Int J Clin Exp Med 2015;8(11):21674-21678
www.ijcem.com /ISSN:1940-5901/IJCEM0013400

Original Article

Prevalence of p.V37I variant of GJB2 among Chinese infants with mild or moderate hearing loss

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Received July 25, 2015; Accepted October 16, 2015; Epub November 15, 2015; Published November 30, 2015

Abstract: GJB2 accounts for more than 80% of recessive forms of hereditary hearing loss (HL); however, the correlation between the p.V37I variant of GJB2 and hearing phenotype is controversial. This study aimed to investigate the clinical and epidemiological characteristics of the p.V37I variant in sensorineural hearing loss in Chinese infants (0-3 months). Hearing and gene tests were conducted in 300 infants (aged 0-3 months) with sensorineural hearing impairment and 484 normal infants (aged 0-3 months). Among the 300 hearing-impaired infants, 16 (5.33%) exhibited homozygous p.V37I variation and 7 (2.34%) showed a compound-heterozygous p.V37I variation, whereas no homozygous p.V37I (0%) or compound-heterozygous p.V37I (0%) condition was found among the 484 normal infants. The hearing impairment ranged from mild to profound in all patients exhibiting the homozygous p.V37I or the compound-heterozygous p.V37I condition, although most patients (61.54%) exhibit mild or moderate HL. Our results indicated that the p.V37I variation of GJB2 mutation is mainly associated with mild or moderate hearing impairment. Therefore, otolaryngologists should also screen the p.V37I variant of GJB2 in patients with mild or moderate HL.

Keywords: GJB2, p.V37I, Chinese, hearing loss

Introduction

Hearing loss (HL) is prevalent worldwide, and nearly 70 million individuals are recently estimated to suffer from HL, which affects their ability to communicate; moreover, 1-3 individuals in every 1,000 newborns exhibit permanent sensorineural hearing loss (SNHL), and half of such cases are associated with hereditary factors [1]. Identifying genes for HL has elucidated the genetic architecture of hearing function. Several regulatory transcription factors (e.g., POU3F4, EYA1, and PAX3), structural proteins maintaining the integrity of the hair cell, gap junction proteins, ion channel genes maintaining solute composition, as well as proteins with yet-to-be-uncovered functions, have been identified [2-4]. Despite the large number of genes implicated in non-syndromic HL, a particular gap junction protein, GJB2, coding for protein connexin 26 accounts for nearly 50% of all autosomal recessive HL and for 15%-18% of HL in all deaf individuals [5].

To date, more than 150 mutations, polymorphism, and unclassified variants have been discovered in the GJB2 gene; moreover, 35delG, 235delC, and 167delT are the most common GJB2 mutations that cause HL in Caucasian, Asian, and Ashkenazi populations, respectively [6]. In addition, the p.V37I variant of GJB2 mutations is commonly reported in HL at a carrier rate of 11.6% in Taiwan, although the pathogenic role of p.V37I remains controversial. p.V37I was previously considered to be a polymorphism without pathogenicity; however, research findings increasingly indicate that p.V37I is associated with mild to moderate hearing impairment [7-9]. The present study aimed to investigate the prevalence of the p.V37I variant of GJB2 mutation in Chinese SNHL infants (aged 0-3 months), as well as the clinical features of the homozygous p.V37I and the compound-heterozygous p.V37I conditions.

Materials and methods

Patients

A total of 300 infants aged 0-3 months diagnosed with SNHL from September 2013 to May 2015 were included in this study. All patients

Table 1. Distribution of p.V37I mutation in SNHL and control infants (0-3 months)

Group	p.V37I/-	p.V37I/p.V37I	p.V37I/other mutation	No p.V37I mutation	Total
SNHL	3 (1.0%)	16 (5.33%)	7 (2.33%)	274 (91.33%)	300
Normal	14 (2.89%)	0 (0%)	0 (0%)	470 (97.11%)*	484

*, P<0.05.

Table 2. Distribution and hearing levels of p.V37I mutation in SNHL infants

Cases	Allele 1	Allele 2	Hearing level (two ears)
3	p.V37I	p.V37I	mild/moderate
1	p.V37I	p.V37I	moderate/profound
2	p.V37I	p.V37I	mild/normal
4	p.V37I	p.V37I	moderate/moderate
3	p.V37I	p.V37I	profound/profound
3	p.V37I	p.V37I	mild/mild
2	p.V37I	c.79G>A	mild/mild
2	p.V37I	c.235delC	severe/profound
1	p.V37I	c.235delC	mild/moderate
1	p.V37I	c.299-300delAT	moderate/severe
1	p.V37I	79G>A+341A>G	moderate/moderate

were from the Otolaryngology Department of the Children's Hospital of Fudan University. Infants were excluded from the study if their onset age was not within 0-3 months or when their auditory brainstem response (ABR) results could not be obtained. A total of 484 normal infants aged 0-3 months served as the control group. This study was approved by the Ethics Committee of the Children's Hospital of Fudan University and was conducted in accordance with the ICH Guidelines for Good Clinical Practice and with the Declaration of Helsinki. All of the subjects of the study signed informed consent forms.

Audiological evaluation

ABR was performed on all SNHL infants under general anesthesia, with the patient lying on a bed in an acoustically and electrically shielded room. The severity of SNHL 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).

Gene testing

Gene testing of the exon 2 of GJB2 were performed on all infants. DNA was extracted from whole blood samples and purified using a commercially available extraction kit (Qiagen Inc., China). The primer sequences used in mutation analysis of GJB2 were as follows: forward: 5'-TCTTTTCCAGAGCAAACCGC-3', reverse: 5'-CTGGGCAATGCG-TTAAACTGG-3'. The PCR protocol was set as follows: predenaturation 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 a total of 35 cycles followed by a post-PCR extension step at 72°C for 1 min. The PCR products with 725 bp covering the entire coding region of GJB2 was sequenced using the ABI PRISM BigDye Terminator Cycle Sequencing Kit and ABI 3730 DNA Automatic Sequencer (USA).

Statistical analysis

The differences in gene phenotypes between the SNHL and normal infants were evaluated using fisher's exact test. Values with P<0.05 were considered statistically significant.

Results

Among the 300 SNHL infants, 26 (8.67%) exhibited more than one p.V37I of the GJB2 mutations, including 16 homozygous (5.33%), 3 heterozygous (1.0%), and 7 compound-heterozygous (2.33%). Among 484 normal infants, 14 (2.89%) heterozygous p.V37I were found and no patient exhibited the homozygous or compound-heterozygous p.V37I condition. The distribution of homozygous and compound-heterozygous p.V37I mutations was significantly higher in the patient group than in the control group (7.66% vs. 0%, P<0.05), indicating that the homozygous and compound-heterozygous p.V37I mutations are more frequently related to infant SNHL (**Table 1**).

Table 2 shows the severity of HL caused by p.V37I. The mean threshold of the ears was 76.07 ± 32.59, and the severity of HL ranged from mild to profound. A total of 16 (69.6%) infants exhibited mild to moderate HL. Two infants showed unilateral HL, whereas the rest exhibited bilateral HL. Moreover, 75.0% (12/16)

exhibited the homozygous p.V37I condition, whereas 57.14% (4/7) showed compound-heterozygous p.V37I condition and a mild to moderate hearing level. No difference in the severity of hearing level was observed between the two groups (P=0.71).

Discussion

The p.V37I mutation, which was first reported by Kelly in 1998, can cause a valine to isoleucine replacement. The carrier frequency of this mutation is very high among Chinese (6.2%) [7], Thai (4.3%) [10], Japanese (1.0%) [11], and Koreans (0.6%) [12]. The pathogenicity of p.V37I is controversial; p.V37I was initially considered a polymorphism but was recently found in a multicenter study to be related to mild and moderate HL [8]. Pu Dai found that the carrier frequency of p.V37I among Chinese Han patients is 6.7%, which is significantly higher than in the control population, demonstrating that p.V37I is a pathogenic mutation and not an allele variant [6]. Kim also reported that 18.2% of Koreans with mild HL are carriers of p.V37I; this fraction is significantly higher than the number of carriers with severe-profound HL (1.2%) and with normal hearing (1.0%), indicating that p.V37I is associated with mild deafness [13]. However, Chai found that the p.V37I mutation is related to a variety of hearing phenotypes ranging from profound HL to normal hearing and from congenital onset to delayed onset [14].

The present study found that 29 (8.67%) SNHL infants (0-3 months) are carriers of the p.V37I mutations, and the carrier frequency was similar to that reported by Shasha Huang (7.83%) [15], although the mean age of the patients in that study was 19.6 years old. Our study found 16 patients (5.33%) carrying homozygous p.V37I and 7 patients (2.33%) carrying compound-heterozygous p.V37I; these frequencies were significantly higher than those in the control group (0%). Moreover, no frequency difference in single heterozygous p.V37I condition was found between the SNHL and normal groups (1.0% vs. 2.89%, P=0.07). The result revealed that the homozygous and compound-heterozygous p.V37I conditions are pathogenic mutation in infants whose onset age was 0-3 months. Most genotype-phenotype correlation studies have indicated that GJB2 mutations are associated with non-progressive, bilateral, severe, or profound HL [16]. By contrast, this study revealed that some hearing impairments caused by GJB2 mutations could be mild or moderate, especially for non-truncating mutations, such as p.V37I.

All hearing phenotypes were found in our study, although most patients (69.6%) carrying the homozygous or compound-heterozygous p.V37I mutation exhibited a mild or moderate HL. Compared with the homozygous p.V37I condition, the compound-heterozygous p.V37I mutation did not cause more serious hearing impairment. Our study found seven patients with compound-heterozygous p.V37I mutation. The severity of HL depends on which types of the other mutations were compounded. The c.79G>A or c.79G>A+341A>G in GJB2 are considered as polymorphism [11], and we found that a patient with heterozygous p.V37I plus c.79G>A condition and another patient with heterozygous p.V37I plus c.79G>A+341A>G condition exhibited mild to moderate hearing levels. The most common pathogenic mutation of GJB2 is the c.235delC mutation, whereas c.299-300delAT is less common [11], although both contribute to SNHL. Our study found four patients with p.V37I plus c.235delC or c.299-300delAT, and three of them showed worse hearing levels than those with homozygous p.V37I condition, suggesting that p.V37I plus c.235delC or c.299-300delAT produces worse phenotype.

Other studies have found homozygous p.V37I or compound-heterozygous p.V37I condition in a certain proportion of the normal hearing population; moreover, p.V37I has been reported to delay the onset of HL [14]. However, our study did not show any normal infant (0-3 months) carrying the homozygous p.V37I or compound-heterozygous p.V37I condition; thus, these conditions may not be associated with delayed HL, if any, among the 484 normal infants. Our results were highly similar to those of Shasha Huang [15], who did not find these mutations among the Chinese Han whose age ranged from 7 months old to 54 years old. Huang speculated that the absence of the pathogenic p.V37I among the normal hearing group in their study is caused by the strict inclusion criteria for the normal controls and their higher average age. The average age of the 600 control individuals was 32 years old, and the hearing level of the normal young people who carry the p.V37I mutation may decline with age. Hao Wu

[7] screened 1516 control newborns and found homozygous p.V37I and one compound-heterozygous p.V37I (0.4%), which is a very low frequency. This finding may explain the absence of any homozygous p.V37I or compound-heterozygous p.V37I among the 484 infants in our control group.

Some studies have reported that SNHL may be caused by mutations in two alleles of different genes, such as GJB2 plus SLC26A4 or mitochondrial gene; one patient with enlarged vestibular aqueduct syndrome was reported to carry both the SLC26A4 and GJB2 mutations [17, 18]. Chai found that the p.V37I mutation is related to a variety of hearing phenotypes, ranging from profound HL to normal hearing [14]. Our study found all hearing phenotypes, except the epigenetic factors, another reason might be p.V37I plus yet-to-be discovered genes simultaneously. We detected another two common genes associated with HL (SLC26A4 and 12rRNA) among seven infants with compound-heterozygous p.V37I mutation, and no other mutations of the SLC26A4 and 12rRNA genes were found. Further studies are still needed, including those with a larger number of subjects and those focused on detecting other genes.

In summary, the results of our study demonstrate the significant potential for clinical application of genetic testing for HL. The p.V37I mutation of GJB2 is also strongly correlated with SNHL in infants, whose hearing phenotypes ranged from mild to profound. In addition, 69.6% of p.V37I carriers exhibit mild to moderate HL, indicating that even patients with mild or moderate HL must be tested for GJB2.

Acknowledgements

This study was supported by Science and Technology Program of Shanghai, China (134-119a070113) and Youth research project of Shanghai Municipal Bureau of health.

Disclosure of conflict of interest

None.

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References

[1] Dufresne D, Dagenais L, Shevell MI and Consortium R. Epidemiology of severe hearing impairment in a population-based cerebral palsy cohort. Pediatr Neurol 2014; 51: 641-644.

[2] Drozniewska M and Haus O. PAX3 gene deletion detected by microarray analysis in a girl with hearing loss. Mol Cytogenet 2014; 7: 30.

[3] Namba A, Abe S, Shinkawa H, Kimberling WJ and Usami SI. Genetic features of hearing loss associated with ear anomalies: PDS and EYA1 mutation analysis. J Hum Genet 2001; 46: 518-521.

[4] Schild C, Prera E, Lublinghoff N, Arndt S, Aschendorff A and Birkenhager R. Novel mutation in the homeobox domain of transcription factor POU3F4 associated with profound sensorineural hearing loss. Otol Neurotol 2011; 32: 690-694.

[5] Minami SB, Mutai H, Nakano A, Arimoto Y, Taiji H, Morimoto N, Sakata H, Adachi N, Masuda S, Sakamoto H, Yoshida H, Tanaka F, Morita N, Sugiuchi T, Kaga K and Matsunaga T. GJB2-associated hearing loss undetected by hearing screening of newborns. Gene 2013; 532: 41-45.

[6] Dai P, Yu F, Han B, Liu X, Wang G, Li Q, Yuan Y, Liu X, Huang D, Kang D, Zhang X, Yuan H, Yao K, Hao J, He J, He Y, Wang Y, Ye Q, Yu Y, Lin H, Liu L, Deng W, Zhu X, You Y, Cui J, Hou N, Xu X, Zhang J, Tang L, Song R, Lin Y, Sun S, Zhang R, Wu H, Ma Y, Zhu S, Wu BL, Han D and Wong LJ. GJB2 mutation spectrum in 2,063 Chinese patients with nonsyndromic hearing impairment. J Transl Med 2009; 7: 26.

[7] Li L, Lu J, Tao Z, Huang Q, Chai Y, Li X, Huang Z, Li Y, Xiang M, Yang J, Yao G, Wang Y, Yang T and Wu H. The p.V37I exclusive genotype of GJB2: a genetic risk-indicator of postnatal permanent childhood hearing impairment. PLoS One 2012; 7: e36621.

[8] Snoeckx RL, Huygen PL, Feldmann D, Marlin S, Denoyelle F, Waligora J, Mueller-Malesinska M, Pollak A, Ploski R, Murgia A, Orzan E, Castorina P, Ambrosetti U, Nowakowska-Szyrwinska E, Bal J, Wiszniewski W, Janecke AR, Nekahm-Heis D, Seeman P, Bendova O, Kenna MA, Frangulov A, Rehm HL, Tekin M, Incesulu A, Dahl HH, du Sart D, Jenkins L, Lucas D, Bitner-Glindzicz M, Avraham KB, Brownstein Z, del Castillo I, Moreno F, Blin N, Pfister M, Sziklai I, Toth T, Kelley PM, Cohn ES, Van Maldergem L, Hilbert P, Roux AF, Mondain M, Hoefsloot LH,

Cremers CW, Lopponen T, Lopponen H, Parving A, Gronskov K, Schrijver I, Roberson J, Gualandi F, Martini A, Lina-Granade G, Pallares-Ruiz N, Correia C, Fialho G, Cryns K, Hilgert N, Van de Heyning P, Nishimura CJ, Smith RJ and Van Camp G. GJB2 mutations and degree of hearing loss: a multicenter study. Am J Hum Genet 2005; 77: 945-957.

[9] Dahl HH, Tobin SE, Poulakis Z, Rickards FW, Xu X, Gillam L, Williams J, Saunders K, Cone-Wesson B and Wake M. The contribution of GJB2 mutations to slight or mild hearing loss in Australian elementary school children. J Med Genet 2006; 43: 850-855.

[10] Wattanasirichaigoon D, Limwongse C, Jariengprasert C, Yenchitsomanus PT, Tocharoenthanaphol C, Thongnoppakhun W, Thawil C, Charoenpipop D, Pho-iam T, Thongpradit S and Duggal P. High prevalence of V37I genetic variant in the connexin-26 (GJB2) gene among non-syndromic hearing-impaired and control Thai individuals. Clin Genet 2004; 66: 452-460.

[11] Abe S, Usami S, Shinkawa H, Kelley PM and Kimberling WJ. Prevalent connexin 26 gene (GJB2) mutations in Japanese. J Med Genet 2000; 37: 41-43.

[12] Han SH, Park HJ, Kang EJ, Ryu JS, Lee A, Yang YH and Lee KR. Carrier frequency of GJB2 (connexin-26) mutations causing inherited deafness in the Korean population. J Hum Genet 2008; 53: 1022-1028.

[13] Kim SY, Park G, Han KH, Kim A, Koo JW, Chang SO, Oh SH, Park WY and Choi BY. Prevalence of p.V37I variant of GJB2 in mild or moderate hearing loss in a pediatric population and the interpretation of its pathogenicity. PLoS One 2013; 8: e61592.

[14] Chai Y, Chen D, Sun L, Li L, Chen Y, Pang X, Zhang L, Wu H and Yang T. The homozygous p.V37I variant of GJB2 is associated with diverse hearing phenotypes. Clin Genet 2015; 87: 350-355.

[15] Huang S, Huang B, Wang G, Yuan Y and PD. The Relationship between the p.V37I Mutation in GJB2 and Hearing Phenotypes in Chinese Individuals. PLoS One 2015; 10: e129662.

[16] Wu BL, Lindeman N, Lip V, Adams A, Amato RS, Cox G, Irons M, Kenna M, Korf B, Raisen J and Platt O. Effectiveness of sequencing connexin 26 (GJB2) in cases of familial or sporadic childhood deafness referred for molecular diagnostic testing. Genet Med 2002; 4: 279-288.

[17] Huang S, Wang G, Jiang Y, Yuan Y, Han D, Song Y and Dai P. Phenotype and genotype of deaf patients with combined genomic and mitochondrial inheritance models. Mitochondrion 2013; 13: 791-794.

[18] Huang S, Han D, Wang G, Yuan Y, Song Y, Han M, Chen Z and Dai P. Sensorineural hearing loss caused by mutations in two alleles of both GJB2 and SLC26A4 genes. Int J Pediatr Otorhinolaryngol 2013; 77: 379-383.