Original Article

DOI: 10.5582/irdr.2015.01018

A study of deafness-related genetic mutations as a basis for strategies to prevent hereditary hearing loss in Hebei, China

Junzhen Zhu[1,*], Qinying Cao[2,*], Ning Zhang[1], Jun Ge[2], Donglan Sun[2,], Qingqi Feng[3]**

[1] *Center for Exceptional Care in 3 Areas, The People's Hospital of Hebei Province, Shijiazhuang, Hebei Province, China;*
[2] *The Fourth Hospital of Shijiazhuang, Shijiazhuang, Hebei Province, China;*
[3] *Xinji Maternal and Child Health Hospital, Xinji, Hebei Province, China.*

Summary **Hearing loss is the most common sensory disorder, and at least 50% of cases are due to a genetic etiology. Two-thirds of individuals with congenital deafness are nonsyndromic. Among the nonsyndromic forms, the large majority are monogenic autosomal recessive traits. The current work summarizes mutations in the *GJB2*, *SLC26A4*, *12SrRNA*, and *GJB3* and their prevalence in 318 students with autosomal recessive nonsyndromic hearing loss at schools for the deaf or special needs schools in 9 cities in Hebei Province, China. Deafness gene mutations were identified in 137 students *via* a gene chip, time-of-flight mass spectrometry, fluorescence quantitative PCR, and gene sequencing. Mutations were detected at a rate of 43.08%. A homozygous mutation of the *GJB2* gene was found in 16 students (5.03%), a heterozygous mutation of that gene was found in 38 (11.95%), a homozygous mutation of the *SLC26A4* gene was found in 22 (6.92%), a heterozygous mutation of that gene was found in 59 (18.55%), and a heterozygous mutation of the mitochondrial *12SrRNA* gene was found in 2 (0.63%). In addition, there were 15 families in which a student's parents had normal hearing. Compound heterozygous mutations of the *GJB2* gene were found in 3 families (20%) and mutations of the *SLC26A4* gene were found in 9 (60%). Thus, this study has provided a molecular diagnostic basis for the causes of deafness, and this study has also provided a scientific basis for the early prevention of and intervention in deafness.**

Keywords: Hereditary hearing loss; gene mutation; gene chip; time-of-flight mass spectrometry; sequencing

1. Introduction

Congenital deafness is an irreversible condition due to intrauterine dysplasia or genetic factors. An estimated 30,000 babies are born with congenital hearing impairment per 20 million live births every year in China, and this impairment seriously affects their quality of life. Worldwide, the incidence of congenital deafness, including deafness caused by many genetic and environmental factors, is about 1/1000 (*1*). An estimated 80,000 new patients appear each year because of the clinical use of a large number of antibiotics. People with disabilities in Hebei number about 519.5 million, accounting for 1.86% of the province's population. People with disabilities include 126.0 million with a hearing disability and 7.7 million with a speech disability, and these 2 types of disabilities account for 25.74% of all disabilities.

Gap junction protein beta-2 (*GJB2*) (MIM 220290) was the first gene in which mutations were reported to cause autosomal recessive nonsyndromic hearing loss (ARNSHL) in 1997 (*2*). Although mutations in *GJB3* (MIM 603324) and *GJB6* (MIM604418) were subsequently discovered, *GJB2* remains the most common cause of hereditary deafness in many populations. Mutations in *GJB2* were discovered and were shown to cause up to 50% of ARNSHL in Caucasian populations, but their frequency is much lower in other parts of the world (*3-5*). The c.235delC

*These authors contributed equally to this works.

***Address correspondence to:*
Dr. Donglan Sun, The Fourth Hospitals of Shijiazhuang, No. 212, Zhong Shan Dong Lu, Chang-an Qu, Shijiazhuang, Hebei Province, China.
E-mail: s-d-l2007@163.com

mutation is the most frequent pathogenic variant in Japanese (*6*), while the c.35delG mutation is the most frequent pathogenic variant in the majority of Caucasian populations (70%) (*7*), and the c.167delT mutation is the most common in Ashkenazi Jews (*8*). Indeed, mutations in other connexin genes, such as *GJB6* for Cx30 and *GJB3* for Cx31, have been identified and shown to cause hearing impairment (*9,10*).

The *SLC26A4* gene encodes pendrin, which is a transmembrane anion exchanger that belongs to the solute carrier 26 family and that exchanges chloride, iodide, bicarbonate, and formate. Pendrin is expressed in different tissues, including the thyroid, the kidneys, and the inner ear. In the cochlea, pendrin is found in the apical membrane of the outer sulcus and spiral prominence epithelial cells that border the endolymph, in the spiral ganglion, and in supporting cells (*11*). DNA sequencing has identified more than 100 different mutations in *SLC26A4* (*12-15*). *SLC26A4* mutations may account for as much as 10% of the hereditary deafness in diverse populations (*16*). Four of these mutations, IVS7-2A>G, 2168A>G, 84C>A, 1975G>C, 754 T>C, and IVS 9+1G>A, were previously reported in patients with hearing loss (*17-19*), and IVS7-2A >G is the most prevalent mutation in China (*20*).

Although most cases of hereditary hearing loss are caused by nuclear gene defects, a study has shown that mutations in mitochondrial DNA (mtDNA) can also cause nonsyndromic hearing loss (*21,22*). The 1555A>G mutation is the best studied of these mutations in the mitochondrial *12S rRNA* gene. The second mutation identified in the mitochondrial *12S rRNA* gene is the 1494C>T in the conserved stem structure of *12SrRNA* (*23*). Other nucleotide changes at positions 961 and 1095 in the *12S rRNA* gene have been shown to be associated with hearing loss, but their pathogenic mechanisms of action in the predisposition of carriers to aminoglycoside toxicity are much less clear (*24,25*). The mtDNA 1555A>G mutation accounts for a small fraction of nonsyndromic hearing loss, with a prevalence of 3.43% in China, 3% in Japan, and 3.43% in Indonesia (*26-28*); this mutation is less evident in Caucasian populations, with a prevalence between 0.6% and 2.5% (*29-31*).

The present study comprehensively analyzed 4 prominent deafness-related genes, *GJB2*, *GJB3*, *SLC26A4*, and mtDNA *12SrRNA*, in 318 students at schools for the deaf or special needs schools and their parents in 9 cities in Hebei Province, China.

2. Subjects and Methods

Potential subjects were students at schools for the deaf or special needs schools in 9 cities in Hebei Province. Subjects were 318 students with non-syndromic deafness (with 30-48 subjects per city). Subjects consisted of 64 males and 154 females ranging in age from 2 months to 58 years, with an average age of 10.48 years. Once parental consent was obtained, 15 families were studied (Figure 1). After subjects and their guardians agreed to voluntarily participate in genetic testing, they provided informed consent in writing. Subjects were asked to provide basic personal information, including marriage information, family history, pregnancy history, gestation history, medication history, history of infection, whether abnormalities occurred during pregnancy, whether birth

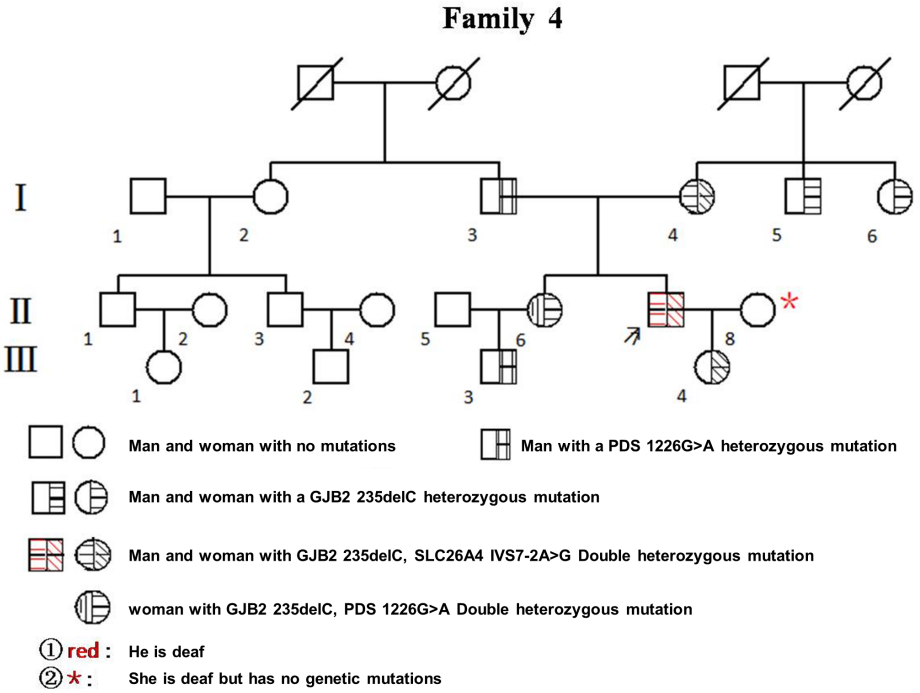

Figure 1. Family 4 was studied.

Table 1. Mutations and the number of students with those mutations

Gene name	Mutation	Type of mutation (no. students)		
		Homozygous mutant	Heterozygous mutation	Homogeneous mutation
GJB2	35delG		1	
	235delC	16	18	
	299-300delAT		13	
	176del 16bp		6	
GJB3	0	0	0	
SLC26A4	IVS7-2A>G	18	33	
	2168 A>G	4	13	
	589G>A		1	
	1174A>T		1	
	1226G>A		3	
	1229C>T		7	
	2027T>A		1	
12SrRNA	1555A>G			2

was premature, whether the neonate had a low birth weight, whether there was an obvious history of head injury before deafness, the use of ototoxic drugs, and detailed medical records. Specimens were then collected.

Three to 5 mL of peripheral blood was collected with a vacuum blood collection tube. Specimens were numbered and blood was dropped on filter paper. DNA was extracted from 2 specimens per subject. UV spectrophotometry was used to quantify and test the purity of the specimen. Specimens were recorded and stored separately by city. Nine gene loci - *GJB2* 35delG, 176del16, 235delC, 299-300delAT, *GJB3* 538, *SLC26A4* 2168A>G, and IVS7-2A>G, *12SrRNA* 1494C>T, and 1555A>G - were detected in 4 common genes (*GJB2*, *GJB3*, *SLC26A4*, and mtDNA *12SrRNA*) using the GeneChip (Beijing Boao Bio Co., Ltd.). Twenty other gene loci - *GJB2* 167delT, *GJB3* 547G>A, *SLC26A4* 281C>T, 589G>A, 1174A>T, 1226 G>A, 1229C>T, IVS15+5 G>A, 1975G>C, 2027T>A, and 2162C>T - were detected with time-of-flight mass spectrometry. Fourteen gene loci - *GJB2* 35delG, 176del16, 235delC, 299-300delAT, 155delTCTG, 512insAACG, *SLC26A4* 2168A>G, IVS7-2A>G 1229C>T, and 1174A>T, *GJB3* 538C>G, 547G>A, *12SrRNA* 1494C>T, and 1555A>G - in the 4 genes were analyzed in 36 of the 318 students using fluorescence quantitative PCR (Jinan Yingsheng Biology). Exons of *GJB2* were analyzed in 3 students using gene sequencing.

All of the members of 15 families underwent acoustic immittance testing, and 13 of the 20 family members with mutations or variants in *SLC26A4* underwent a temporal bone computed tomography (CT) scan for diagnosis of enlarged vestibular aqueducts or inner ear malformation.

3. Results

Gene mutations were detected in 137 (43.08%) of 318 students. *GJB2* mutations were detected in 54 students (16.98%), *SLC26A4* gene mutations were detected in 81 (25.47%), and mitochondrial *12SrRNA* gene mutations were detected in 2 (0.63%). No *GJB3* mutations were detected. A homozygous mutation was detected in 40 students (12.58%). A homozygous mutation in *GJB2* was detected in 16 students (5.03%), a homozygous mutation in *SLC26A4* was detected in 22 (6.92%), and a homogeneous mutation in mtDNA *12rRNA* was detected in 2 (0.63%). Heterozygous mutations were detected in 97 students (30.50%). Heterozygous mutations in *GJB2* were detected in 38 students (11.95%), heterozygous mutations in *SLC26A4* were detected in 59 (18.55%) (Table 1), compound heterozygous mutations were detected in 23 (7.23%), and double heterozygous mutations were detected in 23 (7.23%). A compound heterozygous mutation was detected in 19 students (*GJB2* in 11 students and *SLC26A4* in 8 students) (5.97%), and a double heterozygous mutation was detected in 3 (1.26%) (Tables 2 and 3).

Table 2. Compound heterozygous mutations and the number of students with those mutations

Gene name	Mutation		No. students
GJB2	235delC	299-300delAT	7
	235delC	176-191del16	3
	299-300delAT	176-191del16	1
SLC26A4	IVS7-2A>G	2168 A>G	4[#]
	IVS7-2A>G	589G>A	1
	IVS7-2A>G	2027T>A	1
	IVS7-2A>G	2162C>T	1
	1975G>C	2168 A>G	1
	1226G>A	2168 A>G	1
	IVS7-2A>G	235delC	2
	1226G>A	235delC	1

[#] 1 case was *SLC26A4* IVS7-2A>G/2168 A>G and *GJB2* 235delC compound heterozygous mutation

Table 3. Double heterozygous mutations and the number of students with those mutations

Gene name	Mutation		No. students
SLC26A4/GJB2	IVS7-2A>G	235delC	2
	1226G>A	235delC	1

Heterozygous mutations in the form of exon

Table 4. Twenty-six new polymorphic *GJB2* mutations detected in 36 students

Gene name	Mutation			No. students
GJB2	79G>A Heterozygote			6
	79G>A Heterozygote	341A>G Heterozygote		11
	79G>A Heterozygote	109 G>A Heterozygote	341A>G Heterozygote	1
	79G>A Homozygote	341A>G Heterozygote		3
	79G>A Homozygote	341A>G Homozygote		1
	608T>C Heterozygote			4

polymorphisms in the *GJB2* gene were detected in 36 students. Gene sequencing indicated that 26 students had mosaic or compound heterozygous mutations (66.67%), with 1 student exhibiting a 79G>A homozygous mutation and 1 exhibiting a heterozygous mutation in the form of 341A>G polymorphism. A 79G>A homozygous mutation was detected in 5 students (13.89%), a 608T>C heterozygous mutation was detected in 4 (11.11%), and a complex polymorphic mutation was detected in 15 (41.67%) (Table 4).

A family study found that the proband's parents had normal hearing in 15 pedigrees (Table 5). Pedigrees 1, 2, and 14 exhibited *GJB2* mutations, with pedigree 2 exhibiting a heterozygous mutation in *GJB2* and pedigrees 1 and 14 exhibiting a compound heterozygous mutation in *GJB2*. These 3 pedigrees accounted for 20% of the 15 pedigrees. *SLC26A4* gene mutations were found in 9 pedigrees: 3, 6, 7, 8, 9, 10, 11, 12, and 13 (including pedigrees 6, 7, 8, 9, and 13 with a homozygous mutation and pedigrees 3, 10, 11, and 12 with a heterozygous mutation). These 9 pedigrees accounted for 4.5% of 60 pedigrees in total. Pedigrees with a double *GJB2*/*SLC26A4* heterozygous mutation (including a *GJB2*/235delC homozygous mutation in pedigree 5 and a double heterozygous *SLC2/6A4* mutation and double heterozygous mutations of *GJB2* 235delC and IVS7-2 A>G in pedigree 4) accounted for 13.33% of the pedigrees. Pedigree 15 had a *SLC26A4* 1229C>T and 2168A>G compound heterozygous mutation.

4. Discussion

Congenital deafness is one of the most common birth defects in humans, with an incidence of about 1 ‰ to 3 ‰ (*32*). This condition seriously affects an individual's quality of life. About 50-60% of these cases have a genetic cause. The cause is an autosomal recessive condition in 80% and an autosomal dominant condition in 10% to 20%. These conditions are sex-linked at a rate between 1% and 2%. Thus far, at least 44 deafness-related genes have been identified. The most common gene is *GJB2*, which is located in 13q11-12. In 1998, Xia *et al*. (*33*) first reported *GJB3*, a gene located in 1p33-35. A mutation in *SLC26A4*, which is located in q22-31.1, has been found to be associated with large vestibular conduit syndrome. A mutation in *12SrRNA* in mtDNA has been proven to be associated with drug-induced deafness. At present, strategies to prevent deafness universally include newborn hearing screening, which is one of the keys to the early detection and diagnosis of hearing impairment (*34*), and these strategies have achieved remarkable developments. Although genetic testing enables an estimation of the chance of reoccurrence, there are many other reasons why children with congenital hearing loss should undergo genetic evaluation and receive genetic counseling (*32*). Providers of genetic testing and counseling services have an important role to play in reducing hearing loss in newborns and young children.

Genetic screening detected mutations in 137 (43.08%) of 318 students. Genetic mutations were identified in 13 of 51 deaf children (25.49%) in Qianghu, Anhui (*35*). Gene mutations at 9 sites of 4 genes, including *GJB2*, *GJB3*, *SLC26A4*, and *12SrRNA*, were found in a total of 22 (34.4%) of 64 patients with nonsyndromic hearing impairment in Henan (*36*). Furthermore, patients in Shanxi and Liaoning provinces tested positive for mutations in *SLC26A4*, *GJB2*, or *12SrRNA* 1555 A>G/1494C>T at a rate of 33.3% and 42.5%, respectively (*37,38*). Thus, research has shown that the rate of mutations in deafness genes is higher in Hebei than in other regions. In the current study, mutations in *SLC26A4* were identified in 25.47% of students (81/318) with hearing impairment in Hebei of China. Thirty of the 81 students had 2 mutant alleles while 51 had 1 mutant allele. The most common mutation was IVS7-2A>G. The spectrum of *SLC26A4* mutations in Hebei is similar to that reported in the overall Chinese population, with IVS7-2A>G being a hotspot mutation. In Japan, H723R is the most prevalent mutation (*16*). In South Korea, IVS7-2A>G and H723R are the two most prevalent (*39*). A recent study of 109 unrelated probands with enlarged vestibular aqueducts in a Danish population found that the most frequent mutation was 1246A>C. This implies that Danish and Chinese populations are of different ancestry (*40*). In the current study, *GJB2* gene mutations were detected in 54 students (16.98%). 35delG is most common mutation in Caucasians. Yuan *et al*. (*41*) reported that 235delC accounted for 71.64% of *GJB2* mutant alleles in China, and this figure agrees with the findings of the current study (71.43%, 50/70 students). This mutation is detected at the highest rates in Asian populations, with a prevalence of approximately 41% and 57%

Table 5. The relationship between 50 family mutations and deafness

Family	Patient	Family member	Gene	Mutational bits	Mutational types	Items
1	II_2		*GJB2*	235delC, 299-300delAT	Heterozygous mutation	Some remaining hearing
		I_1		235delC	Heterozygous mutation	Normal hearing
		I_2		299-300delAT	Heterozygous mutation	Normal hearing
2	III_1		*GJB2*	299-300delAT	Heterozygous mutation	Passed
		I_1		235delC, 299-300delAT	Heterozygous mutation	Normal hearing
		I_2		235delC	Heterozygous mutation	Normal hearing
		I_3		299-300delAT	Heterozygous mutation	Normal hearing
		II_1		235delC, 299-300delAT	Heterozygous mutation	Some remaining hearing
		II_2		299-300delAT	Heterozygous mutation	serious hearing
3	II_1		*SLC26A4*	IVS7-2A>G	Heterozygous mutation	Nerve deafness
		II_2		IVS7-2A>G	Heterozygous mutation	Nerve deafness
		II_3		IVS7-2A>G	Heterozygous mutation	Nerve deafness
		I_1		Negative		Normal hearing
		I_2		IVS7-2A>G	Heterozygous mutation	Nerve deafness
4	II_7		*GJB2/SLC26A4*	235delC, IVS7-2A>G	Heterozygous mutation	serious hearing
		I_4	*GJB2/SLC26A4*	235delC, IVS7-2A>G	Heterozygous mutation	Normal hearing
		I_3	*SLC26A4*	1226G>A	Heterozygous mutation	Normal hearing
		I_5	*GJB2*	235delC	Heterozygous mutation	Normal hearing
		I_6	*SLC26A4*	IVS7-2A>G	Heterozygous mutation	Normal hearing
		II_6	*GJB2/SLC26A4*	235delC, 1226G>A	Heterozygous mutation	Normal hearing
		II_8		Negative		serious hearing
		III_3	*SLC26A4*	1226G>A	Heterozygous mutation	Normal hearing
		III_4		IVS7-2A>G	Heterozygous mutation	Normal hearing
5	II_2		*GJB2/*	235delC	Homozygous mutation	Mid-serious deafness
		$I_{1,2}$	*SLC26A4*	IVS7-2A>G	Heterozygous mutation	Mid-serious deafness
		II_1		Negative		Normal hearing
		III_1		Negative		Some remaining hearing
			GJB2	235delC	Heterozygous mutation	Normal hearing
6-7	II_1		*SLC26A4*	IVS7-2A>G	Homozygous mutant	Severe deafness
		$I_{1,2}$		IVS7-2A>G	Heterozygous mutation	Normal hearing
8	II_1			IVS7-2A>G	Homozygous mutant	Severe deafness
		I_1		IVS7-2A>G	Heterozygous mutation	Normal hearing
		I_2		Negative		Normal hearing
9	II_1			IVS7-2A>G	Homozygous mutant	serious hearing
		$I_{1,2}$		IVS7-2A>G	Heterozygous mutation	Normal hearing
10	II_1			IVS7-2A>G	Heterozygous mutation	Nerve deafness
		I_1		Negative		Normal hearing
		I_2		IVS7-2A>G	Heterozygous mutation	Normal hearing
11	II_1			IVS7-2A>G	Heterozygous mutation	Nerve deafness but ability to hear speech
		I_1		1229C>T	Heterozygous mutation	Normal hearing
		I_2		IVS7-2A>G	Heterozygous mutation	Normal hearing
12	II_1			IVS7-2A>G	Heterozygous mutation	Severe nerve deafness
		$I_{1,2}$		Negative		Normal hearing
13	II_1			IVS7-2A>G	Homozygous mutant	Severe nerve deafness
		I_1		IVS7-2A>G	Heterozygous mutation	Normal hearing
		I_2		IVS7-2A>G	Heterozygous mutation	Normal hearing
14	II_1		*GJB2*	235delC 299-300delAT	Heterozygous mutation	Severe nerve deafness
		I_1		299-300delAT	Heterozygous mutation	Normal hearing
		I_2		235delC	Heterozygous mutation	Normal hearing
15	II_1		*SLC26A4*	1229C>T and 2168A>G	Heterozygous mutation	Very severe hearing loss
		I_1		2168A>G	Heterozygous mutation	Normal hearing
		I_2		1229C>T	Heterozygous mutation	Normal hearing

according to 2 Japanese studies, 67% according to 1 Taiwanese study, and 73% according to 1 South Korean study (*41-46*). *12SrRNA* mutations were detected in 2 students (0.63%), although this rate is lower than their prevalence nationally (2.83%) (*47*). No *GJB3* mutations were noted in students.

Studies have shown that the *SLC26A4* gene, the *GJB2* gene, and the mitochondrial *12SrRNA* gene are prominent mutations that lead to most of the hereditary deafness in Asia. Screening for mutations in these 3

genes is crucial to identifying nonsyndromic hereditary hearing loss and drug-induced deafness. The current study identified 15 pedigrees of mutations. Genetic testing can provide a scientific basis for guiding and advising deaf patients and their family members. One example is the members of pedigree 4. Two individuals were lovers who sought consultation before marriage (*48*). The first question they asked was whether they could get married. The second question was whether their child would be deaf like them. They consented to undergo genetic testing. The woman's test results were negative for mutations while the man's *GJB2* genetic testing revealed double heterozygous mutations of 235delc and *SLC26A4* IVS7-2 A>G. Given a scientific estimate regarding the potential for them to have a hearing child, they decided to marry and gave birth to a baby girl with normal hearing. When the gene chip was used to detect 9 loci of 4 genes, it only found *GJB2* and *SLC26A4* gene mutations, but subsequent time-of-flight mass spectrometry found 20 mutations (4 gene loci) in 11 students (3.46%). The S*LC26A4* gene mutation of 1226 G> A was detected in 3 students, the gene mutation of 1229 C>T was detected in 7, and the gene mutation of 2027 T>A was detected in 1. Genetic information can provide more comprehensive information for genetic counseling. Thus, this study suggests that high-risk families should choose 2 methods of genetic testing to avoid a false negative for mutations.

This study detected polymorphisms of the *GJB2* gene. Four polymorphisms were detected in 36 students, including heterozygous mutations in 109 G>A in 1 (2.78%) and in 79 G>A in 22 (61.11%). Homozygous mutations were detected in 1 student. Heterozygous and homozygous mutations in 341A>G were detected in 14 students (38.89%). A heterozygous mutation in 608 T>C was detected in 4 students (11.11%). A study by Liu *et al*. (*49*) found that 79G>A, 341A>G, 109G>A, and 608T>C were common polymorphisms in the Dai and Han ethnic groups. A study by Li *et al*. (*50*) detected *GJB2* mutations in neonates and identified the 4 types of mutation they considered to be polymorphisms. Other studies have shown that 79G>A, 341A>G, and 608T>C are found in the general population but do not cause deafness (*51-53*). The current study found that these changes are common *GJB2* gene polymorphisms. A change in genetic polymorphism means that the structure of DNA molecules changes in an individual in a population, but the aspects of gene expression and gene function remain the same. A change in polymorphism is a normal phenomenon, spontaneously occurring at a rate of around 1% (*54*). Currently, the 109 G>A mutation is assumed to be a mutation resulting in substitution of G for A. A point mutation will result in substitution of the encoded amino acid (valine replaced with isoleucine). However, the issue of whether a mutation substituting G for A at locus 109 of the *GJB2* gene can directly lead to hereditary deafness remains controversial in domestic and foreign literature. Kelly *et al*. (*55*) has detected the 109 G>A mutation in normal populations, implying that the polymorphic change does not lead directly to hereditary deafness. However, a study by Abe *et al*. (*45*) in Japan reached the opposite conclusion. There is no clear consensus in academic circles on whether a mutation at a specific locus leads to hereditary deafness. Thus, whether the 109 G>A mutation in *GJB2* leads to deafness must be studied further.

Hereditary deafness is a common form of severe hearing loss. Genetic testing is a useful way to dispel misinformation or alleviate concerns that parents have about what may have caused hearing loss. Although gene screening plays an important role in decreasing the birth rate of deaf infants, many problems still need to be solved, such as the widespread shortage of technical personnel in genetic testing laboratories. Another problem is the lack of guidelines indicating whether genetic testing for deafness should be performed prior to marriage, prior to pregnancy, prior to birth, or after birth. The lack of solutions constrains the development of methods of genetic screening for deafness. Under current conditions, genetic screening still has a long way to go to facilitate the detection of deafness genes. In other words, the development of prenatal diagnosis and genetic counseling will greatly reduce the birth rate of deaf children, decrease the number of deaf patients, improve the quality of births, and reduce the social and family burden of deafness.

References

1. Corlin RJ, Toriello HV, Cohen MM. Hereditary hearing loss and its syndrome. New York: Oxford University Press, NY, USA, 1995.
2. Kelsell DP, Dunlop J, Stevens HP, Lench NJ, Liang JN, Parry G, Mueller RF, Leigh IM. Connexin 26 mutations in hereditary nonsyndromic sensorineural deafness. Nature. 1997; 387:80-83.
3. Seeman P, Bendova O, Raskova D, Malikova M, Groh D, Kabelka Z. Double heterozygosity with mutations involving both the *GJB2* and *GJB6* genes is a possible, but very rare, cause of congenital deafness in the Czech population. Ann Hum Genet. 2005; 69:9-14.
4. Oguchi T, Ohtsuka A, Hashimoto S, Oshima A, Abe S, Kobayashi Y, Nagai K, Matsunaga T, Iwasaki S, Nakagawa T, Usami S. Clinical features of patients with *GJB2* (connexin 26) mutations: Severity of hearing loss is correlated with genotypes and protein expression patterns. J Hum Genet. 2005; 50:76-83.
5. Tekin M, Xia XJ, Erdenetungalag R, Cengiz FB, White TW, Radnaabazar J, Dangaasuren B, Tastan H, Nance WE, Pandya A. *GJB2* mutations in Mongolia: Complex alleles, low frequency, and reduced fitness of the deaf. Ann Hum Genet. 2010; 74:155-164.
6. Ohtsuka A, Yuge I, Kimura S, Namba A, Abe S, Van Laer L, Van Camp G, Usami S. *GJB2* deafness gene shows a specific spectrum of mutations in Japan, including a

frequent founder mutation. Hum Genet. 2003; 112:329-333.
7. Snoeckx RL, Huygen PL, Feldmann D, *et al*. *GJB2* mutations and degree of hearing loss: A multicenter study. Am J Hum Genet. 2005; 77:945-957.
8. Morell RJ, Kim HJ, Hood LJ, Goforth L, Friderici K, Fisher R, Van Camp G, Berlin CI, Oddoux C, Ostrer H, Keats B, Friedman TB. Mutations in the connexin 26 gene (*GJB2*) among Ashkenazi Jews with nonsyndromic recessive deafness. N Engl J Med. 1998; 339:1500-1505.
9. Yu F, Han DY, Dai P, Kang DY, Zhang X, Liu X, Zhu QW, Yuan YY, Sun Q, Xue DD, Li M, Liu J, Yuan HJ, Yang WY. Mutation of the *GJB2* gene in Chinese patients with nonsyndromic hearing impairment: Analysis of 1,190 cases. Natl Med J China. 2007; 87:2814-2819. (in Chinese)
10. Grifa A, Wagner CA, D'Ambrosio L, Melchionda S, Bernardi F, Lopez-Bigas N, Rabionet R, Arbones M, Monica MD, Estivill X, Zelante L, Lang F, Gasparini P. Mutations in *GJB6* cause nonsyndromic autosomal dominant deafness at DFNA3 locus. Nat Genet. 1999; 23:16-18.
11. Yoshino T, Sato E, Nakashima T, Teranishi M, Yamamoto H, Otake H, Mizuno T. Distribution of pendrin in the organ of corti of mice observed by electron immunomicroscopy. Eur Arch Otorhinolaryngol. 2006; 263:699-704.
12. Wilcox SA, Saunders K, Osborn AH, Arnold A, Wunderlich J, Kelly T, Collins V, Wilcox LJ, McKinlay Gardner RJ, Kamarinos M, Cone-Wesson B, Williamson R, Dahl HH. High frequency hearing loss correlated with mutations in the *GJB2* gene. Hum Genet. 2000; 106:399-405.
13. Prasad S, Kolln KA, Cucci RA, Trembath RC, Van Camp G, Smith RJ. Pendred syndrome and DFNB4-mutation screening of *SLC26A4* by denaturing high-performance liquid chromatography and the identification of eleven novel mutations. Am J Med Genet A. 2004; 124:1-9.
14. Albert S, Blons H, Jonard L, Feldmann D, *et al*. *SLC26A4* gene is frequently involved in nonsyndromic hearing impairment with enlarged vestibular aqueduct in Caucasian populations. Eur J Hum Genet. 2006; 14:773-779.
15. Wang QJ, Zhao YL, Rao SQ, Guo YF, Yuan H, Zong L, Guan J, Xu BC, Wang DY, Han MK, Lan L, Zhai SQ, Shen Y. A distinct spectrum of *SLC26A4* mutations in patients with enlarged vestibular aqueduct in China. Clin Genet. 2007; 72:245-54.
16. Park HJ, Shaukat S, Liu XZ, *et al*. Origins and frequencies of *SLC26A4* (PDS) mutations in East and South Asians: Global implications for the epidemiology of deafness. J Med Genet. 2003; 40:242-248.
17. Yao G, Li S, Chen D, *et al*. Compound heterozygous mutations of *SLC26A4* in 4 Chinese families with enlarged vestibular aqueduct. Int J Pediatr Otorhinolaryngol. 2013; 77:544-549.
18. Jiang L, Feng Y, Chen H, He C, Mei L. An investigation of *SLC26A4* gene mutation in nonsydromic hearing impairment in Hunan province of China. Journal Clinical Otorhinolaryngology and Head and Neck Surg. 2010; 24:587-591. (in Chinese)
19. Huang S, Han D, Yuan Y, Wang G, Kang D, Zhang X, Yan X, Meng X, Dong M, Dai P. Extremely discrepant mutation spectrum of *SLC26A4* between Chinese patients with isolated Mondini deformity and enlarged vestibular aqueduct. J Transl Med. 2011; 9:167.
20. Xin F, Yuan Y, Deng X, Han M, Wang G, Zhao J, Gao X, Liu J, Yu F, Han D, Dai P. Genetic mutations in patients of Chinese minority groups and the Han ethnic group with nonsyndromic deafness in Yunnan, China. J Transl Med. 2013; 11:312. (in Chinese)
21. Fischel-Ghodsian N. Mitochondrial genetics and hearing loss: The missing link between genotype and phenotype. Proc Soc Exp Biol Med. 1998; 218:1-6.
22. Hutchin TP, Cortopassi GA. Mitochondrial defects and hearing loss. Cell Mol Life Sci. 2000; 57:1927-1937.
23. Zhao H, Li R, Wang Q, Yan Q, Deng JH, Han D, Bai Y, Young WY, Guan MX. Maternally inherited aminoglycoside-induced and nonsyndromic deafness is associated with the novel C1494T mutation in the mitochondrial *12S rRNA* gene in a large Chinese family. Am J Hum Genet. 2004; 74:139-152.
24. Castillo FJ, Rodriguez-Ballesteros M, Martin Y, Arellano B, Gallo-Teran J, Morales-Angulo C, Ramirez-Camacho R, Cruz Tapia M, Solanellas J, Martinez-Conde A, Villamar M, Moreno-Pelayo MA, Moreno F, Del Castillo I. Heteroplasmy for the 1555A>G mutation in the mitochondrial *12S rRNA* gene in six Spanish families with non-syndromic hearing loss. J Med Genet. 2003; 40:632-636.
25. Li R, Xing G, Yan M, Cao X, Liu XZ, Bu X, Guan MX. Cosegregation of C-insertion at position 961 with the A1555G mutation of the mitochondrial *12S rRNA* gene in a large Chinese family with maternally inherited hearing loss. Am J Med Genet A. 2004; 124:113-117.
26. Liu X, Dai P, Huang DL, Yuan HJ, Li WM, Cao JY, Yu F, Zhang RN, Lin HY, Zhu XH, He Y, Yu YJ, Yao K. Large-scale screening of mtDNA A1555G mutation in China and its significance in prevention of aminoglycoside antibiotic induced deafness. Zhong hua Yi Xue Za Zhi. 2006; 86:1318-22. (in Chineset)
27. Usami S, Abe S, Akita J, Namba A, Shinkawa H, Ishii M, Iwasaki S, Hoshino T, Ito J, Doi K, Kubo T, Nakagawa T, Komiyama S, Tono T, Komune S. Prevalence of mitochondrial gene mutations among hearing impaired patients. J Med Genet. 2000; 37:38-40.
28. Malik SG, Pieter N, Sudoyo H, Kadir A, Marzuki S. Prevalence of the mitochondrial DNA A1555G mutation in sensorineural deafness patients in island Southeast Asia. J Hum Genet. 2003; 48:480-483.
29. Kupka S, Toth T, Wrobel M, Zeissler U, Szyfter W, Szyfter K, Niedzielska G, Bal J, Zenner HP, Sziklai I, Blin N, Pfister M. Mutation A1555G in the *12S rRNA* gene and its epidemiological importance in German, Hungarian, and Polish patients. Hum Mutat. 2002; 19:308-309.
30. Ostergaard E, Montserrat-Sentis B, Gronskov K, Brondum-Nielsen K. The A1555G mtDNA mutation in Danish hearing-impaired patients: Frequency and clinical signs. Clin Genet. 2002; 62:303-305.
31. Tekin M, Duman T, Bogoclu G, Incesulu A, Comak E, Fitoz S, Yilmaz E, Ilhan I, Akar N. Frequency of mtDNA A1555G and A7445G mutations among children with prelingual deafness in Turkey. Eur J Pediatr. 2003; 162:154-158.
32. White KR. Early hearing detection and intervention programs: Opportunities for genetic services. AJMG. 2004; 130A:29-36.
33. Xia JH, Liu CY, Tang BS, *et al*. Mutations in the gene encoding gap junction Protein beta-3 associated with

autosomal dominant hearing impairment. Nat Genet. 1998; 4:370-373.
34. Burton SK, Withrow K, Arnos KS, Kalfoglou AL, Pandya A. A focus group study of consumer attitudes toward genetic testing and newborn screening for deafness. Genet Med. 2006; 8:779-783.
35. Liu SF, Bu LN, Wu LJ, Wang CX, Feng G. Analysis of common genetic mutations in 51 patients with profound deafness. Anhui Medical Journal. 2014; 35:566-568. (in Chinese)
36. Zhao HR, Liao SX, Ding XB, Wang L, Yang K. Use of a gene chip in the genetic analysis of 64 patients of the Han ethnic group with nonsyndromic hearing impairment (NSHI) in Henan Province. Journal Shanxi Medical University. 2012; 43:649-651. (in Chinese)
37. Hu ZP, Li J, Li XZ, Zhang Y, Liang F, Wei W. Genetic testing of 108 deaf children at a school in Changzhi. Chinese J Birth Health & Heredity. 2014; 22:42-44.
38. Qu CH, Zhang N, Cao DH, Hao DM. Screening for common deafness genes in 240 deaf patients. Acad J Chin PLA Med Sch. 2014; 35:1019-1021. (in Chinese)
39. Tsukamoto K, Suzuki H, Harada D, Namba A, Abe S, Usami S. Distribution and frequencies of PDS (*SLC26A4*) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: A unique spectrum of mutations in Japanese. Eur J Hum Genet. 2003; 11:916-922.
40. Rendtorff N, Schrijver I, Lodahl M, Rodriguez-Paris J, Johnsen T, Hansen E, Nickelsen L, Tumer Z, Fagerheim T, Wetke R, Tranebjaerg L. *SLC26A4* mutation frequency and spectrum in 109 Danish Pendred syndrome/DFNB4 probands and a report of nine novel mutations. Clin Genet. 2013; 84:388-391.
41. Yuan YY, You YW, Huang DL, Cui JH, Wang Y, Wang Q, Yu F, Kang DY, Yuan HJ, Han DY, Dai P. Comprehensive molecular etiology analysis of nonsyndromic hearing impairment from typical areas in China. Journal of Translational Medicine. 2009; 7:79.
42. Park HJ, Hahn SH, Chun YM, Park K, Kim HN. Connexin26 mutations associated with nonsyndromic hearing loss. Laryngoscope. 2000; 110:1535-1538.
43. Ohtsuka A, Yuge I, Kimura S, Namba A, Abe S, Van Laer L, Van Camp G, Usami S. *GJB2* deafness gene shows a specific spectrum of mutations in Japan, including a frequent founder mutation. Hum Genet. 2003; 112:329-333.
44. Hwa HL, Ko TM, Hsu CJ, Huang CH, Chiang YL, Oong JL, Chen CC, Hsu CK. Mutation spectrum of the connexin 26 (*GJB2*) gene in Taiwanese patients with prelingual deafness. Genet Med. 2003; 5:161-165.
45. Abe S, Usami S, Shinkawa H, Kelley PM, Kimberling WJ. Prevalent connexin 26 gene (GJB2) mutations in Japanese. J Med Genet. 2000; 37:41-43.
46. Wang YC, Kung CY, Su MC, Su CC, Hsu HM, Tsai CC, Lin CC, Li SY. Mutations of Cx26 gene (*GJB2*) for prelingual deafness in Taiwan. Eur J Hum Genet. 2002; 10:495-498.
47. Dai P, Liu X, Yu F, Zhu QW, Yuan YY, Yang SZ, Sun Q, Yuan HJ, Yang WY, Huang DL, Han DY. Molecular etiology of patients with nonsyndromic hearing loss from deaf-mute schools in 18 provinces of China. Chin J Otol. 2006; 1:1-5. (in Chinese)
48. Cao QY, Ge J, Zhang WX, Zhang N, Ma HQ, Guo SH, Zhu JZ. An analysis of premarital genetic counseling for and the fertility of the deaf. Chinese Journal Birth Health & Heredity. 2013; 11:79-88. (in Chinese)
49. Liu DS, Wang JL, Sa YL, Yan XM, Wang XJ, Shen T, Peng ZG. Analysis of the *GJB2* gene in non-syndromic deaf patients of the Dai and Han ethnic groups. Journal of Kunming Medical University, 2012; 10:49-52. (in Chinese)
50. Li LQ, Tan JJ, Zhou Y, Yu JL. Does congenital cytomegalovirus infection lead to hearing loss by inducing mutation of the *GJB2* gene? Pediatr Res. 2013; 74:121-126.
51. Dai P, Yu F, Han B, *et al*. *GJB2* mutation spectrum in 2,063 Chinese patients with nonsyndromic hearing impairment. J Transl Med. 2009; 7:26
52. Hilgert N, Smith RJ, Van Camp G. Forty-six genes causing nonsyndromic hearing impairment: Which ones should be analyzed in DNA diagnostics? Mutat Res. 2009; 681:189-96.
53. Wang C, Cai X, Zou G, Tie L, Li Y. Genetic mutations of *GJB2* in non-syndromic deaf patients of the Kazak ethnic group in Xinjiang. Journal Clinical Otorhinolaryngology and Head and Neck Surg. 2014; 28:676-679. (in Chinese)
54. Fei Yu, Pu Dai, Dongyi Han. *GJB2* gene mutations and hereditary non-syndromic deafness prior to speech. Foreign Medical Sciences: Otolaryngology. 2005; 6:359-361. (in Chinese)
55. Kelly PM, Harris DJ, Comer BC, Askew JW, Fowler T, Smith SD, Kimberling WJ. Novel mutations in the connexin 26 gene (*GJB2*) that cause autosomal recessive (DFNBI) hearing loss. Am J Hum Genet. 1998; 62:792-799.

(Received May 19, 2015; Revised July 26, 2015; Accepted August 5, 2015)