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Original Article

Molecular genetics study of the locus of the midrange sensorineural deafness unique to the non-syndromes of ethnic minorities in Yunnan province

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Abstract: Deafness is a common sensory disorder, 70% of which are non-syndromic deafness (NSHL). There are currently more than 70 cloned genes associated with NSHL. This study used SNaPshot technology to explore the four hot spot mutations of deafness genes in 150 moderate-to-sense non-syndromic hereditary neurotic deafness cases in Yunnan, and to elucidate the epidemic distribution of deafness genes in ethnic minority NSHL population in Yunnan Province. This study enrolled 150 cases of Bai, Hani, and Yi non-syndromic deaf patients and 50 cases of Han NSHL patients as control in the special education school and the outpatient clinic of Yunnan province from January 2015 to January 2017. Medical history collection, hearing and imaging examinations, and 21 hot spot mutations of 4 deafness genes detection were performed. Molecular epidemiological control study was adopted. All patients underwent an otolaryngology specialist examination to diagnose the severity of sensorineural hearing loss. The deafness associated with the screening site in the control group was 36.0% (18/50), while it was 20.7% (31/150) in the experimental group. The mutation rates of three deafness-related genes, including GJB2, SLC26A4, and 12SrRNA in 150 ethnic minority deaf populations were 51.6% (16/31), 29% (9/31), and 19.4% (6/31), respectively. However, the GJB3 gene mutation was not detected. Mitochondrial 12SrRNA, GJB2-235delC, and SLC26A4-IVS7-2A > G were hotspot gene mutation.

Keywords: Non-syndromic, deafness, gene, mutation

Introduction

Deafness is the most common sensory neurological dysfunction in human. The World Health Organization (WHO) announced in March 2013 that the proportion of people with hearing impairment in the global increased from 4.2% (278 million) to 5.3% (360 million) between 2005 and 2012. One out of 500-1000 newborns per year is affected [1, 2]. Hearing and speech disability accounts for the first of all kinds of disabilities in China and has reached more than 20 million with nearly 30,000 new cases each year [3]. In addition, 70% of patients with deafness are non-syndromic hereditary sensorineural deafness [4]. The etiology of deafness is complex, including environmental and genetic factors [5-7]. Following the development of genetic testing technology and the deepening of deafness research, the important role of genetic factors in the pathogenesis of deafness has been confirmed [8, 9]. Deafness exhibits a distinct genetic heterogeneity. Genetic patterns include autosomal dominant inheritance, autosomal recessive inheritance, X-linked inheritance, or mitochondrial inheritance.

The molecular pathological mechanism of hereditary deafness has been fully explored in the past decade. Almost all genes of common syndrome deafness have been cloned, and more than 40 genes of NSHL have been cloned. Molecular epidemiological survey showed that there were higher positive rates of GJB2, GJB3, SLC26A4, and mitochondrial 12SrRNA mutations in Chinese deaf patients. However, there were significant differences in gene mutation rates among deaf patients from different ethnic

groups and regions [10, 11]. GJB2 gene mutation is the most common cariogenic factor in Chinese deaf population. GJB3 gene is the first deafness gene that is independently cloned by Chinese scholars, whose mutation may lead to dominant or recessive hereditary NSHL [12]. The SLC26A4 gene is recessive inheritance, and both of its biallelic pathological mutation or the compound heterozygous mutation can cause hereditary deafness. This gene mutation is closely related to large vestibular aqueduct syndrome and cochlear malformation [13]. The mutation of mitochondrial 12SrRNA gene is mainly 1555A > G and 1494C > T in China, which is mitochondrial maternal inheritance that can affect the offspring of maternal members. The deafness caused by its mutation is mainly related to the use of aminoglycoside drugs.

At present, the research is mainly carried out in the Han population. There are few epidemiological studies on the deafness gene in the minority deaf people, and most are carried out at several sites of a single gene. Yunnan contains 52 ethnic groups, among which the three ethnic groups including Bai, Hani, and Yi have a total population of more than 1 million. In this study, we will conduct molecular epidemiological studies on common deafness genes in endemic ethnic groups in Yunnan minority areas to form a preliminary understanding of the prevalence of deafness genes in ethnic minority populations and ethnic minority areas in China. It may identify the corresponding mutation spectrum and hot spot mutations to provide a basis for large-scale screening.

Materials and methods

Research objects

150 cases of Bai, Hani, and Yi non-syndromic deaf patients and 50 cases of Han NSHL patients as control in the special education school and the outpatient clinic of Yunnan Province from January 2015 to January 2017 were enrolled in The First Affiliated Hospital of Kunming Medical University (Kunming, Yunnan, China). All patients underwent an otolaryngology specialist examination to diagnose the severity of sensorineural hearing loss. There was no statistical difference on gender between the two groups.

The study protocol was approved by the Research Ethics Committee of The First Affiliated Hospital of Kunming Medical University (Kunming, Yunnan, China), and all patients gave their informed consent before study commencement.

Inclusion criteria

The analysis and judgment criteria for deafness phenotype are based on the Recommendation for Non-Syndromic Hereditary Deafness Genetics and Audiology Descriptive Terminology published by Van Camp, et al. in 2003. First, non-syndromic deafness and syndromic deafness were distinguished mainly based on abnormalities in other organs. Second, the cases were classified according to the nature of hearing loss, including sensorineural hearing loss, conductive deafness, and mixed deafness, and then reclassified according to the language development stage as pre-linguistic deafness and post-linguistic deafness. Finally, the cases were classified according to the frequency as high frequency, low frequency, medium frequency and full frequency hearing loss types. The degree of hearing loss was evaluated based on the average hearing threshold of better ears (0.5~4 kHz), including mild, moderate, severe, and extremely severe hearing loss. Auditory behavioral response audiometry, game audiometry, pure tone audiometry, binaural air conduction hearing threshold, and bone conduction hearing threshold were tested. The tympanometry and the sacral muscle reflex were detected using an acoustic impedance tester (Medical TSM300, Denmark) to check the function of the middle ear. Imaging, high-resolution tibial computed tomography (CT) and magnetic resonance imaging (MRI) were performed to exclude patients with middle ear and inner ear malformation.

Medical history inquiry

The medical history and family history were collected to clarify the family history of deafness in three generations. All the cases were traced back to three generations without a history of marriage with other ethnic groups, and there was no blood relationship between them. All subjects were enrolled by the guardian under the guidance of the doctor to complete the deaf patient information registration form, including

Table 1. Comparison of the proportion of deafness related to screening sites in two groups

	Proportion	χ^2	*P*
Control	36.0% (18/50)	4.77	*P < 0.05*
Experimental group	20.7% (31/150)		

P < 0.05.

general information, birth history, age of onset, family history, personal history (history of infection before deafness, history of application of ototoxic drugs, history of trauma, etc.). All the patients or parents had signed informed consent.

Sample collection

The peripheral venous blood was collected. 2-3 ml of EDTA-K2 anticoagulated venous blood was extracted from each specimen and frozen at -80°C for cryopreservation.

Methods

This study screened qualified enrolled patients for deafness gene detection, including 21 hot spot mutation sites of 4 deafness genes. Total DNA (containing mt DNA) was extracted from peripheral blood using QIAamp DNA Blood Mini Kit (QIAGEN, 51106), quantified and purified by DU®640 UV spectrophotometer (Beckman), and stored at -80°C. ExoSAP-IT (Thermo Fisher, 78201) was used for PCR amplification and purification. The SnaPshot reaction was analyzed on ABI 3500×1 DX Genetic Analyzer using the reagent Hi-Di Formamide (Thermo Fisher, 4211320). The detection genes include: (1) GJB2 gene 35delG, 167delT, 235delC, 176-191dell6, 299-300delAT, 508-511 dupAACG mutation; (2) GJB3 gene 538C > T, 547G > A mutation; (3) SLC26A4 gene IVS7-2A > G, 2168A > G, 1174A > T, 1229C > T, 1226G > A, 1975G > C, 2027T > A, 2162C > T, 281C > T, 589G > A, IVS15 + 5G > A mutation; (4) 1494C > T and 1555A > G mutations in mitochondrial 12SrRNA.

Statistical analysis

Statistical analysis was performed using PASW Statistics 18.0 software. The comparison of differences between two groups was performed by chi-square test and P < 0.05 indicated statistical significance.

Results

Lower proportion of deafness related to screening sites in experimental group

The deafness associated with the screening site in the control group was 36.0% (18/50), while it was 20.7% (31/150) in the experimental group.

The proportion of non-syndromic hereditary deafness carrying hot spot mutations in the Han population accounted for 36.0% (18/50), and it was 20.7% (31/150) in Bai, Hani and Yi non-syndromic hereditary deafness, including 10 cases of Bai, 9 cases of Hani, and 9 cases of Yi. There was a significant difference in the proportion of patients with mutation sites between the two groups. The proportion in experimental group was significantly lower than the control group (**Table 1**, $\chi^2 = 4.77$, P < 0.05).

Mutation of three deafness-related genes GJB2, SLC26A4, and 12SrRNA

In this study, genetic analysis of four genes and mitochondrial DNA in 150 patients with moderate-to-severe NSHL in Yunnan province showed that 16 patients carry the GJB2 gene mutation (16/150, 10.7%), including 5 cases of Bai, 6 cases of Hani, and 5 cases of Yi (**Figure 1**). There were 5 cases of 235delC homozygous mutation, 4 cases of 235delC single heterozygous mutation, 4 cases of 299-300delAT single heterozygous mutation, 1 case of heterozygous mutation: 235delC/299-300delAT, 2 cases of 35delG single heterozygous mutation, no detectable 167delT, 176-191dell6 or 508-511 dupAACG site mutations. Further analysis demonstrated 9 cases (9/150, 6.0%) carrying SLC26A4 gene mutation in the experimental group, including 3 cases of Bai, 2 cases of Hani, and 4 cases of Yi. Among them, 2168A > G homozygous mutation in 1 case, 2168A > G single heterozygous mutation in 2 cases, IVS7-2A > G homozygous mutation in 4 cases, single heterozygous mutation in 2 cases, and 1174A > T, 1229C > T, 1226G > A, 1975G > C, 2027T > A, 2162C > T, 281C > T, 589G > A and IVS15 + 5G > A mutation were undetected. In addition, it was revealed that there were 6 cases (6/150, 4.0%) carrying 12SrRNA gene mutations, 2 cases of Bai, Hani and Yi, respectively. There were 5 cases of A1555G homogenization mutation and 1 case of 1494C > T mutation.

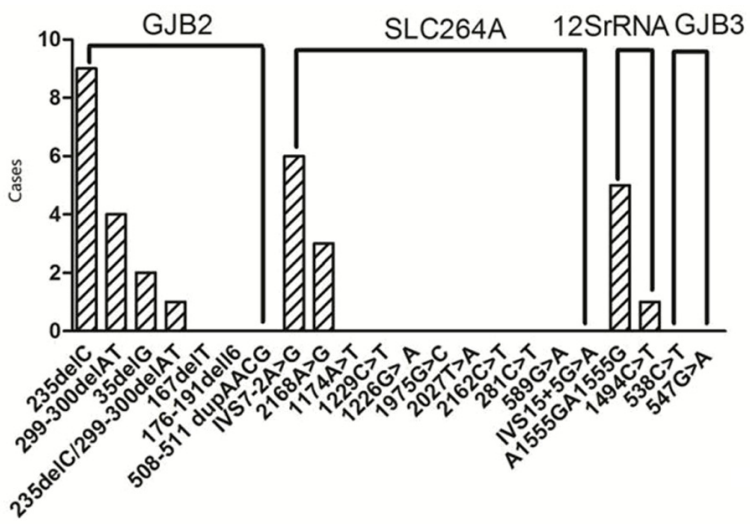

Figure 1. Distribution of GJB2, SLC264A, 12SrRNA and GJB3 gene mutation sites in 150 cases from experimental group.

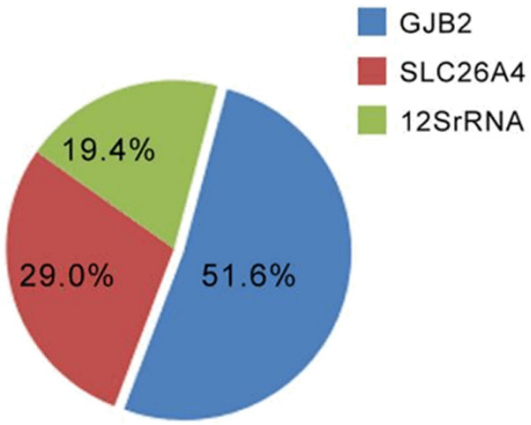

Figure 2. The ratio of mutations in deafness-related genes in 150 cases from ethnic minority.

However, this study failed to find GJB3 gene mutations in 150 NSHL patients. As shown in **Figure 2**, 150 moderate-to-severe NSHL minority patients received 21 hotspot mutation detections in four deafness genes, including GJB2, SLC26A4, 12SrRNA, and GJB3. Seven mutation sites were detected, whereas the GJB3 gene mutation was not detected. The mutation rates of three deafness-related genes, including GJB2, SLC26A4, and 12SrRNA in 150 ethnic minority deaf populations were 51.6% (16/31), 29% (9/31), and 19.4% (6/31), respectively.

Discussion

According to the second national sample survey of disabled people in 2006, the hearing-impaired population accounted for 33.5% of the total disability population in the country. It ranks first with about 27.8 million people. Compared with the first sample survey in 1987, the number of cases showed a significant upward trend mainly caused by genetic factors. It was found that the distribution of deafness is uneven. 50% of deafness is caused by genetic factors, of which 70% of hereditary deafness is NSHL. There are about 72 genes cloned in association with NSHL, including 27 autosomal dominant genes, 40 autosomal recessive genes, 2 mitochondrial genes, and 3 X-linked genes. The national epidemiological survey results reflect the characteristics of mutated gene carrying rate and regional variation in patients with moderate to severe sensorineural deafness in China. The deaf population carrying GJB2 gene mutation is 21%, including 14.5% of SLC26A4 gene mutation and 4.4% of mitochondrial DNA gene mutation. It is known that there are about 52 ethnic minorities in Yunnan. The three ethnic groups of Bai, Hani, and Yi have a total population of more than 1 million, which are rich in characteristic population resources. At present, the study of deafness genes in China is mainly carried out in the Han population. There are few epidemiological studies on deafness genes in ethnic minority deaf people. QW Zhang [14] performed gene test in the screening study of 129 cases severe non-syndromic sensorineural deafness in Shanxi province and observed that the proportion of deaf population with GJB2, SLC26A4, GJB3, and mitochondrial DNA screening sites accounted for 41.09%. Pan J *et al.* detected neurite gene mutations in 380 NSHL patients from northern China (Heilongjiang Province) and found that 19.2% of NSHL patients were caused by mutations in three common deafness genes (GJB2, SLC26A4, and 12S rRNA) [15]. Similarly, Yu H *et al.* adopted genetic screening on hot spots in 103 SNHL children from Shaoxing and found that 46.6% of patients were detected with at least one mutant allele. The most common sites of all detected mutations were GJB2 c.235delC and SLC26A4 c.919. -2A > G [16]. This study indicated that

the proportion of NSHL with 21 hot spot mutation sites in Han nationality account for 36.0%, which was similar to previous studies. Similarly, 150 NSHL patients accounted for 20.7%, which was significantly lower than that in the control group. Sun J *et al.* found that GJB2, SLC26A4, GJB3 and mitochondrial DNA gene mutations in NSHL from Han nationality were higher than Uygur and Hui nationalities [17].

A number of studies exhibited that the 235delC mutation of the GJB2 gene researched 11.8% in patients with severe and extremely severe deafness in China, accounting for 67.7% of all GJB2 mutations [18]. The GJB2 gene encodes Cx26, which is widely distributed in the vascular striate, basal cells, spiral margin, neurosensory epithelium, and cochlear conductive fibers of the cochlea, thus playing an important role in information transmission and material exchange [19, 20]. In this study, the detection rate of GJB2 deafness gene mutation was the highest in the experimental group, accounting for 51.6%. Among them, 235delC, 299-300delAT, and 35delG mutations were detected, whereas 167delT, 176-191dell6, and 508-511 dupAACG site mutations were undetected.

Epidemiological surveys found that the genetic loci associated with loss of hearing ability in the GJB2 gene mutations in the Nordic Jews, Caucasians, and Ghanaian populations were 35delG, 167delT, and 235delC, respectively [21]. The mutations of 235delC in China are various in different regions [22]. Moreover, the ratio between homozygotes and heterozygotes is also different. Mutations in the SLC26A4 gene can lead to autosomal recessive deafness DFNB4 and Pendred syndrome. This gene mutation is closely related to large vestibular aqueduct syndrome and cochlear malformation. In this study, the detection rate of SLC26A4 deafness gene mutation in the experimental group was 29.0%, ranking second, among which 2168A > G and IVS7-2A > G mutations were detected, while 1174A > T, 1229C > T, 1226G > A, 1975G > C, 2027T > A, 2162C > T, 281C > T, 589G > A, and IVS15 + 5G > A mutations were not detected. Inconsistent with Sun J [16], the results showed that the detection rate of SLC26A4 in the hereditary non-syndromic deaf populations from Xinjiang was lower than that in mitochondrial DNA (12SrRNA), only accounting for the third. The similarities and differences between our results may be related to factors such as different regions and ethnic groups. Through the screening of mitochondrial 12SrRNA 1555A > G and 1494C > T mutations, the aminoglycoside-like susceptibility of the whole family can be found by the detection of carriers, which has a relatively high clinical value. In this study, the detection rate of 12SrRNA deafness gene mutation in the experimental group was 19.4%, among which A1555G and 1494C > T mutations were detected, whereas the GJB3 gene mutation was not detected. YB Ji [21] demonstrated that GJB3 gene mutation is not common in the Xinjiang Uygur and Han hereditary non-syndromic deaf population, suggesting that GJB3 gene mutation may not be the main cause of the disease in Xinjiang. In contrast, Chai F *et al.* detected 26 patients with mutations in deafness susceptibility among 501 newborns in Shenzhen, including 3 cases of GJB3 (547G > A, 538C > T) mutations [23]. However, many studies generally believed that the mutation frequency of this gene is low in the normal population [24].

In the past five years, the research on deafness genes in Yunnan Province has just started. The researches focused on the census of deaf children, single gene in a single ethnic group from a certain region. Xin F *et al.* reported that the mutation rates of deafness genes in Kunming Han and other ethnic NSHL patients were 38.09% and 35.23%, respectively. There was no statistical difference between the two groups, and the minority groups included various nationalities [25]. In this study, we conducted a molecular epidemiological study on 21 common loci of 4 deafness genes in the ethnic minorities (Bai, Hani, and Yi) from Yunnan minority areas. It provided a basis for large-scale deafness screening and may find new deafness gene loci through in-depth screening and comparison based on the prevalence of the distribution, understanding of the corresponding mutation spectrum and hotspot mutations. There were statistically significant differences in the proportion of deafness patients with hot spot loci in the Han area compared with the experimental group in Yunnan. In this study, 7 mutation sites were detected from 150 ethnic minority (Bai, Hani, Yi) NSHL patients, but the GJB3 gene mutation was not detected. Although we analyzed ethnic groups, limited by

the size, the number of cases with deafness-related gene mutation sites detected in each group was small. Moreover, the difference among the three groups was not compared. There is a lack of further research on the genetic and molecular mechanisms that contribute to the disease.

Conclusion

The mutation rates of three deafness-related genes, including GJB2, SLC26A4, and 12SrRNA in 150 ethnic minority deaf populations were 51.6% (16/31), 29% (9/31), and 19.4% (6/31), respectively. GJB2 exhibited the highest detection rate. Mitochondrial DNA-A1555G, GJB2-235delC, and SLC26A4-IVS7-2A > G were hotspot gene mutation in moderate-to-severe NSHL.

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Disclosure of conflict of interest

None.

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