Concurrent Hearing and Genetic Screening of 180,469 Neonates with Follow-up in Beijing, China

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Concurrent hearing and genetic screening of newborns is expected to play important roles not only in early detection and diagnosis of congenital deafness, which triggers intervention, but also in predicting late-onset and progressive hearing loss and identifying individuals who are at risk of drug-induced HL. Concurrent hearing and genetic screening in the whole newborn population in Beijing was launched in January 2012. This study included 180,469 infants born in Beijing between April 2013 and March 2014, with last follow-up on February 24, 2018. Hearing screening was performed using transiently evoked otoacoustic emission (TEOAE) and automated auditory brainstem response (AABR). For genetic testing, dried blood spots were collected and nine variants in four genes, *GJB2*, *SLC26A4*, mtDNA *12S rRNA*, and *GJB3*, were screened using a DNA microarray platform. Of the 180,469 infants, 1,915 (1.061%) were referred bilaterally or unilaterally for hearing screening; 8,136 (4.508%) were positive for genetic screening (heterozygote, homozygote, or compound heterozygote and mtDNA homoplasmy or heteroplasmy), among whom 7,896 (4.375%) passed hearing screening. Forty (0.022%) infants carried two variants in *GJB2* or *SLC26A4* (homozygote or compound heterozygote) and 10 of those infants passed newborn hearing screening. In total, 409 (0.227%) infants carried the mtDNA *12S rRNA* variant (m.1555A>G or m.1494C>T), and 405 of them passed newborn hearing screening. In this cohort study, 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, indicating that concurrent screening provides a more comprehensive approach for management of congenital deafness and prevention of ototoxicity.

Introduction

Hearing loss (HL) is the most common human neurosensory disorder. The reported incidence ranges from 1.33 to 1.86 per 1,000 newborns;[1,2] in more than half of these newborns, HL has a genetic etiology.[1,2] Newborn hearing screening is widely performed worldwide and has played an important role in early detection, diagnosis, and intervention. The average age at which HL is confirmed by hearing screening has dropped from 24–30 months to 3 months.[3] However, some neonates pass newborn hearing screening but then show delayed-onset, progressive

HL or susceptibility to ototoxic drugs.[4] Studies have revealed that the prevalence of permanent HL continues to increase during childhood and reaches a rate of about 2.7 per 1,000 children before the age of 5 years and 3.5 per 1,000 during adolescence.[2,5]

Hereditary HL is extremely heterogeneous. We provide a detailed overview of deafness genes and the pattern of variants in China,[6–8] and we demonstrate that nine variants in four genes are the most common causes of nonsyndromic HL, including c.235delC (p.Leu79Cysfs*3), c.299_300delAT (p.His100Argfs*14), c.176del16 (p.Gly59Alafs*18)/ c.35delG (p.Gly12Valfs*2) of *GJB2* (MIM: 121011);

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c.919-2A>G and c.2168A>G (p.His723Arg) of *SLC26A4* (MIM: 605646); m.1555A>G and m.1494C>T of mtDNA *12S rRNA* (MIM: 561000); c.538C>T (p.Arg180*) of *GJB3* (MIM: 603324)[6,8,9] (Table S1). Demonstrating this allows for the development of a DNA microarray kit for genetic testing.[10] The prevalence of these pathogenic variants has led to tremendous interest in the clinical use of genetic screening to identify newborns who have or may develop hereditary HL. In 2000, Green et al.[11] proposed that neonates should be screened for deafness genes. In 2006, we suggested that concurrent hearing and genetic screening is an optimal screening strategy for newborns.[5]

Since January 2012, over 1.5 million neonates have undergone concurrent hearing and genetic screening in Beijing, China. We report herein the concurrent hearing and genetic screening data of 180,469 infants born in Beijing from April 2013 to March 2014, with hearing and habilitation follow-up. This project seeks to demonstrate a practical concurrent screening strategy to improve detection rates of HL in newborns, identify newborns at high risk for HL, and introduce a newborn HL prevention strategy in a megacity (a metropolitan areas with a total population of 10 million or more) with one of the largest populations (20.7 million) worldwide.

Material and Methods

Clinical Data

Concurrent hearing and genetic screening were initiated in January 2012 in Beijing; during each year since, 175,973–288,884 neonates have been screened. Up to December 21, 2018, a total of 1,585,892 neonates in Beijing underwent concurrent hearing and genetic screening. Both conventional newborn hearing screening and concurrent genetic screening were conducted within 72 h after birth for all neonates at no charge. Between April 2013 and March 2014, 180,469 infants born in Beijing who received concurrent hearing and genetic screening were enrolled, and the clinical data of newborns who screened positive for deafness-associated variants were followed up systematically.

In this project, positive cases were referred to the six designated top-ranking hospitals (including Chinese People's Liberation Army [PLA] General Hospital, Beijing TongRen Hospital, China Rehabilitation Research Center for Hearing and Speech Impairment, Peking Union Medical College Hospital, Peking University Third Hospital, and Beijing Children's Hospital) for diagnostic audiological testing; genetic testing; and counseling, hearing, and habilitation follow-up.

Questionnaire Survey

Comprehensive hearing and habilitation information was collected from all parents through the use of a unified common questionnaire designed specifically for this investigation (Table S2). Data were statistically analyzed.

Hearing Screening

Initial screening was performed using transiently evoked otoacoustic emission (TEOAE) testing. For those referred after initial testing, a repeat TEOAE test combined with automated auditory brainstem response (AABR) analysis was performed by the age of 42 days (Figure S1). The relevant test parameters are as follows: TEOAE, acoustic stimulation—click; stimulus intensity—70 ~75 dB sound pressure level (SPL); signal superposition—500 ~2,080 times; background noise < 40 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. AABR, acoustic stimulation—click; stimulus intensity—35 dB nHL; 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).

Genetic Screening

The Deafness Gene Variant Detection Array Kit (CapitalBio)[10] was used to identify nine variants in four genes, including c.235delC (p.Leu79Cysfs*3), c.299_300delAT (p.His100Argfs*14), c.176del16 (p.Gly59Alafs*18), and c.35delG (p.Gly12Valfs*2) of *GJB2*; c.919-2A>G and c.2168A>G (p.His723Arg) of *SLC26A4*; c.538C>T(p.Arg180*) of *GJB3*; and m.1555A>G and m.1494C>T of mtDNA *12S rRNA*. The microarray strategy for detecting heteroplasmy of m.1555A>G of mtDNA *12S rRNA* is shown in Figure S2, and data for validating the range of heteroplasmy is shown in Table S3. Dried blood spots from all newborn infants were collected from all 132 maternal and child care service centers in Beijing where hearing screening is routinely performed. Genetic screening was performed (as described in detail previously)[10] at six designated top-ranking hospitals in which genetic screening laboratories were authorized by the Beijing Municipal Health Commission. Results were recorded as pass (all wild-type genotypes), refer (homozygote or compound heterozygote of *GJB2* or *SLC26A4*, mtDNA *12S rRNA* variants), or carrier (heterozygote of *GJB2* or *SLC26A4* and heterozygote or homozygote of *GJB3*). Those genotypes with homozygous and compound heterozygous variants in *GJB2* or *SLC26A4* were diagnosed as deafness-causing genotypes, and those with mtDNA *12S rRNA* variants were diagnosed as drug-susceptible.

There were three major procedures applied to make sure that the families received results and notification for further follow-up: (1) For all newborns participating in the genetic screening, Beijing Municipal Health Commission informed the families with the confirmed results of the genetic screening via a short message notice sent through mobile phones within 60 work days after birth, and meanwhile the parents could access the results by logging into the website of the Beijing Municipal Health Commission (as shown in Figure S3) by typing in the screening ID and the name of the mother to download the report. (2) For those infants who were screened positive by genetic testing (heterozygote, homozygote, or compound heterozygote and mtDNA homoplasmy or heteroplasmy), the healthcare professional for the maternal and child care service centers informed the families of the genetic screening results by phone and advised them for further followup. If a family was lost to follow-up (*e.g.*, due to a change in phone number), their data was then transferred to the community center who then followed up with the infants at their home addresses. 3) In this project, positive cases were referred to the six designated top-ranking hospitals. The physicians and genetic counselors at the six hospitals were trained and certified by the Beijing Municipal Health Commission, and guaranteed positive cases received appropriate diagnostic audiological testing, genetic testing and counseling, and hearing and habilitation follow-up.

Table 1. Results of Hearing Screening

Results	Number of Cases	Percentage (%)
Initial Hearing Screening		
pass	168,672	93.46
unilateral refer	7,204	3.99
bilateral refer	4,593	2.55
Second Hearing Screening		
pass	178,554	98.94
unilateral refer	1,038	0.58
bilateral refer	877	0.48
total	180,469	-

Hearing Diagnosis

Infants who did not pass two-step hearing screenings were evaluated using a series of diagnostic audiological tests by the age of 3 months in one of six authorized institutions in Beijing. Auditory brainstem response (ABR), auditory steady state response (ASSR), distortion product otoacoustic emission (DPOAE), and acoustic immittance were performed to determine the degree of HL, which was diagnosed according to WHO 1997 criteria. If infants were older than 6 months, the infantile behavioral audiometry was then added.

Ethics Statement

This study was approved by the Beijing Municipal Health Commission and Chinese PLA General Hospital Research Ethics Committee. Fully informed written consent was obtained from the parents of all neonates for evaluation and publication of their clinical data.

Results

Demographic Characteristics

In this study, 98% (213,110/217,441) of infants born in Beijing from April 2013 to March 2014 underwent concurrent hearing and genetic screening between 72 h after birth and hospital discharge. Ultimately, 180,469 neonates were recruited, and 32,641 cases were excluded due to incomplete or unmatched information (Table S4).

Results of Hearing Screening of 180,469 Neonates

A total of 180,469 neonates were screened using the TEOAE test, and those who were referred (6.54%; 11,797/180,469) were screened again at the age of 42 days through the use of otoacoustic emission (OAE) and AABR tests. A total of 1,915 (1.061%) neonates did not pass the second hearing screening either bilaterally or unilaterally (Table 1).

Results of Genetic Screening and Associations Between Hearing and Genetic Screening of 180,469 Neonates

Genetic screening data for the nine deafness-associated variants evaluated in the 180,469 neonates and the variant spectra are shown in Table 2.

Table 2. Results of Genetic Screening

Variants	Number of Cases	Percentage (%) (n = 8,136)	Percentage (%) (n = 180,469)
Refer			
12S rRNA m.1555A>G	382	4.70	0.212
12S rRNA m.1494 C>T	27	0.33	0.015
GJB2 homozygote	20	0.25	0.011
GJB2 compound heterozygote	13	0.16	0.007
SLC26A4 homozygote	7	0.09	0.004
total	449	5.53	0.249
Carrier			
GJB2 heterozygote	4,231	52.00	2.344
SLC26A4 heterozygote	2,814	34.59	1.559
GJB3 heterozygote	539	6.62	0.299
GJB2 heterozygote with *SLC26A4* heterozygote	72	0.88	0.040
GJB2 heterozygote with *GJB3* heterozygote	24	0.29	0.013
SLC26A4 heterozygote with *GJB3* heterozygote	7	0.09	0.004
total	7,687	94.47	4.259
Total for All Screenings			
total	8,136	100	4.508

n, number: The number 8,136 accounts for those infants who were screened positive genetically (heterozygote, homozygote, or compound heterozygote and mtDNA homoplasmy or heteroplasmy); and 180,469 is the number of all infants included in this study.

The frequencies of the nine variants in the population under this study are c.235delC (1.80%), c.299_300delAT (0.50%), c.176del16 (0.12%), c.35delG (0.01%), c.919-2A>G (1.34%), c.2168A>G (0.27%), c.538C>T (0.32%), m.1555A>G (0.21%), and m.1494C>T (0.02%). Of the 180,469 infants, 8,136 (4.508%) screened positive for deafness-associated variants, 449 (0.249%) were genetically referred, and 7,687 (4.259%) were, or were suspected of being, genetic deafness-associated variant carriers. Among genetically referred infants, 33 (0.018%) had two variants in *GJB2*, seven (0.004%) had two variants in *SLC26A4*, and 409 (0.227%) carried the mtDNA *12S rRNA* variants. The range for heteroplasmy of m.1555A>G of mtDNA *12S rRNA* detected by our array was validated by next-generation sequencing (Ion Torrent, Thermo Fisher Scientific) as 1.85-89.14% (Table S3). Among the deafness-associated variant carriers, 4,231 (2.344%) were heterozygote carriers of *GJB2*, 2,814 (1.559%) were heterozygote carriers of *SLC26A4*, and 539 (0.299%) had the *GJB3* heterozygous or homozygous variant. In this study, we also identified 103 (0.057%) infants who were double heterozygotes in *GJB2*, *SLC26A4*, or *GJB3* (Table 2). Significantly, we found that 25% (10/40) of the infants with two pathogenic

Table 3. Associations between Hearing and Genetic Screening of 180,469 Neonates

Genotypes	Pass Hearing Screening Number (%)	Refer Hearing Screening Number (%)	Total Number (%)
GJB2 homozygote/compound heterozygote	9 (0.0050)	24 (0.0133)	33 (0.0183)
GJB2 heterozygote	4,071 (2.2558)	160 (0.0887)	4,231 (2.3444)
SLC26A4 homozygote	1 (0.0005)	6 (0.0033)	7 (0.0038)
SLC26A4 heterozygote	2,778 (1.5393)	36 (0.0199)	2,814 (1.5592)
GJB3 heterozygote	529 (0.2931)	10 (0.0055)	539 (0.2986)
mtDNA *12s rRNA* homoplasmy variants	312 (0.1729)	4 (0.0022)	316 (0.1751)
mtDNA *12s rRNA* heteroplasmy variants	93 (0.0515)	0 (0)	93 (0.0515)
GJB2 heterozygote with *SLC26A4* heterozygote	72 (0.0399)	0 (0)	72 (0.0399)
GJB2 heterozygote with *GJB3* heterozygote	24 (0.0133)	0 (0)	24 (0.0133)
SLC26A4 heterozygote with *GJB3* heterozygote	7 (0.0038)	0 (0)	7 (0.0038)
total	7,896 (4.375)	240 (0.133)	8,136 (4.508)
wild type	170,658 (94.564)	1,675 (0.928)	172,333 (95.492)
total	178,554 (98.939)	1,915 (1.061)	180,469 (100)

combinations of *GJB2* or *SLC26A4* variants and 99% (405/409) of infants with a m.1555A>G or m.1494C>T variant passed initial or second-tier newborn hearing screening.

Associations between hearing and genetic screening are summarized in Table 3. Of 1,915 neonates who did not pass hearing screening, either bilaterally or unilaterally, 240 (12.53%) were also referred from genetic screening. In addition, 7,896 (4.375%) neonates who passed hearing screening were screened positive by genetic screening. Among the deafness-associated variant carriers in this study, 160 (0.0887%) of 4,231 heterozygous variant carriers of *GJB2* referred on the second hearing screen (21 cases were lost to follow-up, 34 cases did not accept the hearing diagnosis, and 105 cases accepted the hearing diagnosis, including 21 cases with HL), 36 (0.0199%) heterozygous variant carriers of *SLC26A4* referred on the second hearing screen (five cases were lost to follow-up, four cases did not accept the hearing diagnosis, and 27 cases underwent hearing diagnosis, including seven cases with HL), 10 (0.0055%) heterozygous or homozygous variant carriers of *GJB3* referred on the second hearing screen but confirmed later to have normal hearing, and all of the 103 (0.056%) with digenic heterozygous variants of the above three genes (*GJB2* heterozygote with *SLC26A4* heterozygote, *GJB2* heterozygote with *GJB3* heterozygote, and *SLC26A4* heterozygote with *GJB3* heterozygote) passed initial hearing screening.

For those with only one pathogenic variant, recommendations were made according to the hearing screening results: (1) additional testing to analyze the whole coding sequence of *GJB2* or *SLC26A4* and (2) avoidance of all situations that could potentially influence hearing, such as trauma, infection, and ototoxic drugs. The recommendation was made that when HL occurred, immediate management should be pursued. In addition, for those infants

not passing hearing screening, it was recommended that diagnostic audiological testing should be performed before three months of age and any HL should be followed up and managed appropriately. For those infants who passed hearing screening, the recommendation was for diagnostic audiological testing to be performed before one year of age and periodic audiological testing to be performed on an annual basis through three years of age.

Hearing Follow-up for Infants with Pathogenic Variants in *GJB2, SLC26A4*, and *GJB3*

Among the entire cohort, we further analyzed the clinical and genetic profiles of 40 infants with deafness-causing genotypes (either homozygous or compound heterozygous variants in *GJB2* or *SLC26A4*). Their clinical and genetic characteristics are shown in Table S5. Notably, nine neonates with etiologic variants in *GJB2*, and one with a homozygous pathogenic variant in *SLC26A4*, passed both the initial and second hearing screens. Further follow-up showed that eight of these nine cases carrying *GJB2* pathogenic variants suffered from varying degrees (mild to severe) of HL (one lost to follow-up). The age at which HL was subsequently identified in these nine children (including one with homozygous pathogenic variants in *SLC26A4*) ranged from 6–60 months (Figure S4). These data suggest that 27.27% (9/33) of infants with pathogenic combinations of *GJB2* variants and 14.28% (1/7) of infants with pathogenic combinations of *SLC26A4* variants may pass newborn hearing screening, and most of them will develop HL at an early age (<5 years old, Table S5). In addition, 570 neonates (570/180,469, 0.32%) were found to have a *GJB3* c.538C>T variant. All of them passed hearing screening except for 10 neonates (four bilateral and six unilateral referrals), who were all later verified by diagnostic audiological testing to have normal hearing.

Hearing Follow-up for Infants with Mitochondrial Variants

Variants of mtDNA *12S rRNA* have been shown to be associated with aminoglycoside-induced HL, and these variants reveal highly variable penetrance and expressivity of deafness. Variants of mtDNA *12S rRNA* were detected in 409 neonates, of whom 382 had an m.1555A>G variant (290 homoplasmic and 92 heteroplasmic) and 27 had an m.1494C>T variant (26 homoplasmic and one heteroplasmic). These neonates are all potentially sensitive to aminoglycoside antibiotics, and their hearing may be compromised by even small amounts of such drugs. Among these 409 infants, 405 passed hearing screening, two infants with m.1555A>G did not pass the second hearing screening bilaterally (one was verified to have normal hearing later, and one was lost to follow-up), and two were shown later to have profound unilateral HL. No infants had exposure to any aminoglycoside. A total of 142 infants (aged 2–3 years) had mtDNA *12S rRNA* variants, and their parents responded to recall requests and visited the Chinese PLA General Hospital. All 142 infants had normal hearing, consistent with their physical examination, parental interviews, and TEOAE testing.

Hearing and Habilitation Follow-up Data on Infants Referred for a Second-Tier Hearing Screen

Of the 1,915 children who did not pass the second-tier hearing screen, 1,644 were followed up and 1,265 underwent diagnostic audiological testing. Audiological results showed that 226 infants (17.87%) had decreased hearing and 1,039 (82.13%) had normal hearing. Therefore, the positive predictive value (PPV, probability that subjects who screen positive are deaf) is 17.87%. Of the 226 children with HL, 49 (21.68%) were fitted with hearing aids or cochlear implants. Among them, 24 were only fitted with hearing aids at an average age of 11.26 months; 25 children received cochlear implants at an average age of 12.23 months, among whom 11 received both hearing aids and cochlear implants (bi-modal habilitation) at average ages of 7.55 and 15.90 months, respectively.

Hearing and Habilitation Follow-up Data on Infants with Deafness-Causing Genotypes in *GJB2* and *SLC26A4*

Of 40 children with deafness-causing genotypes (either homozygous or compound heterozygous variants at *GJB2* or *SLC26A4*), 37 were followed up and underwent diagnostic audiological testing (31 with *GJB2* and six with *SLC26A4* variants) and showed various degrees of HL. For this panel, the overall PPV of genetic screening is over 90%. Twenty-three (62.16%) were fitted with hearing aids or received cochlear implants. Among them, 11 were fitted with hearing aids at an average age of 7.59 months, four received cochlear implants at an average age of 14.80 months, and eight received both hearing aids and cochlear implants (bi-modal habilitaion) at 8.13 and 15.75 months, respectively.

Discussion

Significance and Limitations of the Newborn Hearing Screening

Hearing screening for neonates was mandated in China by the *Maternal and Infant Health Care Act* of 2000, and has since been applied nationwide. Delayed speech and language development caused by deafness can be avoided in most cases through early diagnosis, timely intervention, and habilitation. Although considered to be a tremendously successful public health program worldwide, conventional newborn hearing screening has certain limitations. In particular, not all types of HL can be identified by hearing screening immediately after birth. The onset of HL may be delayed due to later expression of the genetic etiology or may be triggered by environmental factors such as trauma or certain medications. Even individuals with two pathogenic *GJB2* variants, which are well known to be etiologic in congenital severe deafness, have been reported to experience delayed mild HL and may even exhibit normal hearing at birth, where the rate of non-penetrance at birth is 3.8% to 28%.[12–15] Therefore, some neonates with inherited HL may pass the standard newborn hearing screening and experience delayed intervention.[16] However, citywide data on the frequency of variants in deafness genes and delayed diagnosis of HL are rarely reported.

Clinical Significance of Newborn Genetic Screens

Many studies have shown that newborn hearing screening combined with genetic screening detects not only congenital deafness but also some cases of delayed-onset HL caused by genetic or environmental factors[17–24] (Tables S6 and S7). Unlike conventional newborn hearing screening, genetic screens allow early identification of the molecular etiology of HL, leading to prevention and timely intervention.[21] Studies have shown that the genetic findings have played an important guiding role for hearing aid fitting and cochlear implants. For example, individuals with *GJB2*- or *SLC26A4*-related HL tend to have good prognoses after cochlear implantation.[25–27] From January 2012 to December 21, 2018, a total of 1,585,892 neonates underwent combined screening in Beijing. According to the 2010 census, 95.69% of Beijing's residents were Han Chinese. Of the remaining 4.31%, another 55 ethnicities were included. In this study, we performed a comprehensive data analysis for one fiscal year (April 2013 to March 2014) of the Beijing concurrent screening data. We detected 33 cases with *GJB2* deafness-causing genotypes and seven with *SLC26A4* deafness-causing genotypes in one year of screening for 180,469 neonates in Beijing. Importantly, as soon as a molecular etiology is confirmed, 62.16% (23/37) of infants with deafness-causing genotypes (either homozygous or compound heterozygous variants at *GJB2* or *SLC26A4*) entered a habilitation program and were fitted with hearing aids or cochlear implants in

a timely manner (Table S5). Compared with a 21.68% (49/226) intervention rate in infants who were diagnosed with HL by audiological tests, genetic testing appears to have a positive impact on parental compliance and children's habilitation. Ten carriers of homozygous or compound heterozygous variants in *GJB2* or *SLC26A4* passed initial hearing screening. Nine cases were followed up, and all developed mild-to-moderate HL after 6–60 months. Newborn genetic screening clearly shortens time to diagnosis and intervention, reveals the etiology of genetic deafness, ensures timely habilitation of infants and young children, and identifies additional newborns who have deafness or are at risk for deafness.[22]

The PPV of newborn hearing screening in our project is 17.87%, which is similar to the 16.3% PPV of the hearing screening program from the Centers for Disease Control and Prevention (CDC) of the United States and to the 16.28%–21.19% PPVs of other studies.[17,24]

A study of 58,397 Chinese newborns screened for 20 common pathogenic variants in four genes identified a genetic carrier rate of 5.52% and detected 20.59% (7/34) of newborns with deafness-causing genotypes (*GJB2* or *SLC26A4*) who passed hearing screening, which is comparable to our study.[18] Another study of 5,173 Chinese newborns screened for *GJB2* c.235delC and c.109G>A (p.V37I) identified 736 (14.2%) heterozygous carriers for *GJB2* c.109G>A and 42 (53.85%) of 78 newborns with two variants of *GJB2* who passed hearing screening. The number 53.85% is much higher than those found in other reports; this is likely because *GJB2* c.109G>A shows significantly variable penetrance.[21] The above two studies did not have follow-up of diagnostic audiological testing. In another recent similar study, data on 12,778 carriers of 20 variants in four genes out of 1,172,234 Chinese newborns were analyzed, and 43 (38.39%) of 112 newborns with deafness-causing genotypes (*GJB2* or *SLC26A4*) passed hearing screening. Among those 43 newborns, nine were confirmed later via phone interview to have HL,[20] indicating that the PPV of genetic screening is 20.93%. Data from our project show that among 10 (25%) of 40 newborns with deafness-causing genotypes (*GJB2* or *SLC26A4*) and who passed hearing screening, nine (the remaining one was lost to follow-up) were confirmed by audiological testing to have HL; these results indicate that the PPV of genetic screening is over 90%. The obvious difference in PPV of genetic screening between the previous study (20.93%)[20] and ours (> 90%) lies in some limitations of follow-up via phone interview. These limitations include: (1) Population movement in modern society, which results in changes of telephone numbers; (2) Poor compliance in follow-up due to distrust and privacy protection; and (3) Phone interviews performed without validating diagnostic audiological testing, thus potentially resulting in underreporting of mild-to-moderate HL.

In comparison, our data come from the six designated top-ranking diagnostic centers. The design and workflow of our study greatly reduce the LTF/D (lost-to-follow-up/

documentation) and likely ensure collection of more accurate data. Our results demonstrate the importance and significance of concurrent genetic screening for timely diagnosis and habilitation (in addition, other results are shown in Table S6). Overall, variability in detection rates among studies might also reflect differences in sample size, variants screened, and screening methodologies.

Regarding study design, our project was prospectively planned by a multidisciplinary expert team (including otologists, pediatricians, obstetricians, audiologists, health economists, and statisticians) assembled by the Beijing Municipal Health Commission. All participating hospitals were required to adopt the laboratory equipment, testing kit, and unified method of investigation recommended by the expert team. All laboratory staff and genetic counselors were trained and certified by the Beijing Municipal Health Commission. Screening covered all maternal and child care service centers in Beijing at no cost for all of the participants, and provided a good representation of the Beijing population.

Notably, the detection of two heterozygous variants on an array is insufficient for a genetic diagnosis. As shown in Table S5, among 40 infants with deafness-causing genotypes, 27 were found to have homozygous variants, and 13 infants had two variants in *GJB2* or *SLC26A4*. Thirteen families were advised to go on to parental validation, but only four families' results were available, and the two variants in the probands from these four families proved to be inherited from both parents. In addition, according to the literature,[28] the six variants (c.235delC, c.299_300delAT, c.176del16, and c.35delG in *GJB2* and c.919-2A>G and c.2168A>G in *SLC26A4*, which were included in the Deafness Gene Variant Detection Array Kit of this study) were confirmed to be variants consistent with a founder effect (i.e., genetic alteration observed with high frequency in a group that is or was geographically or culturally isolated, in which one or more of the ancestors was a carrier of the altered gene, NCI Dictionary of Genetics Terms), meaning that these variants segregated from generation to generation and are unlikely to be *de novo* variants. Based on analysis of our previous clinical data from 1,569 *GJB2* and 1,668 *SLC26A4* families (probands and parents underwent genetic testing together in these 3,237 families, and all probands had one or two of the above six variants in homozygous or compound heterozygous combination), all probands with two variants were in *trans* by parental validation, and no *de novo* cases were detected. Therefore, we presume that the two variants each in gene *GJB2* or *SLC26A4* of a newborn are inherited from both parents in the Chinese population. Furthermore, in this study, nearly all newborns (except for three cases lost to follow-up) with two variants in *GJB2* or *SLC26A4* were audiologically diagnosed to have HL, which confirmed the above deduction and proved these variants should be in *trans* and pathogenic in combination.

The heterozygous c.538C>T variant in *GJB3* has been reported to be associated with late-onset high-frequency

sensorineural HL.[9,29] We studied the c.538C>T variant in a previous study which showed that it had a very low incidence in the Chinese population, and there was no clear evidence to support a role of the variant in the autosomal dominant form of non-syndromic HL.[30] In this study, 98% of neonates (560/570) with the *GJB3* c.538C>T variant passed hearing screening, and another 10 neonates referred based on hearing screening results were all verified later to have normal hearing. Genetic counseling and long-term follow-up were recommended for these individuals.

Genetic screening also identifies those at risk of drug-induced HL and facilitates appropriate preventive measures. We found 409 infants carrying mtDNA *12S rRNA* variants, 405 of whom passed initial hearing screens. These neonates are all potentially sensitive to aminoglycoside antibiotics, and their hearing may be compromised by even small amounts of such drugs. Antibiotic selection instruction cards (Figure S5) were distributed to these children's families, and they were instructed to show these cards to their doctors during clinical visits to avoid exposure to aminoglycosides. The reason why the vast majority of carriers had normal hearing is because they had not had any exposure to aminoglycoside antibiotics. Previous epidemiological studies found that each individual with an m.1555A>G variant had about 10 maternal relatives with normal hearing.[31] Therefore, the one-year genetic screening in Beijing would theoretically identify about 4,090 subjects at risk for ototoxic HL. Concurrent hearing and genetic screening were initiated in January 2012 in Beijing; during each year since, 175,973–288,884 neonates were screened. The coverage of the concurrent hearing and genetic screening for the newborns in Beijing was 98.80% (179,974/182,167), 99.06% (206,394/208,345), 99.27% (259,364/261,276), 98.34% (175,973/178,944), 99.25% (288,884/291,070), 99.36% (262,301/263,991), and 99.62% (213,002/213,818) for the years from 2012 to 2018, respectively; this represents a total of 1,585,892 newborns concurrently screened by this project. A total of 3,806 neonates (0.24%) were found to carry mtDNA *12S rRNA* variants (m.1555A>G or m.1494C>T). Based on this ratio and the total populations of Beijing (20.7 million) and China (1.39 billion), it is estimated that the number of carriers of mtDNA *12S rRNA* variants (m.1555A>G or m.1494C>T) in Beijing and China is 49,680 and 3,336,000, respectively. If such concurrent screening is promoted nationwide, screening results will be recorded in the Electronic Medical Record (EMR), which is readily available at the time when gentamicin or other aminoglycoside antibiotics would be scheduled to be administered. This would promote selection of alternative antibiotics, and the risk of side effects of aminoglycosides could be safely avoided with a positive impact on personalized medicine. This translates into over three million people who could potentially benefit from concurrent genetic screening as a component of newborn hearing screening in the whole of China.

Population-based neonatal gene screening is generally considered to be a tertiary form of deafness prevention.

However, the fact that such screening prevents aminoglycoside-induced deafness by allowing precautions to be taken immediately could be considered primary prevention. Our study and previous studies highlight the importance of newborn genetic testing for the future well-being and development of the children with appreciation for the impact of any discrepant initial information to a family. Such screening will aid in development of novel follow-up strategies for concurrent hearing and genetic screening, promoting both timely diagnosis and habilitation.

According to the literature, congenital cytomegalovirus (CMV) is estimated to be a cause of ~10% of congenital deafness.[32] However, until now, there were no systematical data on and comprehensive evaluation of the relationship between CMV infection and congenital deafness in the Chinese population; such data and evaluation is indispensable for implementation of city-wide or nation-wide newborn screening. In recent years, some ear-nose-and-throat (ENT) doctors, otologists, and pediatricians in Beijing began to recommend CMV testing in infants who did not pass hearing screening or who had confirmed HL, but the data have not been systematically collected. When systematic evaluation of the results and significance of CMV testing in deafness of Chinese newborns is accomplished, CMV testing may well be added into a future government-initiated newborn screening project. In addition, the feasibility and cost-benefit ratio of CMV screening for the whole newborn population of Beijing must be evaluated by a multidisciplinary expert team (including otologists, pediatricians, obstetricians, audiologists, health economists, and statisticians) assembled by the Beijing Municipal Health Commission before its implementation.

The Advantage of Using a DNA Microarray Platform for Large-Scale Genetic Screening

Large-scale nationwide deafness-related molecular epidemiological surveys in China[33,34] have identified hotspot variants associated with non-syndromic HL, and this has facilitated the development of a DNA microarray kit for genetic testing. According to our epidemiological data and investigation of clinical molecular diagnoses, nine hotspot variants of the four genes used in this study account for 80.18% and 62.33% of pathogenic variants in *GJB2* and *SLC26A4*, and these two genes account for about 66% of genetic HL (Tables S8 and S9). Therefore, screening nine hotspot variants would produce a relatively high detection rate for the majority of instances of genetic HL.

The performance of the allele-specific PCR-based universal array (ASPUA) was validated.[10] The kit can be used to simultaneously detect nine variants in four common deafness-related genes, and the kit was approved by the Chinese Food and Drug Administration (CFDA) in 2009; CFDA approval is a mandatory condition for the application of gene testing kits in Chinese hospitals. Only small amounts of various clinical materials (e.g., dried blood spots, amniotic fluid, or buccal swabs) are required. DNA extraction, PCR, hybridization, washing, scanning, and

interpretation of results together require approximately 5 h. Rapid turnaround and low cost make the screening of large populations feasible.[35] In addition, the chip is versatile and can be easily modified to screen for other hereditary diseases; thus, many clinical applications are possible. Notably, the frequency of heteroplasmic mitochondrial variants was high in the present study, in part because the detection sensitivity of the microarray platform is excellent (i.e., able to distinguish heteroplasmic variants in <5% of mtDNA *12S rRNA*).

Since 2010, next generation sequencing (NGS) has been used in genetic testing for deafness and has been shown to increase the diagnostic rate. Although NGS could expand the number of HL-related genes evaluated, it is still challenging to apply NGS to large-scale newborn screening due to high cost, long turnaround time, and increased interpretation burden. In addition, NGS currently results in an appreciably large number of variants of uncertain significance and thus poses risks of underestimation and overestimation; on the other hand, this project found that eight common variants screened are definitely pathogenic and one variant (*GJB3* c.538C>T) is confirmed to be related to late-onset HL.[9]

The cost-benefit ratio of the project was calculated by the China Health Economics Association (CHEA) to be 1:7.27, meaning that $1 spent on genetic screening for hearing would eventually result in an economic savings of $7.27.[36] The calculation is based on concurrent screening data from 155,206 newborns screened from April 2012 to January 2013 in Beijing. The ratio 1:7.27 was from the cost of the project (screening and counseling costs for all screened cases plus diagnosis, treatment, and habilitation costs for HL cases) versus the benefit of the project (avoiding treatment and habilitation costs for preventable HL cases, reducing training, and allowing for disabled individuals with savings in labor).

Implementation of concurrent hearing and genetic screening requires the collaboration of hospitals, maternal and child care service centers, certified laboratories, and genetic testing and counseling centers. Beijing, as the capital of China, serves as a leading role model in this project. After successful implementation in Beijing, concurrent hearing and genetic screening in newborns with the same strategy have been adopted by 21 cities in 16 provinces in China including Sichuan, Henan, Shanxi, Shandong, Guangdong, Jilin, Jiangsu, Hunan, Fujian, Anhui, Xinjiang, Gansu, Ningxia, Zhejiang, Shanghai, and Chongqing. Newborn hearing screening is implemented in rural areas, albeit not systematically, and we believe that in the future, following leadership in the large cities, concurrent hearing and genetic screening will also be implemented in rural areas.

Limitations

This study had several limitations. First, nine hotspot variants of the four genes used in this study do not account for all possible deafness-associated variants. A total of 19.82% and 37.67% of confirmed pathogenic variants in *GJB2* and *SLC26A4*, respectively, were not detected (Tables S8 and S9), and the possibility remains that other untested genes, including "non-rare" new deafness genes not identified previously, and variants therein may contribute to the risk of HL. To address this issue to some extent, a CFDA-approved new microarray with an additional six common variants in *SLC26A4* (c.1174A>T [p.Asn392Tyr], c.1226G>A [p.Arg409His], c.1229C>T [p.Thr410Met], c.1975G>C [p.Val659Leu], c.2027T>A [p.Leu676Gln], and c.1707+5G>A) is now being employed for screening. Second, infants who screened positive for deafness-associated variants should be followed-up with long-term to determine whether they later experience HL. Third, the economic burden on the healthcare system and potential psychosocial impact on families of implementing population-wide newborn genetic screening for deafness should be evaluated prospectively in the future. Fourth, this study was based on the population in one area. There are clear genetic differences in HL among various racial and ethnic groups, thus the findings in this study may or may not apply to other racial and ethnic groups.

Conclusions

In summary, the highlights and strength of our study lie in performing concurrent hearing and genetic screening for 98%–99% of newborns annually for six years (initiated from April 2012 and including up to 1.58 million newborns screened through the end of 2018) in Beijing, a megacity with a population of 20.7 million. In this study, we report the systematic hearing and habilitation five-year follow-up of 180,469 newborns concurrently screened from April 2013 to March 2014 (one fiscal year). Concurrent newborn hearing and genetic screening in Beijing has revealed a significant level of hereditary HL in a large Chinese population. The interpretation and application of the current results have allowed us to develop preliminary deafness prevention strategies. Although our study focused on the prevention and control of deafness, it did not escape our attention that it potentially serves as a model for the diagnosis and prevention of other genetic diseases.

Supplemental Data

Supplemental Data can be found online at https://doi.org/10.1016/j.ajhg.2019.09.003.

Author Contributions

Acknowledgments

Declaration of Interests

The authors declare no competing interests.

Web Resources

Beijing Municipal Health Commission, http://wjw.beijing.gov.cn/
Centers for Disease Control and Prevention (CDC), https://www.cdc.gov/ncbddd/hearingloss/ ehdi-data2015
NCI Dictionary of Genetics Terms, https://www.cancer.gov/publications/dictionaries/ genetics-dictionary
OMIM, https://www.omim.org

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