article

The prevalence of the 235delC *GJB2* mutation in a Chinese deaf population

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Purpose: Mutations in the *GJB2* gene are the most frequently found mutations in patients with nonsyndromic hearing impairment in populations studied to date. However, the prevalence of mutations varies among different ethnic groups. In most areas of China, genetic testing for nonsyndromic hearing impairment is currently not available because of the lack of information regarding the molecular cause of nonsyndromic hearing impairment. The purpose of this study is to determine the prevalence of a common *GJB2* mutation, 235delC, in Chinese deaf children. **Methods:** We collected DNA specimens from 3004 patients with nonsyndromic hearing impairment from 26 regions of China; 368 Han Chinese and 98 Uigur controls, and screened for the 235delC mutation. The coding exon of the *GJB2* gene was polymerase chain reaction amplified, followed by restriction enzyme digestion with *ApaI* and analysis by agarose gel. **Results:** Overall, 488 patients (16.3%) were determined to carry at least one 235delC mutant allele, with 233 (7.8%) homozygotes and 255 (8.5%) heterozygotes. Therefore, within the subpopulations examined, the frequency varies from 0% to 14.7% for 235delC homozygotes and from 1.7% to 16.1% for heterozygotes. On the basis of this survey of the patient cohort as stated, Chinese patients with nonsyndromic hearing impairment appear to have a relatively higher 235delC frequency than that of other Asian populations. **Conclusion:** These results demonstrate that an easy and fast genetic testing method for this well-known *GJB2* gene mutation can be made available for at least 2 million Chinese patients and family members with nonsyndromic hearing impairment. By screening for the common *GJB2* 235delC mutation, the molecular cause in as high as 15% of patients with nonsyndromic hearing impairment in certain regions of China can be identified. In addition, patients who are negative for the 235delC mutation would be candidates for further mutational analysis of *GJB2* or other deafness-related genes. ***Genet Med* 2007:9(5):283–289.**

Key Words: *235delC mutation, GJB2 mutation, nonsyndromic hearing loss, Chinese nonsyndromic hearing loss, epidemiology.*

Hearing impairment is the most common neurosensory disorder in humans with an incidence of approximately 1 in 1000 children.[1] Approximately two thirds of cases have a genetic cause.[1] Hereditary deafness is genetically heterogeneous. Nonsyndromic deafness accounts for 60% to 70% of inherited hearing impairment and involves more than 100 different genes demonstrating autosomal dominant (DFNA), autosomal recessive (DNFB), X-linked (DFN), and maternal inheritance.[2] For many populations, the most common cause of nonsyndromic autosomal recessive hearing loss is mutations in Connexin 26, a gap junction protein encoded by the *GJB2* gene.[3–10] Approximately 30% to 40% of hereditary deafness is syndromic, presenting with other clinical features in addition to hearing impairment.

To date, more than 100 mutations, polymorphisms, and unclassified variants have been described in the *GJB2* gene (http://davinci.crg.es/deafness). The mutation spectrum and prevalence of mutations vary significantly among different ethnic groups. Three mutations, 35delG, 167delT, and 235delC, are found to be the most frequent mutations in white, Ashkenazi Jewish, and Asian populations, respectively.[3,4,6–15] A recent multicenter study reported that the 35delG mutation accounted for 72.4% of *GJB2* mutant alleles in 1718 white patients with biallelic DFNB1 mutations, including del*GJB6-D13S1830*.[16] In the Ashkenazi Jewish population, 35delG and 167delT accounted for 96% of the *GJB2* mutant alleles.[17] However, the 35delG mutation is rarely found in Asian patients.

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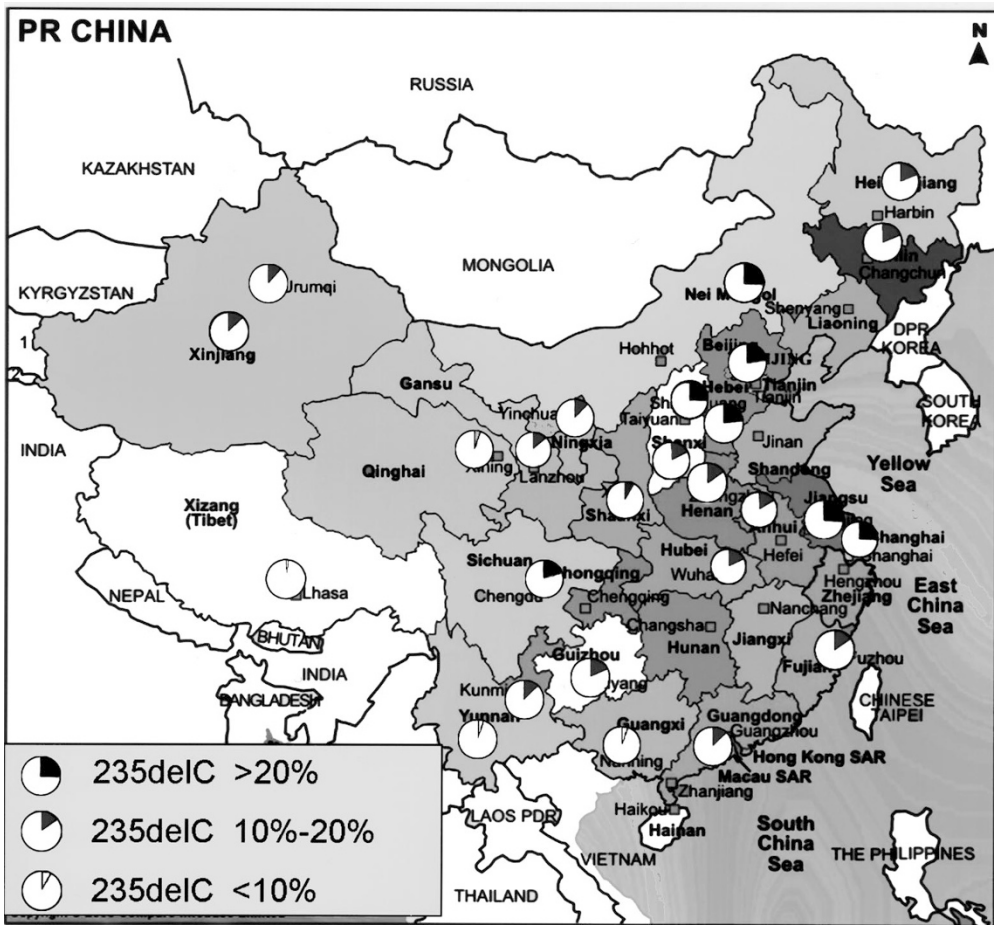

Fig. 1. Geographic distribution and proportion of patients carrying at least one 235delC mutant allele in each region studied.

Instead, the 235delC mutation was the most prevalent in Japanese, Korean, and Taiwanese.[7,8,11,18,19]

In China, it is estimated that 30,000 babies are born with congenital hearing impairment every year.[20] Because of the lack of an established molecular cause in Chinese deaf children, genetic testing has not been offered in most areas of China. Recent reports demonstrated that 235delC is the most prevalent *GJB2* mutation in Taiwanese, a subpopulation of Chinese. A large number of Han people from mainland China's coastal provinces of Fujian and Guangdong emigrated to Taiwan in the 16th century, and more recently in 1949, there was another influx of Chinese from mainland China during the retreat of Kuo-Ming-Dong army led by Chiang. Thus, approximately 98% of the Taiwanese population are Han Chinese (http://www.gio.gov.tw/taiwan-website/5-gp/yearbook/p028.html). China is a large country with more than 56 different subracial groups (e.g., Han, Man, Mon, Hui, Zang, Miao, Yi, Wa, Bai, Zhuang, Wei, Qiang, and NaXi) clustered in different parts of the country. The Han people make up 91.9% of the Chinese population of approximately 1.3 billion (http://education.yahoo.com/reference/factbook/ch/popula.html). Therefore, we hypothesize that the 235delC mutation is likely to be the most prevalent mutation in mainland China. Our first step toward a comprehensive genetic analysis of deaf children in different regions of China is to determine the frequency of the 235delC mutation in different regions of China and Chinese subpopulations. In this study, we report the results of screening the 235delC mutation in 3004 patients with nonsyndromic hearing impairment (NSHI) from 26 different regions of China (Fig. 1).

MATERIALS AND METHODS

Patients and DNA samples

A total of 3004 unrelated students with NSHI from 26 different regions of China were included in this study. Parents were not included. Overall, the patients included in this study consisted of 1706 males and 1298 females from 2 to 30 years with an average age of 13.8 $\pm$ 4.5 (standard deviation) years. Although the majority of patients are Han Chinese, depending on the geographic location, the proportion of patients with minority ethnic backgrounds may be different. To broadly sample as many regions of China as possible, we included the remote northwestern provinces including Xinjiang (translates to New Territory), Tibet, and Qinghai, where minorities make up a significant part of the local population. We also included samples from the southwestern provinces of Yunnan and Guizhou, where the population is made up of a number of minorities originating from various native tribes. We included the ap-

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Table 1
The prevalence of 235delC homozygote, heterozygote, and allele frequency

City, Province	Population size (M)[a]	Han (%)[b]	No. of NSHI	235delC homozygotes	235delC heterozygotes	Patients with 235delC	235delC allele frequency
Nantong, Jiangsu	7.7	99.6	196	21 (10.7%)	31 (15.8%)	52 (26.5%)	18.6%
Datong, Shanxi	3.0	99.9	172	22 (12.8%)	23 (13.4%)	45 (26.2%)	19.5%
Shanghai	13.5	99.4	31	3 (9.7%)	5 (16.1%)	8 (25.8%)	17.7%
Chifeng, Inner Mongol	4.5	79.2	128	14 (10.9%)	19 (14.8%)	33 (25.8%)	18.4%
Gaobeidian Zhuozhou, Hebei	1.2	95.6	69	7 (10.1%)	9 (13.0%)	16 (23.2%)	16.7%
Beijing	11.8	95.7	156	23 (14.7%)	13 (8.3%)	36 (23.1%)	18.9%
Chengdu, Sichuan	10.6	95.0	109	10 (9.2%)	12 (11.0%)	22 (20.2%)	14.7%
Jilin, Jilin	4.3	90.8	57	7 (12.3%)	4 (7.0%)	11 (19.3%)	15.8%
Mudanjiang, Heilongjiang	2.7	95.1	42	3 (7.1%)	5 (11.9%)	8 (19.1%)	13.1%
Wuhan, Hubei	7.9	95.6	87	9 (10.3%)	7 (8.1%)	16 (18.4%)	14.4%
Guiyang, Guizhou	3.5	62.2	138	16 (11.6%)	9 (6.5%)	25 (18.1%)	14.9%
Yuncheng, Shanxi	4.9	99.9	189	12 (6.4%)	20 (10.6%)	32 (16.9%)	11.6%
Fuyang, Anhui	9.0	99.3	36	2 (5.6%)	4 (11.1%)	6 (16.7%)	11.1%
Anyang, Henan	5.3	98.7	152	11 (7.2%)	12 (7.9%)	23 (15.1%)	11.2%
Fuzhou, Fujian	6.1	98.3	148	13 (8.8%)	9 (6.1%)	22 (14.9%)	11.8%
Lanzhou, Gansu	3.1	91.2	56	3 (5.4%)	5 (8.9%)	8 (14.3%)	9.8%
Kunming, Yunnan	5.0	87.4	160	10 (6.3%)	11 (6.9%)	21 (13.1%)	9.7%
Foshan, Guangdong	3.5	98.5	168	13 (7.7%)	9 (5.4%)	22 (13.1%)	10.4%
Korla, Xinjiang	0.4	69.9	117	10 (8.6%)	5 (4.3%)	15 (12.8%)	10.7%
Yinchuan, Ningxia	1.3	65.4	202	9 (4.5%)	16 (7.9%)	25 (12.4%)	8.4%
Urumqi, Xinjiang	1.9	75.4	190	11 (5.8%)	11 (5.8%)	22 (11.6%)	8.7%
Xi'an, Shaanxi	7.3	99.5	57	1 (1.8%)	4 (7.0%)	5 (8.8%)	5.3%
Xining, Qinghai	1.8	54.0	67	1 (1.5%)	3 (4.5%)	4 (6.0%)	3.7%
Liuzhou, Guangxi	3.5	61.6	88	1 (1.1%)	4 (4.6%)	5 (5.7%)	3.4%
Lincang, Yunnan	2.2	62.1	71	1 (1.4%)	3 (4.2%)	4 (5.6%)	3.5%
Lhasa, Tibet	0.4	6.1	118	0	2 (1.7%)	2 (1.7%)	0.9%
Total	126.4		3004	233 (7.8%)	255 (8.5%)	488 (16.3%)	12.0%

M, millions.
[a]http://www.xzqh.org/quhua/index.htm.
[b]Percentage of Han people in the population of each region studied (http://www.cdjsw.gov.cn/Article_Show.asp?ArticleID=462).
In regions of Nantong, Datong, Shanghai, Gaobeidian-Zhuozhou, Beijing, Chengdu, Jilin, Mudanjiang, Wuhan, Yuncheng, Fuyang, Anyang, Fuzhou, Lanzhou, Foshan, and Xi'an City, Han people constitute more than 90% of the population. Minorities constitute 20.8% of the population in Chifeng (most are Mon), 30.1% in Korla (most are Uigur), 24.6% in Urumqi (most are Uigur), 34.6% in Yinchuan (most are Hui), 46.0% in Xining (most are Hui and Zang), and 93.9% in Lhasa (most are Zang). In the southwestern region of China, minorities constitute 36.4% of the population in Liuzhou, 37.8% in Guiyang, 12.6% in Kunming, and 37.9% in Lincang. Most minorities originated from various native tribes.

proximate percentage of Han and minorities in the remote provinces in Table 1. In the eastern and southeast coastal provinces, the population consists of 90% to 98% Han Chinese.

The patients were identified through schools. Because the students attending the schools are from the same city or province in this study, they are reasonably representative of the general population in that region.

This study was performed according to a protocol approved by the ethics committee of the Chinese PLA General Hospital. Informed consent was obtained from parents of students aged less than 18 years or from adult patients themselves aged more than 18 years before blood sampling. The ethnic subgroup was categorized according to the information recorded in each individual's permanent residency documentation. Parents were interviewed to obtain family history information and a maternal pregnancy history in addition to the patients' age at diagnosis and clinical history, including infections, possible head or brain injuries, and exposure to aminoglycoside antibiotics.

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Only the patients with nonsyndromic hearing loss were included in this study. These patients showed moderate to profound bilateral sensorineural hearing impairment on audiograms. DNA specimens from all patients were analyzed by restriction fragment length polymorphism (RFLP) for the presence of the 235delC mutation in the *GJB2* gene. In addition, we analyzed 368 control individuals with normal hearing, all Han Chinese from the capital, Beijing (Northern) and Jiangsu Province (Eastern), two densely populated regions, and 98 control individuals from Uigur of Xinjiang, the northwestern remote region of China. All DNA was extracted from peripheral blood leukocytes using a commercially available DNA extraction kit (Watson Biotechnologies Inc., Shanghai, China).

Mutational analysis

The 235delC mutation in *GJB2* gene was analyzed by polymerase chain reaction (PCR) amplification using forward (5'-TTGGTGTTTGCTCAGGAAGA-3′) and reverse (5′-GGCCTACAGGGGTTTCAAAT-3′) primers located (115 base pair [bp] and 110 bp) upstream and downstream, respectively, of the single coding exon, followed by RFLP analysis. The 944-bp PCR product of a normal control DNA was digested with *Apa*I, producing two fragments with 585 bp and 359 bp. The 235delC mutation results in the loss of the *Apa*I site. A heterozygous mutation results in three DNA bands with 944, 585, and 359 bp, whereas the homozygous mutation produces a single 944-bp band (Fig. 2).

Statistical analysis

The statistical analysis was performed using SAS 9.1.3 software (SAS Inc., Cary, NC). Because there is a statistically significant difference in the whole comparison of 235delC mutant allele frequency of all subethnic groups in this study, the comparison of each two subethnic groups must use a corrected significance level: $\alpha' \approx 0.00227$ $\{\alpha' = \alpha/[0.5k(k-1)+1] = 0.05/[0.5 \times 7 \times (7-1)+1] = 0.00227$, k is the number of group, $\alpha = 0.05\}$. All comparisons between two subgroups in this study used this *P* value for the significance level.

RESULTS

Geographic distribution of patients

In China, approximately 94% (http://www.gxu.edu.cn/administration/gxdxjsb/zzcl/rkfb.htm) of the population is concentrated in the Southern (e.g., Guangdoung and Fujian provinces) and the Eastern (e.g., Beijing, Shanghai, Nanjing) parts of the country. The western half of the country is mostly sparsely populated desert (e.g., Xinjiang) and elevated plateau (e.g., Tibet). We studied patients from 26 regions, including the northwestern province, Xinjiang, the middle-west province Qinghai, and the northeastern province, Heilongjiang, as shown in Fig. 1. The population of each area studied is given in the second column of Table 1 (http://www.xzqh.org/quhua/index.htm). The number of patients studied from each region ranges from 31 (Shanghai) to 202 (Ningxia), with a total of 3004.

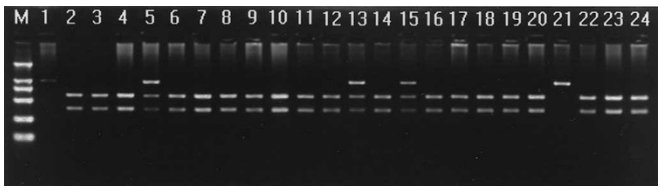

Fig. 2. PCR/RFLP analysis of the 235delC mutation.. The DNA markers are 2000, 1000, 750, 500, 250, and 100 bp. Lane 1 is a homozygous 235delC control, lanes 2 and 3 are normal controls, lanes 5, 13, and 15 are heterozygous for 235delC, lane 21 is homozygous for 235delC, and other lanes are normal cases. The heterozygous 235delC mutation produces three bands; 944, 585, and 359 bp.

Frequency of homozygous and heterozygous 235delC *GJB2* mutation in different regions of China

PCR/RFLP analysis (Fig. 2) of all 3004 patients for the presence of the 235delC mutation revealed a broad distribution of homozygote, heterozygote, and allele frequencies in different regions of China (Table 1). Among the 3004 patients, 233 (7.8%) were homozygous and 255 (8.5%) were determined to be heterozygous for the 235delC mutation. The frequency of homozygous 235delC varied from 0% in Lhasa, Tibet, to 14.7% in Beijing. Heterozygosity for 235delC varied from 1.7% in Lhasa, Tibet, to 16.1% in Shanghai. The total number of patients with NSHI carrying at least one 235delC mutant allele was 488 (16.3%), with a frequency as high as 26.5% in Nantong City, Jiangsu, and as low as 1.7% in Lhasa, Tibet. The 235delC mutant allele frequency varied with regions from 0.9% to 19.5%, yielding a nationwide average frequency of 12.0% based on the populations studied. Variability of distribution of 235delC homozygote, heterozygote, and mutant allele frequency among different regions of China was therefore evident. Six of seven cities where the 235delC frequency was more than 20% (20.2%–26.5%) were located in the eastern provinces (Fig. 1). In most regions, the proportion of 235delC homozygotes and heterozygotes was approximately equal. However in Beijing, Jilin, Korla (Xinjiang), and Guiyang, the number of 235delC homozygotes was approximately twice that of the heterozygotes, whereas, in Yuncheng, Fuyang, and Lanzhou, it was half that of the heterozygotes. In Xi'an, Liuzhou, Xining, and Lincang, the number of homozygotes represented only approximately 20% that of the number of heterozygotes. These regions also have the lowest 235delC allele frequency. These observations suggest that mutations other than 235delC in the *GJB2* gene or other genes are responsible for NSHI in these patients.

We attribute part of the high variability of the frequency of the 235delC mutation in different regions to the makeup of the general population in the regions studied. To examine whether the difference in the frequency of the 235delC mutation among different subethnic populations is statistically significant, we performed a statistical comparison between two subethnic groups. The total populations of Han, Tibetan, Hui, Man, Mon, minorities in Xinjiang, and minorities in Southwestern China are 1137.4 million, 5.4 million, 9.8 million, 10.7 million, 5.8 million, 10.8 million, and 57.1 million, respectively

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Table 2
Statistical results of 235delC allele frequency among different ethnic deaf groups

	Han (655/5032, 13.0%)[a]	Tibetan (2/244, 0.8%)	Hui (25/248, 10.1%)	Man (9/38, 23.7%)	Mon (11/60, 18.3%)	Minority in Xinjiang (7/138, 5.1%)
Tibetan (2/244, 0.8%)	P = .000					
Hui (25/248, 10.1%)	P = .206	P = .000				
Man (9/38, 23.7%)	P = .084	P = .000	P = .008			
Mon (11/60, 18.3%)	P = .245	P = .000	P = .114	P = .609		
Minority in Xinjiang (7/138, 5.1%)	P = .004	P = .013	P = .122	P = .002	P = .006	
Minority in Southwest (10/244, 4.1%)	P = .000	P = .036	P = .013	P = .000	P = .001	P = .797

[a]Number of 235delC alleles/number of total alleles = % allelic frequency; the corrected significance level is .00227. Underlined numbers represent significant differences. The populations of Han, Tibetan, Hui, Man, Mon, minorities in Xinjinag, and minorities in Southwestern China are 1137.4, 5.4, 9.8, 10.7, 5.8, 10.8, and 57.1 million, respectively; http://www.cnmuseum.com/intro/renkou_intro.asp, http://www.xzqh.org/quhua/index.htm).

(http://www.cnmuseum.com/intro/renkou_intro.asp, http://www.xzqh.org/quhua/index.htm). The 235delC frequency of Man, Mon, Han, and Hui minorities in Xinjiang and minorities in southwestern China and Tibet is 23.7%, 18.3%, 13.0%, 10.1%, 5.1%, 4.1%, and 0.8%, respectively (Table 2). When the 235delC allele frequency among different Chinese ethnic groups was compared, there was a statistically significant difference in the comparison of the whole subethnic groups, so we used a corrected *P* value of approximately .00227 for the cutoff of significance in the paired comparison. The Tibetan subethnic group has the lowest 235 delC mutant allele frequency (0.8%), which is statistically significant when compared with other subethnic groups, Han, Man, Mon, and Hui. The Man subethnic group has the highest (23.7%) 235delC mutant allele frequency, which is statistically significant when compared with Tibetan and minorities in Xinjiang and the Southwest. As shown in Table 2, the distribution of the 235delC mutant allele among different Chinese ethnic groups is variable. The 235delC mutation occurs at a much lower frequency in the minorities in the southwest and the northwest regions than in the majorities Han and Man. These results suggest that sequencing of the entire *GJB2* gene is necessary to establish the mutation spectrum and mutation frequency within the Chinese subpopulations.

Comparison of the prevalence of 235delC in Asian populations

Previous reports have suggested that the prevalence of *GJB2* common mutations among different ethnic groups varies.[3,4,6–15] The 235delC is the most common *GJB2* mutation in Asian populations, and its frequency also varies among intra-Asian ethnic groups and subpopulations. Overall, Chinese patients with NSHI as a group appear to carry the highest 235delC mutant allele frequency compared with Korean and Japanese patients with NSHI (Table 3). Thus the frequency of 235delC seems to decline from China (12.0%) to Korea (9.3%) to Japan (3.9%) (Table 3). It should be noted that this comparison is based on the limited reports on studies of particular regions in the countries.

DISCUSSION

This study represents the largest study of the common *GJB2* mutation, 235delC, in patients with NSHI. Accordingly, we analyzed a total of 6008 *GJB2* alleles for the presence of a 235delC mutation. Our results demonstrate that the 235delC mutation in the *GJB2* gene accounts for a significant portion of Chinese patients with NSHI. An estimated 2 million in the Chinese population has NSHI.[20] By assuming that the 235delC

Table 3
The occurrence of the 235delC *GJB2* mutation among Asian patients with nonsyndromic hearing impairment

	This study	Taiwan, Hwa[18]	Korea, Park[8]	Japan, Ohtsuka[7]
Total no. patients	3004	324	147	1227
Total no. alleles	6008	648	274	2454
235delC homozygote (%)	233 (7.8)	19 (5.9)	7 (4.8)	NA
235delC heterozygote (%)	255 (8.5)	29 (9.0)	9 (6.1)	NA
No. patients (%) with 235delC	488 (16.3)	48 (14.8)	16 (10.9)	NA
235delC allele frequency	12.0	10.3	9.3	96 (3.9)

NA, not available.

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mutation is present in approximately 16% of the Chinese population with NSHI, then approximately 320,000 Chinese patients would be expected to test positive for this mutation. The PCR/RFLP diagnostic method is relatively straightforward, rapid, and inexpensive, enabling this genetic service to be readily available to Chinese patients with NSHI and their family members. For the sake of comparison, we would estimate that the average cost of *GJB2* 235delC genotyping per sample by PCR/RFLP is 18.3 Ren-Min-Bi (RMB), equal to approximately 2.5 U.S. dollars, which is approximately half of the cost of the Universal Newborn Hearing Screening in China. Most general hospitals in China have laboratories and equipment necessary for this testing. The *GJB2* gene is small with a high mutation rate. If the 235delC mutation is detected in a heterozygous form, it should prompt analysis of the *GJB2* gene for the second mutant allele. We screened 368 normal controls from Beijing and Jiangsu areas and identified five individuals with the heterozygous 235delC mutation; meanwhile, we screened 98 normal Uigur controls and identified no individuals with the heterozygous 235delC mutation. Thus, the carrier frequency of the 235delC mutation in the Han population from Beijing and Jiangsu is approximately 1.4%. This carrier frequency in the general population is similar to that reported for the Asian carrier rate.[21] Because the carrier frequency of the 235delC mutation may be different in different subethnic groups, it is necessary to analyze controls from various regions and populations of China to determine the accurate carrier frequencies in overall and subethnic groups.

Previous reports[3,4,6–15] suggested that the most frequent *GJB2* mutation in whites, 35delG, represents a hot spot mutation. However, the prevalence of specific *GJB2* mutations depends on the ethnic origin and founder effect: 167delT in Ashkenazi Jews[6,17], R143W (c.427C>T) in a restricted village in Africa,[22] and 235delC in Asian populations.[7,18,23] These European, Jewish, and African common mutations have not been reported in Asian populations. Our results demonstrate that 235delC also occurs at a high frequency in the Chinese population with NSHI. Whether mutations other than 235delC in the region of the *GJB2* gene are responsible for the Chinese patients with NSHI in regions where the frequency of the 235delC mutation is low is currently under investigation. Our preliminary results reveal that other *GJB2* mutations account for an additional 6.1% of patients with NSHI from Xining, where the frequency of the 235delC mutation is only 3.7%. Nevertheless, sequencing analysis of the entire coding region of *GJB2* gene in patients from Liuzhou, where the frequency of the 235delC mutation is 3.4%, reveals no additional mutations. These results have two important implications: the *GJB2* gene needs to be sequenced in its entirety, and mutations in genes other than *GJB2* responsible for NSHI should be looked for in patients who harbor one or no mutations in the *GJB2* gene.

China today is a melting pot of Chinese people from more than 56 different ethnic backgrounds. The Han Chinese originating from the middle and eastern parts of China make up the majority of the population in China. Today the Man population from the northeastern part, the Zang and the Hui from the western part, the Mon from Mongolia, and the many aborigines from the southwestern part are admixing with Hans. Consequently the frequency of homozygotes can be expected to transiently decline according to Wahlund's principles.[24,25] It is not clear whether the 235delC is a founder mutation. Several studies investigating the possible founder effect of 235delC in Asian populations have been reported.[7,26,27] However, a definite conclusion was never reached because of the small size of samples. Further studies are necessary to determine whether the common mutation 235delC in China is a founder effect or a mutation hot spot.

The results of this study will facilitate the establishment of DNA diagnostic testing for the common *GJB2* mutation in Chinese patients with NSHI. The variability in the prevalence of the 235delC mutation among different Asian populations suggests that other mutations in the *GJB2* gene may be responsible for patients with NSHI from regions where the occurrence rate of the 235delC mutation is low. It is also possible that other genes, such as *GJB6 or SLC26A4*, may play a role. Since the molecular cause in the majority of the patients with NSHI in China remains unidentified, many patients will require sequencing of the entire coding region of the *GJB2* gene or evaluation for mutations in other genes such as connexin 30, 31, 32, and *SLC26A4*.

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