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SHORT COMMUNICATION

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Connexin26 gene (*GJB2*): prevalence of mutations in the Chinese population

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Abstract The connexin26 gene (*GJB2*) has been shown to be responsible for DFNB1 and DFNA3 (Autosomal Recessive Hereditary Nonsyndromic Deafness Locus 1 and Autosomal Dominant Hereditary Nonsyndromic Deafness Locus 3). Two hundred ten independently ascertained Chinese probands with nonsyndromic hearing loss (NSHL) were evaluated for mutations in *GJB2*, including 43 probands from families with more than one sib with NSHL, likely indicating dominant inheritance, and sporadic cases of NSHL, compatible with recessive inheritance. Of the 210 probands, 43 (20%) were homozygous or heterozygous for mutations in *GJB2*. Four different mutations were identified: 35delG, 109G-A, 235delC, and 299–300delAT. It was confirmed that *GJB2* mutations are an important cause of hearing loss in this population. Of these four mutations, 235delC was the most prevalent at 93%; yet the 35delG mutation, which is the most common *GJB2* mutation in Caucasian subjects (Europeans and Americans), was found in low frequency in the present study. It appears from our limited data and reports from other East Asians that 235delC is the most prevalent *GJB2* mutation in these populations. *GJB2* mutations are consistent with ethnic predilections.

Key words Nonsyndromic hearing loss · *GJB2* · Connexin26 (Cx26) · Mutation · Chinese

Introduction

Genetic causes are believed to account for at least 60% of cases of hearing impairment. Close to 80 human loci causing nonsyndromic hearing loss (NSHL) have been mapped and 31 identified; 24 genes have been determined to cause NSHL (Van Camp and Smith 2002, http://www.iro.es/deafness/). Prominent genes are those encoding gap junctions. Connexin26 is believed to play a critical role in the recycling of potassium ions at their entry into hair cells during sensory transduction from the endolymph through to the stria vascularis where other potassium channels pump potassium back into the endolymph (Scott et al. 1998). Connexin 26 gene (*GJB2*) mutations were first reported by Kelsell et al. in 1997. Mutations of *GJB2*, which is responsible for DFNB1 and DFNA3 (Autosomal Recessive Hereditary Nonsyndromic Deafness Locus 1 and Autosomal Dominant Hereditary Nonsyndromic Deafness Locus 3), are the most frequent cause of inherited hearing loss (Cohn et al. 1999; Denoyell et al. 1998; Fuse et al. 1999; Kelley et al. 1998). In some regions, mutations in *GJB2* account for about 50% of autosomal recessive prelingual NSHL (Denoyelle et al. 1997; Estivill et al. 1998; Zelante et al. 1997).

The prevalence of NSHL is high in the Chinese population. To estimate the proportion of inherited deafness attributed to *GJB2* in the Chinese population, we checked the coding DNA sequence (CDS) of *GJB2* with polymerase chain reaction (PCR), single-strand conformational polymorphism (SSCP), and restriction fragment length polymorphism (RFLP) in 210 independently ascertained probands with NSHL.

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Subjects and methods

All 210 independently ascertained probands with bilateral sensorineural NSHL were from different regions of China, and were 5–54 years old. Among them, 43 probands were from families with more than one sib with NSHL, likely indicating dominant inheritance; the others were sporadic cases of hearing loss, compatible with recessive inheritance. Informed consent was obtained from all participants and parents of patients younger than 18 years. Excluded from this study were individuals with acquired hearing loss (environmental causes: infection, noise, drug ototoxicity, and trauma). Evaluation included a complete case history, physi-

cal examination, and audiometry. All individuals underwent pure-tone audiometry by diagnostic audiometer (Siemens Danplex DA 74 Clinical Diagnostic Audiometer, NJ, USA) in a soundproof room. Pure-tone averages of more than 25 dBHL (mean dBHL at 500, 1000, and 2000 Hz) were defined as hearing loss. The degree of hearing impairment ranged from mild to profound, and 81 probands showed no measurable hearing level. The control population consisted of 200 randomly selected normal Chinese.

DNA was isolated from peripheral-blood lymphocytes according to standard protocols. Three PCR primer pairs from the *GJB2* sequence were designed to cover 681 bp of the coding region. Amplification was performed with the following primer pairs: 1F 5′-TCT TTT CCA GAG CAA ACC GC-3′, 1R 5′-GAC ACG AAG ATC AGC TGC AG-3′; 2F 5′-GCT GCA AGA ACG TGT GCT AC-3′, 2R 5′-TGG GTT TTG ATC TCC TCG AT-3′; and 3F 5′-CGA GGA GAT CAA AAC CCA GA-3′, 3R 5′-GGA CAC AAA GCA GTC CAC AG-3′.

PCR was performed using 100 ng of human DNA in a 25-μl PCR reaction, which contained 2.5 μl PCR buffer (100 mM of tris-hydrochloride, pH 8.8, 500 mM of potassium chloride, 15 mM of magnesium chloride, 0.01% wt/vol gelatin), 200 μM mixed deoxyribonucleoside triphosphates, and 25 pM primer. Samples were denatured at 95°C for 5 min followed by 30 cycles at 94°C for 40 s, 60°C for 30 s, and 72°C for 30 s, with a final extension for 7 min at 72°C.

The PCR products were screened by SSCP for mutations in *GJB2*, followed by sequencing after purification (Bio-applied Corporation, Shanghai, China) if SSCP shifts were observed. After finding mutations, all samples were checked with RFLP (Table 1).

Results

Four different *GJB2* mutations (three frameshift mutations and a missense mutation) were identified among 210 probands (Tables 1 and 2); 43 (20%) carried mutations in *GJB2*, including 4 with and 39 without deaf sibs. Three single-nucleotide polymorphisms (SNPs), 79G-A, 257–258GC-CG, and 341A-G, were detected. 79G-A and 341A-G were reported previously, whereas one novel SNP, 257–258GC-CG (no amino acid change), was observed in all sequencing samples of 18 probands and 6 controls. Among 200 hearing controls from the general Chinese population, one individual was heterozygous for 235delC. No control carried any other mutations.

The frameshift mutation 235delC was found in 40 probands, 3 in homozygotes whose parents are NSHL and homozygous, 24 in homozygotes with sporadic hearing loss, and 13 in heterozygotes with sporadic hearing loss in which no other mutation of *GJB2* was detected. Three heterozygous mutations, 35delG, 109G-A, and 299–300delAT, were detected in three probands, respectively. No other mutation in *GJB2* was detected. The same heterozygous mutation, 299–300delAT, was also found in the proband whose mother has NSHL. The homozygous mutations in *GJB2*, which were observed in 13% of all probands, accounted for 16% (27/210–43 + 3) of all autosomal recessive deafness in the tested populations. The mutation 109G-A could not be checked with RFLP because there is no appropriate restriction endonuclease site for it. The 235delC mutation causes a frameshift at codon 79, resulting in a truncated polypeptide. 299–300delAT causes a frameshift leading to an altered amino acid sequence starting from codon 100, followed by a stop at codon 113.

Discussion

Mutations in *GJB2* are responsible for a substantial proportion (13%) of hearing loss in the Chinese. Four different mutations appear in 43 probands, 27 homozygous for the same allele. The carrier frequency of these mutations in the

Table 1. Mutations checked with RFLP

GJB2 mutation	Restriction endonucleases (U)		PCR product (μl)	Buffer (μl)	Reaction temperature	Reaction time
35delG	*Bsl* I	(3)	12.5	1.7	55°C	4 h
235delC	*Apa* I	(2.5)	5	1	37°C	4 h
299–300delAT	*Hsp*92II	(5)	5	2	37°C	16 h

RFLP, Restriction fragment length polymorphism; PCR, polymerase chain reaction

Table 2. *GJB2* mutations in Chinese probands with nonsyndromic hearing loss

Mutation	Effect	Deaf probands with mutations		Allele frequency among deaf probands
		Homozygotes	Heterozygotes	
35delG	Frameshift	0	1	0.002
109G-A	V37I, missense	0	1	0.002
235delC	Frameshift	27	13	0.16
299–300delAT	Frameshift	0	1	0.002
All		27	16	0.166

general Chinese population is as low as 1 in 200 controls, consistent with the high frequency of deafness resulting from mating among deaf people.

The present result indicates that the most frequent mutation in *GJB2* is 235delC, which is found in 40 of 43 affected individuals with *GJB2*-detectable mutations. It has also been found in the Japanese, Korean, and Mongolian populations (Abe et al. 2000; Fuse et al. 1999; Kudo et al. 2000; Park et al. 2000; A. Pandya and W.E. Nance unpublished). Therefore, mutation in *GJB2* is an important contributor to recessively inherited NSHL in the Chinese population, as also shown in other ethnic groups (Cohn et al. 1999).

Another mutation, 299–300delAT, has also been reported, mainly in East Asians (Abe et al. 2000; Kudo et al. 2000; Park et al. 2000), although it does not appear to be common in any population yet studied. No second mutation in *GJB2* was detected from the proband and his mother with a heterozygous 299–300 delAT mutation, leading us to question whether this mutation was dominantly inherited. It is more likely that the apparently normal allele in both individuals in fact carried an inactivating mutation that escaped our analysis. Although dominant *GJB2* mutations have been described, leading to DFNA3 hearing loss, these all produce a full-length but abnormal protein, which can probably exert a dominant negative effect. Numerous other chain-terminating mutations in *GJB2* are known (http://www.iro.es/deafness/). They typically act as null alleles, and heterozygotes have normal hearing.

Although numerous mutations of this gene occur that cause deafness, a single mutation, 35delG, predominates in Europe and the United States (Denoyelle et al. 1997; Gabriel et al. 2001; Gasparini et al. 1997; Green et al. 1999; Shahin et al. 2002). 35delG is absent in the Japanese (Abe et al. 2000; Fuse et al. 1999; Kudo et al. 2000) and rare in Koreans (Park et al. 2000). Our data show that the 35delG mutation is not common in the Chinese population, consistent with the case in other East Asians. *GJB2* mutations are consistent with ethnic predilections.

Recent studies show a shared origin of the mutation in *GJB2*, such as the 167delT mutation in Ashkenazi Jews and the R143W mutation in several families in a Ghanaian village (Brobby et al. 1998; Morell et al. 1998). Haplotyping needs to be performed with 235delC, the most prevalent mutation in Chinese and other East Asians, to evaluate the hypotheses relating to its origin.

These data support the use of screening programs in future, such as appropriate genetic tests, as a valuable complement to audiometric screens to identify neonates with heritable congenital hearing impairment in non-endogamous Chinese. Applying this test may facilitate earlier habilitation in a substantial percentage of deaf infants and ultimately provide parents with precise clinical diagnosis, appropriate prognostic genetic counseling, and proper medical management information.

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