Original Article

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Optimized concurrent hearing and genetic screening in Beijing, China: A cross-sectional study

Cheng Wen[1,2,3,§], Xiaozhe Yang[1,2,3,§], Xiaohua Cheng[1,2,3], Wei Zhang[1,2,3], Yichen Li[4], Jing Wang[4], Chuan Wang[5], Yu Ruan[1,2,3], Liping Zhao[1,2,3], Hongli Lu[6], Yingxin Li[6], Yue Bai[6], Yiding Yu[1,2,3], Yue Li[1,2,3], Jinge Xie[1,2,3], Bei-er Qi[1,2,3], Hui En[1,2,3], Hui Liu[1,2,3], Xinxing Fu[1,2,3], Lihui Huang[1,2,3,*], Demin Han[1,2,3,*]

[1] Otolaryngology-Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing, China;
[2] Beijing Institute of Otolaryngology, Beijing, China;
[3] Key Laboratory of Otolaryngology Head and Neck Surgery, Ministry of Education, Beijing, China;
[4] Maternal and Child Health, Beijing Obstetrics and Gynecology Hospital, Capital Medical University. Beijing Maternal and Child Health Care Hospital, Beijing, China;
[5] Maternal and Child Health Hospital of Chao Yang District, Beijing, China;
[6] CapitalBio Corporation & National Engineering Research Center for Beijing Biochip Technology, Beijing, China.

SUMMARY Concurrent screening has been proven to provide a comprehensive approach for management of congenital deafness and prevention of ototoxicity. The *SLC26A4* gene is associated with late-onset hearing loss and is of great clinical concern. For much earlier detection of newborns with deafness-causing mutations in the *SLC26A4* gene, the Beijing Municipal Government launched a chip for optimized genetic screening of 15 variants of 4 genes causing deafness based on a chip to screen for 9 variants of 4 genes, and 6 variants of the *SLC26A4* gene have now been added. To ascertain the advantage of a screening chip including 15 variants of 4 genes, the trends in concurrent hearing and genetic screening were analyzed in 2019 and 2020. Subjects were 76,460 newborns who underwent concurrent hearing and genetic screening at 24 maternal and child care centers in Beijing from January 2019 to December 2020. Hearing screening was conducted using transiently evoked otoacoustic emissions (TEOAEs), distortion product otoacoustic emissions (DPOAE), or the automated auditory brainstem response (AABR). Dried blood spots were collected for genetic testing and 15 variants of 4 genes, namely *GJB2*, *SLC26A4*, mtDNA *12S rRNA*, and *GJB3*, were screened for using a DNA microarray platform. The initial referral rate for hearing screening decreased from 3.60% (1,502/41,690) in 2019 to 3.23% (1,124/34,770) in 2020, and the total referral rate for hearing screening dropped form 0.57% (236/41,690) in 2019 to 0.54% (187/34,770) in 2020, indicating the reduced false positive rate of newborn hearing screening and policies to prevent hearing loss conducted by the Beijing Municipal Government have had a significant effect. Positivity according to genetic screening was similar in 2019 (4.970%, 2,072/41,690) and 2020 (4.863%,1,691/34,770), and the most frequent mutant alleles were c.235 del C in the *GJB2* gene, followed by c.919-2 A > G in the *SLC26A4* gene, and c.299 del AT in the *GJB2* gene. In this cohort study, 71.43% (5/7) of newborns with 2 variants of the *SLC26A4* gene were screened for newly added mutations, and 28.57% (2/7) of newborns with 2 variants of the *SLC26A4* gene passed hearing screening, suggesting that a screening chip including 15 variants of 4 genes was superior at early detection of hearing loss, and especially in early identification of newborns with deafness-causing mutations in the *SLC26A4* gene. These findings have clinical significance.

Keywords Deafness-related genes, Newborn genetic screening, Newborn hearing screening, Concurrent screening, Hearing loss

1. Introduction

Hearing loss is the most common human neurosensory disorder. The World Report on Hearing published by the World Health Organization (WHO) indicates that > 1.5 billion people currently experience some degree of hearing loss, which could grow to 2.5 billion by 2050 (*1*). The WHO estimates that over 400 million people,

including 34 million children, live with disabling hearing loss, which affects their health and quality of life (*1*). The reported incidence of hearing loss ranges from 1 to 2 per 1,000 newborns; in more than half of these newborns, it has a genetic etiology (*2,3*). A universal newborn hearing screening (UNHS) program has been implemented in China since the 1990s and has contributed to early hearing loss detection, diagnosis, and interventions, with good social benefits (*4,5*). The UNHS program is considered to be an extraordinarily successful public health program worldwide, but it has some limitations. The conventional UNHS is limited by its ability to detect children with late-onset or progressive sensorineural hearing loss after birth, and these children may not benefit from improved outcomes conferred from early identification and intervention by UNHS alone (*6*).

In 2006, Morton *et al.* pointed out the limitations of traditional newborn hearing screening and proposed the concept of combined newborn hearing screening and genetic screening for deafness for the first time (*7*). In 2007, Chinese scholars Wang *et al.* preliminarily discussed the protocol and strategy for concurrent newborn hearing and genetic screening and proposed that hearing screening and genetic screening should be conducted for prelingual hearing loss, delayed-onset high-risk children, or carriers of deafness-related genes and combined with regular follow-up and monitoring; they also advocated for extensive simultaneous newborn hearing screening and genetic screening; this has become a highly powerful screening strategy (*8*). In 2011, the Beijing Municipal Health Bureau conducted a successful pilot project on genetic screening of newborns for deafness at Beijing Tongren Hospital and the Chinese People's Liberation Army General Hospital. In 2012, with the support of the Beijing Municipal Government, the former Beijing Municipal Health Bureau initiated a project for genetic screening of newborns for deafness. The project screened for 9 mutations in 4 common deafness-related genes, including c.235delC (p.Leu79Cysfs*3), c.299_300delAT (p.His100Argfs*14), c.176_191del16 (p.Gly59Alafs*18), and c.35delG (p.Gly12Valfs*2) in *GJB2* (MIM: 121011); c.919-2A>G and c.2168A>G (p.His723Arg) in *SLC26A4* (MIM: 605646); and m.1555A>G and m.1494C>T of mtDNA *12SrRNA* (MIM: 561000); c.538C>T (p.Arg180*) in *GJB3* (MIM: 603324). The genetic screening project was led by Beijing Tongren Hospital, in collaboration with 5 facilities conducting genetic screening of newborns for deafness in Beijing, thus making Beijing the first city in China to genetically screen newborns for deafness. Based on the demonstrated effectiveness in Beijing, other cities like Chengdu, Changzhi, Zhengzhou, and Nantong in about 20 provinces, municipalities, and autonomous regions started including this project in their livelihood projects and they began genetically screening newborns for deafness for free (*9*). After more than 10 years in practice, the concurrent hearing and genetic screening of newborns in China had entered a phase of rapid progress.

In 2019, the current authors' group conducted concurrent hearing and genetic screening of 180,469 neonates with follow-up in Beijing, China. For genetic testing, dried blood spots were collected and 9 variants of 4 genes, namely *GJB2*, *SLC26A4*, mtDNA *12SrRNA*, and *GJB3*, were screened for using a DNA microarray platform (*10*). 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 routine newborn hearing screening [10]. In 2020, the current status of genetic screening of newborns for deafness was analyzed from 2016 to 2017 in multiple regions of China, and results revealed that the genetic screening of newborns for deafness is more extensive in the eastern region of China than in the central and western regions (*9*). In 2021, the China Clinical Multicenter Collaborative Research Group for Genetic Screening and Diagnosis of Deafness and the National Technical Guidance Group for Prevention and Treatment of Deafness promulgated the "Specifications for Genetic Screening for Deafness," which focus on the principles, process, technical methods, interpretation of results, and genetic counseling for deafness, with the aim of providing guidance for professionals engaged in this work and standardizing the workflow of genetic screening for deafness and post-screening in China (*11*). It makes genetic screening for deafness more effective for early diagnosis, treatment and prevention of deafness. Above all, it indicates that after 15 years of clinical practice, concurrent newborn hearing and genetic screening is superior to traditional hearing screening, especially in identifying infants with deafness-gene-caused hearing loss.

Data in China have indicated that *SLC26A4* is the second most common gene that causes non-syndromic hearing loss (NSHL), accounting for 14.5% (*12*). Individuals with mutations in the *SLC26A4* gene may have hearing loss, as well as an enlarged vestibular aqueduct (EVA). In 2017, the current authors' research team retrospectively analyzed 582 subjects with genetic mutations causing deafness, results indicated that *SLC26A4* gene mutations were mainly associated with high-frequency hearing loss and profound-severe hearing loss (*13*). In addition, some patients with *SLC26A4* mutations may develop delayed-onset hearing loss (*14*). For earlier detection of newborns with deafness-causing mutations in the *SLC26A4* gene, the Beijing Municipal Government launched a new chip to genetically screen for 15 variants of 4 genes based on a screening chip including 9 variants of 4 genes. The new chip added 6 variants of the *SLC26A4* gene: 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). To ascertain the advantage of screening for 15 mutations of 4 genes over screening for 9 mutations of 4 genes, the

current study first analyzed trends in concurrent hearing and genetic screening and differences across 2 years. This study also reported the sex-specific and gestational age-specific results of hearing and genetic screening, indicating the sex differences and gestational age differences in concurrent hearing and genetic screening. These findings might provide a reference for the promotion and implementation of hearing and genetic screening.

2. Material and Methods

2.1. Clinical data

Subjects were 76,460 infants born at 24 maternal and child care centers in Beijing who underwent concurrent hearing and genetic screening between January 2019 to December 2020. The clinical data on newborns who were screened for pathogenic deafness-associated variants were followed up systematically (Table S1, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=142*). Conventional newborn hearing screening and concurrent genetic screening were both conducted within 72 h after birth for all neonates at no charge.

2.2. Hearing screening

According to the "Technical Specifications for Newborn Hearing Screening (2010 Edition)," initial screening was conducted using transiently evoked otoacoustic emission (TEOAE) or distortion product otoacoustic emission (DPOAE) testing for normal infants 48-72 h after birth. For high-risk infants, the automated auditory brainstem response (AABR) test was completed prior to discharge from the hospital (*15*). For those referred after initial testing, a repeat otoacoustic emission (OAE) test or OAE test combined with AABR analysis was conducted by the age of 42 days. The relevant test parameters were as follows: TEOAE: acoustic stimulation – click; stimulus intensity – 70-75 dB; sound pressure level (SPL); signal superposition – 500-2,080 times; background noise ≤ 45 dB (A); passing criteria – total reaction intensity ≥10 dB SPL; repetition rate ≥50%; and signal-to-noise ratio (SNR) (at least 3 frequencies) ≥ 3 dB; DPOAE: acoustic stimulation – two consecutive pure tones f_1 and f_2; stimulus intensity – 65 dB and 55/50 dB; SPL; and frequency ratio – 1.1-1.5 (at least 6 frequencies); AABR: acoustic stimulation – click; stimulus intensity – 35 dB n HL; stimulation rate – 93 times/sec; sampling rate – 16 kHz; signal superposition – up to 15,000 times; spectrum range – 700/750 -5,000 Hz; and background noise: ≤ 45 dB (A). The TEOAE, DPOAE and AABR results were automatically determined by the screening device and displayed as "PASS" or "REFER." Those who failed rescreening would be referred to Beijing Tongren Hospital for diagnostic hearing testing within 3 months.

2.3. Genetic screening

The Deafness Gene Variant Detection Array Kit (Capital Bio) was used to identify 15 variants of 4 genes in newborns born between January 2019 to December 2020, including c.235delC (p.Leu79Cysfs*3), c.299_300delAT (p.His100Argfs*14), c.176_191del16 (p.Gly59Alafs*18), and c.35delG (p.Gly12Valfs*2) in *GJB2*; c.919-2A>G, c.2168A>G (p.His723Arg), 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) in *SLC26A4*; c.538C>T(p.Arg180*) in *GJB3*; and m.1555A>G and m.1494C>T in mtDNA *12S rRNA* (Table S2, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=142*, Figure S1, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=142*) (*16*). Dried blood spots from all newborn infants were collected from all 24 maternal and child care centers in Beijing where hearing screening is routinely conducted. Genetic screening was conducted at Beijing Tongren Hospital, which has genetic screening laboratories that were authorized by the Beijing Municipal Health Commission (*10*). Results were recorded in a report card (Table S3, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=142*) as pass (wild-type genotypes), refer (homozygote or compound heterozygote of *GJB2* or *SLC26A4*, mtDNA *12SrRNA* variants), or carrier (heterozygote of *GJB2* or *SLC26A4* and heterozygote or homozygote of *GJB3*). Genotypes with homozygous and compound heterozygous variants of *GJB2* or *SLC26A4* were diagnosed as deafness-causing genotypes, and those with mtDNA *12SrRNA* variants were diagnosed as drug-susceptible.

2.4. Statistical analysis

In the current study, the cohort consisted of 54,359 neonates in 2019 and 39,106 neonates in 2020. Analyses included all participants for whom the variables of interest were available. Missing data were not imputed. Ultimately, this study involved a cohort of 41,690 neonates in 2019 and 34,770 neonates in 2020 (Table S4, *http://www.biosciencetrends.com/action/getSupplementalData.php?ID=142*). The significance of differences was assessed using the χ^2 test for categorical variables and the *t* test or ANOVA for continuous variables. All data analyses with performed using SAS (version 9.4) (SAS Institute, Cary, NC, USA).

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 the parents of all neonates for evaluation and publication of their clinical data.

3. Results

3.1. Baseline characteristics of hearing loss in the 2 years studied

In the current study, 41,690 infants born in 2019 and 34,770 infants born in 2020 underwent concurrent hearing and genetic screening within 72 h after birth and before hospital discharge. Demographic characteristics are shown in Table 1. The mean gestational age was 38.71 weeks in 2019 and 38.67 weeks in 2020. The mean birth weight was 3,294 grams in 2019 and 3,285 grams in 2020. Seven-point-one percent of newborns were born prematurely in 2019 and 7.31% were born prematurely in 2020. In this cohort of newborns, there were more males than females, with a total sex ratio of 1.083:1.000. In 2019, 96.07% of newborns were singleton pregnancies and 96.39% were singleton pregnancies in 2020, 3.93% were multiple pregnancies in 2019, and 3.61% were multiple pregnancies in 2020.

3.2. Hearing screening results in the 2 years studied

Table 1. The baseline characteristics of hearing and genetic screening in different years

Characteristics	Year group	
	2019 (*N* = 41,690)	2020 (*N* = 34,770)
Gestational age, weeks, mean (SD)	38.71 ± 1.66	38.67 ± 1.67
Premature, %	2,961 (7.10)	2,542 (7.31)
Sex, %		
Female	20,014 (48.01)	16,693 (48.01)
Male	21,676 (51.99)	18,075 (51.98)
Unspecified	0 (0.00)	2 (0.01)
Birth weight, g, mean (SD)	3,294.59 ± 488.76	3,285.62 ± 490.21
Fetus		
Single birth	40,052 (96.07)	33,514 (96.39)
Multiple births	1,638 (3.93)	1,256 (3.61)

N, number of newborns.

The initial referral rate for hearing screening decreased in the 2 years studied, from 3.60% (1,502/41,690) to 3.23% (1,124/34,770) (Table 2). Those newborns who were referred were screened again at the age of 42 days; 0.57% (236/41,690) did not pass the second hearing screening either bilaterally or unilaterally in 2019 and 0.54% (187/34,770) did not pass in 2020, so the percentage tended to decline. There were significant differences in trends for both initial screening and second screening between 2020 and 2019 ($P = 0.0057$).

3.3. Genetic screening results in the 2 years studied

Forty-one thousand six hundred and ninety newborns underwent genetic screening in 2019 and 34,770 did so in 2020, and genetic screening data are shown in Table 3. Four-point-nine-seven percent of neonates (2,072/41,690) screened positive for deafness-associated variants in 2019 and 4.863% (1,691/34,770) did so in 2020. The percentages were similar in the 2 years studied. There were no significant differences between 2020 and 2019 ($P = 0.4973$).

Trends in allele frequency in genetic screening are shown in Table 4. The most frequent mutant alleles were those of the *GJB2* gene, *SLC26A4* gene, and *GJB3* gene in the 2 years studied, in descending order. The frequency of mutant alleles in the *GJB2* gene, in descending order over the 2 years studied, was 1.229% (1,025/83,380) in 2019 and 1.150% (800/69,540) in 2020. The frequency of mutant alleles in the *SLC26A4* gene, in descending order over the 2 years studied, was 1.015% (705/69,540) in 2020 and 1.013% (844/83,380) in 2019. The frequency of mutant alleles in the *GJB3* gene, from high to low, was 0.168% (117/69,540) in 2020 and 0.156% (130/83,380) in 2019.

The most frequent mutant alleles were c.235 del C in the *GJB2* gene, followed by c.919-2 A > G in the *SLC26A4* gene, and c.299 del AT in the *GJB2* gene in both years studied. The frequency of the mutant

Table 2. Trends in results of hearing screening

Results	Year group				*P* values for total referrals (%)
	2019		2020		
	N	Percentage (%)	*N*	Percentage (%)	
Initial hearing screening					
Passed	40,188	96.40	33,646	96.77	
Unilateral referral	903	2.16	685	1.97	
Bilateral referral	599	1.44	439	1.26	$P = 0.0057$
Total referrals (%)	1,502	3.60	1,124	3.23	
Second hearing screening					
Passed	41,454	99.43	34,583	99.46	
Unilateral referral	98	0.24	99	0.29	
Bilateral referral	138	0.33	88	0.25	
Total referrals (%)	236	0.57	187	0.54	
Total	41,690	100	34,770	100	

N, number of newborns.

Table 3. Trends in results of genetic screening

Genotypes	Group by year				*P* value for the positive genotype (%)
	2019		2020		
	N	Percentage (%)	*N*	Percentage (%)	
Wild type	39,618	95.030	33,079	95.137	
Positive	2,072	4.970	1,691	4.863	*P* = 0.4973
Total	41,690	100	34,770	100	

N, number of newborns. * Newborns born from April 2013 to March 2014.

Table 4. Trends in allele frequency in genetic screening

Variants	Group by year					
	2019 (15-site chip)			2020 (15-site chip)		
	Heterozygotes (*N*)	Homozygotes (*N*)	Allele frequency (%)	Heterozygotes (*N*)	Homozygotes (*N*)	Allele frequency (%)
GJB2 c.35 del G	6	0	0.007	6	0	0.009
GJB2 c.176 del 16	40	0	0.048	39	0	0.056
GJB2 c.235 del C	761	3	0.920	599	1	0.864
GJB2 c.299 del AT	212	0	0.254	154	0	0.221
GJB3 c.538 C > T	130	0	0.156	117	0	0.168
SLC26A4 c.2168 A > G	109	0	0.131	100	0	0.144
SLC26A4 c.919-2 A > G	567	0	0.680	427	2	0.620
SLC26A4 c.1174 A > T	28	0	0.034	33	0	0.047
SLC26A4 c.1226 G > A	22	0	0.026	29	0	0.042
SLC26A4 c.1229 C > T	23	0	0.028	36	0	0.052
SLC26A4 c.1975 G > C	61	0	0.073	40	0	0.058
SLC26A4 c.2027 T > A	24	0	0.029	22	1	0.035
SLC26A4 c.1707+5 G > A	10	0	0.012	12	0	0.017

N, number of newborns.

allele c.235 del C in the *GJB2* gene in 2019 (0.920%, 767/83,380) was higher than in 2020 (0.864%, 601/69,540). The frequency of the mutant allele c.919-2 A > G in the *SLC26A4* gene in 2019 (0.680%, 567/83,380) was also higher than in 2020 (0.620%, 431/69,540). The frequency of the mutant allele c.299 del AT in the *GJB2* gene in 2019 (0.254%, 212/83,380) was higher than in 2020 (0.221%, 154/69,540).

3.4. Concurrent hearing and genetic screening in the 2 years studied

Associations between hearing and genetic screening are summarized in Table 5. In the 2 years studied, 63 of the 423 neonates who did not pass hearing screening, either bilaterally or unilaterally, were also referred for genetic screening. Moreover, 3,700 neonates who passed hearing screening were positive according to genetic screening. Among infants referred for genetic screening, 4 (0.0052%) had 2 variants of the *GJB2* gene, 7 (0.0091%) had 2 variants of the *SLC26A4* gene, and 222 (0.2903%) carried mtDNA *12SrRNA* variants. Among the deafness-associated variant carriers, 1,817 (2.3764%) were heterozygous carriers of *GJB2*, 1,535 (2.0076%) were heterozygous carriers of *SLC26A4*, and 247 (0.3230%) had the *GJB3* heterozygous or homozygous variant. A point worth noting is that 71.43% (5/7) of newborns with 2 variants of the *SLC26A4* gene were screened for newly added mutations, and as shown in Table S5 (*http://www.biosciencetrends.com/action/getSupplementalData.php?ID=142*), 28.57% (2/7) of newborns with 2 variants of the *SLC26A4* gene passed hearing screening.

In 2019, 35 (14.83%) of 236 neonates who did not pass hearing screening, either bilaterally or unilaterally, were also referred for genetic screening. In 2020, 28 (14.97%) of 187 neonates who did not pass hearing screening, either bilaterally or unilaterally, were also referred for genetic screening. In addition, 2,037 (4.91%, 2,037/41,454) of the neonates who passed hearing screening were positive according to genetic screening in 2019, and 1,663 (4.81%, 1,663/34,583) were positive according to genetic screening in 2020. Among the deafness-associated variant carriers, 31 (1.54%) of 2,017 heterozygous mutation carriers were referred for additional hearing screening in 2019, and 24 (1.47%) of 1,632 heterozygous mutation carriers were referred for additional hearing screening in 2020. In addition, 99.19% (123/124) of infants with a m.1555A>G or m.1494C>T variant in 2019 and 98.98% (97/98) of those newborns in 2020 passed newborn hearing screening.

3.5. Sex-specific results of hearing and genetic screening

Table 5. Associations between hearing and genetic screening

Genotypes	Group by year				Total number (%)
	2019		2020		
	Passed hearing screening *N* (%)	Referred for hearing screening *N* (%)	Passed hearing screening *N* (%)	Referred for hearing screening *N* (%)	
Carrier					
GJB2 heterozygote	998 (2.3939)	21 (0.0504)	779 (2.2404)	19 (0.0546)	1,817 (2.3764)
SLC26A4 heterozygote	830 (1.9909)	8 (0.0192)	694 (1.9960)	3 (0.0086)	1,535 (2.0076)
GJB3 heterozygote	129 (0.3094)	1 (0.0024)	115 (0.3307)	2 (0.0058)	247 (0.3230)
GJB2 heterozygote with *SLC26A4* heterozygote	23 (0.0552)	1 (0.0024)	15 (0.0431)	0 (0.00)	39 (0.0510)
GJB2 heterozygote with *GJB3* heterozygote	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	24 (0.0093)
SLC26A4 heterozygote with *GJB3* heterozygote	6 (0.0144)	(0.00)	5 (0.0144)	0 (0.00)	11 (0.0144)
Pathogenic variants					
GJB2 homozygote	0 (0.00)	3 (0.0072)	0 (0.00)	1 (0.0029)	4 (0.0052)
SLC26A4 homozygote	0 (0.00)	0 (0.00)	1 (0.0029)	2 (0.0058)	3 (0.0039)
SLC26A4 compound Heterozygote	1 (0.0024)	2 (0.0048)	0 (0.00)	1 (0.0029)	4 (0.0052)
Mitochondrial variants					
m.1494 C>T homoplasmy	10 (0.0240)	0 (0.00)	7 (0.0201)	0 (0.00)	17 (0.0222)
m.1555A>G homoplasmy	66 (0.1583)	1 (0.0024)	56 (0.1611)	0 (0.00)	123 (0.1609)
m.1555A>G heteroplasmy	47 (0.1127)	0 (0.00)	34 (0.0978)	1 (0.0029)	82 (0.1072)
Non-wild type (n)	2,037 (4.8861)	35 (0.0840)	1,663 (4.7829)	28 (0.0805)	3,763 (4.9215)
Wild type (n)	39,417 (94.5479)	201 (0.4821)	32,920 (94.6793)	159 (0.4573)	72,697 (95.0785)
Total for all screened	41,454 (99.4339)	236 (0.5661)	34,583 (99.4622)	187 (0.5378)	76,460 (100.00)

N, number of newborns.

The sex-specific results of hearing and genetic screening in 2019 and 2020 were analyzed in Table 6. Of the screened newborns, 51.99% were males and 48.01% were females in both 2019 and 2020. In 2019, 4.97% of newborns were referred for genetic screening and 0.57% were referred for hearing screening. In 2020, 4.86% newborns were referred for genetic screening and 0.54% were referred for hearing screening. The referral rates for genetic screening for females in 2019 (5.07%) and 2020 (4.97%) were higher than the referral rates for males (4.88% in 2019 and 4.76% in 2020) but not significantly so (P = 0.384, P = 0.354, respectively). The referral rates for hearing screening for females in 2019 and 2020 were 0.47% and 0.44%, both of which were lower than referral rates for males (0.66% in 2019 and 0.63% in 2020), and the rates differed significantly (P = 0.0117 and P = 0.0138, respectively). Taken together, there were significant differences between females and males only in hearing screening results.

3.6. Gestational age-specific results of hearing and genetic screening

The gestational age-specific and gestational age-standardized results of hearing and genetic screening in 2019 and 2020 were analyzed in Table 7. In 2019, non-premature babies accounted for 92.90% of the 41,690 neonates, and the remaining 7.10% were premature neonates. In 2020, non-premature babies accounted for 92.69% of the 34,770 neonates and 7.31% were premature ones. The referral rate for genetic screening for non-premature newborns was 5.02% in 2019, which was higher than the rate for premature newborns (4.36%) but not significantly so (P = 0.111). However, positivity according to genetic screening for nonpremature newborns was 4.83% in 2020, which was lower than the positivity for premature newborns (5.31%) but not significantly so (P = 0.276). The referral rate for hearing screening for nonpremature newborns was 0.57% in 2019 and 0.54% in 2020, both of which were higher than the referral rates for premature newborns (0.51% in both 2019 and 2020) but not significantly so (P = 0.6544, P = 0.850, respectively).

4. Discussion

This study analyzed concurrent newborn hearing and genetic screening results for 76,460 neonates in total. This study also reported the sex-specific and gestational age-specific hearing and genetic screening results in the 2 years studied. Here, the trends in hearing screening, genetic screening, and concurrent screening in the years studied are discussed. Also discussed is the association between sex, gestational age, and concurrent hearing and genetic screening.

4.1. Hearing screening during the 2 years studied

In 2004, the former Ministry of Health issued the "Technical Specifications for Newborn Hearing

Table 6. Sex-specific results of hearing and genetic screening in Beijing

	Group by year						
	2019				2020		
Genetic screening		Male	Female	Total		Male	Female	Total
N	Passed	20,618	19,000	39,618	Passed	17,215	15,863	33,078
(%)		49.46	45.57	95.03		49.51	45.63	95.14
Row (%)		52.04	47.96			52.04	47.96	
Column (%)		95.12	94.93			95.24	95.03	
N	Referred	1,058	1,014	2,072	Referred	860	830	1,690
(%)		2.54	2.43	4.97		2.47	2.39	4.86
Row (%)		51.06	48.94			50.89	49.11	
Column (%)		4.88	5.07			4.76	4.97	
	Total	21,676	20,014	41,690	Total	18,075	16,693	34,768
		51.99	48.01	100		51.99	48.01	100
	2019				2020			
Hear screening		Male	Female	Total		Male	Female	Total
N	Passed	21,534	19,920	41,454	Passed	17,961	16,620	34,581
(%)		51.65	47.78	99.43		51.66	47.80	99.46
Row (%)		51.95	48.05			51.94	48.06	
Column (%)		99.34	99.53			99.37	99.56	
N	Referred	142	94	236	Referred	114	73	187
(%)		0.34	0.23	0.57		0.33	0.21	0.54
Row (%)		60.17	39.83			60.96	39.04	
Column (%)		0.66	0.47			0.63	0.44	
	Total	21,676	20,014	41,690	Total	18,075	16,693	34,768
		51.99	48.01	100		51.99	48.01	100

N, number of newborns; two newborns of an unspecified sex in the 2020 cohort were excluded from the analysis.

Table 7. Gestational age-specific and gestational age-standardized results of hearing and genetic screening in Beijing

	Group by year						
	2019				2020		
Genetic screening		Non-premature	Premature	Total		Non-premature	Premature	Total
N	Passed	36,786	2,832	39,618	Passed	30,672	2,407	33,079
(%)		88.24	6.79	95.03		88.21	6.92	95.14
Row (%)		92.85	7.15			92.72	7.28	
Column (%)		94.98	95.64			95.17	94.69	
N	Referred	1,943	129	2,072	Referred	1,556	135	1,691
(%)		4.66	0.31	4.97		4.48	0.39	4.86
Row (%)		93.77	6.23			92.02	7.98	
Column (%)		5.02	4.36			4.83	5.31	
	Total	38,729	2,961	41,690	Total	32,228	2,542	34,770
		92.90	7.10	100		92.69	7.31	100
	2019				2020			
Hearing screening		Non-premature	Premature	Total		Non-premature	Premature	Total
N	Passed	38,508	2,946	41,454	Passed	32,054	2,529	34,583
(%)		92.37	7.07	99.43		92.19	7.27	99.46
Row (%)		92.89	7.11			92.69	7.31	
Column (%)		99.43	99.49			99.46	99.49	
N	Referred	221	15	236	Referred	174	13	187
(%)		0.53	0.04	0.57		0.5	0.04	0.54
Row (%)		93.64	6.36			93.05	6.95	
Column (%)		0.57	0.51			0.54	0.51	
	Total	38,729	2,961	41,690	Total	32,228	2,542	34,770
		92.90	7.10	100		92.69	7.31	100

N, number of newborns.

Screening (2004 Edition)," which set clear requirements for institutional settings, personnel, housing and equipment, as well as clear regulations for hearing screening, diagnosis, interventions, and quality control (*17*). In 2005, the Beijing Children's Hearing Care Expert Steering Group summarized early hearing detection and interventions for children ages 0-6 years in Beijing and it further standardized the hearing screening and diagnosis for children ages 0-6 years (*18*). At this point, newborn hearing screening and diagnosis constitutes a system that is being widely implemented and gradually standardized in all regions. In 2010, the former Ministry of Health promulgated the "Technical Specifications for Newborn Hearing Screening (2010 Edition)," which further promoted the standardization of the program. The World Health Organization has paid increasing attention to the Chinese UNHS program and reported in 2017 that the UNHS program is effective in high-income countries, including China, at identifying serious problems promptly (*19*).

In 2020, Wen *et al*. (*20*) studied the current status of the UNHS program at 26 facilities in China, and results revealed that the total referral rate for initial screening in 2017 (9.21%) was lower than that in 2016 (10.26%). In the current study, the initial referral rate for hearing screening decreased in the 2 years studied, from 3.60% to 3.23%; these rates are lower than those reported by Wen *et al*. The reason may be that the newborns included in the study by Wen *et al.* were from different regions in east, central, and west China, while the newborns included in the current study were all from Beijing. The UK's Newborn Hearing Screening Programme Standards 2016 to 2017 mentioned that a referral rate within 15% at initial screening in a community program was acceptable, that a rate within 13.5% was achievable, and that the rate is a negative polarity standard, meaning that a lower percentage is considered better (*21*). The initial referral rate for hearing screening was 3.60% and 3.23% in the 2 years studied, both of which were within the 13.5% recommended by the UK's UNHS guidelines and which were in line with international recommendations. In 2019, Dai *et al.* reported that 6.54% neonates were referred at initial screening and 1.061% of 180,469 neonates were referred bilaterally or unilaterally for hearing screening (*10*). Wang *et al.* (*22*) reported an initial screening referral rate of 6.87% and an overall failure rate in newborn hearing screening of 0.748% in 9,755 newborns born in Beijing from January 2017 to December 2017. The initial referral rate in the current study was lower than 6.54% and 6.87%; the reason may be due to the quality control of newborn hearing screening by the Beijing expert group in recent years and the Beijing Municipal Government's emphasis on concurrent newborn hearing screening and genetic screening for deafness, which improved the quality of screening in 2019 and 2020 compared to 2013. The overall failure rate of newborn hearing screening in the current study was 0.57% in 2019 and 0.54% in 2020, both of which were much lower than the 1.061% reported by Dai *et al.* and the 0.748% reported by Wang *et al.* (*10,22*) In the current study, after follow-up, all newborns who failed the initial screening were rescreened at the age of 42 days, and the decrease in the overall failure rate of newborn hearing screening was associated with a decrease in the initial screening failure rate.

4.2. Trends in genetic screening during the 2 years studied

Genetic screening of newborns for deafness has been implemented in China for more than 10 years. In 2011, Wang *et al*. (*23*) reported genetic screening for 3 common genes, mtDNA *12S rRNA*, *GJB2*, and *SLC26A4*, and positivity was 2.05% (306/14,913). In 2013, Zhang *et al*. analyzed the concurrent hearing and genetic screening results of 58,397 neonates born in Tianjin. Twenty common hearing loss-associated mutations of *GJB2*, *GJB3*, *SLC26A4*, and mtDNA *12S rRNA* were screened for, and they found that 5.52% of infants carried at least one mutant allele (*24*). Wu *et al.* conducted simultaneous hearing screening and genetic screening for 4 common deafness-related mutations in 5,173 newborns and found that 1.6% had conclusive genotypes and 16.2% had a *GJB2* or *SLC26A4* mutation (*25*). Later in 2017, Lu *et al*. reported that 1.2% of 1,716 newborns had conclusively positive genotypes on genetic screening and 20.10% had a *GJB2* or *SLC26A4* mutation (*26*). In 2019, Dai *et al.* reported that 4.508% of 180,469 neonates born from April 2013 to March 2014 were positive according to genetic screening (*10*). Positivity in genetic screening for deafness was higher in both 2019 (4.970%) and 2020 (4.863%) than the rate reported previously (4.508%), and this is probably because screening included 6 more mutations in 2019 and 2020 than in 2013. Positivity in genetic screening in the current study was higher than the 2.05% reported by Wang *et al*. in 2011 and lower than the 5.52% reported by Zhang *et al*. in 2013; this may due to differences in genes and mutations that were screened for. Similarly, positivity in genetic screening for deafness in the current study was lower than that reported by Taiwanese researchers; this is probably due to their genetic screening targeting four common deafness mutations including p.V37I of *GJB2* gene, which has a high allele frequency in Chinese population. In summary, the current study reported increasing positivity in genetic screening for deafness using more powerful gene microarray chips in 2019 and 2020 than in 2013. These findings may provide a reference for the development of genetic screening for deafness in the Chinese Han population in other regions.

The *GJB2* gene is the most common gene that causes non-syndromic hearing loss (NSHL) (*27*). Researches have found that *SLC26A4* is the second most common

gene that causes NSHL and is related to an EVA (*12*). The most frequent mutant alleles were those of the *GJB2* gene, *SLC26A4* gene, and *GJB3* gene in the 2 years studied, in descending order, and this finding was consistent with the results of previous studies. In 2019 and 2020, 6 mutations of the *SLC26A4* gene were added to a chip screening for 9 variants of 4 genes. Therefore, the frequency of mutant alleles in the *SLC26A4* gene in 2019 (1.013%) and 2020 (1.015%) was higher than in 2013 (0.809%), suggesting that a microarray to screen for 15 variants of 4 genes can screen more newborns for an EVA and may yield better societal benefits.

Early in 2007, Dai *et al*. conducted a study on the prevalence of the c.235delC mutation in *GJB2* in the Chinese deaf population, and they found that the c.235delC mutation in the *GJB2* gene caused NSHL in as much as 15% of patients in certain regions of China (*28*). Later in 2008, Dai *et al*. reported that the c.919-2A>G mutation in the *SLC26A4* gene alone would identify the molecular cause in up to 8–12% of individuals with sensorineural hearing loss in a few eastern and central regions of China (*29*). A large population-based cohort study by Zhang *et al.* also proved that the c.235delC mutation in the *GJB2* gene was the most common variant and that the second most common variant was the c.919-2A>G mutation in the *SLC26A4* gene in the Chinese population (*30*). The most frequent mutant allele was c.235 del C in the *GJB2* gene, followed by c.919-2 A > G in the *SLC26A4* gene in the 2 years studied, and this finding was consistent with the results of previous research.

4.3. Concurrent hearing and genetic screening during the 2 years studied

Genetic screening of newborns for deafness makes up the deficiency of the conventional UNHS program and allows for early detection of congenital hereditary hearing loss, drug-sensitive newborns, and carriers of common deafness-related genes. In 2011, Schuelke *et al*. (*31*) conducted two-step DPOAE screening and newborn genetic screening for deafness among 1,017 newborns, who were screened for p.V37I and c.235delC in *GJB2*, c.919-2A>G in *SLC26A4*, and mitochondrial m.1555A>G. They found that 27.27% (3/11) of babies who were homozygous for p.V37I, 83.33% (5/6) who were compound heterozygous for p.V37I and c.235delC, and 100% (1/1) who were homoplasmic for m.1555A>G passed hearing screening at birth. Later in 2017, Wu *et al*. reported that 56.1% (46/82) of 5,173 newborns with conclusive genotypes passed hearing screening at birth and that long-term follow-up identified progressive hearing loss in children with the *GJB2* p.V37I/p.V37I and p.V37I/c.235delC genotypes (*25*). Dai *et al.* reported that among 4.508% newborns who were born between 2013 and 2014 and who were positive according to genetic screening, 4.375% passed hearing screening, 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 routine newborn hearing screening (*10*).

Significantly, 71.43% of newborns with 2 variants of the *SLC26A4* gene were identified by genetic screening for the newly added mutations, and 28.57% of newborns with 2 variants of the *SLC26A4* gene passed hearing screening. This indicates that the chip to screen for 15 variants of 4 genes is superior for concurrent hearing and newborn screening, and particularly in the early identification of newborns with deafness-causing mutations in the *SLC26A4* gene because of the 6 newly added *SLC26A4* gene mutations. In the current study, 2 newborns with c.2168 A > G/c.2027 T > A compound heterozygous mutations in the *SLC26A4* gene in 2019 and a newborn with a c.919-2A > G/ c.1229 C > T compound heterozygous mutation in the *SLC26A4* gene in 2020 failed the newborn hearing screening bilaterally and were later diagnosed with an EVA bilaterally; they underwent hearing management at the age of three months. Of note, there was a newborn with c.2168 A > G/c.1975 G > C compound heterozygous mutation in 2019 who passed the newborn hearing screening bilaterally and who was later diagnosed with moderately severe hearing loss in the left ear. In addition, there was a newborn with a c.2027 T > A homozygous mutation in 2020. According to the Deafness Variation Database (http://deafnessvariationdatabase.org/), c.2027 T > A is pathogenic and is associated with deafness. However, the newborn with a c.2027 T > A homozygous mutation passed the newborn hearing screening bilaterally and was later diagnosed with normal hearing at the age of 11 months. Computed tomography of the temporal bone revealed no enlargement of the vestibular aqueduct bilaterally and poor medial parietal morphology of the cochlea bilaterally. This case is currently being followed further. The mutations c.2027 T > A, c.1229 C > T, and c.1975 G > C, which were newly added to genetic screening in 2019, were identified, which allowed these families to directly benefit from early etiological diagnosis and early intervention.

Moreover, 4.91% and 4.81% of neonates who passed hearing screening but were positive according to genetic screening in the 2 years studied and 28.57% of newborns with 2 variants of the *SLC26A4* gene passed hearing screening. After hearing follow-up, a newborn with 2 pathogenic combinations of *SLC26A4* variants was found to exhibit sensorineural hearing loss, implying that the baby's hearing at birth might have been normal or near normal and could not have been detected by newborn hearing screening. Wang *et al.* also reported that genetic screening identified 13% more hearing-impaired infants than hearing screening alone and that it identified 0.23% of newborns predisposed to preventable ototoxicity undetectable by hearing screening (*32*). In 2020, Zhang *et al.* analyzed 22 studies related to concurrent hearing

and genetic screening in neonates in China and reported a pooled prevalence of passing the UNHS while failing genetic screening of 0.22%, while the pooled prevalence of passing the UNHS with the MT-RNR1 variant was 0.20% (*33*). The range of variation in this rate of neonates passing hearing screening but testing positive according to genetic screening was small and relatively stable over the 2 years studied. However, the wide range of variation in the rate of newborns with 2 pathogenic combinations of *GJB2* or *SLC26A4* variants who passed newborn hearing screening may be due to differences in the sample size of the 2-year period studied and few newborns carrying the pathogenic mutations. Six newborns screened positive for pathogenic mutations in 2019 and 5 screened positive in 2020. The rates in the current study were all lower than the 83.33% and 56.1% reported by Wu *et.al*. (*25, 31*); the reason might be the differences in the screened population and the variants screened for. The screened population reported by Wu *et al*. was the Taiwanese population and the mutations screened for were p.V37I and c.235delC in *GJB2*, c.919-2A>G in *SLC26A4*, and mitochondrial m.1555A>G, whereas p.V37I was mainly associated with mild to moderate hearing loss (*34*). In addition, 98.98% and 99.19% of infants with a m.1555A>G or m.1494C>T variant passed newborn hearing screening in the current study, and this finding was consistent with the 100% reported by Schuelke *et al.* (*31*). These newborns are all potentially sensitive to aminoglycoside antibiotics, and their hearing may be compromised by even small amounts of such drugs (*10*). This indicates that genetic screening for deafness can identify such newborns early, guide medication, and minimize the incidence of drug-related deafness.

4.4. Association between sex and gestational age and hearing loss

According to the World Report on Hearing published by the World Health Organization in 2021, causative factors that lead to hearing loss across the course of one's life include genetic factors and intrauterine infections during the prenatal period, hypoxia or birth asphyxia, hyperbilirubinemia, a low birth weight, perinatal infections, and receiving ototoxic medicines during the perinatal period (*1*).The 12 separate factors f hearing loss were listed by the Joint Committee on Infant Hearing in 2019 and included 9 predominantly perinatal risk factors and 3 postnatal risk factors, including family members being deaf or hard of hearing with onset in childhood, infants requiring care in the NICU or special care nursery for more than 5 days, hyperbilirubinemia, aminoglycoside administration for more than 5 days, perinatal asphyxia, and in-utero infections (*35*).

Nie *et al*. explored the risk indicators of newborn hearing loss and found that there were 3 high-risk indicators associated with newborn hearing loss: a family history of hearing loss, craniofacial anomalies, and receiving care in the NICU (*36*). Yu *et al.* investigated the correlation between genetic abnormalities causing deafness and high-risk factors for hearing loss, and they reported that detection of gene mutations causing deafness was highest among children with a family history of congenital hearing loss (*37*). The referral rates for genetic screening for females and males in the 2 years studied did not differ significantly, suggesting that sex may not be directly associated with the results of genetic screening for deafness, but a family history of deafness that may be relevant was not included in the analysis in the current study. Studies have reported that passing rates on the UNHS were higher for female infants than for male infants (*38,39*). Fitzgibbons *et al*. predicted hearing loss from 10 years of universal newborn hearing screening results and risk factors, and they found that factors significantly associated with permanent childhood hearing loss included being female and bilateral referral as a result of screening (*40*). The finding that the referral rate for hearing screening was lower for females than for males in the 2 years studied was consistent with results reported by Yan *et al*. and Li *et al*. but was inconsistent with the results reported by Fitzgibbons *et al*. This may be due to differences in newborn hearing screening protocols, with the TEOAE or DPOAE technique being used for initial screening in this study, whereas Fitzgibbons *et al*. used a two-stage AABR screening protocol.

There were no significant differences in referral rates after the results of genetic and hearing screening were stratified by prematurity, suggesting that there may not necessarily be an association between prematurity and positivity in genetic screening for deafness. Sabbagh *et al*. reported that the main risk factors for hearing loss included a low gestational age (<35 weeks) (*41*). In the current study, preterm delivery was defined as less than 37 weeks of gestation. The current results indicated that preterm birth may not be a risk factor for hearing loss. Further study is warranted to investigate a more detailed definition of preterm delivery specifically for hearing and genetic screening.

5. Conclusions

In summary, the highlights and strengths of the current study lies in its analysis of the trends in concurrent newborn hearing and deafness genetic screening in different years. For the first time, a study has analyzed the advantages of a chip to screen for 15 variants of 4 genes. Findings suggest that the quality of newborn hearing screening improved in 2019 and 2020 and that a chip to screen for 15 variants of 4 genes has advantages in early identification of newborns with deafness-causing mutations in the *SLC26A4* gene. This chip can screen more newborns for large vestibular aqueduct syndrome at an early stage. These findings provided a reference

for other regions where genetic screening for deafness is proposed.

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[§]These authors contributed equally to this work.
**Address correspondence to:*
Lihui Huang and Demin Han, Otolaryngology-Head and Neck Surgery, Beijing Tongren Hospital, Capital Medical University, Beijing 100730, China; Beijing Institute of Otolaryngology, Beijing 100005, China; Key Laboratory of Otolaryngology Head and Neck Surgery, Ministry of Education, Beijing 100005, China.
E-mail: huanglihui@ccmu.edu.cn; deminhan_ent@hotmail.com

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