mutations in *GJB2* and *SLC26A4* for PD in couples with a family history of these two forms of gene mutation-related HL.

2. Materials and methods

2.1. Subject recruitment

This study was supported by the Xiangya Hospital Ethics Committee of Central South University, and written informed consent was obtained prior to participating in this study from all couples. A total of 15 unrelated families were enrolled at our hearing clinic from 2015 to 2016. These probands were all diagnosed with NSHL by analyzing their medical history and family history and through comprehensive audiological examinations, physical examinations and temporal bone imaging inspections. Peripheral blood was collected from all probands, and DNA was extracted by a routine phenol-chloroform method and fetal DNA was isolated from maternal amniotic fluid at 16 weeks of gestation using a cultured cell DNA kit (MagCares™, Genphar, China).

2.2. Genetic screening

A fluorescence polymerase chain reaction (PCR)-based congenital deafness gene detection kit (Jinan Ying Sheng Biology, China) was used to initially screen hot-spot mutations (*GJB2* 235delC, 299-300delAT, 176del16bp, 512insAAGG and *SLC26A4* IVS7-2A > G, 1174A > T, 1229C > T 2168A > G) among all probands. For the probands with monoallelic mutations in the preliminary screening, we then applied Sanger sequencing to sequence exon 2 of *GJB2* and adopted multiple PCR-based target gene capture and high-throughput sequencing to sequence all the exons of *SLC26A4*, *FOXI1*, and *KCNJ10*. It was necessary to verify the identified pathogenic variants in *SLC26A4*, *FOXI1*, and *KCNJ10* using Sanger sequencing.

If the pathogenic variants detected in the probands were verified by Sanger sequencing, then the corresponding couples were enrolled in subsequent PD. In the process of PD, the DNA of immediate relatives of the fetus, including fetal parents and siblings, was first screened using Sanger sequencing to completely delineate the pathogenic variant within the family. At this point, we could confirm that the pathogenic variant played a decisive role in causing HL in the family. Finally, the fetal DNA underwent screening via Sanger Sequencing for the respective pathogenic variants.(Fig. 1).