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Original Article

Analysis of Hearing Loss-Associated Gene Mutations Using MALDI-TOF Mass Spectrometry in Neonates from Shaoxing, China

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Abstract ***Purpose:*** The aim of our study was to evaluate the application of concurrent standard newborn hearing screening (NHS) and genetic screening for neonates in Shaoxing. ***Methods:*** According to whether passed NHS, 257 neonates and 514 neonates were divided into experimental group and control group, respectively. Twenty hotspot hearing-associated mutations of four common deafness-susceptibility genes (12S rRNA, GJB2, GJB3, SLC26A4) were analysed with heel blood samples by MALDI-TOF mass spectrometry. ***Findings:*** In the experimental group, 24 neonates were detected with hearing loss-associated gene mutations (9.34%, 24/257), and there were 5 pathogenic mutations. In the control group, 25 neonates were detected with hearing loss-associated gene mutations (4.86%, 25/514), there were 3 pathogenic mutations. The rates of gene mutations, pathogenic mutations and heterozygous mutations in the experimental group were statistically higher than the control group (P<0.05). ***Conclusions:*** Hearing loss susceptibility gene screening should be carried out for the neonates who did not pass NHS. Concurrent NHS and genetic screening should be undertaken in the areas where conditions permit.

Key words *Gene mutation; Hearing loss; Hearing screening; Neonates; MALDI-TOF*

Introduction

Hearing loss (HL) is the most prevalent sensory impairment in both childhood and adulthood.[1] According to statistics, one in every 1000 neonates suffers from pre-lingual deafness and more than half of these cases result from genetic factors.[2,3] Although serious hearing problems during childhood are rare, early testing ensures that any problems are picked up and managed as early as possible.[4] However, children with permanent hearing loss continue to increase with age.[5] An important reason for the rising incidence is the limitations or defects present in newborn hearing screening (NHS), drug-induced deafness and late-onset deafness, which could not be detected in NHS.[6] The deafness-susceptibility gene screening could compensate for the limitations of NHS.

Methods

All enrolled neonates were from Shaoxing Maternal and Child Health Care Hospital. A two-stage screening approach with GSI distortion product otoacoustic emissions (DPOAE) was performed in the hearing screening on the 2~3 days after birth. If the neonates failed the initial test, a repeated DPOAE test and an automatic auditory

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brainstem repose (AABR) assessment were performed at 42 days of age. The results are presented in the form of passed or not passed. According to whether passed NHS, 257 neonates and 514 neonates were divided into experimental group and control group, respectively. The study was approved and conducted in accordance with the protocol of the Institutional Medical and Ethics Committees of the Shaoxing Maternal and Child Health Care Hospital. We obtained informed consent from all participants who volunteered to participate in this detection study. Genomic DNA was extracted from heel blood samples of neonates with AxyPrep Genomic Blood DNA Extraction Kit (AXYGEN, Shanghai, China).

Hearing loss-associated genes were detected using MALDI-TOF mass spectrometry as previously described,[7] including twenty hotspot hearing-associated mutations of four common deafness - susceptibility genes (12S rRNA, GJB2, GJB3, SLC26A4). The hotspot mutations were 235delC, 299_300delAT, 35delG, 176_191del16, 167delT mutation in GJB2 gene; 538C>T, 547G>A mutation in GJB3 gene; IVS7-2A>G, c.2168A>G, c.1174A>T, c.1229C>T, c.1226G>A, c.1975G>C, c.2027T>A, c.2162C>T, c.281C>T, c.589G>A, IVS15+5G>A mutation in SLC26A4 gene, and 1494C>T, 1555A>G in mitochondrial DNA (mtDNA) gene. Confirmation of validation was obtained with both wild and mutant types previously confirmed by Sanger sequencing.

Comparisons of mutation frequencies of GJB2, GJB3, mtDNA 12S rRNA, and SLC26A4 genes were conducted by Chi-square test. The statistical data analysis was carried out by SPSS 19.0 software. If the P-value is less than 0.05, differences were considered as statistically significant.

Results

Most of the neonates with genetic mutations were profound hearing loss (25/49), have no family history (44/49), and with no malformations(40/49). Clinical characteristics of 49 infants detected to have gene mutations were shown in Table 1.The rate of hearing loss-associated gene mutations was 6.36% (49/771). In the experimental group, 24 neonates were detected with hearing loss-associated gene mutations (9.34%, 24/257), and there were five pathogenic mutations (one GJB2 235delC homozygous mutation, four GJB3 538C>T heterozygous mutations). In the control group, 25 neonates were detected with hearing loss-associated gene mutations (4.86%, 25/514), and there were three pathogenic mutations (one 12S rRNA 1555A>G homoplasmic mutation, one GJB3 538C>T heterozygous mutation, one GJB3 547G>A heterozygous mutation). The rates of gene mutations, pathogenic mutations and heterozygous mutations in the experimental group were statistically higher than the control group (P<0.05).

All 49 cases of hearing loss-associated mutations were heterozygous except one case of GJB2 235delC homozygous mutation, and one case of 12S rRNA 1555A>G homoplasmic mutation. The rate of mutations in GJB2, SLC26A4, GJB3 and mtDNA was 55.1% (27/49), 32.65% (16/49), 10.20% (5/49) and 2.05% (1/49), respectively. The rate of GJB2 gene mutations was significantly higher than the SLC26A4 mutation, GJB3 mutation and mtDNA, there was a significant difference among them (P<0.01). The results of hearing loss-associated gene mutations were shown in Table 2. The rates of the hearing loss-associated gene mutations, pathogenic mutations and heterozygous carrying in the experimental group, were significantly higher than those of the control group, as shown in Table 3.

Discussion

Hearing loss is the most common sensory defect.[8] Various genetic as well as environmental factors have been shown

Table 1 Clinical characteristics of 49 infants detected to have gene mutations

Parameters	N(%)
Sex	
Male	28 (57.14)
Female	21 (42.86)
Degree of hearing loss	
Mild	1 (2.04)
Moderate	9 (18.37)
Severe	14 (28.57)
Profound	25 (51.02)
Family history	
Yes	5 (10.20)
No	44 (89.80)
Malformations	
None	40 (81.63)
Enalrged vestibular aqueduct	4 (8.16)
Aplasia of the auditory nerve	3 (6.12)
Inner ear malformation	2 (4.08)
Cochlear hair cell degeneration	1 (0.1)

to contribute the onset of hearing loss.[5,9,10] Genetic factors are thought to cause more than 50% of all incidents of congenital hearing loss in children.[11] Also, a large number of late-onset and gradual worsening hearing impairment may be due to defective genes disease or genetic defect and polymorphism associated with susceptible environmental factors.[5] Currently, NHS has been carried out in our country for many years, and made significant achievements. However, the present commonly used DPOAE and Automated Auditory Brainstem Response (AABR) could only screen the hearing situation at the moment, we could not detect the hearing loss with no phenotype. So concurrent NHS and genetic screening is the most powerful strategy for pre-lingual or delayed hearing loss in children and high-risk hearing loss-associated gene carriers.

In this study, twenty hotspot hearing-associated mutations of four common hearing loss- susceptibility.

Genes were analysed by MALDI-TOF-MS which sensitivity and specificity were >99.9%.[12] GJB2 mutations were detected in 3.5% (27/771) of all participants. GJB2 mutations are a major cause of autosomal recessive nonsyndromic hearing loss in many populations, could cause moderate, severe, profound severe hearing loss.[11,13] In this study, one case of GJB2 gene homozygous mutations was diagnosed as bilateral hearing loss. Three major GJB2 gene mutations, 235delC, 299_300delAT and 176_191del16 were detected; other two mutations, 35delG and 167delT were not detected.

GJB3 is considered to be related to high-frequency hearing decrease, can cause autosomal dominant or recessive hereditary non-syndromic hearing loss.[14] We

Table 2 Hearing loss-associated gene mutations distribution of 771 neonates

Gene	Mutation	Case	Rate (%)	Experimental (n=257)		Control (n=514)	
				Case	Rate (%)	Case	Rate (%)
GJB2	235delC homozygous	1	0.13	1	0.39	0	0.00
	235delC heterozygous	19	2.46	9	3.50	10	1.95
	299_300delAT heterozygous	4	0.52	2	0.78	2	0.39
	176_191del16 heterozygous	3	0.39	2	0.78	1	0.19
	Subtotal	27	3.50	14	5.45	13	2.53
SLC26A4	IVS7-2A>G heterozygous	8	1.04	3	1.17	5	0.97
	1174A>T heterozygous	2	0.26	1	0.39	1	0.19
	2168A>G heterozygous	2	0.26	1	0.39	1	0.19
	1229C>T heterozygous	2	0.26	2	0.78	0	0.00
	2027T>A heterozygous	1	0.13	0	0.00	1	0.19
	2162C>T heterozygous	1	0.13	0	0.00	1	0.19
	Subtotal	16	2.08	7	2.72	9	1.75
GJB3	538C>T heterozygous	4	0.52	3	1.17	1	0.19
	547G>A heterozygous	1	0.13	0	0.00	1	0.19
	Subtotal	5	0.65	3	1.17	2	0.39
mtDNA	1555A>G homoplasmic	1	0.13	0	0.00	1	0.19
Total		**49**	**6.36**	**24**	**9.34**	**25**	**4.86**

Table 3 The rate of hearing loss-associated gene mutations in the two groups

	Experimental group (n=257)		Control group (n=514)			
	Gene mutation	Rate (%)	Gene mutation	Rate (%)	X^2	P
Pathogenic mutations	5	1.95	3	0.58	4.69	<0.05
Heterozygous carrying	19	7.39	22	4.28	4.78	<0.05
Total	**24**	**9.34**	**25**	**4.86**	**8.14**	**<0.01**

detected five GJB3 gene heterozygous mutations, and two passed DPOAE and AABR.

mtDNA mutations are responsible for both maternally inherited syndromic and nonsyndromic hearing loss and play a role in predisposition to aminoglycoside-induced ototoxicities, such as the 1494C>T and 1555A>G mutation.[5] We detected one mtDNA 12S rRNA 1555A> G homoplasmic mutation in control group, suggesting we should pay high attention to this neonate avoiding the use of ototoxic drugs lifelong.

Recessive mutations in SLC26A4 are associated with Pendred syndrome, and autosomal recessive hearing loss with the enlarged vestibular aqueduct, and/or incomplete partition of the cochlea.[15] In this study, the rate of SLC26A4 gene mutations was 2.08%, just following GJB2 gene mutations. Timely detection of SLC26A4 gene mutations could guide patients to avoid strenuous exercise, brain trauma and other environmental factors that lead to late-onset progressive hearing loss in daily life.

The total rate of hearing loss-associated gene mutations was 6.36%, and the rate of gene mutations, pathogenic mutations in the experimental group were statistically higher than the control group, suggesting neonates who can't pass the NHS could undergo common hearing loss-associated gene screening.

Conclusion

Concurrent NHS and genetic screening should be undertaken in the areas where conditions permit. Early detection, early diagnosis and early intervention of deaf children could achieve the purpose of early hearing and speech rehabilitation.

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Conflict of Interest

The authors declare no conflict of interest.

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