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MAIN ARTICLE

Prevalence of *GJB2* gene mutation in 330 cochlear implant patients in the Jiangsu province

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Abstract

Objective: *GJB2* gene mutations are highly prevalent in pre-lingual hearing loss patients from China. Pre-lingual deafness is a sensorineural disorder that can only be treated with cochlear implantation.

Method: The prevalence of *GJB2* gene mutations was examined in 330 randomly selected patients treated with cochlear implantation.

Results: Overall, 276 patients (83.64 per cent) carried variations in the *GJB2* gene. Seventeen different genotypes were identified, including 10 confirmed pathogenic mutations (c.235delC, c.299delAT, c.176del16, p.E47X, p.T123N, p.V167M, p.C218Y, p.T86R, p.V63L and p.R184Q), 3 polymorphisms (p.V27I, p.E114 G and p.I203 T) and 2 unidentified mutations (p.V37I and c.571 T > C).

Conclusion: A total of 103 patients (31.2 per cent) carried 2 confirmed pathogenic mutations. The frequency of c.235delC was higher than that reported previously in the Jiangsu province. The two novel mutations identified, 69C > G and 501G > A, are likely to be polymorphisms.

Key words: Deafness; GJB2 Protein, Human; Mutations; Cochlear Implant

Introduction

In China alone, there are 27 800 000 hearing loss patients (as per the China National Sample Survey on Disability, 2006). More than 60 per cent of these patients suffer from pre-lingual hearing loss due to genetic factors.[1] Cochlear implantation is an efficient means to treat hereditary hearing loss, a sensorineural disorder affecting 1 in 1000 newborns.[2] The majority of hearing loss cases are non-syndromic, and about 70 per cent are autosomal recessive inherited.[3] To date, more than 100 genes related to deafness have been identified. Among these, mutation of the *GJB2* gene is known to play an important role in autosomal recessive non-syndromic hearing loss in China.

GJB2 was the first non-syndromic hearing loss and deafness (autosomal recessive) gene identified. Mutations in this gene contribute to approximately 50 per cent of non-syndromic, autosomal recessive deafness cases. The gene encodes connexin 26 protein (CX26),[4] which belongs to the gap junction protein family. The function of this protein is to recycle potassium ions to the endolymph, which is responsible for auditory signal transduction.[5,6] Malfunction of CX26 triggers accumulation of potassium ions in the endolymph, resulting in hair cell dysfunction.

The Nanjing Drum Tower Hospital ENT department is devoted to cochlear implantation surgery and has helped hundreds of patients recover hearing. In the current investigation, we examined 330 randomly selected patients with unknown aetiologies treated with cochlear implantation, with the aim of identifying the prevalence and spectrum of *GJB2* gene mutations. Data from the current study should provide guidance with regard to pre-conception diagnosis, prenatal diagnosis and the genetic counselling of individuals.

Materials and methods

Patients and samples

During the period from January 2010 to March 2014, 330 individuals from unrelated families (probands), who had agreed to undergo cochlear implantation in Nanjing Drum Tower Hospital, participated in *GJB2* testing. All patients were from the Jiangsu province. Patients were randomly selected from all the individuals scheduled to undergo cochlear implant surgery at our hospital. The group consisted of 217 males and 113 females aged 1 to 15 years. Parents were not included in this study. Participants had either congenital hearing loss or progressive, early-onset idiopathic hearing loss, and showed bilateral sensorineural

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hearing loss in the audiogram. Children with known aetiologies of hearing loss were not included in our study population.

Prior to surgery, each patient was subjected to otological and audiological evaluation, including pure tone audiometry (using an Orbiter 922 clinical audiometer; Madsen, Copenhagen, Denmark), immittance testing (Zodiac 901 middle-ear analyser; Madsen), auditory brainstem response testing (GSI Audera system; Grason-Stadler, Madison, Wisconsin, USA) and transient evoked otoacoustic emissions testing (Celesta 503 cochlear emissions analyser; Madsen).

Tests were performed with the permission of the families. Authorisation from parents and signed consent forms were obtained. As approved by the Nanjing Drum Tower Hospital ENT department, 3 ml peripheral blood was obtained from each patient for laboratory testing. Nanjing Medical University provided the technology for this test and performed detection of gene expression for all participants.

GJB2 mutational analysis

Blood samples were obtained from patients using standard procedures, and were screened for *GJB2* gene mutations using specific amplification primers and conditions. The two primer pairs used were as follows: exon 2 F(5′TTGGTGTTTGCTCAGGAAGA3′), R(5′GGCCTACAGGGGTTTCAAAT3′) and exon 1 F(5′TGGGGGCACTTGGGGAACTCA3′), R(5′GCAGAAACGCCCGCTCCAGAA3′).

Polymerase chain reaction products were purified and sequenced with primers using QIAquick spin columns (Qiagen, Valencia, California, USA), the BigDye™ Terminator Cycle Sequencing Kit (version v.3.1) and Applied Biosystems 3730 DNA Analyzer (Foster City, California, USA). Data were analysed using Applied Biosystems Sequencing Analysis Software (version 3.7) and compared with the reference sequence of wild-type *GJB2* (GenBank number: NG_008358.1).

Results

The *GJB2* coding sequence was screened in 330 non-syndromic hearing impairment patients, resulting in the identification of 17 different mutations, 2 of which were novel.

No disease-causing mutations were detected in 16.36 per cent of patients (54 out of 330); that is, 83.64 per cent of patients (276 out of 330) carried at least one mutation.

Overall, 66 of the 330 patients (20.00 per cent) carried a single *GJB2* mutation, while up to 177 patients (53.64 per cent) carried 2 mutations, and 15 (4.55 per cent) and 18 (5.45 per cent) were identified as having 3 and 4 mutations, respectively (Table I).

As shown in Table II, 17 different genotypes were detected. These included 10 confirmed pathogenic mutations (c.235delC, c.299delAT, c.176del16, p.E47X, p.T123N, p.V167M, p.C218Y, p.T86R, p.V63L and p.R184Q), 3 polymorphisms (p.V27I, p.E114 G and p.I203 T) and 2 unidentified mutations (p.V37I and c.571 T > C). All the mutations were autosomal recessive, single mutation was not the pathogenic factor.

TABLE I
FREQUENCY OF *GJB2* MUTATIONS IN COHORT PATIENTS

Number of mutations	Patients (*n*)	Frequency (%)
Total	330	100
None	54	16.36
One	66	20
Two	177	53.64
Three	15	4.55
Four	18	5.45

The most prevalent single gene mutation in the Jiangsu province was c.235delC, present in 119 of the 330 probands, with an allele frequency of 28.48 per cent. As shown in Table III, 72 patients carried homozygous pathogenic mutations, 31 had compound heterozygous pathogenic mutations and the remainder had heterozygous pathogenic mutations.

The second most frequent mutation was the nucleotide variant, c.79G > A (122 patients, 21.06 per cent), presenting as 8 heterozygous pathogenic mutations and 8 unidentified mutations, with 106 polymorphisms in total. Another common nucleotide variant was c.341A > G (94 patients, 16.82 per cent), which mainly manifested as polymorphisms.

The third most frequent pathogenic mutation in our study was c.299delAT (25 patients, 4.24 per cent), followed by a still controversial genotype, c.109G > A (20 patients, 3.33 per cent).

Eight rare variants (p.E47X, p.T123N, p.V167M, p.F191L, p.C218Y, p.T86R, p.V63L and p.R184Q) were identified in the cohort, and their influence on *GJB2* protein predicted.

The two novel mutations c.69C > G and c.501G > A have not been described before. Both were carried by separate patients, and manifested as heterozygous pathogenic mutations.

Discussion

GJB2 gene mutations are the most prevalent in Chinese autosomal recessive non-syndromic hearing loss patients. Dai *et al*. reported a 4–30.4 per cent prevalence of *GJB2* mutations in China, which varied among different sub-ethnic groups.[7] In order to update and evaluate the spectrum and prevalence of *GJB2* mutations in the Jiangsu area, 330 autosomal recessive non-syndromic hearing loss patients scheduled to undergo cochlear implantation were recruited for the study.

Overall, 83.64 per cent of patients ($n = 276$) were identified with variations, and 31.2 per cent ($n = 103$) carried two confirmed pathogenic mutations (according to the Deafness Variation Database).[8] Within these patients, c.235delC, c.299delAT and

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TABLE II
GJB2 MUTATIONS IN 330 UNRELATED AUTOSOMAL RECESSIVE NON-SYNDROMIC HEARING LOSS PATIENTS

Genotype	Amino acid change	Location	Effect	Patients (*n*)	Allele frequency (%)
c.235delC	Frameshift	TM2	Pathogenic	119	28.48
c.79G > A	p.V27I	TM1	Polymorphism	122	21.06
c.341A > G	p.E114G	IC2	Polymorphism	94	16.82
c.299delAT	Frameshift	IC2	Pathogenic	25	4.24
c.109G > A	p.V37I	TM1	Unidentified	20	3.33
c.176del16	Frameshift	EC1	Pathogenic	18	2.73
c.608 T > C	p.I203 T	TM4	Polymorphism	15	2.27
c.139G > T	p.E47X	EC1	Pathogenic	3	0.45
c.368C > A	p.T123N	IC2	Pathogenic	3	0.45
c.499G > A	p.V167M	EC2	Pathogenic	2	0.30
c.571 T > C	p.F191L	TM4	Unidentified	2	0.30
c.653G > A	p.C218Y	IC3	Pathogenic	1	0.15
c.257C > G	p.T86R	TM2	Pathogenic	1	0.15
c.187G > T	p.V63L	EC1	Pathogenic	1	0.15
c.551G > A	p.R184Q	EC2	Pathogenic	1	0.15
c.69C > G	Novel			1	0.15
c.501G > A	Novel			1	0.15

c.176del16 were the most prevalent pathogenic mutations in the Jiangsu province, and accounted for 36.97 per cent of all the mutant alleles identified. These findings indicate that frameshift truncation is the leading cause of pathogenic mutations. Frameshift mutation was more commonly associated with severe deafness, as this type of substitution resulted in a defective chromosome that could not be translated to the correct protein. A previous investigation revealed a high frequency of c.299delAT and c.176del16 in Chinese individuals.[7] Interestingly, in our research, the respective allele frequencies were 4.24 per cent and 2.73 per cent, indicating that these are not particularly prevalent. Thus, prevalence of the c.299delAT and c.176del16 alleles appears to markedly differ according to region.[9]

Although the spectrum of *GJB2* mutations varies among different regions of China, c.235delC has

TABLE III
GENOTYPES OF PATIENTS WITH *GJB2* MUTATIONS

Category	Allele 1	Allele 2	Affected patients (*n*)
Homozygous pathogenic mutations	c.235delC	c.235delC	67
	c.299delAT	c.299delAT	3
	c.235delC	c.235delC, c.499G > A	1
	c.235delC	c.235delC, c.501G > A	1
Compound heterozygous pathogenic mutations	c.235delC	c.299delAT	13
	c.235delC	c.176del16	12
	c.235delC	c.139G > T	3
	c.299delAT	c.176del16	2
	c.299delAT	c.257C > G	1
Heterozygous pathogenic Mutations	c.235delC	No variant	17
	c.299delAT	No variant	6
	c.176del16	No variant	4
	c.235delC	c.79G > A	3
	c.79G > A	c.368C > A	3
	c.499G > A	No variant	1
	c.653G > A	No variant	1
	c.235delC	c.109G > A	1
	c.79G > A, c.341A > G	c.187G > T	1
	c.235delC	c.109G > A, c.69C > G	1
	c.79G > A, c.341A > G	c.551G > A, c.109G > A	1
Unidentified mutations	c.109G > A	No variant	7
	c.109G > A	c.79G > A, c.341A > G	7
	c.109G > A	c.109G > A	2
	c.571 T > C	No variant	1
	c.109G > A	c.79G > A	1
	c.571 T > C	c.608 T > C	1
Polymorphisms	c.79G > A	c.341A > G	63
	c.79G > A	No variant	20
	c.79G > A, c.341A > G	c.79G > A, c.341A > G	17
	c.608 T > C	No variant	8
	c.79G > A	c.341A > G, c.608 T > C	4
	c.79G > A	c.608 T > C	2
	c.341A > G	No variant	1

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been commonly identified as the most prevalent mutation.[7] Among all 330 patients, the c.235delC mutation was detected in 28.48 per cent of the alleles. This frequency was higher than that reported in a previous study of individuals from the Jiangsu province (20.6 per cent of alleles in a non-syndromic deafness population).[7] One possible explanation is that the individuals in our study had severe-to-profound sensorineural hearing loss and were randomly selected from patients treated with cochlear implantation.

Our findings indicate that p.V27I and p.E114 G are the most common polymorphism variants in the Jiangsu province, with respective rates of 21.06 per cent and 16.82 per cent. p.V27I and p.E114 G were initially recorded as a pathogenic mutation in the *GJB2* mutation database.[3] Subsequently, Tekin and colleagues considered the allele less likely to cause profound congenital deafness.[9]

The effects of the p.V37I mutation are still controversial. Kelley *et al*. initially identified pV371 as a polymorphism variant.[10] Huang and co-workers investigated 3864 hearing loss patients, including 106 with homozygous p.V37I or compound p.V37I variations, and concluded that the milder phenotype is possibly attributable to p.V37I mutation.[11] In our study, p.V37I was detected with an allele frequency of 3.33 per cent within the Jiangsu province, while previous studies from this region have reported a frequency of 4.5 per cent.[10–13]

The two novel mutations, c.501G > A and c.69C > G, were carried by one patient with homozygous pathogenic and one with heterozygous pathogenic mutation genotypes separately. So far, there is no evidence to suggest that these two variations are associated with profound sensorineural hearing loss. c.501G > A is a same-sense mutation, whereby the residue cannot be changed owing to codon degeneracy. Further studies are required to elucidate whether or not the mutation is a polymorphism.

Major mutations identified in other ethnic groups, such as c.35delG in Caucasians and c.167delT in Ashkenazi Jews, were absent, consistent with previous reports.[11,15,16]

- **_GJB2_ gene mutations are quite common in Chinese pre-lingual hearing loss patients**
- **To update the spectrum of _GJB2_ gene mutation in Jiangsu province, 330 children treated with cochlear implantation were studied**
- **A higher rate (31.2 per cent) of patients with two confirmed mutations was found, and two novel mutations were identified**

One major limitation of our study was the lack of a positive control, without which normal individuals cannot be compared to deaf patients. Overall, data from the current investigation have facilitated updating of the unique *GJB2* mutation spectrum in non-syndromic hearing loss patients from the Jiangsu region and have led to the identification of two novel mutations in the gene.

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