Concurrent Hearing and Genetic Screening of 18,001 Neonates with Hearing Diagnose in Nantong, China

Jianhua Chen  
Affiliated Maternity and Child Health Care Hospital of Nantong University

Qingwen Zhu (✉️ 18912251305@163.com)  
Nantong Maternal and Children Hospital

Jingyu Li  
Nantong Maternal and Child Health Hospital

Jing Wang  
Nantong Maternal and Child Health Hospital

Wenjun Bian  
Nantong Maternal and Child Health Hospital

Mengsi Lin  
Nantong Maternal and Child Health Hospital

Wei Lei  
CapitalBio Technology Inc.

Research

Keywords: Hearing loss (HL), genetic screening, hearing screening, newborns, mtDNA

DOI: https://doi.org/10.21203/rs.3.rs-404100/v1

License: ©️ This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

Objectives: Concurrent hearing and genetic screening of newborns is expected to play an important role in the early detection and diagnosis of congenital deafness, which triggers an intervention, as well as in predicting late-onset and progressive hearing loss and identifying individuals who are at risk of drug-induced hearing loss (HL).

Methods: A Deafness Gene Variant Detection Array Kit covering fifteen variants in four genes was used to screen for deafness genes in 18001 infants.

Results: A total of 108 neonates did not pass the second hearing screening. In addition, 912 (5.07%) screened positive for deafness-associated variants, including 78 (0.43%) genetically referred and 834 (4.63%) genetic deafness-associated variant carriers. Of the 912 screened positive cases, 880 passed the hearing screening, and 32 failed. A total of 62 (0.34%) cases carried the mtDNA 12S rRNA variants. A total of 108 cases did not pass the hearing screening and underwent a hearing diagnostic examination. An expanded DNA test identified 17 patients who possessed deafness gene mutations, increasing the detection rate to 5.16%.

Conclusion: Early detection, diagnosis, and interventions are necessary for newborns who are susceptible to deafness. A good strategy is to use a small panel to quickly screen all subjects and then apply an extended panel to study the cause of deafness in affected patients.

1. Introduction

Hearing loss (HL) is the most common human neurosensory disorder. The reported incidence is approximately 1.9 per 1,000 children [1, 2]; this prevalence continues to increase during childhood and reaches 2.7 per 1,000 children before the age of 5 years and 3.5 per 1,000 during adolescence. HL has a primary genetic etiology [3] and it exerts a negative effect on speech, language, and cognitive development. Its early identification and management are of paramount importance for improving the language, communication, mental health, and employment prospects of hearing-impaired children [4].

Newborn hearing screening is widely performed worldwide and has played an important role in early detection and diagnosis, and allowing for early intervention [5]. Previous studies have, however, reported that hearing screening can miss newborns with severe to profound hearing loss [6, 7]. Previous epidemiological studies have shown that a majority of nonsyndromic hearing loss (NSHL) is caused by a limited number of genes with recurrent mutations in the Chinese population, including the gap junction beta-2 protein (GJB2), gap junction beta-3 protein (GJB3), solute carrier family 26 member 4 (SLC26A4), and mitochondrial 12S rRNA genes [8–11]. Thus, rapid screening of a limited number of hotspot variants is expected to provide inexpensive and timely clinical benefits [3, 12]. Many studies have evaluated concurrent focused genetic screening and hearing screening in the Chinese population and demonstrated that focused genetic screening could identify newborns who are otherwise missed by hearing screening alone and can identify individuals predisposed to ototoxicity risk [13–16].
In this study, we used a Deafness Gene Variant Detection Array Kit (CapitalBio), CFDA registration permit No. 20173401343, that tests for 15 variants in four genes. From 2018 to May 2020, a total of 18001 neonates were tested; all neonates underwent universal newborn hearing screening and genetic screening, and neonates who failed universal newborn hearing screening were advised to undergo a hearing diagnostic examination. We retrospectively analyzed the medical records of the universal newborn hearing screening results, genetic screening results and hearing diagnosis results, and compared the outcomes of hearing screening and genetic screening among infants with different results, and provided counseling, hearing tests, and follow-up. We emphasize the need for universal adoption of such a practice.

2. Materials And Methods

Subjects and newborn hearing screenings

From 2018 to May 2020, a total of 18 001 neonates were included in this study. Both conventional newborn hearing screening and concurrent genetic screening were conducted within 72 h after birth for all neonates. Neonates who failed the first hearing screening underwent a second hearing screening 42 days later. The clinical data of newborns who screened positive for deafness-associated genetic variants were followed up systematically. The diagnostic audiology evaluations included acoustic immittance testing, distortion product evoked otoacoustic emission, auditory brainstem response (ABR), and auditory steady-state response.

Genetic Screening

The Deafness Gene Variant Detection Array Kit (CapitalBio) was used to screen for fifteen 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, and c.2168A > G (p.His723Arg), IVS7-2 A > G, 1226 G > A (p.Arg409His), 1174 A > T (p.Asn392Tyr), 1229 C > T (p.Thr410Met), 1975 G > C (p.Val659Leu), 2027 T > A (p.Leu676Gln), and IVS15 + 5 G > A of SLC26A4; c.538C > T (p.Arg180*) of GJB3; and m.1555A > G and m.1494C > T of mtDNA 12S rRNA. Dried blood spots from all newborn infants were collected from Nantong Maternal and Child Health Hospital and six other hospitals in Nantong City. Genetic screening was performed for the 15 variants according to the test kit's instructions. The results were recorded as passes (all wild-type genotypes), refers (homozygotes or compound heterozygotes of GJB2 or SLC26A4, mtDNA 12S rRNA variants), or carriers (heterozygotes of GJB2 or SLC26A4 and heterozygotes or homozygotes 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.

Hearing Diagnosis

Infants who did not pass the two-step hearing screenings were evaluated using a series of diagnostic audiological tests by the age of 3 months. 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 the infants were older than 6 months, infantile behavioral audiometry was added to the examination. The severity of hearing loss was graded as profound (≥ 90 dB HL), severe (70–89 dB HL), moderate (50–69 dB HL), or mild (30–49 dB HL).

Expanded DNA Testing

Patients diagnosed with hearing loss were randomly selected for verification. We used an expanded panel including 227 deafness genes to perform whole-exome sequencing to study the genotype of patient deafness. Dried blood spots from all randomly selected patients underwent DNA extraction and quality inspection, and a total of 26 samples passed the quality inspection. We performed library construction and sequencing analysis of these 26 samples. Sanger verification of all positive samples was performed.

3. Results

Demographic Characteristics

In this study, all of the infants born between January 2018 and May 2020 underwent concurrent hearing and genetic screening between 72 h after birth and hospital discharge. Ninety-six percent of the infants were from Nantong Maternal and Child Health Hospital, and the remaining infants came from six other hospitals in Nantong City.

Results of Hearing Screening of 18001 Neonates

A total of 18,001 neonates were screened for hearing, and those who failed (1.99%, 359/18001) in the initial hearing screening were screened again at the age of 42 days. A total of 108 neonates did not pass the second hearing screening (Table 1).

Results of the Genetic Screening

Genetic screening data for the fifteen deafness-associated variants in the 18001 neonates and the variant spectra are shown in Table 2.

Of the 18,001 infants, 912 (5.07%) screened positive for deafness-associated variants, 78 (0.43%) were genetically referred, and 834 (4.63%) were suspected of being genetic deafness-associated variant carriers. Among the genetically referred infants, 10 (0.056%) had two variants in GJB2, six (0.033%) had two variants in SLC26A4, and 62 (0.34%) carried the mtDNA 12S rRNA variants. Among the deafness-associated variant carriers, 461 (2.56%) were heterozygote carriers of GJB2, 321 (1.78%) were heterozygote carriers of SLC26A4, and 46 (0.26%) had the GJB3 heterozygous variant. In this study, we also identified 6 (0.03%) infants who were double heterozygotes for variants in GJB2 and SLC26A4 (Table 2).
Associations between Hearing and Genetic Screening

Associations between the hearing and genetic screening are shown in Table 2. Of the 912 screened positive cases, 880 passed the hearing screening, and 32 failed. For the DNA-positive cases who passed the hearing screening, there was 1 case of GJB2 compound heterozygosity, c.235delC (p.Leu79Cysfs*3), c.299_300delAT (p.His100Argfs*14). Furthermore, 62 patients with mtDNA 12S rRNA variants passed the hearing screening. In addition, 817 carriers passed, including 453 GJB2 heterozygotes, 315 SLC26A4 heterozygotes, 46 GJB3 heterozygotes and 3 compound GJB2 heterozygotes with SLC26A4 heterozygotes.

There were 32 cases of positive cases who did not pass the hearing screening, including 15 of the referred cases (6 GJB2 homozygotes, 3 SLC26A4 homozygotes, 4 GJB2 compound heterozygotes and 2 SLC26A4 compound heterozygotes) and 17 carrier cases (8 GJB2 heterozygotes, 6 SLC26A4 heterozygotes, and 3 compound GJB2 heterozygotes with SLC26A4 heterozygotes). None of the infants who passed the hearing screening underwent the full hearing diagnosis examination. In addition, 76 genetically negative cases failed the hearing screening.

Hearing Diagnosis

Infants who did not pass the two-step hearing screenings were evaluated using a series of diagnostic audiological tests by the age of 3 months, and acoustic immittance was performed to determine the degree of HL. There were 108 infants who failed the hearing screening. Hearing diagnosis identified 50 cases graded as profound (≥ 90 dB HL), 40 cases graded as severe (70–89 dB HL), 13 cases graded as moderate (50–69 dB HL), and 4 cases graded as mild (30–49 dB HL). In addition, 1 case was graded as normal hearing (Table 4).

Expanded DNA testing results

A total of 26 qualified samples underwent expanded DNA verification testing. We used a panel testing for 227 hereditary deafness genes and detected 17 patients who possessed deafness gene mutations, resulting in a detection rate of 65.4% (17/26). Of the 17 cases, there were 10 cases of compound heterozygosity, 2 cases of homozygosity, and 5 cases of heterozygosity. If applied to the entire group, the detection rate would have been expected to increase to 5.16% (929/18001). The genetic test results of the 17 positive patients are shown in Table 4. A total of 27 heterozygous loci distributed on 14 genes and 2 homozygous loci distributed on 2 genes were detected. The highest frequency loci among the 17 babies was c.109G > A of the GJB2 gene. Following the American Society of Medical Genetics and Genomics (ACMG) guidelines, the pathogenicities of the variants were classified. Variants c.235delC and c.109G > A of GJB2 were classified as pathogenic, and variant c.571T > C of GJB2 was classified as likely pathogenic. All others were VUS. In addition, we followed these patients and obtained new hearing tests and information about the interventions applied. Two patients received cochlear implants, 14 patients wore a hearing aid, and 1 patient was lost to contact. None of the patients had any other apparent abnormalities aside from hearing impairment.
4. Discussion

Hearing screening for neonates was mandated in China by the Maternal and Infant Health Care Act of 2000. Delayed speech and language development caused by deafness can be avoided in most cases through early diagnosis, timely interventions, and habilitation. Conventional newborn hearing screening has certain limitations; for example, some types of HL may be missed by hearing screening shortly after birth [7, 17–19]. 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 [14, 15, 20, 21]. In addition, genetic findings also play an important guiding role in hearing aid fitting and the selection of candidates for cochlear implants [22, 23].

In this study, we performed simultaneous hearing screening and genetic screening for deafness in 18,001 newborns. There were 108 infants who failed the hearing screening, and we identified 912 babies (5.07%) with deafness gene mutations. Clinicians could provide genetic counseling and medical guidance according to concurrent hearing and genetic screening, which plays important roles in the early detection and diagnosis of congenital deafness. We emphasize the need for universal adoption of such a practice, and a good strategy is to use a small DNA panel to test all subjects and then apply an extended DNA panel to study the cause of deafness in selected patients.

We quantified a genetic carrier rate of 5.07% in this study, which is similar to that previously reported. A study of 58,397 Chinese newborns screened for 20 common pathogenic variants in four genes identified a genetic carrier rate of 5.52% [21], and a 180,469 study that screened for 9 common pathogenic variants in four genes identified a genetic carrier rate of 4.508% [13]. Of the 912 positive infants in this study, 78 were referred for additional testing and 834 were carrier cases. Of the 78 infants referred for additional testing, 63 passed the hearing screening and 15 failed. In the referred cases, there was 1 case of GJB2 compound heterozygosity c.235delC (p.Leu79Cysfs*3), c.299_300delAT (p.His100Argfs*14) who passed the hearing screening. In a previous study of 5,173 Chinese newborns screened for GJB2 c.235delC and c.109G > A (p.V37I), 42 (53.85%) of 78 newborns with two variants of GJB2 passed the hearing screening. A full hearing diagnosis was performed for this baby in the present study, and the results were normal. Similarly, 43 (38.39%) of 112 newborns with deafness-causing genotypes (GJB2 or SLC26A4) passed hearing screening in a study of 1,172,234 Chinese newborns [15]. Among those 43 newborns, nine were confirmed later via phone interview to have HL. Dai’s [13] study also showed that among 10 (25%) of 40 newborns with deafness-causing genotypes (GJB2 or SLC26A4) who passed hearing screening, nine were confirmed by audiological testing to have HL.

Thus, 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. Therefore, 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 [7, 17, 18]. We need to continue to pay attention to this child’s hearing over time.
Among the 834 carrier infants, 817 passed the hearing screening, and 17 failed, including 3 GJB2 heterozygotes with SLC26A4 heterozygotes, 6 SLC26A4 heterozygotes and 8 GJB2 heterozygotes. The 17 cases of GJB2 heterozygosity or SLC26A4 heterozygosity were found to have hearing loss in the full hearing diagnosis. In addition, there were 76 cases with negative results on the genetic screening but were found to have hearing loss in the full hearing diagnosis in the present study. The main reason for this may be that there are other heterozygotes that were not included in our 15 tested sites.

Therefore, to obtain more genetic information about the 76 cases who had negative results by the initial genetic screening but had hearing loss, we randomly selected some patients for a broader genetic panel. Thus, a total of 26 qualified samples underwent genetic panel detection and revealed 17 cases of genetic deafness mutations. The c.109G > A of the GJB2 gene was a high-frequency locus in this group. The missense variant of c.109G > A/p. Val37Ile was first identified as a polymorphism in a heterozygous control [24] and later found to be homozygous or in trans with known pathogenic GJB2 variants in affected individuals [25, 26]. Hearing loss may be unilateral or bilateral, from mild to profound, affecting different frequency ranges, even in individuals with the same genotype. There are some publications reporting individuals with c.109G > A/p.Val37Ile. However, case-level genotype and phenotype data are limited. Previous studies concluded that the average hearing threshold of infants with the c.109G > A homozygous genotype was 50 dB HL [27]. In the present study, there were 2 cases of GJB2 compound heterozygosity, one c.235delC with c.109G > A and one c.571T > C with c.109G > A, and there was one homozygous mutation of the c.109G > A variant among these 17 cases. The hearing diagnoses of these two compound heterozygous patients were 70 dB HL and 50 dB HL. The homozygous mutation of the patient’s hearing diagnosis was 70 dB HL. Therefore, more studies are needed to provide data about the case-level genotype and phenotype.

In addition, the addition of an expanded DNA panel improved the overall detection rate to 5.16%, and could also identify pathogenic information other than common mutations. However, it is not feasible from an economic perspective to use large panel genetic testing for all subjects. Obviously, the cost of larger panels is much higher. Therefore, it is a relatively good strategy to use a small panel to screen the subjects and to use a large panel to study the cause of deafness in selected patients only.

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 [28]. Neonates possessing mtDNA 12S rRNA are all potentially sensitive to aminoglycoside antibiotics, and their hearing may be compromised by even small amounts of such drugs. Because all of the participants were infants, they had not had any exposure to aminoglycoside antibiotics. It is advisable to distribute antibiotic selection instruction cards to these children's families, and they should be instructed to show these cards to their doctors during clinical visits to avoid exposure to aminoglycosides. In addition, individuals with m.1555A > G may develop hearing loss without exposure to aminoglycoside antibiotics [29]. Thus, identifying newborns at risk for ototoxicity will alert them and their family members to methods that can prevent or slow the progression of hearing loss and to monitor their hearing status to receive timely interventions when needed.
In this study, we selected 15 mutation sites of 4 genes to detect neonatal deafness genes, but this approach may not work in a more culturally diverse population. There are ethnic and regional differences in hotspot mutations. For example, in Brazil, the population is unevenly distributed in 5 main geographic regions. The 35delG mutation in the \textit{GJB2} gene is screened first in Brazil. Then, they perform sequencing of the entire coding region of the \textit{GJB2} gene and test for the A1555G and A827G mitochondrial mutations in the \textit{MTRNR1} gene [30].

Congenital deafness accounts for the overwhelming majority of cases among the prelingual deafness population. The timing of the identification of hearing impairment is very important because it can affect language capacity. Therefore, early detection, diagnosis, and intervention are necessary for newborns who are susceptible to deafness. Although our study only investigated a Chinese population, concurrent genetic and hearing screening may also prove beneficial in other populations.

**Abbreviations**

HL Hearing loss; NSHL nonsyndromic hearing loss; ABR Auditory brainstem response; ASSR auditory steady state response; DPOAE distortion product otoacoustic emission

**Declarations**

*Ethics approval and consent to participate*

This study was approved by the Ethics Committee of Nantong Municipal Maternal and Child Health Hospital

*Consent for publication*

The authors declare that they have no competing interests and the patients of their parents in this case report had provided their consent for publication.

*Availability of data and material*

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

*Competing interests*

The authors declare no conflicts of interest.

*Funding*

This work was financially supported by Maternal and Child Health in Jiangsu Province (No. F201673).
Authors' contributions

All authors have materially participated in the study and manuscript preparation. Qingwen Zhu designed the work; Jianhua Chen collected the clinical data and drafted the manuscript; Jingyu Li, Jing Wang, Wenjun Bian participated in the clinical data collection and carried out all the molecular genetic analyses; Mengsi Lin and Wei Lei revised the manuscript. All authors have approved the final article.

Acknowledgements

None.

References

1. Kennedy C, McCann D: Universal neonatal hearing screening moving from evidence to practice. Archives of disease in childhood Fetal and neonatal edition 2004, 89(5):F378-383.
2. 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 (Clinical research ed) 2001, 323(7312):536-540.
3. Morton CC, Nance WE: Newborn hearing screening—a silent revolution. The New England journal of medicine 2006, 354(20):2151-2164.
4. Yoshinaga-Itano C, Sedey AL, Coulter DK, Mehl AL: Language of early- and later-identified children with hearing loss. Pediatrics 1998, 102(5):1161-1171.
5. 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. The New England journal of medicine 2006, 354(20):2131-2141.
6. Dedhia K, Kitsko D, Sabo D, Chi DH: Children with sensorineural hearing loss after passing the newborn hearing screen. JAMA otolaryngology–head & neck surgery 2013, 139(2):119-123.
7. Minami SB, Mutai H, Nakano A, Arimoto Y, Taiji H, Morimoto N, Sakata H, Adachi N, Masuda S, Sakamoto H et al: GJB2-associated hearing loss undetected by hearing screening of newborns. Gene 2013, 532(1):41-45.
8. Dai P, Yu F, Han B, Liu X, Wang G, Li Q, Yuan Y, Liu X, Huang D, Kang D et al: GJB2 mutation spectrum in 2,063 Chinese patients with nonsyndromic hearing impairment. Journal of translational medicine 2009, 7:26.
9. Dai P, Yuan Y, Huang D, Zhu X, Yu F, Kang D, Yuan H, Wu B, Han D, Wong LJ: Molecular etiology of hearing impairment in Inner Mongolia: mutations in SLC26A4 gene and relevant phenotype analysis. Journal of translational medicine 2008, 6:74.
10. Xia JH, Liu CY, Tang BS, Pan Q, Huang L, Dai HP, Zhang BR, Xie W, Hu DX, Zheng D et al: Mutations in the gene encoding gap junction protein beta-3 associated with autosomal dominant hearing impairment. Nature genetics 1998, 20(4):370-373.
11. Yuan Y, You Y, Huang D, Cui J, Wang Y, Wang Q, Yu F, Kang D, Yuan H, Han D et al: Comprehensive molecular etiology analysis of nonsyndromic hearing impairment from typical areas in China. *Journal of translational medicine* 2009, 7:79.

12. Shearer AE, Shen J, Amr S, Morton CC, Smith RJ: A proposal for comprehensive newborn hearing screening to improve identification of deaf and hard-of-hearing children. *Genet Med* 2019, 21(11):2614-2630.

13. Dai P, Huang LH, Wang GJ, Gao X, Qu CY, Chen XW, Ma FR, Zhang J, Xing WL, Xi SY et al: Concurrent Hearing and Genetic Screening of 180,469 Neonates with Follow-up in Beijing, China. *Am J Hum Genet* 2019, 105(4):803-812.

14. Wang QJ, Zhao YL, Rao SQ, Guo YF, He Y, Lan L, Yang WY, Zheng QY, Ruben RJ, Han DY et al: Newborn hearing concurrent gene screening can improve care for hearing loss: a study on 14,913 Chinese newborns. *International journal of pediatric otorhinolaryngology* 2011, 75(4):535-542.

15. Wang Q, Xiang J, Sun J, Yang Y, Guan J, Wang D, Song C, Guo L, Wang H, Chen Y et al: Nationwide population genetic screening improves outcomes of newborn screening for hearing loss in China. *Genet Med* 2019, 21(10):2231-2238.

16. Wu CC, Tsai CH, Hung CC, Lin YH, Lin YH, Huang FL, Tsao PN, Su YN, Lee YL, Hsieh WS et al: Newborn genetic screening for hearing impairment: a population-based longitudinal study. *Genet Med* 2017, 19(1):6-12.

17. Norris VW, Amos KS, Hanks WD, Xia X, Nance WE, Pandya A: Does universal newborn hearing screening identify all children with GJB2 (Connexin 26) deafness? Penetrance of GJB2 deafness. *Ear and hearing* 2006, 27(6):732-741.

18. Pagarkar W, Bitter-Glindzicz M, Knight J, Sirimanna T: Late postnatal onset of hearing loss due to GJB2 mutations. *International journal of pediatric otorhinolaryngology* 2006, 70(6):1119-1124.

19. Chan DK, Schrijver I, Chang KW: Connexin-26-associated deafness: phenotypic variability and progression of hearing loss. *Genet Med* 2010, 12(3):174-181.

20. Chu CW, Chen YJ, Lee YH, Jaung SJ, Lee FP, Huang HM: Government-funded universal newborn hearing screening and genetic analyses of deafness predisposing genes in Taiwan. *International journal of pediatric otorhinolaryngology* 2015, 79(4):584-590.

21. Zhang J, Wang P, Han B, Ding Y, Pan L, Zou J, Liu H, Pang X, Liu E, Wang H et al: Newborn hearing concurrent genetic screening for hearing impairment-a clinical practice in 58,397 neonates in Tianjin, China. *International journal of pediatric otorhinolaryngology* 2013, 77(12):1929-1935.

22. Wu CM, Ko HC, Tsou YT, Lin YH, Lin JL, Chen CK, Chen PL, Wu CC: Long-Term Cochlear Implant Outcomes in Children with GJB2 and SLC26A4 Mutations. *PloS one* 2015, 10(9):e0138575.

23. Yan YJ, Li Y, Yang T, Huang Q, Wu H: The effect of GJB2 and SLC26A4 gene mutations on rehabilitative outcomes in pediatric cochlear implant patients. *European archives of oto-rhinolaryngology : official journal of the European Federation of Oto-Rhino-Laryngological Societies (EUFOS) : affiliated with the German Society for Oto-Rhino-Laryngology - Head and Neck Surgery* 2013, 270(11):2865-2870.
24. Kelley PM, Harris DJ, Comer BC, Askew JW, Fowler T, Smith SD, Kimberling WJ: Novel mutations in the connexin 26 gene (GJB2) that cause autosomal recessive (DFNB1) hearing loss. Am J Hum Genet 1998, 62(4):792-799.

25. Wilcox SA, Saunders K, Osborn AH, Arnold A, Wunderlich J, Kelly T, Collins V, Wilcox LJ, McKinlay Gardner RJ, Kamarinos M et al: High frequency hearing loss correlated with mutations in the GJB2 gene. Hum Genet 2000, 106(4):399-405.

26. Abe S, Usami S, Shinkawa H, Kelley PM, Kimberling WJ: Prevalent connexin 26 gene (GJB2) mutations in Japanese. J Med Genet 2000, 37(1):41-43.

27. Ma Y, Yang T, Li Y, Tao Z, Huang Z, Li X, Chai Y, Ouyang Z, Shen X, Wu H: Genotype-phenotype correlation of two prevalent GJB2 mutations in Chinese newborn infants ascertained from the Universal Newborn Hearing Screening Program. Am J Med Genet A 2010, 152a(11):2912-2915.

28. Barbarino JM, McGregor TL, Altman RB, Klein TE: PharmGKB summary: very important pharmacogene information for MT-RNR1. Pharmacogenetics and genomics 2016, 26(12):558-567.

29. Estivill X, Govea N, Barceló E, Badenas C, Romero E, Moral L, Scozzri R, D’Urbano L, Zeviani M, Torroni A: Familial progressive sensorineural deafness is mainly due to the mtDNA A1555G mutation and is enhanced by treatment of aminoglycosides. Am J Hum Genet 1998, 62(1):27-35.

30. Nivoloni Kde A, da Silva-Costa SM, Pomílio MC, Pereira T, Lopes Kde C, de Moraes VC, Alexandrino F, de Oliveira CA, Sartorato EL: Newborn hearing screening and genetic testing in 8974 Brazilian neonates. International journal of pediatric otolarhinolaryngology 2010, 74(8):926-929.

Tables

Due to technical limitations, table 1 to 4 is only available as a download in the Supplemental Files section.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- tables.xlsx