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Effect of *GJB2* 235delC and 30-35delG genetic polymorphisms on risk of congenital deafness in a Chinese population

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ABSTRACT. Congenital deafness is a serious and irreversible condition in humans. The *GJB2* gene is implicated in the pathogenesis of autosomal recessive nonsyndromic hearing loss. Its 235delC and 30-35delG polymorphisms are reported to be associated with risk of hereditary deafness. However, the effect of the interaction between *GJB2* 235delC and 30-35delG and environmental factors on congenital deafness has not been described. Therefore, we performed a case-control study to investigate the influence of these polymorphisms on congenital deafness risk, and their interaction with maternal and other environmental factors in the development of this disease. Between March 2014 and May 2015, 118 patients with congenital deafness and 242 healthy controls were enrolled into our study. Compared with the GG genotype, the adjusted odds ratios (ORs) [and 95% confidence intervals (CIs)] for the 235delC GC and CC genotypes were 4.66 (1.77-13.07)

and 8.28 (2.06-47.52), respectively. Individuals harboring the GC+CC genotypes were at a greatly increased risk of congenital deafness compared to those with the GG genotype (OR = 5.65, 95%CI = 2.54-13.18). However, no significant relationship was established between the 30-35delG variant and this disease. The 235delC polymorphism exhibited an interaction with use of aminoglycoside antibiotics during pregnancy in conferring susceptibility to congenital deafness (chi-square = 8.76, P = 0.003). In conclusion, our study suggests that the *GJB2* 235delC polymorphism, but not the 30-35delG variant, contributes to congenital deafness susceptibility in the Chinese population examined, and demonstrates an interaction with consumption of aminoglycoside antibiotics during pregnancy in exerting this effect.

Key words: *GJB2* 235delC; *GJB2* 30-35delG; Polymorphism; Congenital deafness; Aminoglycoside antibiotics

INTRODUCTION

Congenital deafness in humans is a serious and irreversible condition, and it is estimated that approximately 30,000 newborns are diagnosed with congenital hearing impairment every year in China (Feng and Song, 2013). This disease greatly influences the quality of life and social adaptation of affected individuals (Feng and Song, 2013) and has been reported to have a global incidence of around 1/1000 (Gorlin et al., 1995). The development of congenital deafness involves many environmental and genetic factors. The former include several maternal influences to which sufferers may have been exposed during pregnancy, such as infection (with rubella virus, *Toxoplasma*, *Treponema pallidum*, herpes simplex virus, or cytomegalovirus), use of aminoglycoside antibiotics, and hypothyroidism (Leibovici et al., 2008; Faundes et al., 2012). In addition, fetal or neonatal hypoxia, premature birth, head trauma, low birth weight, and neonatal hemolytic jaundice contribute to the development of this disease (Leibovici et al., 2008; Faundes et al., 2012). However, congenital deafness does not occur in all infants born to mothers exposed to such risk factors, suggesting that the development of this condition also has a genetic component (Leibovici et al., 2008; Faundes et al., 2012). To date, several studies have revealed that many genetic factors contribute to the development of this disease, including the genes *MYO3A*, *SLC26A4*, *CDH23*, and *PTPRQ* (Adhikary et al., 2015; Menezes et al., 2015; Mizutari et al., 2015; Sakuma et al., 2015; Qu et al., 2016).

Gap junction protein beta-2 (*GJB2*) is located on chromosome 13q11-12 and is 4804 bp in length (Kikuchi et al., 1995). In 1997, Kelsell et al. (1997) reported that the *GJB2* gene is associated with the pathogenesis of autosomal recessive nonsyndromic hearing loss. Although further studies have indicated that *GJB3* and *GJB6* mutations also correlate with susceptibility to hereditary deafness, *GJB2* remains an important heritable factor in this condition (Wangemann, 1995; Estivill et al., 1998; Scott et al., 1998; Martin and Evans, 2004). The distribution of *GJB2* mutations differs widely between ethnicities. The *GJB2* 30-35delG variant is found in 60% of Northern Europeans, Caucasians, and Turks suffering from hereditary deafness (Wangemann, 1995; Martin and Evans, 2004). The *GJB2* 167delT variation has been recorded at a frequency of 53% among Jewish people suffering from hereditary deafness and the 30-35delG polymorphism at 18% (Wangemann, 1995; Martin and Evans,

2004). In the Chinese population, the *GJB2* 235delC genetic polymorphism is present in 12.2-33% of individuals with hereditary deafness (Estivill et al., 1998; Scott et al., 1998; Maeda et al., 2005). However, no study has reported the interaction between the 235delC and 30-35delG variants and environmental factors in affecting risk of congenital deafness. Therefore, we performed a case-control study to investigate the role of the *GJB2* 235delC and 30-35delG genetic polymorphisms and their interaction with maternal and other environmental factors in susceptibility to this condition.

MATERIAL AND METHODS

All patients and controls voluntarily participated in this study and signed an informed consent form prior to enrollment. This study was approved by the Ethics Committee of Nanfang Hospital of Southern Medical University and was performed according to the Declaration of Helsinki of 1964.

Subjects

Between March 2014 and May 2015, a total of 118 patients with congenital deafness were enrolled. They were recruited from clinics at the Nanfang Hospital and Boai Hospital of Zhongshan, both of Southern Medical University. Patients were confirmed as suffering from congenital deafness, defined as deafness at birth or mutism. Those with deafness caused by otitis media, head injury, ear trauma, infection, or drugs were excluded.

During the same period, a total of 242 healthy controls were recruited from pediatrics clinics at the same hospitals. All control subjects were confirmed to have normal hearing by otological examination and hearing, auditory brainstem response, and acoustic impedance tests.

Data concerning demographic characteristics and other environmental factors were collected from medical records and structured questionnaires. The questionnaire items concerned age, premature birth, low birth weight, neonatal hemolytic jaundice, and various aspects of pregnancy, including infections, use of aminoglycoside antibiotics, alcohol and tobacco consumption, and hypothyroidism.

The following organisms were considered in our definition of infection during pregnancy: rubella virus, *Toxoplasma*, *T. pallidum*, herpes simplex virus, and cytomegalovirus.

DNA extraction and genotyping

Each participant provided 5 mL peripheral venous blood for DNA extraction. Samples were kept at -20°C, and genomic DNA was extracted using a Tiangen Blood DNA Mini Kit (Tiangen Biotech, Beijing, China) according to the manufacturer instructions.

The *GJB2* 235delC and 30-35delG polymorphisms were analyzed by the polymerase chain reaction (PCR)-restriction fragment length polymorphism method. The primers used were designed with the Primer Premier 5.0 software (PREMIER Biosoft, Palo Alto, CA, USA). The sequences of the reverse and forward primers for *GJB2* 235delC were 5'-TGTGTGCAT TCGTCTTTTCCAG-3' and 5'-GGTTCCTCATCCCTCTCAT-3', respectively. Those of the reverse and forward primers for *GJB2* 30-35delG were 5'-CTTTTCCAGAGCAAACCGCC C-3' and 5'-TGCTGGTGGAGTGTTTGTTCAC-3', respectively. Each PCR comprised a 25-μL mixture containing 2.5 μL deoxynucleotides (at 2 M), 2 μL each primer (at 10 pM), 1.5

μL $MgCl_2$ (at 2 M), 1.5 U *Taq* DNA polymerase, and 100 ng template DNA. PCRs were performed in a thermocycler, beginning with denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 45 s, before a final extension at 72°C for 7 min. The restriction enzymes used to type the *GJB2* 235delC and 30-35delG polymorphisms were *Apa*I and *Pst*I, respectively. Amplification of both regions was verified by electrophoresis on 2% agarose gels, followed by staining with ethidium bromide.

Statistical analysis

Differences between study groups regarding demographic characteristics and maternal and other environmental factors were ascertained using the chi-square test or Student *t*-test. The goodness-of-fit chi-square test was used to establish whether *GJB2* 235delC and 30-35delG genotype distributions deviated from Hardy-Weinberg equilibrium (HWE). The association between these polymorphisms and risk of congenital deafness was analyzed by multiple logistic regression analysis, using odds ratios (ORs) and their 95% confidence intervals (CIs) to estimate the effect. Gene-environment interactions were assessed by the chi-square test. All statistical analysis was carried out with SPSS Statistics for Windows Version 20.0 (IBM Corp., Armonk, NY, USA). P values < 0.05 were considered statistically significant.

RESULTS

The demographic characteristics of patients and control subjects, and the maternal and other environmental factors to which they were exposed are shown in Table 1. In comparison with the mothers of control subjects, those of the patients were more likely to have taken aminoglycoside antibiotics during pregnancy (chi-square = 4.90, P = 0.03). However, no significant differences were observed between patients with congenital deafness and controls in terms of age (t = 1.30, P = 0.10), gender (chi-square = 3.46, P = 0.06), premature birth (chi-square = 1.65, P = 0.20), low birth weight (chi-square = 2.76, P = 0.10), neonatal hemolytic jaundice (chi-square = 0.41, P = 0.52), infection (chi-square = 1.39, P = 0.08), alcohol consumption (chi-square = 1.85, P = 0.40), tobacco use (chi-square = 0.8, P = 0.67), or hypothyroidism during pregnancy (chi-square = 0.24, P = 0.62).

GJB2 235delC and 30-35delG genotype distributions are shown in Table 2. Using the chi-square test, we observed that the frequencies of the GG, GC, and CC genotypes of *GJB2* 235delC significantly differed between patients and control subjects, whereas those of the GG, GA, and AA genotypes of *GJB2* 30-35delG did not. The distribution of *GJB2* 235delC genotypes was consistent with HWE among both patients (chi-square = 3.64, P = 0.14) and controls (chi-square = 5.49, P = 0.06), although that of *GJB2* 30-35delG genotypes deviated from HWE in both patient (chi-square = 21.83, P < 0.001) and control groups (chi-square = 35.09, P < 0.001).

Using multiple logistic regression analysis, we observed that the *GJB2* 235delC GC and CC genotypes were significantly associated with a higher risk of congenital deafness compared to the GG genotype (Table 3), with adjusted ORs (and 95%CIs) of 4.66 (1.77-13.07) and 8.28 (2.06-47.52), respectively. In addition, individuals harboring the GC+CC genotypes were found to be exposed to a greatly increased risk of congenital deafness in comparison to those with the GG genotype (OR = 5.65, 95%CI = 2.54-13.18). However, no significant association was established between the *GJB2* 30-35delG polymorphism and risk of this disease.

Table 1. Demographic characteristics of patients with congenital deafness and control subjects, and maternal and other environmental factors to which they were exposed.

Variables	Patients (N = 118)	%	Controls (N = 242)	%	Chi-square or *t*	P
Age, years	2.43 ± 1.05		2.57 ± 0.91		1.30	0.10
Gender						
Female	74	62.71	146	60.33		
Male	44	37.29	96	39.67	3.46	0.06
Infection during pregnancy						
No	99	83.90	228	94.21		
Yes	19	16.10	14	5.79	1.39	0.08
Alcohol consumption during pregnancy						
No	79	66.95	177	73.14		
Yes	6	5.08	13	5.37		
Former	33	27.97	52	21.49	1.85	0.40
Tobacco use during pregnancy						
No	90	76.27	194	80.17		
Yes	4	3.39	8	3.31		
Former	24	20.34	40	16.53	0.80	0.67
Hypothyroidism during pregnancy						
No	108	91.53	225	92.98		
Yes	10	8.47	17	7.02	0.24	0.62
Use of aminoglycoside antibiotics during pregnancy						
No	111	94.07	238	98.35		
Yes	7	5.93	4	1.65	4.90	0.03
Premature birth						
No	105	88.98	225	92.98		
Yes	13	11.02	17	7.02	1.65	0.20
Low birth weight						
No	101	85.59	221	91.32		
Yes	17	14.41	21	8.68	2.76	0.10
Neonatal hemolytic jaundice						
No	108	91.53	226	93.39		
Yes	10	8.47	16	6.61	0.41	0.52

Table 2. Genotype distributions of *GJB2* 235delC and 30-35delG polymorphisms among patients with congenital deafness and control subjects.

Variant	Patients	%	Controls	%	Chi-square	P	Chi-square (HWE)	P	Chi-square (HWE)	P
							Patients		Controls	
GJB2 235delC										
GG	93	78.81	231	95.45						
GC	15	12.71	8	3.31						
CC	10	8.48	3	1.24	24.92	<0.001	3.64	0.14	5.49	0.06
GJB2 30-35delG										
GG	108	91.53	230	95.04						
GA	7	5.93	9	3.72						
AA	3	2.54	3	1.24	1.79	0.41	21.83	<0.001	35.09	<0.001

HWE = Hardy-Weinberg equilibrium.

Table 3. Associations between*GJB2* 235delC and 30-35delG polymorphisms and risk of congenital deafness.

Variant	Patients	%	Controls	%	OR (95%CI)[1]	P
GJB2 235delC						
GG	93	78.81	231	95.45	1.0 (Ref.)	-
GC	15	12.71	8	3.31	4.66 (1.77-13.07)	<0.001
CC	10	8.48	3	1.24	8.28 (2.06-47.52)	<0.001
GC+CC	25	21.19	11	4.55	5.65 (2.54-13.18)	<0.001
GJB2 30-35delG						
GG	108	91.53	230	95.04	1.0 (Ref.)	-
GA	7	5.93	9	3.72	1.66 (0.51-5.14)	0.32
AA	3	2.54	3	1.24	2.13 (0.28-16.12)	0.35
GA+AA	10	8.47	12	4.96	1.77 (0.66-4.63)	0.19

[1]Adjusted for age, gender, and use of aminoglycoside antibiotics during pregnancy. OR = odds ratio; CI = confidence interval; Ref. = reference.

Correlations between the *GJB2* 235delC polymorphism and demographic characteristics and maternal and other environmental factors associated with risk of congenital deafness are shown in Table 4. We observed that the *GJB2* 235delC variant exhibited an interaction with use of aminoglycoside antibiotics during pregnancy in affecting susceptibility to this condition (chi-square value = 8.76; P = 0.003). However, no interaction between this polymorphism and gender (chi-square = 2.08, P = 0.15), premature birth (chi-square = 0.40, P = 0.53), low birth weight (chi-square = 1.58, P = 0.21), neonatal hemolytic jaundice (chi-square = 2.65, P = 0.10), or pregnancy-related factors, including infection (chi-square = 2.70, P = 0.10), alcohol consumption (chi-square = 1.07, P = 0.59), tobacco use (chi-square = 3.35, P = 0.18), and hypothyroidism (chi-square = 2.35, P = 0.13) was noted.

Table 4. Correlations between the *GJB2* 235delC polymorphism and demographic characteristics and maternal and other environmental factors in risk of congenital deafness.

Variable	*GJB2* 235delC		Chi-square	P
	GG (N = 324)	GC+CC (N = 36)		
Sex				
Male	194	26		
Female	130	10	2.08	0.15
Infection during pregnancy				
No	297	30		
Yes	27	6	2.70	0.10
Alcohol consumption during pregnancy				
No	228	28		
Yes	17	2		
Former	79	6	1.07	0.59
Tobacco use during pregnancy				
No	256	28		
Yes	9	3		
Former	59	5	3.35	0.18
Hypothyroidism during pregnancy				
No	302	31		
Yes	22	5	2.35	0.13
Use of aminoglycoside antibiotics during pregnancy				
No	317	32		
Yes	7	4	8.76	0.003
Premature birth				
No	296	34		
Yes	28	2	0.40	0.53
Low birth weight				
No	292	30		
Yes	32	6	1.58	0.21
Neonatal hemolytic jaundice				
No	303	31		
Yes	21	5	2.65	0.10

DISCUSSION

Increasingly, many genetic studies have been adopted to identify genes involved in the initiation and progression of congenital deafness (Jiang et al., 2014; Dai et al., 2015; Zheng et al., 2015; Xia et al., 2016). We performed the case-control study described here to investigate the role of the *GJB2* 235delC and 30-35delG polymorphisms in susceptibility to this disease, finding that the GC and CC genotypes of the former were associated with increased risk, but that no such relationship was evident with the latter.

GJB2 is expressed by two cell types: sensory epithelial cells, including internal tooth, groove inside, supporting, ditch outside, and spiral ligament root cells (Mukherjee et

al., 2003); and connective tissue cells (Mukherjee et al., 2003). Mutations within the *GJB2* coding region can cause frameshifts affecting protein translation, influencing gap junction protein structure, and resulting in connexin defects (Mesolella et al., 2004; Matsunaga et al., 2006; Blanchard et al., 2012). Moreover, such polymorphisms may also affect the opening and closing of these channels, blocking or disrupting intercellular exchange. Abnormal connection channels can influence the movement of potassium ions back into the endolymph, altering potassium concentration to the point of intoxication of the organ of Corti, ultimately resulting in sensorineural deafness (Lefebvre and Van De Water, 2000). To date, many studies have indicated that mutations in the gene *GJB2* can interrupt the transmission of second messengers such as inositol trisphosphate and calcium ions between cells, and the former is known to play an important role in auditory physiology (Beltramello et al., 2005; García et al., 2016).

Previous investigations have reported that approximately 35% of patients with congenital deafness carry *GJB2* 233-235delC variants (Jiang et al., 2014; Dai et al., 2015; Zheng et al., 2015; Xia et al., 2016). These variations comprise the deletion of a cytosine residue in the *GJB2* coding region at positions 233-235, leading to a frameshift mutation. The encoded protein lacks part of the M2 region, and the CL, M3, E2, and M4 regions are entirely absent. Such proteins may compromise the regulation of gap junctions by pH and decrease their permeability, causing impaired auditory function.

Some studies have examined the association between the *GJB2* 235delC polymorphism and development of congenital deafness, but their results have been inconsistent (Dai et al., 2007; Chen et al., 2009; Padma et al., 2009; Dzhemileva et al., 2010; Yang et al., 2013). In a study of a Chinese population, Dai et al. (2007) reported that this genetic variant is significantly associated with deafness. Similarly, Chen et al. (2009) found it to be more common among Chinese sufferers of nonsyndromic deafness. Padma et al. (2009) investigated an Indian population, showing that the *GJB2* 235delC mutation is more prevalent among patients with hearing impairments than healthy controls. In a Eurasian population, Dzhemileva et al. (2010) found that the 235delC and 30-35delG sequence variations are more frequently observed in patients with hereditary hearing impairments. In addition, Yang et al. (2013) reported that the 235delC polymorphism is the most prevalent mutation in Tibet patients with hearing loss. However, certain authors have reported inconsistent findings. Li et al. (2007) performed a study of deaf patients in the Xinjiang region of China, establishing that the *GJB2* 35delG genetic variant correlates with deafness, whereas the 235delC mutation does not.

Here, we have shown for the first time that in its effects on risk of congenital deafness, the *GJB2* 235delC polymorphism significantly correlates with use of aminoglycoside antibiotics during pregnancy. However, no previous study has reported the interaction between this sequence variant and environmental factors, and further investigations are greatly needed to confirm our findings.

Two limitations of the present study should be mentioned. First, it is possible that genes other than *GJB2* play a role in the development of congenital deafness. Thus, gene-gene interactions should be considered in future analyses. Two, the sample size of this study was relatively small. Large-scale investigations based in different locations need to be performed to validate our results.

In conclusion, our data suggest that the *GJB2* 235delC polymorphism, but not the 30-35delG variant, contributes to congenital deafness susceptibility and interacts with use of aminoglycoside antibiotics during pregnancy in so doing. Further, large studies in other geographic regions need to be carried out to verify our findings.

Conflicts of interest

The authors declare no conflict of interest.

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