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The homozygote p.V27I/p.E114G variant of *GJB2* is a putative indicator of nonsyndromic hearing loss in Chinese infants

Wen-xia Chen [1], Yue Huang [1], Xiao-lin Yang, Bo Duan, Ping Lu, Yan Wang, Zheng-min Xu *

Department of Otolaryngology-Head and Neck Surgery, Children's hospital of Fudan University, Shanghai, China

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ABSTRACT

The gap junction β2 (*GJB2*) gene is associated with more than half of the recessive forms of hereditary hearing loss (HHL). However, the correlation between p.V27I and p.E114G variants of *GJB2* and hearing phenotype remains controversial. This study aimed to clarify possible roles of these variants in Chinese infants with nonsyndromic hearing loss (NSHL). Hearing and gene tests were conducted in 300 infants (aged 0–3 months) with NSHL and 484 normal infants (aged 0–3 months). The p.V27I and p.E114G variants appeared frequently in both NSHL patients and normal controls. The allele and haplotype frequencies of p.V27I and p.E114G in patients and controls were compared, but no significant difference was observed ($p = 0.44$ and $p = 0.26$, respectively). Moreover, genotype frequencies of the p.V27I variant showed no significant difference between the two groups ($p = 0.66$). Interestingly, more homozygote p.V27I/p.E114G subjects were found in NSHL infants than in controls (5/484 and 13/300, respectively), most of whom (61.54%) had mild or moderate hearing losses. Our results indicate that homozygote p.V27I/p.E114G is associated with mild and moderate HHL.

1. Introduction

Hearing loss is the most common sensory disorder in humans, affecting one newborn in a thousand, with hearing loss impairment significant enough to compromise the development of normal language skills. An estimated 50% of all childhood hearing loss is due to hereditary hearing loss (HHL) [1]. Numerous genes have been shown to cause hearing loss, among which gap junction β2 (*GJB2*) plays a major role in more than 50% of the recessive forms of HHL [2,3]. To date, more than 200 different mutations in *GJB2* have been identified (http://davinci.crg.es/deafness/). Some *GJB2* mutations are common, such as the c.35delG mutation, which is the most common in the Caucasian population and the c.235delC and p.V37I mutations, which are the most prevalent in the Asian population [4]. Moreover, the p.V27I and p.E114G variants are commonly reported in the East Asian population with allele frequencies of 28.3 and 18.3%, respectively [5,6].

The p.V27I mutation involves a sequence change of Val to Ile at amino acid residue 27, whereas p.E144G involves a sequence change of Glu to Gly at amino acid residue 114. Both variants have been discussed in some studies and are considered as genetic risk indicators of HHL, as well as determinants of disease severity. However, other studies considered them benign polymorphisms, because they were found in people with normal hearing as well. To resolve these conflicting data, we investigated the prevalence of *GJB2* mutations p.V27I and p.E114G in Chinese NSHL children (aged 0–3 months), as well as the clinical features of different p.V27I and p.E114G genotypes.

2. Materials and methods

2.1. Patients

Three hundred infants aged 0−3 months with a diagnosis of nonsyndromic hearing loss (NSHL) were recruited between September 2013 and May 2015 from the Otolaryngology Department of Children's Hospital of Fudan University. Infants were excluded from the study if their onset age was not within 0−3 months, their auditory brainstem response (ABR) results could not be obtained, or there were environmental causes for HL. Four hundred and eighty-four normal infants aged 0–3 months comprised the control group. This work was approved by the Ethic Committee of Children's Hospital of Fudan University, and was conducted in accordance with the ICH guidelines for Good Clinical Practice and the Declaration of

* Corresponding author at: Department of Otolaryngology-Head and Neck Surgery, Children's Hospital of Fudan University, 399 Wan Yuan Road, Shanghai 201102, China. Tel.: +86 021 64931926; fax: +86 021 64931901.
E-mail addresses: xuzhengmin@sh163.net, 13916320945@163.com (Z.-m. Xu).
[1] These two authors contributed equally to this work.

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Helsinki. Parents of all study subjects signed informed consent forms.

2.2. Audiological evaluation

ABR testing was carried out in all NSHL infants. It was performed under general anesthesia with the patient lying on a bed in an acoustically and electrically shielded room. The severity of NSHL and asymmetry were documented. Severity levels were classified as mild (35–55 dB HL), moderate (56–70 dB HL), severe (71–90 dB HL), or profound (>91 dB HL).

2.3. Gene testing

Gene testing of *GJB2* exon 2 was performed in all infants. DNA was extracted from whole blood samples and purified by a commercially available extraction kit (Qiagen Inc. China). Primer sequences used for mutation analysis of *GJB2* were as follows: Forward: 5′-TCTTTTCCAGAGCAAACCGC-3′, Reverse: 5′-CTGGGC-AATGCGTTAAACTGG-3′. The PCR protocol was set as follows: pre-denaturation at 94 °C for 2 min, denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and extension at 72 °C for 45 s for 35 cycles, followed by post-extension at 72 °C for 1 min. PCR products with 725 bp, the size of the entire coding region of *GJB2*, were sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Kit (source) and an ABI 3730 DNA Automatic Sequencer (company, USA).

2.4. Statistical analysis

Statistical analysis was performed with SPSS statistical software (IBM SPSS Statistics 22.0). Allele frequencies and genotype frequencies in patients and controls were calculated; the chi-square test was used to evaluate the significance of inter-group differences. $p < 0.05$ was considered statistically significant.

3. Results

No statistically significant differences in p.V27I allele frequencies were observed between NSHL infants and controls (22.0 and 20.4%, respectively, $p = 0.44$), as well as in p.E114G allele frequencies between the two groups (16.3 and 14.2%, respectively, p = 0.26) (Table 1). Moreover, no significant differences in the p.V27I genotype frequencies were observed between NSHL infants and controls ($p = 0.66$), but p.E114G variant genotype frequencies showed significant differences between the two groups ($p = 0.0098$) (Table 1). In addition, more homozygote p.V27I/p.E114G subjects were found among NSHL infants than among controls (5/484 and 13/300, respectively) (Table 3).

Table 2
Haplotype frequencies of p.V27I and p.E114G in nonsyndromic hearing loss (NSHL) patients and normal hearing controls.

Haplotypes	Controls (%)	Patients (%)	*p*-value
n	968	600	
WT	771 (78.6%)	468 (78.0%)	0.436
p.V27I	59(6.1%)	34 (5.6%)	0.727
p.E114G	0 (0.0%)	0 (0.0%)	–
p.V27I/p.E114G	138 (14.3%)	98(16.3%)	0.26

Table 2 shows the haplotype frequency distributions of p.V27I and p.E114G in NSHL patients and controls. Four haplotype frequencies (wild type, p.V27I, p.E114G and p.V27I/p.E114G) were calculated and no significant difference in haplotype frequencies was observed between the two groups.

The sequencing results of *GJB2* show (Table 3, 11 and 5) different genotypes harboring p.V27I or p.E114G among the 300 NSHL infants and 484 normal infants, respectively. The data indicated that p.V27I and p.E144G mutations were common in both NSHL patients and the normal population. The p.V27I mutation was found alone or together with the p.E114G mutation in most cases, while p.E114G was found together with the p.V27I variant in most cases. Genotype frequencies were similar in all groups, except that of the homozygote p.V27I/p.E114G, which interestingly was significantly higher in NSHL groups than in controls, indicating its pathogenic role in HHL (Table 3).

4. Discussion

p.V27I and p.E114G are commonly reported mutations in the Asian population [5,6]. In a recent study, 1067 Chinese subjects with NSHL were analyzed, and the allele frequencies of p.V27I and p.E114G were 25.2 and 19.7%, respectively [7]. The carrying rates of variants p.V27I and p.E114G in our study were estimated to be 20.4 and 14.2%, respectively, which are similar to published reports [7,8]. The p.V27I mutation was found alone or together with the p.E114G mutation in most cases, whereas p.E114G was rarely alone and was always found with p.V27I. These findings are in agreement with those of the genetic study of the *GJB2* mutation by Dai et al., wherein only two patients carried c.155-158delTCTG and p.E114G and one patient carried c.235delC and p.E114G [8].

Table 1
Allele and genotype frequencies (%) of p.V27I and p.E114G variants in nonsyndromic hearing loss (NSHL) patients and normal hearing controls.

Variables	Amino acid	Controls (%)	NSHL patients (%)	P value	Odds ratio (95%CI)
n		484	300		
p.V27I					
Allele	V	771 (79.6%)	468 (78.0%)	0.436	1.104 (0.861–1.415)
C	I	197 (20.4%)	132 (22.0%)		
T				0.661	
Genotypes	VV	319 (65.9%)	193 (64.3%)		
CC	VI	133 (27.5%)	82 (27.3%)		
CT	II	32 (6.6%)	25 (8.3%)		
TT					
p.E114G					
Allele					
T	E	830(85.7%)	502(83.7%)	0.264	1.174 (0.886–1.556)
C	G	138(14.2%)	98(16.3%)		
Genotypes				0.0098	
TT	EE	351(72.5%)	215(71.7%)		
TC	EG	128(26.4%)	72(24.0%)		
CC	GG	5(1.0%)	13(4.3%)		