Original Article

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Genetic screening of newborns for deafness over 11 years in Beijing, China: More infants could benefit from an expanded program

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SUMMARY Genetic screening of newborns for deafness plays an important role in elucidating the etiology of deafness, diagnosing it early, and intervening in it. Genetic screening of newborns has been conducted for 11 years in Beijing. It started with a chip to screen for 9 variants of 4 genes in 2012; the chip screened for 15 variants of those genes in 2018, and it now screens for 23 variants of those genes. In the current study, a comparative analysis of three screening protocols and follow-up for infants with pathogenic variants was performed. The rates of detection and hearing test results of infants with pathogenic variants were analyzed. Subjects were 493,821 infants born at 122 maternal and child care centers in Beijing from April 2012 to August 2023. Positivity increased from 4.599% for the chip to screen for 9 variants to 4.971% for the chip to screen for 15 variants, and further to 11.489% for the chip to screen for 23 variants. The carrier frequency of the *GJB2* gene increased from 2.489% for the chip to screen for 9 variants and 2.422% for the chip to screen for 15 variants to 9.055% for the chip to screen for 23 variants. The carrier frequency of the *SLC26A4* gene increased from 1.621% for the chip to screen for 9 variants to 2.015% for the chip to screen for 15 variants and then to 2.151% for the chip to screen for 23 variants. According to the chip to screen for 9 variants and the chip to screen for 15 variants, the most frequent mutant allele was c.235delC. According to the chip to screen for 23 variants, the most frequent mutant allele was c.109G>A. The chip to screen for 15 variants was used to screen 66.67% (14/21) of newborns with biallelic variants in the *SLC26A4* gene for newly added mutations. The chip to screen for 23 variants was used to screen 92.98% (53/57) of newborns with biallelic variants in the *GJB2* gene (52 cases were biallelic c.109G>A) and 25% (1/4) of newborns with biallelic variants in the *SLC26A4* gene for newly added mutations. Among the infants with pathogenic variants (biallelic variants in *GJB2* or *SLC26A4*), 20.66% (25/121) currently have normal hearing. In addition, 34.62% (9/26) of newborns who passed the hearing screening were diagnosed with hearing loss. Findings indicate that a growing number of newborns have benefited, and especially in the early identification of potential late-onset hearing loss, as the number of screening sites has increased. Conducting long-term audiological monitoring for biallelic variants in individuals with normal hearing is of paramount significance.

Keywords deafness genes, genetic screening of newborns, hearing screening for newborns, hearing loss, follow-up

1. Introduction

Hearing loss (HL) is the most common human neurosensory disorder, with a lifelong impact that may be ameliorated by early detection and intervention (*1*). The reported incidence of HL ranges from 1.33 to 1.86 per 1,000 newborns; more than half of these newborns have a genetic etiology (*2,3*).

In 2012, the Beijing Municipal Government launched a large-scale project for the genetic screening of

newborns, which included 9 variants of 4 genes associated with deafness, including c.235delC (p.Leu79Cysfs*3), c.299_300delAT (p.His100Argfs*14), c.176_191del16 (p.Gly59Alafs*18), and c.35delG (p.Gly12Valfs*2) of *GJB2* (MIM: 121011); c.919-2A>G and c.2168A>G (p.His723Arg) of *SLC26A4* (MIM: 605646); m.1555A>G and m.1494C>T of mtDNA *12SrRNA* (MIM: 561000); c.538C>T (p.Arg180*) of *GJB3* (MIM: 603324). Genetic screening of newborns can effectively increase the early rate of detection of hereditary HL, ototoxicity-related deafness, and delayed-onset HL, providing an early warning and diagnosis of HL and facilitating early intervention. Dai *et al.* conducted a study on 180,469 newborns born between 2013 and 2014 who underwent concurrent hearing and deafness genetic screening using the chip to screen for 9 variants (*4*). Results revealed that 25% of infants with pathogenic combinations of *GJB2* or *SLC26A4* variants and 99% of infants with an m.1555A>G or m.1494C>T variant passed the routine hearing screening for newborns. This highlights the importance of genetic screening in identifying infants at risk for HL, even when they may initially pass conventional hearing screening.

In 2018, to further enhance the early detection of large vestibular aqueduct syndrome (LVAS), the Beijing Municipal Government used an enhanced genetic screening chip. Based on the chip to screen for 9 variants, 6 additional variants of the *SLC26A4* gene were added to provide a chip to screen for 15 variants of the 4 genes, including c.1975G>C (p.Val659Leu), c.1707+5G>A, c.1229C>T (p.Thr410Met), c.1226G>A (p.Arg409His), c.2027T>A (p.Leu676Gln), and c.1174A>T (p.Asn392Tyr). Wen *et al.* analyzed 76,460 newborns who underwent concurrent hearing and deafness genetic screening using the chip to screen for 15 variants in 2019 and 2020 and found that 28.57% (2/7) of the newborns with the *SLC26A4* mutation causing deafness passed hearing screening (*5*).

In December 2022, the Beijing Municipal Government began using a chip to screen for 23 variants of the 4 genes. Based on the chip to screen for 15 variants, 5 additional variants of the *GJB2* gene and 3 variants of the *SLC26A4* gene were included: c.109G>A (p.Val37Ile), c.257C>G (p.Thr86Arg), c.512insAACG, c.427C>T (p.Arg143Trp) and c.35insG of *GJB2*; c.589G>A (p.Gly197Arg), c.917insG and c.281C>T (p.Thr94Ile) of *SLC26A4*. This upgrade has significantly broadened the scope of the screening, allowing for the identification of a greater variety of deafness-causing mutations and contributing to more comprehensive and accurate diagnoses of hereditary HL in newborns. Few studies have evaluated the performance of the chip to screen for 23 variants and few have compared the rate of detection with other protocols within the same category.

Since April 2012, the genetic screening of newborns in Beijing has been in clinical practice for more than a decade, with the screening protocols being continually updated. It started with a chip to screen for 9 variants of 4 genes in 2012; the chip screened for 15 variants of those genes in 2018, and the current chip now screens for 23 variants of those genes. This study involved an analysis of 493,821 newborns from Beijing born from April 2012 to August 2023. This study compared positivity, rates of detection of various genotypes, carrier frequencies, allele frequencies, and pathogenic genotypes according to three distinct screening protocols. In addition, the hearing phenotypes of individuals with pathogenic variants were analyzed. The aim of this study was to analyze the results of three screening protocols and hearing testing of infants with pathogenic variants in order to optimize protocols for current genetic screening and to improve interventions and management strategies for HL.

2. Materials and Methods

2.1. Clinical data

Subjects were 493,821 infants born at 122 maternal and child care centers in Beijing who underwent concurrent hearing and genetic screening from April 2012 to August 2023. Both hearing screening and genetic screening for newborns were conducted within 72 h after birth for all neonates at no charge.

A chip was used to screen 273,647 of those newborns born from April 2012 to December 2017 for 9 variants of 4 genes, which is referred to here as the chip to screen for 9 variants. A chip was used to screen 195,767 newborns born from January 2018 to November 2022 for 15 variants of those genes, which is referred to here as the chip to screen for 15 variants. A chip was used to screen 24,407 newborns born from December 2022 to August 2023 for 23 variants of those genes, which is referred to here as the chip to screen for 23 variants. The newborns who were screened for pathogenic variants were followed up systematically to obtain hearing screening and hearing test results. Figure 1 shows the study flow. Hearing screening was conducted using the otoacoustic emissions (OAEs) or automated auditory brainstem response (AABR) test.

2.2. Genetic screening

Dried blood spots were collected for genetic testing of genes associated with deafness: *GJB2*, *SLC26A4*, mtDNA *12SrRNA*, and *GJB3*. The Deafness Gene Variant Detection Array Kit (CapitalBio) was used to identify 9 variants of 4 genes in newborns born from April 2012 to December 2017, including c.235delC (p.Leu79Cysfs*3), c.299_300delAT (p.His100Argfs*14), c.176_191del16 (p.Gly59Alafs*18), and c.35delG (p.Gly12Valfs*2) of *GJB2* (MIM: 121011); c.919-2A>G and c.2168A>G (p.His723Arg) of *SLC26A4* (MIM: 605646); c.538C>T

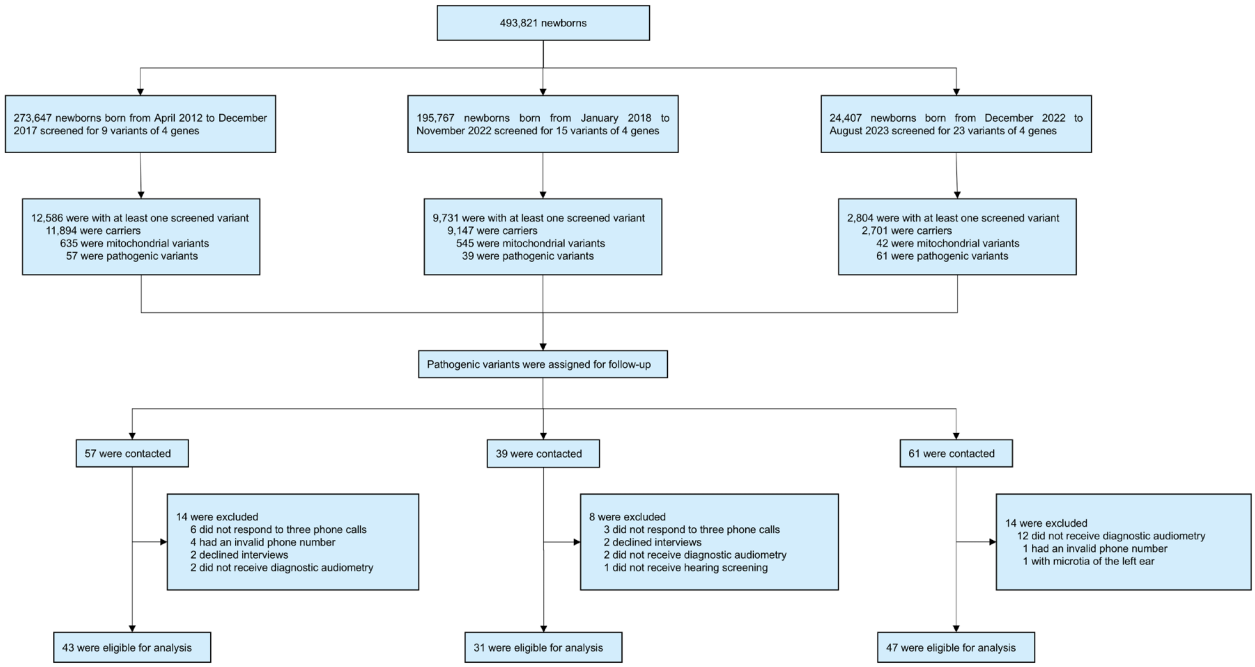

Figure 1. Participation in genetic screening and outcomes for 493,821 newborns. Genetic screening results were classified as follows: pathogenic variants (homozygote or compound heterozygote for *GJB2* or *SLC26A4*), carriers (heterozygote for *GJB2* or *SLC26A4* and heterozygote or homozygote for *GJB3*), mitochondrial variants (any mtDNA*12SrRNA* variant), and wild (no variant). Newborns with pathogenic variants were followed up.

(p.Arg180*) of *GJB3* (MIM: 603324); and m.1555A>G and m.1494C>T of mtDNA *12SrRNA* (MIM: 561000). The Deafness Gene Variant Detection Array Kit (CapitalBio) was used to identify 15 variants of 4 genes in newborns born from January 2018 to November 2022. Based on the chip to screen for 9 variants, 6 variants of the *SLC26A4* gene have been added: c.1975G>C (p.Val659Leu), c.1707+5G>A, c.1229C>T (p.Thr410Met), c.1226G>A (p.Arg409His), c.2027T>A (p.Leu676Gln), and c.1174A>T (p.Asn392Tyr). The Deafness Gene Variant Detection Array Kit (CapitalBio) was used to identify 23 variants of 4 genes in newborns born from December 2022 to August 2023. Based on the chip to screen for 15 variants, 5 variants of the *GJB2* gene and 3 variants of the *SLC26A4* gene have been added: c.109G>A(p.Val37Ile), c.257C>G(p.Thr86Arg), c.512insAACG, c.427C>T(p.Arg143Trp), c.35insG of *GJB2*; c.589G>A (p.Gly197Arg), c.917insG, c.281C>T (p.Thr94Ile) of *SLC26A4*. Genetic screening was conducted at Beijing Tongren Hospital, where genetic screening laboratories were authorized by the Beijing Municipal Health Commission (*4*). Results were recorded on a report card as wild or positive. Positive included carriers (heterozygous for *GJB2* or *SLC26A4* and heterozygous or homozygous for *GJB3*), pathogenic variants (homozygous or compound heterozygous for *GJB2* or *SLC26A4*) and mitochondrial variants (any mtDNA *12SrRNA* variant). Positive infants in genetic screening were referred to Beijing TongRen Hospital for diagnostic audiological testing, genetic counseling, and genetic diagnosis.

Genotypes with homozygous and compound heterozygous variants in *GJB2* or *SLC26A4* were diagnosed as deafness-causing genotypes, and those with mtDNA *12SrRNA* variants were diagnosed as drug-susceptible. Each subject was categorized into only one group based on genetic results. When a subject's results fell into more than one category, the subject was assigned to a group in the following order of priority: pathogenic variants, mitochondrial variants, carriers, and wild.

2.3. Hearing testing

Audiological evaluations of infants with pathogenic variants were performed at the Pediatric Hearing Diagnostic Center at Beijing Tongren Hospital. These assessments included objective and subjective hearing tests to ensure a thorough analysis of auditory function. Objective audiological tests consisted of the auditory brainstem response (ABR), distortion product otoacoustic emissions (DPOAEs), auditory steady state response (ASSR), and acoustic immittance. Subjective audiological tests included pediatric behavioral audiometry and pure tone audiometry.

According to the WHO 1997 criteria, the degree of HL was classified based on the average hearing thresholds of pediatric behavioral audiometry at 500 Hz, 1,000 Hz, 2,000 Hz, and 4,000 Hz for the better ear: normal (≤ 25 dB HL), mild (26-40 dB HL), moderate (41-60 dB HL), severe (61-80 dB HL), and profound (>80dB HL).

2.4. Statistical analysis

The software SPSS 27.0 was used to perform chi-square tests to compare positivity, rates of detection of various genotypes, carrier frequencies, and allele frequencies among the three screening protocols.

2.5. Ethics statement

This study was approved by the Ethics Committee of the Beijing Institute of Otolaryngology. Fully informed written consent was obtained from parents before screening.

3. Results

3.1. Comparison of positivity according to three genetic screening protocols

As shown in Table 1, total positivity was 5.087% (25,121/493,821). Positivity with the protocol for the chip to screen for 9 variants was 4.599% (12,586/273,647), and positivity with the protocol for the chip to screen for 15 variants was 4.971% (9,731/195,767), and positivity with the protocol for the chip to screen for 23 variants was 11.489% (2,804/24,407). Positivity according to the three screening protocols increased significantly ($P < 0.001$).

3.2. Comparison of the rates of detection of various genotypes according to three genetic screening protocols

As shown in Table 2, the rates of detection of pathogenic variants among three screening protocols, from highest to lowest, were as follows: the chip to screen for 23 variants (0.250%, 61/24,407), the chip to screen for 9 variants (0.021%, 57/273,647), and the chip to screen for 15 variants (0.020%, 39/195,767). The rates differed significantly ($P < 0.001$).

The rates of detection of mitochondrial variants, from highest to lowest, were as follows: the chip to screen for 15 variants (0.279%, 545/195,767), the chip to screen for 9 variants (0.232%, 635/273,647), and the chip to screen for 23 variants (0.176%, 42/24,407). The rates differed significantly ($P < 0.001$).

The rates of detection of carriers, from highest to lowest, were as follows: the chip to screen for 23 variants (11.066%, 2,701/24,407), the chip to screen for 15 variants (4.672%, 9,147/195,767), and the chip to screen for 9 variants (4.346%, 11,894/273,647). The rates

Table 1. Positivity according to three genetic screening protocols

Genotypes	Chip to screen for 9 variants *n* (%)	Chip to screen for 15 variants *n* (%)	Chip to screen for 23 variants *n* (%)	Total number (%)	*P* value
Positive	12,586 (4.599)	9,731 (4.971)	2,804 (11.489)	25,121 (5.087)	< 0.001
Wild type	261,061 (95.401)	186,036 (95.029)	21,603 (88.511)	468,700 (94.913)	
Total	273,647 (100.000)	195,767 (100.000)	24,407 (100.000)	493,821 (100.000)	

n: number of newborns.

Table 2. Rates of detection of various genotypes according to three genetic screening protocols

Genotypes	Chip to screen for 9 variants *n* (%)	Chip to screen for 15 variants *n* (%)	Chip to screen for 23 variants *n* (%)	*P* value
Pathogenic variants				
GJB2 homozygote/compound heterozygote	48 (0.018)	18 (0.009)	57 (0.234)	
SLC26A4 homozygote/compound heterozygote	9 (0.003)	21 (0.011)	4 (0.016)	
total	57 (0.021)	39 (0.020)	61 (0.250)	< 0.001
Mitochondrial variants	635 (0.232)	545 (0.279)	42 (0.176)	< 0.001
Carrier				
GJB2 heterozygote	6,615 (2.417)	4,597 (2.348)	2,096 (8.588)	
SLC26A4 heterozygote	4,287 (1.567)	3,783 (1.932)	471 (1.930)	
GJB3 heterozygote/homozygote	846 (0.309)	632 (0.323)	80 (0.328)	
Multiple gene heterozygote	146 (0.053)	135 (0.069)	54 (0.221)	
total	11,894 (4.346)	9,147 (4.672)	2,701 (11.066)	< 0.001
Total for all screened	12,586 (4.599)	9,731 (4.971)	2,804 (11.489)	

n: number of newborns. According to the protocol for the chip to screen for 9 variants, there were 25 cases of mitochondrial variants heterozygous for *GJB2* or *SLC26A4* or *GJB3*, and 146 cases of multiple gene heterozygotes who were heterozygous for *GJB2* and *SLC26A4* or heterozygous for *GJB2* and *GJB3* or heterozygous for *SLC26A4* and *GJB3*. According to the protocol for the chip to screen for 15 variants, there was 1 case of a *GJB2* homozygote with mitochondrial variants, 1 case of an *SLC26A4* homozygote heterozygous for *GJB2*, 29 cases of mitochondrial variants heterozygous for *GJB2* or *SLC26A4* or *GJB3*, and 1 case of a multiple gene heterozygote who was heterozygous for *GJB2* and *SLC26A4* and *GJB3*. According to the protocol for the chip to screen for 23 variants, there was 1 case of a *GJB2* homozygote with mitochondrial variants, 1 case of a *GJB2* compound heterozygote heterozygous for *GJB3*, 1 case of a *GJB2* compound heterozygote heterozygous for *SLC26A4*, and 5 cases of mitochondrial variants who were heterozygous for *GJB2* or *SLC26A4*.

differed significantly ($P < 0.001$).

3.3. Comparison of carrier frequency according to three genetic screening protocols

As shown in Table 3, the carrier frequency for the 4 screened genes, from highest to lowest, was the *GJB2* gene, *SLC26A4* gene, *GJB3* gene, and mtDNA *12SrRNA* gene. The carrier frequency for the *GJB2* gene was highest according to the chip to screen for 23 variants (9.055%, 2,210/24,407), followed by the chip to screen for 9 variants (2.489%, 6,811/273,647), and then the chip to screen for 15 variants (2.422%, 4,742/195,767). The differences were significant ($P < 0.001$).

The carrier frequency for the *SLC26A4* gene was highest according to the chip to screen for 23 variants (2.151%, 525/24,407), followed by the chip to screen for 15 variants (2.015%, 3,944/195,767), and then the chip to screen for 9 variants (1.621%, 4,435/273,647). The differences were significant ($P < 0.001$).

The carrier frequency of the *GJB3* gene was 0.320% (876/273,647) according to the chip to screen for 9 variants, 0.340% (666/195,767) according to the chip to screen for 15 variants, and 0.361% (88/24,407) according to the chip to screen for 23 variants. The differences were not significant ($P = 0.346$).

The carrier frequency of the mtDNA *12SrRNA* gene was highest according to the chip to screen for 15 variants (0.279%, 546/195,767), followed by the chip to screen for 9 variants (0.232%, 635/273,647), and then the chip to screen for 23 variants (0.176%, 43/24,407). The differences were significant ($P < 0.001$).

3.4. Comparison of allele frequency according to three genetic screening protocols

The allele frequencies according to three screening protocols are shown in Table 4. According to the

Table 3. Carrier frequencies according to three genetic screening protocols

Gene	Chip to screen for 9 variants *n* (%)	Chip to screen for 15 variants *n* (%)	Chip to screen for 23 variants *n* (%)	*P* value
GJB2	6,811 (2.489)	4,742 (2.422)	2,210 (9.055)	< 0.001
SLC26A4	4,435 (1.621)	3,944 (2.015)	525 (2.151)	< 0.001
GJB3	876 (0.320)	666 (0.340)	88 (0.361)	0.346
mtDNA *12S rRNA*	635 (0.232)	546 (0.279)	43 (0.176)	< 0.001
Total	12,586 (4.599)	9,731 (4.971)	2,804 (11.489)	

n: number of newborns. According to the protocol for the chip to screen for 9 variants, there were 171 cases of digenic gene variants. According to the protocol for the chip to screen for 15 variants, there were 165 cases of digenic gene variants and 1 case of trigenic gene variants. According to the protocol for the chip to screen for 23 variants, there were 62 cases of digenic gene variants. Therefore, the sum of the carrier frequencies for each gene is greater than the overall positivity according to genetic screening.

Table 4. Allele frequencies according to three genetic screening protocols

Variants	Chip to screen for 9 variants			Chip to screen for 15 variants			Chip to screen for 23 variants			*P* value
	Het (*n*)	Hom (*n*)	AF (%)	Het (*n*)	Hom (*n*)	AF (%)	Het (*n*)	Hom (*n*)	AF (%)	
GJB2 c.235delC	4,998	27	0.923	3,537	11	0.909	424	0	0.869	0.425
GJB2 c.299_300delAT	1,378	2	0.253	945	1	0.242	120	1	0.250	0.591
GJB2 c.176_191del16	381	1	0.070	217	0	0.055	37	0	0.076	0.014
GJB2 c.35delG	42	0	0.008	37	0	0.009	5	0	0.010	0.519
GJB2 c.109G>A	/	/	/	/	/	/	1,579	30	3.358	
GJB2 c.257C>G	/	/	/	/	/	/	6	0	0.012	
GJB2 c.512insAACG	/	/	/	/	/	/	15	0	0.031	
GJB2 c.427C>T	/	/	/	/	/	/	8	0	0.016	
GJB2 c.35insG	/	/	/	/	/	/	11	0	0.023	
SLC26A4 c.919-2A>G	3,719	7	0.682	2,597	6	0.666	350	3	0.729	0.243
SLC26A4 c.2168A>G	709	1	0.130	513	0	0.131	57	0	0.117	0.710
SLC26A4 c.1174A>T	/	/	/	165	0	0.042	24	0	0.049	0.480
SLC26A4 c.1226G>A	/	/	/	133	0	0.034	12	0	0.025	0.281
SLC26A4 c.1229C>T	/	/	/	125	0	0.032	24	0	0.049	0.061
SLC26A4 c.1975G>C	/	/	/	244	0	0.062	20	0	0.041	0.069
SLC26A4 c.2027T>A	/	/	/	110	1	0.029	14	0	0.029	0.993
SLC26A4 c.1707+5G>A	/	/	/	64	0	0.016	8	0	0.016	0.994
SLC26A4 c.589G>A	/	/	/	/	/	/	7	0	0.014	
SLC26A4 c.917insG	/	/	/	/	/	/	1	0	0.002	
SLC26A4 c.281C>T	/	/	/	/	/	/	6	0	0.012	
GJB3 c.538C>T	875	1	0.160	666	0	0.170	88	0	0.180	0.357

n: number of newborns, Het: heterozygote, Hom: homozygote, AF: allele frequency.

protocol for the chip to screen for 9 variants, the most frequent mutant alleles were c.235delC (0.923%, 5,052/547,294), c.919-2A>G (0.682%, 3,733/547,294), and c.299_300delAT (0.253%, 1,382/547,294), in descending order. According to the protocol for the chip to screen for 15 variants, the most frequent mutant alleles were c.235delC (0.909%, 3,559/391,534), c.919-2A>G (0.666%, 2,609/391,534), and c.299_300delAT (0.242%, 947/391,534). According to the protocol for the chip to screen for 23 variants, the most frequent mutant alleles were c.109G>A (3.358%, 1,639/48,814), c.235delC (0.869%, 424/48,814), and c.919-2A>G (0.729%, 356/48,814).

The allele frequencies of 7 sites (No.1-4, 10-11 and 21 in Table 4) according to three screening protocols were compared. The allele frequency of c.176_191del16 according to the chip to screen for 23 variants (0.076%, 37/48,814) was higher than that according to the chip to screen for 9 variants (0.070%, 383/547,294) and that according to the chip to screen for 15 variants (0.055%, 217/391,534); the difference was significant ($P = 0.014$). There were no differences in the other 6 sites among the three genetic screening protocols.

The allele frequencies for the 6 sites (No.12-17 in Table 3) according to the chip to screen for 15 variants and the chip to screen for 23 variants were compared, and no significant differences were found.

3.5. Comparison of pathogenic genotype according to three genetic screening protocols

The comparison of pathogenic genotypes according to three genetic screening protocols is shown in Table 5. According to the protocol for the chip to screen for 9 variants, the rate of detection of the c.235delC/c.235delC genotype was the highest (47.37%, 27/57), followed by c.235delC/c.299_300delAT (17.54%, 10/57). According to the protocol for the chip to screen for 15 variants, the rate of detection of the c.235delC/c.235delC genotype was the highest (28.21%, 11/39), followed by c.919-2A>G/c.919-2A>G (15.38%, 6/39). According to the protocol for the chip to screen for 23 variants, the rate of detection of the c.109G>A/c.109G>A genotype was the highest (49.18%, 30/61), followed by c.235delC/c.109G>A (24.59%, 15/61).

A point worth noting is that according to the protocol for the chip to screen for 15 variants, 66.67% (14/21) of newborns with biallelic variants in the *SLC26A4* gene were detected with newly added mutations based on the chip to screen for 9 variants. According to the protocol for the chip to screen for 23 variants, 92.98% (53/57) of newborns with biallelic variants in the *GJB2* gene were detected with the newly added mutations based on the protocol for the chip to screen for 15 variants, 52 of whom were biallelic c.109G>A variant. In addition, 25%

Table 5. Pathogenic genotypes according to three genetic screening protocols

Genotypes	Chip to screen for 9 variants *n* (%)	Chip to screen for 15 variants *n* (%)	Chip to screen for 23 variants *n* (%)
c.235delC/c.235delC	27	11	0
c.235delC/c.299_300delAT	10	5	0
c.235delC/c.176_191del16	5	1	2
c.299_300delAT/c.176_191del16	2	0	0
c.299_300delAT/c.35delG	1	0	0
c.299_300delAT/c.299_300delAT	2	1	1
c.176_191del16/c.176_191del16	1	0	0
c.235delC/c.35delG	0	0	1
c.919-2A>G/c.919-2A>G	7	6	3
c.2168A>G/c.2168A>G	1	0	0
c.919-2A>G/c.2168A>G	1	1	0
c.2027T>A/c.2027T>A	/	1	0
c.919-2A>G/c.1229C>T	/	5	0
c.1229C>T/c.1975G>C	/	3	0
c.2168A>G/c.2027T>A	/	2	0
c.2168A>G/c.1975G>C	/	1	0
c.919-2A>G/c.1226G>A	/	1	0
c.919-2A>G/c.1975G>C	/	1	0
c.109G>A/c.109G>A	/	/	30
c.235delC/c.109G>A	/	/	15
c.299_300delAT/c.109G>A	/	/	6
c.109G>A/c.35insG	/	/	1
c.235delC/c.35insG	/	/	1
c.919-2A>G/c.281C>T	/	/	1
Total	57	39	61

n: number of newborns. According to the protocol for the chip to screen for 15 variants, there was 1 case of a c.299_300delAT homozygote with m.1555A>G homoplasmy and 1 case of a c.2027T>A homozygote with a c.235delC heterozygote. According to the protocol for the chip to screen for 23 variants, there was 1 case of a c.109G>A homozygote with m.1555A>G homoplasmy, 1 case of c.235delC/c.109G>A with a c.538C>T heterozygote, and 1 case of c.235delC/c.109G>A with a c.919-2A>G heterozygote.

(1/4) of newborns with biallelic variants in the *SLC26A4* gene were detected with newly added mutations based on the protocol for the chip to screen for 15 variants.

3.6. Hearing testing of individuals with pathogenic variants

Among 121 newborns with pathogenic variants, 43 were identified by the protocol for the chip to screen for 9 variants, 31 were identified by the protocol for the chip to screen for 15 variants, and 47 were identified by the protocol for the chip to screen for 23 variants (Figure 2). Of the 121 newborns, 95 (78.51%, 95/121) failed the hearing screening, while 26 (21.49%, 26/121) passed.

All 95 newborns who failed the hearing screening underwent hearing testing, and the age at hearing testing ranged from 2 to 8 months. Results revealed that 87 newborns (91.58%, 87/95) were diagnosed with HL and 8 (8.42%, 8/95) were diagnosed with normal hearing. Among those with HL, 77 (88.51%, 77/87) had bilateral HL and 10 (11.49%, 10/87) had unilateral HL. All 8 newborns with normal hearing were detected by the protocol for the chip to screen for 23 variants, with the following genotypes: c.109G>A/c.109G>A in 4 newborns, c.235delC/c.109G>A in 1, c.235delC/c.109G>A/c.919-2A>G in 1, c.299_300delAT/c.109G>A in 1, and c.109G>A/c.35insG in 1.

As shown in Table 6, all 26 newborns who passed the hearing screening underwent hearing testing, and the age at hearing testing ranged from 2 to 12 months. Results revealed that 9 newborns (34.62%, 9/26) were diagnosed with HL and 17 (65.38%, 17/26) were diagnosed with normal hearing. Among those with HL, 8 (88.89%, 8/9) had bilateral HL and 1 (11.11%, 1/9) had unilateral HL. Among the 17 newborns with normal hearing, 3 were detected by the protocol for the chip to screen for 15 variants and 14 were detected by the protocol for the chip to screen for 23 variants, with the following genotypes: c.2027T>A/c.2027T>A/c.235delC in 1 newborn, c.1229C>T/c.1975G>C in 1, c.919-2A>G/c.1229C>T in 1, c.919-2A>G/c.919-2A>G in 1, c.109G>A/c.109G>A in 4, c.235delC/c.109G>A in 6, c.235delC/c.109G>A/c.538C>T in 1, c.299_300delAT/c.109G>A in 2.

4. Discussion

This study analyzed the genetic screening of 493,821 newborns for deafness in Beijing according to three different screening protocols over 11 years since the initiation of a project for the genetic screening of newborns in 2012. Here, positivity, rates of detection of various genotypes, carrier frequencies and allele frequencies, pathogenic genotypes of genetic screening, and hearing testing of infants with pathogenic variants

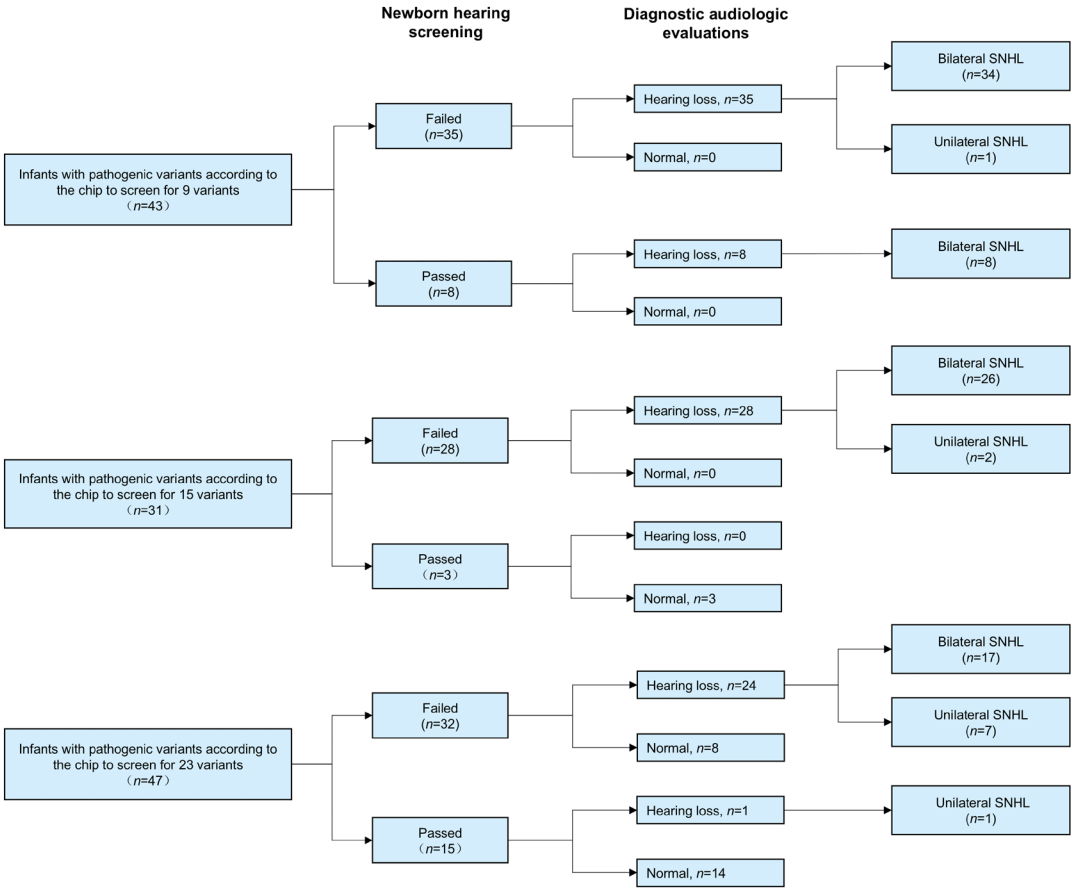

Figure 2. Auditory performance of infants with pathogenic variants.

Table 6. Hearing test results for 26 infants with pathogenic variants who passed hearing screening

No.	Sex	Year	Genetic screening protocol	Genotypes	Age at diagnosis (months)	Hearing test results	
						R	L
1	F	2013	Chip to screen for 9 variants	c.235delC/c.176_191del16	unavailable	Severe	Severe
2	F	2013	Chip to screen for 9 variants	c.235delC/c.176_191del16	unavailable	Severe	Moderate
3	F	2014	Chip to screen for 9 variants	c.235delC/c.235delC	2	Moderate	Moderate
4	M	2015	Chip to screen for 9 variants	c.919-2A>G/c.919-2A>G	3	Normal	Moderate
5	F	2016	Chip to screen for 9 variants	c.299_300delAT/c.299_300delAT	3	Severe	Profound
6	F	2016	Chip to screen for 9 variants	c.919-2A>G/c.2168A>G	22	Profound	Severe
7	M	2016	Chip to screen for 9 variants	c.176_191del16/c.176_191del16	3	Profound	Profound
8	M	2017	Chip to screen for 9 variants	c.235delC/c.235delC	3	Severe	Mild
9	M	2020	Chip to screen for 15 variants	c.2027T>A/c.2027T>A/c.235delC	12	Normal	Normal
10	F	2020	Chip to screen for 15 variants	c.1229C>T/c.1975G>C	10	Normal	Normal
11	F	2022	Chip to screen for 15 variants	c.919-2A>G/c.1229C>T	3	Normal	Normal
12	F	2022	Chip to screen for 23 variants	c.919-2A>G/c.919-2A>G	2	Normal	Normal
13	M	2022	Chip to screen for 23 variants	c.109G>A/c.109G>A	4	Normal	Normal
14	F	2022	Chip to screen for 23 variants	c.235delC/c.109G>A	6	Normal	Normal
15	F	2023	Chip to screen for 23 variants	c.299_300delAT/c.109G>A	2	Mild	Normal
16	F	2023	Chip to screen for 23 variants	c.299_300delAT/c.109G>A	4	Normal	Normal
17	F	2023	Chip to screen for 23 variants	c.109G>A/c.109G>A	3	Normal	Normal
18	M	2023	Chip to screen for 23 variants	c.235delC/c.109G>A/ c.538C>T	4	Normal	Normal
19	F	2023	Chip to screen for 23 variants	c.109G>A/c.109G>A	6	Normal	Normal
20	M	2023	Chip to screen for 23 variants	c.299_300delAT/c.109G>A	6	Normal	Normal
21	M	2023	Chip to screen for 23 variants	c.235delC/c.109G>A	3	Normal	Normal
22	F	2023	Chip to screen for 23 variants	c.235delC/c.109G>A	3	Normal	Normal
23	M	2023	Chip to screen for 23 variants	c.235delC/c.109G>A	3	Normal	Normal
24	M	2023	Chip to screen for 23 variants	c.235delC/c.109G>A	4	Normal	Normal
25	F	2023	Chip to screen for 23 variants	c.109G>A/c.109G>A	3	Normal	Normal
26	F	2023	Chip to screen for 23 variants	c.235delC/c.109G>A	4	Normal	Normal

are discussed.

4.1. Positivity according to genetic screening

Positivity with the protocol for the chip to screen for 9 variants was reported to be 4.508% (8,136/180,469) according to Dai *et al.* (*4*). The current authors' research team reported that 4.510% (3,412/75,649) of infants carried at least one mutant allele according to genetic screening with the chip to screen for 9 variants (*6*). Gao *et al.* (*7)* analyzed 10 years of data from genetic screening of newborns at Peking Union Medical College Hospital and found that positivity with the protocol for the chip to screen for 9 variants was 4.629% (4,262/92,080). The results of the current study indicated that positivity with the protocol for the chip to screen for 9 variants was 4.599%, which was consistent with the findings of the aforementioned studies.

Positivity with the screening protocol for the chip to screen for 15 variants was reported to be 4.922% (3,763/76,460) from 2019 to 2020 according to Wen *et al*. (*5*). Gao *et al*. (*7*) reported on the genetic screening of 73,733 newborns at Peking Union Medical College Hospital using the chip to screen for 15 variants, and positivity was 5.095%. The current study noted that the positivity with the screening protocol for the chip to screen for 15 variants was 4.971%, which was consistent with the findings of the aforementioned studies.

There are only few studies on positivity with the screening protocol for the chip to screen for 23 variants. The current study found that positivity with the screening protocol for the chip to screen for 23 variants was 11.489%, which was higher than that with the chip to screen for 9 variants (4.599%) and that with the chip to screen for 15 variants (4.971%). The increase could be attributed to the addition of c.109G>A in the *GJB2* gene, which had a higher carrier frequency in the Chinese population, ranging from 6.930% to 12.500% (*8*).

In summary, the current study reported increasing positivity in genetic screening for deafness using a more powerful microfluidic chip to screen for 23 variants than the chip to screen for 15 variants and the chip to screen for 9 variants. These findings may provide a reference for the development of genetic screening for deafness in the Chinese population in other regions.

4.2. Rate of detection of various genotypes of genetic screening

The results of the current study indicated that screening protocol for the chip to screen for 23 variants had a higher rate of detection of carriers (11.066%) compared to that with the chip to screen for 15 variants (4.672%) and the chip to screen for 9 variants (4.346%). Therefore, an increasing number of individuals with deafness-associated variants were identified by

microfluidics technology. These individuals are at risk for HL. Through reproductive counseling and avoidance of potential triggers (*e.g.*, noise exposure, slapping, and head injury) in individuals with such variants, the incidence of HL could be significantly reduced.

According to the three screening protocols, the rates of detection of *GJB2* heterozygotes were 2.417%, 2.348%, and 8.588%, respectively, in the current study. Recent research on hereditary deafness has indicated that *GJB2* hetero-mutation carriers exhibit heightened auditory sensitivity, potentially attributable to enhanced cochlear amplification (*9*). The increase in cochlear amplification heightens sensitivity to noise. Exposure to daily-level noise can cause permanent hearing threshold shifts in $Cx26^{+/-}$ mice, leading to HL. *GJB2* hetero-mutation carriers are vulnerable to noise and should avoid exposure to noise in daily life. Consequently, offering targeted guidance on strategies to avoid daily exposure to noise to *GJB2* heterozygous carriers could be instrumental in preserving their hearing health.

According to the three screening protocols, the rates of detection of *SLC26A4* heterozygotes were 1.567%, 1.932%, and 1.930%, respectively, in the current study. *SLC26A4* gene mutations are closely related to LVAS (*10*), with 67-90% of patients with enlarged vestibular aqueduct (EVA) having biallelic *SLC26A4* mutations (*11-16*). According to previous studies, however, 7.4-36% of patients with EVA had a monoallelic *SLC26A4* mutation (*17-23*). Proactive counseling needs to be provided to *SLC26A4* hetero-mutation carriers, who are predisposed to EVA. This guidance should address the potential hazards associated with external stimuli, notably strenuous physical activities, to ensure their auditory well-being. Hence, implementing genetic screening can facilitate the early identification of at-risk individuals. This allows for the provision of timely and proactive guidance to mitigate potential hearing impairments.

In addition, all three screening protocols in the current study included the most common mtDNA *12SrRNA* variants in the Chinese population (m.1555A>G and m.1494C>T). Individuals with mtDNA *12SrRNA* variants were susceptible to drug-induced HL. By extending pharmacological counseling to individuals and their maternal lineage, coupled with vigilant avoidance of aminoglycoside antibiotics, the risk of HL could be significantly mitigated.

4.3. Carrier frequency and allele frequency according to genetic screening

According to the three screening protocols, the carrier frequency of the *GJB2* gene was the highest. Specifically, the carrier frequency of the *GJB2* gene according to the screening protocol for the chip to screen for 23 variants (9.055%) was significantly higher than that with the protocols for the chip to screen for 15 variants (2.422%) and that with the chip to screen for 9 variants (2.489%). The *GJB2* gene was reported to be the most common gene associated with over 50% of non-syndromic HL cases (*24*). The current research indicated that the screening protocol for the chip to screen for 23 variants significantly enhances the detection of *GJB2* gene mutations and has an advantage in early identification of newborns with mutations in the *GJB2* gene.

The *SLC26A4* gene was found to be the second most common gene in the screened population. The carrier frequency of the *SLC26A4* gene in the current study was highest according to the chip to screen for 23 variants (2.151%), followed by the chip to screen for 15 variants (2.015%), and then the chip to screen for 9 variants (1.621%). This suggested that the screening protocol for the chip to screen for 23 variants could identify more newborns at risk for EVA.

In the current study, the most frequent variant was *GJB2* c.109G>A, with an allele frequency of 3.358%. Another prevalent variant, c.235delC, was the second most common variant in *GJB2*, with an allele frequency of 0.869%-0.923%. The c.919-2A>G variant was the most prevalent in *SLC26A4*, with an allele frequency of 0.666%-0.729%. Wu *et al*. (*25*) conducted a longitudinal study on genetic screening in 5,173 newborns in Taiwan and found that the allele frequencies, from highest to lowest, were c.109G>A (8.535%), c.235delC (0.638%), and c.919-2A>G (0.580%). Li *et al*. (*26*) reported allele frequencies of c.109G>A and c.235delC in newborns in Shanghai, with 6.168% (187/3,032) and 0.759% (23/3,032), respectively. Zhang *et al*. (*27*) investigated the spectrum of deafness-associated variants in 3,555,336 newborns across 32 provinces nationwide and found that c.235delC and c.919-2A>G were the most common variants in the Chinese population, with allele frequencies of 0.990% and 0.667%, respectively. Our previous study (*5*) on concurrent hearing and genetic screening of newborns in Beijing found that the most frequent mutations were c.235delC and c.919-2A>G, with allele frequencies of 0.920% (767/83,380) and 0.680% (567/83,380), respectively. The allele frequencies of c.235delC and c.919-2A>G in the current study were close to those of the aforementioned studies. The allele frequency of c.109G>A in the current study was lower than those reported by Wu *et al.* and Li *et al*., possibly due to regional differences between Taiwan, Shanghai, and the Beijing area.

4.4. Pathogenic genotypes according to genetic screening

The current study found that screening protocol for the chip to screen for 15 variants detected 66.67% (14/21) more newborns with *SLC26A4* deafness-causing mutations than the protocol with the chip to screen for 9 variants, which was close to the 71.43% (5/7) reported

by Wen *et al.* (*5*).

The screening protocol for the chip to screen for 23 variants detected 92.98% (53/57) more newborns with *GJB2* deafness-causing mutations than the protocol for the chip to screen for 15 variants, 52 of whom had the biallelic c.109G>A variant. A study has reported that the biallelic c.109G>A variant was associated with an increasing incidence of HL with age (*28*). Therefore, more biallelic c.109G>A individuals could benefit from early diagnosis and intervention through the screening protocol for the chip to screen for 23 variants. In addition, the screening protocol for the chip to screen for 23 variants detected 25% (1/4) more newborns with *SLC26A4* deafness-causing mutations than the protocol for the chip to screen for 15 variants, suggesting that the protocol for the chip to screen for 23 variants also had advantages in screening newborns with EVA.

These findings highlighted the significant advantage of utilizing enhanced genetic screening chips, which are crucial to the identification of a broader spectrum of individuals with mutations associated with deafness.

4.5. Hearing testing of children with pathogenic variants

Of the 121 individuals with pathogenic variants in the current study, 21.49% (26/121) of newborns passed hearing screening, 34.62% (9/26) of whom were diagnosed with HL. Dai *et al*. (*4*) reported that 25% (10/40) of newborns with pathogenic variants passed hearing screening. The current results are consistent with the findings reported by Dai *et al*. The data underscores the pivotal role of genetic screening in the early identification of individuals with potential HL, including those who may be missed by conventional hearing screening for newborns. By preemptively identifying such individuals, genetic screening enables timely intervention and management strategies to be implemented.

Among the 121 individuals with pathogenic variants, 96 (79.34%) were diagnosed with HL. Zhu *et al*. (*29*) conducted a concurrent hearing and genetic screening of 32,512 individuals in Nantong and found that 86.36% (19/22) of individuals with biallelic variants in the *GJB2* or *SLC26A4* gene had HL. The results of the current study are similar to those reported by Zhu *et al*. Among the 121 individuals with pathogenic variants, 25 were diagnosed with normal hearing, with the age at diagnosis ranging from 2 to 12 months. Four of the individuals had biallelic variants in the *SLC26A4* gene and 21 had a biallelic c.109G>A variant in the *GJB2* gene. The occurrence of delayed HL in these 25 children remains a significant concern. Fang *et al*. emphasized the importance of incorporating long-term follow-up in the detection of and interventions for children with delayed HL (*30*).

Among the 4 infants with confirmed normal hearing and biallelic variants in the *SLC26A4* gene listed in Table 6 (No. 9-12), 3 underwent computed tomography of the temporal bone. In one, there was no enlargement of the vestibular aqueduct bilaterally and poor medial parietal morphology of the cochlea bilaterally. In another, there was no enlargement of the vestibular aqueduct bilaterally and no obvious abnormalities in the bilateral inner, middle, or outer ear. A subsequent MRI scan of the inner ear was recommended for these two infants. In the third infant, prominent enlargement of the vestibular aqueduct was noted bilaterally. Moreover, continuous and close clinical follow-up is required for all 4 of these infants.

Chai *et al*. (*31*) reported that among hearing impaired subjects with homozygous c.109G>A, the onset of hearing impairment was congenital in 65% (11/17) and delayed in 35% (6/17). Chen *et al*. (*28*) found that among biallelic c.109G>A newborns, 43.91% (18/41) passed hearing screening for newborns or had normal hearing. The incidence of moderate or higher grades of HL in individuals with biallelic c.109G>A increases with age, with 9.52% at ages of 7 to 15, 23.08% at ages of 20 to 40, 59.38% at ages of 40 to 60, and 80.00% at ages of 60 to 85. Therefore, conducting long-term audiological monitoring and regular follow-up for newborns who are homozygous or compound heterozygous for c.109G>A in the *GJB2* gene is imperative.

In conclusion, the key points and highlights of the current study lie in its comparative analysis of three protocols for genetic screening of newborns and follow-up for infants with pathogenic variants. This study first analyzed the advantages of the chip to screen for 23 variants compared to the chip to screen for 15 variants and the chip to screen for 9 variants. Findings suggest that a growing number of newborns have benefited, and especially in the early identification of potential late-onset HL, as the number of screening sites has increased. Individuals with biallelic variants could have normal hearing, indicating that long-term audiological monitoring is imperative. This is of significant clinical importance to improve interventions and management strategies for HL.

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References

1. Kennedy CR, McCann DC, Campbell MJ, Law CM, Mullee M, Petrou S, Watkin P, Worsfold S, Yuen HM, Stevenson J. Language ability after early detection of permanent childhood hearing impairment. N Engl J Med. 2006; 354:2131-2141.
2. Kennedy C, McCann D. Universal neonatal hearing screening moving from evidence to practice. Arch Dis Child Fetal Neonatal Ed. 2004; 89:F378-383.
3. Fortnum HM, Summerfield AQ, Marshall DH, Davis AC, Bamford JM. Prevalence of permanent childhood hearing impairment in the United Kingdom and implications for universal neonatal hearing screening: questionnaire based ascertainment study. BMJ. 2001; 323:536-540.
4. Dai P, Huang LH, Wang GJ, *et al.* Concurrent hearing and genetic screening of 180,469 neonates with follow-up in Beijing, China. Am J Hum Genet. 2019; 105:803-812.
5. Wen C, Yang X, Cheng X, *et al.* Optimized concurrent hearing and genetic screening in Beijing, China: A cross-sectional study. Biosci Trends. 2023; 17:148-159.
6. Ruan Y, Wen C, Zhao XL, Wang XL, Cheng XH, Zhao LP, Zhang W, Huang LH. A follow-up study of 75,649 newborn genetically screened for deafness and patients with confirmed deafness. Chin J Otology. 2019; 17:661-669.
7. Gao RZ, Fan Y, Yang TY, Li DD, Guo Y, Jiang H, Xu YC, Chen XW. Results of neonatal genetic screening for hearing loss in peking union medical college hospital in the past 10 years. Med J Peking Union Med Coll Hospital. 2022; 13:1020-1027.
8. Chinese clinical multicenter research collaborative group for deafness gene screening and diagnosis. A guidelines for genetic screening of hereditary deafness genes. Natl Med J China. 2021; 101:97-102.
9. Liu LM, Liang C, Chen J, Fang S, Zhao HB. Cx26 heterozygous mutations cause hyperacusis-like hearing oversensitivity and increase susceptibility to noise. Sci Adv. 2023; 9:eadf4144.
10. Du Y, Huang L, Wang X, Cui Q, Cheng X, Zhao L, Ni T. Clinical data analysis of genotypes and phenotypes of deafness gene mutations in newborns: A retrospective study. Biosci Trends. 2017; 11:460-468.
11. Mey K, Muhamad AA, Tranebjaerg L, Rendtorff ND, Rasmussen SH, Bille M, Caye-Thomasen P. Association of SLC26A4 mutations, morphology, and hearing in pendred syndrome and NSEVA. Laryngoscope. 2019; 129:2574-2579.
12. Choi BY, Stewart AK, Nishimura KK, *et al.* Efficient molecular genetic diagnosis of enlarged vestibular aqueducts in East Asians. Genet Test Mol Biomarkers. 2009; 13:679-687.
13. Miyagawa M, Nishio SY, Usami S, Deafness Gene Study Consortium. Mutation spectrum and genotype-phenotype correlation of hearing loss patients caused by SLC26A4 mutations in the Japanese: A large cohort study. J Hum Genet. 2014; 59:262-268.
14. Reyes S, Wang G, Ouyang X, Han B, Du LL, Yuan HJ, Yan D, Dai P, Liu XZ. Mutation analysis of SLC26A4 in mainland Chinese patients with enlarged vestibular aqueduct. Otolaryngol Head Neck Surg. 2009; 141:502-508.
15. Wang QJ, Zhao YL, Rao SQ, Guo YF, Yuan H, Zong L, Guan J, Xu BC, Wang DY, Han MK, Lan L, Zhai SQ, Shen Y. A distinct spectrum of SLC26A4 mutations in patients with enlarged vestibular aqueduct in China. Clin Genet. 2007; 72:245-254.
16. Zhao FF, Lan L, Wang DY, Han B, Qi Y, Zhao Y, Zong L, Li Q, Wang QJ. Correlation analysis of genotypes, auditory function, and vestibular size in Chinese children with enlarged vestibular aqueduct syndrome. Acta Otolaryngol. 2013; 133:1242-1249.
17. Wu CC, Lu YC, Chen PJ, Yeh PL, Su YN, Hwu WL, Hsu CJ. Phenotypic analyses and mutation screening of the SLC26A4 and FOXI1 genes in 101 Taiwanese families with bilateral nonsyndromic enlarged vestibular aqueduct (DFNB4) or Pendred syndrome. Audiol Neurootol. 2010; 15:57-66.
18. Zhao J, Yuan Y, Chen J, Huang S, Wang G, Han D, Dai P. SLC26A4 gene copy number variations in Chinese patients with non-syndromic enlarged vestibular aqueduct. J Transl Med. 2012; 10:82.
19. Pang XH, Chai YC, Chen P. The incidence and pathogenic correlation of SLC26A4 mono-allelic mutations in deaf patients with enlarged vestibular aqueduct. Chin Sci J Hearing Speech Rehab. 2015; 3:190-194.
20. Rah YC, Kim AR, Koo JW, Lee JH, Oh SH, Choi BY. Audiologic presentation of enlargement of the vestibular aqueduct according to the SLC26A4 genotypes. Laryngoscope. 2015; 125:E216-222.
21. Tsukamoto K, Suzuki H, Harada D, Namba A, Abe S, Usami S. Distribution and frequencies of PDS (SLC26A4) mutations in Pendred syndrome and nonsyndromic hearing loss associated with enlarged vestibular aqueduct: A unique spectrum of mutations in Japanese. Eur J Hum Genet. 2003; 11:916-922.
22. Pryor SP, Madeo AC, Reynolds JC, Sarlis NJ, Arnos KS, Nance WE, Yang Y, Zalewski CK, Brewer CC, Butman JA, Griffith AJ. SLC26A4/PDS genotype-phenotype correlation in hearing loss with enlargement of the vestibular aqueduct (EVA): Evidence that Pendred syndrome and non-syndromic EVA are distinct clinical and genetic entities. J Med Genet. 2005; 42:159-165.
23. Albert S, Blons H, Jonard L*, et al.* SLC26A4 gene is frequently involved in nonsyndromic hearing impairment with enlarged vestibular aqueduct in Caucasian populations. Eur J Hum Genet. 2006; 14:773-779.
24. Kenneson A, Van Naarden Braun K, Boyle C. GJB2 (connexin 26) variants and nonsyndromic sensorineural hearing loss: A HuGE review. Genet Med. 2002; 4:258-274.
25. Wu CC, Tsai CH, Hung CC, Lin YH, Lin YH, Huang FL, Tsao PN, Su YN, Lee YL, Hsieh WS, Hsu CJ. Newborn genetic screening for hearing impairment: A population-based longitudinal study. Genet Med. 2017; 19:6-12.
26. 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, Wu H. The p.V37I exclusive genotype of GJB2: A genetic risk-indicator of postnatal permanent childhood hearing impairment. PLoS One. 2012; 7:e36621.
27. Zhang J, Wang H, Yan C, Guan J, Yin L, Lan L, Li J, Zhao L, Wang Q. The frequency of common deafness-associated variants among 3,555,336 newborns in China and 141,456 individuals across seven populations worldwide. Ear Hear. 2023; 44:232-241.
28. Chen Y, Wang Z, Jiang Y, *et al.* Biallelic p.V37I variant in GJB2 is associated with increasing incidence of hearing loss with age. Genet Med. 2022; 24:915-923.
29. Zhu QW, Li MT, Zhuang X, Chen K, Xu WQ, Jiang YH, Qin G. Assessment of hearing screening combined with

limited and expanded genetic screening for newborns in Nantong, China. JAMA Netw Open. 2021; 4:e2125544.
30. Fang BX, Cen JT, Yuan T, Yin GD, Gu J, Zhang SQ, Li ZC, Liang YF, Zeng XL. Etiology of newborn hearing impairment in Guangdong Province: 10-year experience with screening, diagnosis, and follow-up. World J Pediatr. 2020; 16:305-313.
31. Chai Y, Chen D, Sun L, Li L, Chen Y, Pang X, Zhang L, Wu H, Yang T. The homozygous p.V37I variant of GJB2 is associated with diverse hearing phenotypes. Clin Genet. 2015; 87:350-355.

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