Original Article

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Clinical data analysis of genotypes and phenotypes of deafness gene mutations in newborns: A retrospective study

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Summary **We retrospectively analyzed newborns with deafness gene mutations and summarized the relationship between genotype and phenotype to provide a basis for genetic counseling. We studied 582 subjects positive for deafness gene mutations that were treated in the otology outpatient department of Beijing Tongren Hospital, Capital Medical University, between April 2012 and April 2016. The subjects were divided into 3 categories: a diagnosed group (group A), which was further subdivided into subgroups A1 (homozygous and compound heterozygous *GJB2* mutations) and A2 (homozygous and compound heterozygous *SLC26A4* mutations); a drug-induced deafness group (group B, mitochondrial (Mt) gene mutations); and a mutation carrier group (group C), which was further subdivided into the subgroups C1 (*GJB2* heterozygous mutations), C2 (*SLC26A4* heterozygous mutations), C3 (*GJB3* heterozygous mutations), and C4 (double gene mutations). Partial sequences positive for *GJB2* or *SLC26A4* were sequenced and analyzed for mutations. Subjects underwent otoscopic examination and comprehensive audiological evaluation, and temporal bone computerized tomography and/or inner ear magnetic resonance imaging were performed. *GJB2* 235delC was the most common mutation locus. The highest proportion of deafness detected during universal newborn hearing screening was for drug-induced deafness, whereas the lowest was for the diagnosed group. *GJB2* gene mutations mainly resulted in flat-type, profound-to-severe sensorineural hearing loss (SNHL). *SLC26A4* gene mutation was mainly associated with high-frequency drop-type and profound-severe SNHL and was closely related to enlargement of the vestibular aqueduct.**

Keywords: Gene, screening, *GJB2*, *SLC26A4*, hearing loss

1. Introduction

Deafness, which refers to varying degrees of hearing loss, is one of the most common sensory disorders. In 2016, the World Health Organization reported that the rate of disabling deafness was as high as 360 million, accounting for approximately 5.14% of the world's population. Among them, more than 32 million are children. Thus, deafness has become a global public health problem.

Although deafness is ascribed to many causes, genetic factors account for approximately 50-60% of cases (*1*), and the incidence of neonatal congenital deafness is approximately 1-3‰ (*2,3*). With rapid advances in science and technology, several deafness genes have been identified. By the end of May 2015, 97 non-syndromic deafness genes and 152 non-syndromic deafness genetic loci were identified. These findings

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underscore the importance of detecting deafness genes in children at a minimized cost.

Green *et al*. (*4*) proposed the use of deafness gene chip screening in the diagnosis of neonatal deafness in 2000. Soon after, Morton *et al*. (*2*) and Wang *et al*. (*3*) proposed deafness gene screening as a part of newborn hearing screening, leading to increased awareness. Using large-scale national deafness disease molecular epidemiology survey data (*5,6*), together with data on mutations for non-syndromic deafness in the Chinese population, allele-specific primer extension polymerase chain reaction (PCR) and universal chip technology were combined to develop gene chip technology for 4 genetic deafness genes (*7*). In these 4 genes, 9 loci were screened, including *GJB2* c.235delC, c.299delAT, c.176dell6, and c.35delG; *GJB3* c.538C>T; *SLC26A4* c.IVS7-2A>G and c.2168A>G; and *Mt 12S rRNA* m.1555A>G and m.1494C>T. The whole experimental process takes approximately 5 h, which is conducive to rapid detection in the clinical setting or for large-scale population screening (*7*).

Newborn deafness gene screening can enable early detection of deafness in children and guide necessary management as soon as possible. In 2007, China put forth the concept of "newborn deafness gene screening" for the first time (*3*), and neonatal deafness gene screening and joint hearing screening were gradually actualized. In 2012, Beijing became the first city in China to implement a neonatal deafness gene screening project in the resident population. In the preliminary statistics of our research group, blood samples of 62,560,000 newborns were screened in Beijing by December 2014. The positivity rate for the 9 common deafness gene mutations was 4.59%. The ototoxic drug susceptibility rate was 2.37‰, and the rate of diagnosed congenital deafness was 0.24‰. Of single heterozygous mutations, 4.34% might be associated with late-onset deafness. Currently, many provinces and cities nationwide are equipped to screen for newborn deafness genes.

Owing to the increasing number of newborns being diagnosed with deafness gene mutations, otologists are facing great challenges. The purpose of this study, therefore, was to investigate the nature, degree, and curves of hearing loss in neonates positive for deafness gene mutations, and to provide the basis for clinical genetic counseling.

2. Materials and Methods

Subjects' parents provided written informed consent for their participation in the study. The protocol was approved by the Declaration of Helsinki principles and Ethics Committee of Beijing Tongren Hospital, Capital Medical University.

2.1. *Subject recruitment*

Between April 2012 and April 2016, 1258 Chinese newborns who underwent deafness gene screening were recruited from among patients seeking genetic testing and counseling at the Department of Otolaryngology, Head and Neck Surgery, Tongren Hospital (Beijing, China). We screened 9 loci in 4 genes, including *GJB2* c.235delC, c.299delAT, c.176dell6, and c.35delG; *GJB3* c.538C>T; *SLC26A4* c.IVS7-2A>G and c.2168A>G; and *Mt 12S rRNA* m.1555A>G and m.1494C>T. In total, 582 cases were finally included in the study.

According to the gene mutations, the subjects were divided into the following 3 groups: diagnosed group (group A), which was further subdivided into A1 (homozygous and compound heterozygous mutations in *GJB2*) and A2 (homozygous and compound heterozygous mutations in *SLC26A4*); drug-induced deafness group (group B): mitochondrial gene mutations; and gene mutation carrier group (group C), which was further subdivided into C1 (*GJB2* heterozygous mutations), C2 (*SLC26A4* heterozygous mutations), C3 (*GJB3* heterozygous mutations), and C4 (double gene non-pathogenic mutations).

2.2. *Clinical evaluation*

The following demographic information was collected for each patient: sex, birth date, pregnancy, relevant family history, and date of initial otolaryngological consultation, major comorbidities, and the result of newborn hearing screening (*8*).

2.3. *DNA analysis*

Genomic DNA was extracted from 2 ml of whole blood from each patient, using the Blood DNA kit (Tiangen Biotech, Beijing, China). All exons and flanking splice sites of the *GJB2* and *SLC26A4* genes were screened for mutations by PCR amplification and bidirectional sequencing.

2.4. *Auditory evaluation*

Subjects underwent physical examination, including otoscopic examination, with special attention to hearing. Comprehensive audiological evaluation included auditory brainstem response (ABR), 40-Hz auditory event-related potential, distortion product otoacoustic emission, auditory steady-state response (ASSR), acoustic immittance, and pediatric behavioral audiometry. According to Liden/Jerger, the classification of acoustic immittance (226 Hz) was as follows: A (including As and Ad), B, and C. A was considered normal. The classification of acoustic immittance (1,000 Hz) was unimodal, bimodal, or flat type (*9,10*).

We evaluated the audiology according to Mazzoli *et al.*, who described the term non-syndromic hearing

Table 1. Comparison of basic parameters among the diagnosed, drug-induced deafness, and mutation carrier groups (n = 582 cases)

Group	Sub-group	Number (case)	Sex (case)		Age (months)		Age at first visit (months)		Family history (case,%)
			Male	Female	Section	Mean	Section	Mean	
A	A1	85	54	31	8-58	35.4 ± 13.02	1-44	8.19 ± 9.14	8 (9.41)
	A2	32	14	18	9-58	36.8 ± 15.69	1-46	13.2 ± 13.74	3 (9.38)
B	B	69	35	34	14-56	42.1 ± 10.29	2-17	3.83 ± 2.73	19 (27.54)
C	C1	188	114	74	11-56	7.15 ± 5.02	1-25	7.15 ± 5.02	15 (7.98)
	C2	133	72	61	11-57	37.4 ± 10.83	2-43	7.28 ± 6.49	7 (5.26)
	C3	48	18	30	16-54	40.58 ± 9.60	2-16	5.46 ± 3.71	1 (2.08)
	C4	27	17	10	16-56	39.5 ± 12.18	1-33	6.26 ± 6.64	5 (18.52)
	Total	582	324	258	/	/	/	/	58 (9.97)

loss in 2003 (*11*). The nature of hearing loss was divided into SNHL, conductive hearing loss (CHL), and mixed hearing loss (MHL). The hearing threshold was calculated as the average hearing level at 0.5, 1.0, 2.0, and 4.0 kHz according to the World Health Organization standard (1997). The severity of hearing impairment was defined as mild (26-40 dB), moderate (41-60 dB), severe (61-80 dB), or profound (>80 dB). Owing to the subjects' young age, the ABR threshold and/or ASSR were recorded, and mean thresholds at frequencies in the 0.5-4 kHz range were averaged to obtain an approximation for directional conditioned reflex. For children lacking behavioral thresholds and ASSR results, the ABR threshold is considered the high-frequency auditory threshold (*12,13*). We excluded patients with discriminated hearing loss curves. Hearing loss curve types are divided into ascending-type, U-type, drop-type, and flat-type curves. When the maximum sound output evoked no response, the default could not be determined (*12*).

2.5. *Image evaluation and statistical analysis*

Computerized tomography of the temporal bone or magnetic resonance imaging of the inner ear was performed. SPSS21.0 statistical software was used for data analysis using the chi-squared (χ^2) test.

3. Results

3.1. *Demographic data*

Of the 582 cases, male and female subjects accounted for 55.67% and 44.33%, respectively. The age ranged from 8 to 58 months (mean, 39.20 ± 11.26 months). The age at first visit ranged from 1 to 46 months (mean, 7.10 ± 6.89 months). In total, 58 (9.97% of the total) subjects had a family history of deafness.

Table 1 presents the clinical characteristics of the 3 groups. With regard to age at first visit, patients in group B visited the clinic the earliest, followed by group A, then C. The rate of family history of mitochondrial gene mutation (group B) was the highest, accounting for 27.54% of the observed mutations.

3.2. *Genetic testing*

Table 2 shows the mutation detection results of the 3 groups. The decreasing order of the proportion of gene detection was as follows: C > A > B, accounting for 68.04% (396/582), 20.10% (117/582), and 11.86% (69/582), respectively. c.235delC was the most common mutation locus, accounting for 22.76% (265/1164) of the mutations. Then, c.IVS7-2A>G accounted for 22.76% (265/1164). The c.35delG mutation was not detected.

3.3. *Comparison of the results of gene detection and universal newborn hearing screening (UNHS)*

Table 3 shows the comparison of the UNHS results of the 3 groups. For UNHS, 378 subjects had the pass outcome, whereas 204 were referred for UNHS, including 40 with single reference.

The UNHS rates of groups A, B, and C were 21.37%, 98.55%, and 77.02%, respectively (χ^2 = 143.47, $P < 0.01$). Pair-wise comparisons between groups showed significant differences: group A, B (χ^2 = 126.75, $P < 0.01$); group A, C (χ^2 = 62.75, $P < 0.01$); and group B, C (χ^2 = 22.917, $P < 0.01$).

3.4. *Genetic testing results and the nature of hearing loss*

Genetic testing results and the nature of hearing loss are shown in Table 4. The normal hearing rates of groups A, B, and C were 8.97%, 97.10%, and 79.17%, respectively (χ^2 = 219.269, $P < 0.01$). Group-wise comparison for any 2 groups showed significant differences ($\alpha = 0.05/3 = 0.01667$; $P1 = P2 = P3 < 0.01$).

A few patients in group B had hearing loss; therefore, this group was not compared with the other groups. Both groups A and C demonstrated mostly SNHL (81.20% and 18.56%, respectively; χ^2 = 76.88, $P < 0.01$). The rates of SNHL in their subgroups A1, A2, C1, and C2 were 85.29%, 70.31%, 78.72%, and 71.05%, respectively. The difference between A1 and A2 was significant (χ^2 = 6.45, $P < 0.01$). However, the comparisons of C1 and C2, A1 and C1, and A2 and C2 showed no significant difference ($P1 = P2 = 0.19$, $P3 = 0.88$).

Table 2. Results of gene mutation analyses of the diagnosed, drug-induced deafness, and mutation carrier groups (*n* = 582 cases)

Group	sub-group	Gene mutation	Number (case,%)	Total (case,%)
A	A1	*GJB2* 109G>A / 235delC CHM	8	85 (14.60)
		GJB2 109G>A / 299delAT CHM	4	
		GJB2 176del16 / 235delC CHM	5	
		GJB2 176del16 / 299delAT CHM	1	
		GJB2 235delC / 299delAT CHM	29	
		GJB2 235delC / 512G>A CHM	1	
		GJB2 235delC Hom M	34	
		GJB2 299delAT Hom M	1	
		GJB2 9G>A/235delC CHM	2	
	A2	*SLC26A4* 1641T>G / 2168AT CHM	1	32 (5.50)
		SLC26A4 IVS7-2A>G / 1226G>A CHM	3	
		SLC26A4 IVS7-2A>G / 2168A>G CHM	5	
		SLC26A4 IVS7-2A>G Hom M	18	
		SLC26A4 IVS7-2A>G / 916dupG CHM	1	
		SLC26A4 IVS7-2A>G / 2000T>C CHM	1	
		SLC26A4 IVS7-2A>G / 2106delG CHM	1	
		SLC26A4 IVS7-2A>G / 1522A>G CHM	1	
B	B	*Mt 12s rRNA* 1494C>T Hom M	4	69 (11.86)
		Mt 12s rRNA 1555A>G Hom M	49	
		Mt 12s rRNA 1555A>G Het M	16	
C	C1	*GJB2* 176del16 Het M	8	188 (32.30)
		GJB2 235delC Het M	133	
		GJB2 299delAT Het M	47	
	C2	*SLC26A4* 2168A>G Het M	29	133 (22.85)
		SLC26A4 IVS7-2A>G Het M	104	
	C3	*GJB3* 538C>T Het M	48	48 (8.25)
	C4	*GJB2* 176del16 Het M / *SLC26A4* IVS7-2A>G Het M	1	27 (4.64)
		GJB2 176del16 Het M / *Mt 12s rRNA* 1555A>G Hom M	1	
		GJB2 235delC Het M / *Mt 12s rRNA* 1555A>G Hom M	1	
		GJB2 235delC Het M / *GJB3* 538C>T Het M	2	
		GJB2 235delC Het M / *SLC26A4* 2168A>G Het M	3	
		GJB2 235delC Het M / *SLC26A4* IVS7-2A>G Het M	13	
		GJB2 299delAT Het M / *Mt 12s rRNA* 1555A>G Het M	1	
		GJB2 299delAT Het M / *SLC26A4* IVS7-2A>G Het M	1	
		GJB3 538C>T Het M / *SLC26A4* 2168A>G Het M	1	
		GJB3 538C>T Het M / *SLC26A4* IVS7-2A>G Het M	2	
		SLC26A4 IVS7-2A>G Het M / *Mt 12s rRNA* 1555A>G Hom M	1	
	Total	/	582	582 (100.00)

Hom M: homozygous mutation: CHM: compound heterozygous mutations: Het M: heterozygous mutation.

Table 3. Comparison of UNHS results of the diagnosed, drug-induced deafness, and mutation carrier groups (*n* = 1,164 ears)

Group	Sub-group	UNHS (ear, %)		Total (case,%)
		Pass	Refer	
A	A1	24 (14.12)	146 (85.88)	170
	A2	26 (40.63)	38 (59.38)	64
	Total (ear,%)	50 (21.37)	184 (78.63)	234
B	B	136 (98.55)	2 (1.45)	138
C	C1	265 (70.48)	111 (29.52)	376
	C2	203 (76.32)	63 (23.68)	266
	C3	94 (97.92)	2 (2.08)	96
	C4	48 (88.89)	6 (11.11)	54
	Total (ear,%)	610 (77.02)	182 (22.98)	792

3.5. *Genetic testing results and degree of hearing loss*

In total, 382 ears had hearing loss (Table 5). Profound, severe, moderate, and mild hearing loss occurred in 169, 113, 62, and 38 ears, respectively. Severe-profound hearing loss was frequent in all groups, and profound hearing loss had the highest prevalence. The rates of profound hearing loss in groups A, B, and C were 44.60%, 50.00%, and 43.64%, respectively (Table 5). Two patients in group B had mild hearing loss, and 2 had profound hearing loss; therefore, group B was not analyzed with the other groups. χ^2 analysis between profound hearing loss in group A and that in group C ($P = 0.13$) revealed no significant difference. Severe-profound hearing loss in group A (80.28%) and group C (66.06%) ($P < 0.01$) differed significantly.

Profound hearing loss was most frequent in A1, C1, and C2, and severe hearing loss was most frequent in A2. There was no significant difference between A1 and A2 or between A2 and C2 ($P1 = 0.06$, $P2 = 0.10$). The difference between C1 and C2 and that between A1 and C1 were statistically significant ($P3 = 0.04$, $P4 < 0.01$).

Table 4. Nature of hearing loss across the diagnosed, drug-induced deafness, and mutation carrier groups (*n* = 1,164 ears)

Group	Sub-group	The nature of Heaing Loss (ear,%)				Total (case,%)
		Normal	SNHL	CHL	MHL	
A	A1	17 (10.00)	145 (85.29)	2 (1.18)	6 (3.53)	170
	A2	4 (6.25)	45 (70.31)	6 (9.38)	9 (14.06)	64
	Total (ear,%)	21 (8.97)	190 (81.20)	8 (3.42)	15 (6.41)	234
B	B	134 (97.10)	3 (2.17)	0 (0.00)	1 (0.72)	138
C	C1	296 (78.72)	74 (19.68)	6 (1.60)	0 (0.00)	376
	C2	189 (71.05)	69 (25.94)	1 (0.38)	7 (2.63)	266
	C3	92 (95.83)	0 (0.00)	3 (3.13)	1 (1.04)	96
	C4	50 (92.59)	4 (7.41)	0 (0.00)	0 (0.00)	54
	Total (ear,%)	627 (79.17)	147 (18.56)	10 (1.26)	8 (1.01)	792

Table 5. Levels of hearing loss across the diagnosed, drug-induced deafness, and mutation carrier groups (*n* = 382 ears)

Group	Sub-group	The curve type of Hearing Loss (ear,%)				Total (case,%)
		Mild	Moderate	Severe	Profound	
A	A1	5 (3.27)	27 (17.65)	49 (32.03)	72 (47.06)	153
	A2	5 (8.33)	5 (8.33)	27 (45.00)	23 (38.33)	60
	Total (ear,%)	10 (4.69)	32 (15.02)	76 (35.68)	95 (44.60)	213
B	B	2 (50.00)	0 (0.00)	0 (0.00)	2 (50.00)	4
C	C1	14 (17.50)	20 (25.00)	17 (21.25)	29 (36.25)	80
	C2	9 (11.69)	8 (10.39)	19 (24.68)	41 (53.25)	77
	C3	3 (75.00)	1 (25.00)	0 (0.00)	0 (0.00)	4
	C4	0 (0.00)	1 (25.00)	1 (25.00)	2 (50.00)	4
	Total (ear,%)	26 (15.76)	30 (18.18)	37 (22.42)	72 (43.64)	165

Table 6. Curve type of hearing loss across three groups (*n* = 382 ears)

Group	Sub-group	The curve type of Hearing Loss (ear,%)					Total (case,%)
		Flat	Drop	U	Ascending	Discriminated	
A	A1	84 (54.90)	19 (12.42)	3 (1.96)	11 (7.19)	36 (23.53)	153
	A2	24 (40.00)	30 (50.00)	0 (0.00)	2 (3.33)	4 (6.67)	60
	Total (ear,%)	108 (50.70)	49 (12.42)	3 (1.96)	13 (7.19)	40 (23.53)	213
B	B	2 (50.00)	2 (50.00)	0 (0.00)	0 (0.00)	0 (0.00)	4
C	C1	44 (55.00)	21 (26.25)	1 (1.25)	5 (6.25)	9 (11.25)	80
	C2	26 (33.77)	37 (48.05)	2 (2.60)	5 (6.49)	7 (9.09)	77
	C3	4 (100.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	4
	C4	3 (75.00)	0 (0.00)	0 (0.00)	1 (25.00)	0 (0.00)	4
	Total (ear,%)	77 (46.67)	58 (35.15)	3 (1.82)	11 (6.67)	16 (9.70)	165

3.6. *Genetic testing results and hearing loss curves*

Genetic testing results were compared with hearing loss curves (Table 6). Of 382 ears with hearing loss, 187 ears, 109 ears, 24 ears, and 6 ears, respectively, had flat-type, drop-type, ascending-type, and *U*-type hearing loss curves, whereas the curves could not be identified in 56 ears.

Few subjects in group B had hearing loss curves; therefore, this group was excluded from the comparative analysis with the other groups. Groups A and C mostly demonstrated the flat-type curve (50.70% and 46.67%, respectively; $\chi^2 = 0.32$, $P = 0.57$). The rates of flat-type hearing loss curves were highest in groups A1 and C1 (54.90% and 55.00%, respectively). The rates of drop-type hearing loss curves were highest in groups A2 and C2 (50.00% and 48.05%, respectively). The differences in these rates were significantly different between A1 and A2, A1 and C1, and C1 and C2 ($P1 < 0.01$, $P2 = 0.04$, $P3 = 0.048$), but not between A2 and C2 ($P4 = 0.38$).

3.7. *Imaging results for SLC26A4 gene mutation*

SLC26A4 mutations were detected in 165 cases – 32 cases in A2 and 133 in C2. Among them, 61 cases had imaging results (A2, 23 cases; C2, 38 cases). In total, 50 cases were abnormal, and 11 cases had no obvious abnormalities. The abnormality was an enlarged vestibular aqueduct (EVA) in 47 (94 ears) cases; thus, the overall abnormality rate was 77.05%. EVA was present in 95.65% (22/23) of patients in group A2 and 65.79% of patients in group C2 (25/38). EVA with Mondini deformity (MD) occurred in 2 (3.28%) cases.

4. Discussion

The number of positive results in newborn deafness gene screening is large, and genetic counseling work is increasingly important. Deafness gene detection can: *i*) clarify the cause of congenital hereditary deafness and improve adherence to deafness intervention; *ii*) detect delayed deafness early to enable intervention and early prevention of hearing loss; *iii*) identify individuals susceptible to drug-induced deafness and prevent the occurrence of deafness in these individuals; and *iv*) allow genetic counseling that reduces the birth of deaf children, thereby reducing the burden on families and society. Deafness gene screening in newborns is important for early detection of deafness in children to provide early guidance for hearing care.

4.1. *Demographic data*

In 2006, Huang *et al*. studied 265 children (0-6 years old) with hearing loss, and found that the mean age at first visit was 28.01 ± 13.41 months (*14*). In 2014, Yang (*15*) studied 122 children diagnosed with an EVA. Among them, 84 had undergone UNHS, and the mean age at first visit was 17.24 ± 17.08 months; however, the mean age at first visit for 37 children who had not undergone UNHS was 30.92 ± 18.21 months.

The age at first visit in our current study was earlier than that of the previous study. These findings indicate that screening of newborn deafness genes in concert with UNHS can advance the timing of the first visit for investigation of hearing loss. Drug-related deafness had the lowest mean age at first visit, and the highest mean age at first visit was observed for the *GJB3* gene heterozygous mutation. This may be associated with the highest proportion of patients with a family history of drug-induced deafness (27.54%), indicating that a similar situation at home can make parents pay more attention to hearing loss.

4.2. *Genetic testing*

GJB2 is the most frequently mutated gene in cases of hereditary hearing loss, but the mutation spectrum varies among ethnic groups. For example, among Caucasians, the most common *GJB2* gene mutation is c.35delG, with a carrier frequency of 2-4% (*16*). However, c.235delC is most frequently observed among Asians, with an allele frequency ranging from 5% to 22% (*17-19*), which is consistent with our present results.

SLC26A4 is the most important pathogenic gene underlying deafness in large vestibular aqueduct syndrome (LVAS), and there are obvious regional and racial differences in hot spot mutations. Wang *et al*. conducted a screening of the *SLC26A4* gene in 107 patients with deafness with LVAS, and found that the C.IVS7-2A>G mutation is the most common mutation in the Chinese population, accounting for 57.63% of the total mutations, followed by c.2168A>G (9.04%). Up to 97.9% of patients had at least one *SLC26A4* mutation, and 88.4% of patients had bi-allelic mutations (*20*). In the Japanese and Korean populations, the main mutation of the *SLC26A4* gene was c.2168A>G, and the c.IVS7-2A>G mutation in the Korean population was also common (*21,22*). In Caucasian populations in Europe and the United States, the most common three mutations were observed to be p. L236P (16%), p. T416P (15%), and IVS8 + lG>A (14%). The sum of the prevalence of these three mutations is approximately half of that of the total mutation, but these three mutations are very infrequent in the East Asian population (*23*).

In this study, the rate of *GJB2* gene mutation reached 46.91%; followed by that of *SLC26A4* gene mutation, accounting for 28.35%. The mutation rate of c.235delC was 22.75%, followed by the c.IVS7-2A>G mutation (14.59%). The c.35delG mutation was not detected.

4.3. *Comparison of the results of gene detection and UNHS*

The most fundamental purpose of UNHS is to realize "early detection, early diagnosis, and early intervention" in children with congenital hearing loss. Therefore, UNHS benefits children, families, and society as a whole. In 2010, China made UNHS a routine inspection. Before the introduction of UNHS, children with hearing loss were diagnosed at an average age of 23 months (8 months later in rural areas than in urban areas) (*14*). Congenital hearing loss, especially moderate or heavy hearing loss, prevents babies from hearing voices and hence leads to speech and cognitive developmental disorders. Newborn deafness gene screening in combination with UNHS effectively identifies children with hearing loss because both approaches complement each other.

The UNHS rates of groups A, B, and C were 21.37%, 98.55%, 77.02%, respectively, differing significantly from one another. The UNHS pass rate in group B was the highest, suggesting that warning against ototoxic drugs was effective. The UNHS pass rate for group A was lower than that of group C, corresponding to the nature of the corresponding gene mutations. However, the homozygous or compound heterozygous mutations of group A are pathogenic. Children in group A might have had hearing loss in theory, but the UNHS pass rate was 21.37%. This could be attributed to late-onset hearing loss or technical and personnel factors when performing UNHS, leading to false pass rates. Thus, positivity for newborn deafness genes should warn parents to pay close attention to the child's hearing even if they pass the UNHS.

4.4. *Genetic testing results and the nature of hearing loss*

The normal hearing rate of group B was the highest,

suggesting that caution was being practiced against the use of deafness-causing drugs. Few patients in group B had hearing loss, and these were not included in the comparison with other groups. Both group A and group C demonstrated mostly SNHL. On comparing SNHL of A1 and A2, C1 and C2, A1 and C1, and A2 and C2, the difference was statistically significant only between A1 and A2. This result may be related to the pathogenic mechanism of the different genes, which is explained below.

GJB2 encodes the connexin-26 gap junction protein (Cx26). Cx26 protein forms a membrane channel with 6 subunits, thus constituting the cell gap junction, and is distributed in the cochlear stria, basal cells, spiral limbus convex, nerve conduction fiber, and cochlear sensory epithelium. It is an important channel for electrolytes, second messengers, and metabolites, playing an important role in the exchange of information and materials (*24*). Therefore, *GJB2* mutations mainly lead to SNHL. However, other factors such as otitis media and ear deformities might result in a different nature of hearing loss.

SLC26A4 encodes pendrin, a protein with complex structure and function. It is expressed mainly in the inner ear lymph sac and lymphatic vessels, mediating the transport of Cl^-, $HCO3^-$, and I- to maintain the ionic balance of the inner ear lymph and playing an important role in inner ear lymph reuptake (*25*). *SLC26A4* mutations can cause syndromic or non-syndromic SNHL (*26*), but the pathogenesis is not clear.

The pathogenesis in group C might be explained as follows. First, some patients might not have undergone gene sequencing, or they might have harbored other deafness mutations. Second, interaction with other genes might have occurred. In an earlier study, a single *GJB2* mutation was detected in 10-42% of patients with deafness (*27*). Other genes might also be involved in the phenotypic expression of deafness. *GJB6* and *GJB2* are both expressed in the cochlea, and their mutations can affect gap junction formation (*27*). Furthermore, the pathogenesis of *SLC26A4* mutations might be related to mutations in *FOXI1* and *KCNJ10* (*28,29*). In addition, other unknown reasons may underlie the pathogenesis, warranting further studies on patients with *SLC26A4* mutations.

4.5. *Genetic testing results and the degree of hearing loss*

Severe-profound hearing loss was the most common across the groups, and profound hearing loss had the highest rate. The rates of profound hearing loss did not differ significantly between group A and group C, whereas severe-profound hearing loss rates were significantly different between group A and group C. Furthermore, the degree of hearing loss but not that of severe-profound hearing loss differed significantly between group A1 and A2. Severe-profound hearing loss significantly differed between C1 and C2, between A1 and C1, and between A2 and C2.

A national molecular epidemiological investigation of deafness showed *GJB2*, *SLC26A4*, and mitochondrial DNA to be the genes most commonly associated with severe-profound non-syndromic hearing loss in China. The hearing loss resulting from *GJB2* mutations is generally congenital, bilateral, non-progressive, and severe or profound (*30*). The hearing loss related to *SLC26A4* mutations is bilateral or non-bilateral, progressive, and with differing degrees (*31*). In the present study, *GJB2* and *SLC26A4* mutations caused severe-profound hearing loss, consistent with the literature. It is noteworthy that mild and moderate deafness was associated with *GJB2* and *SLC26A4* mutations. In addition, to some extent, the degree of hearing loss with *SLC26A4* mutations was milder than that with *GJB2*. Nevertheless, the relationship between genotype and hearing loss varies across patients (*32,33*).

4.6. *Genetic testing results and the hearing loss curve*

In a previous study in 77 deaf individuals, the hearing loss curves of cases positive for *GJB2* mutations were mainly of the drop type and flat type, with little U shape and no ascending curve (*34*). The ascending type was found in 14.93% of 297 cases with *GJB2* mutations, but the *GJB2* hearing loss curve still generally indicated the drop type (26.27%) and flat type (25.16%) (*35*). In this study, the flat type of *GJB2* gene mutations was the most common, followed by drop type, ascending type, and finally the U type was less, which is basically the same pattern as reported in the literature.

SLC26A4 mutations often lead to LVAS, with the hearing loss curve most often being of the drop type, followed by the flat type (*36*). This report showed that the hearing loss curves of *SLC26A4* mutations were predominantly of the drop type, followed by flat type, ascending type, and U type.

4.7. *Analysis of imaging results of the SLC26A4 mutations*

SLC26A4 mutation was found to be associated with LVAS, which is characterized by vestibular aqueduct and SNHL. Huang *et al*, studied MD (28 cases), MD combined with EVA (50 cases), EVA alone (50 cases), and patients with other internal ear deformity (16 cases) by performing *SLC26A4* gene sequence analysis. The results showed that mutations in the *SLC26A4* gene are common in children with or without EVA, but there was no evidence of MD being associated with mutations in the *SLC26A4* gene (*37*). Zhu *et al*. sequenced the *SLC26A4* gene in 14 patients with EVA, 6 patients with MD (with EVA), and 7 patients with other internal ear deformity (without EVA). In total, there were

14 cases of EVA children, 12 cases of double allelic *SLC26A4* mutation, 2 cases of single heterozygous mutation, 6 cases of MD (with EVA) with double mutation of *SLC26A4*, and 7 patients with other internal ear deformity (without EVA) in which the *SLC26A4* mutation was not detected. They concluded that the incidence of MD with EVA or EVA is closely related to the *SLC26A4* mutation (*38*). In this study, of 165 cases with the *SLC26A4* gene mutation, a total of 61 cases had imaging results showing EVA (77.05%), and EVA with MD accounted for 3.28%.

5. Conclusions

Among positive results in genetic testing of deafness, *GJB2* gene mutations are prevalent, and the most common mutation is c.235delC. The drug-induced deafness group, gene mutation carrier group, and diagnosed group had high rates of patients who passed the universal newborn hearing screening. The hearing loss features of the *GJB2* gene mutation suggest a flat type, severe-profound sensorineural effect. The hearing loss features of the *SLC26A4* gene mutation include drop type and severe-profound sensorineural effects, as well as an enlarged vestibular aqueduct.

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