Journal of Multidisciplinary Healthcare

Dovepress
Taylor & Francis Group

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ORIGINAL RESEARCH

Neonatal Hearing Loss Risk Factors and miRNA Biomarkers Identified Through AABR Screening

Min Huang[1,*], Yunlong Jing[1,*], Xiangyue Peng[1], Jiang Xie[1], Qin Zhao[1], Jiang-Long Yin[1], Wen-Du Xiao[1], Si-Jun Zhao[1], Hong-Ju Tian[2]

[1]Department of Otorhinolaryngology, Head and Neck Surgery, The Affiliated Children's Hospital, Xiangya School of Medicine, Central South University (Hunan Children's Hospital), Changsha, Hunan, 410007, People's Republic of China; [2]Obstetrics and Gynecology Center of Hunan Occupational Disease Prevention and Control Institute, Changsha, 410007, People's Republic of China

*These authors contributed equally to this work

Correspondence: Si-Jun Zhao, Department of Otorhinolaryngology, Head and Neck Surgery, The Affiliated Children's Hospital, Xiangya School of Medicine, Central South University (Hunan Children's Hospital), Yuhua District, Changsha, 410007, People's Republic of China, Tel +86 07318535611, Fax +86073185356666, Email zhao_sijun@126.com; Hong-Ju Tian, Obstetrics and Gynecology Center of Hunan Occupational Disease Prevention and Control Institute, Yuhua District, Changsha City, Hunan Province, 410007, People's Republic of China, Tel +86 85303109, Fax +86 85534673, Email tianhongju713@outlook.com

Objective: To determine the prevalence of hearing impairment in neonates and identify associated risk factors through auditory brainstem response (ABR) screening.

Study Design: A prospective observational longitudinal study was conducted involving 158 infants, with Automated Auditory Brainstem Response (AABR) evaluations performed by 6 months of age.

Setting: The study was undertaken in a hospital-based neonatal care unit.

Methods: Infants with significant hearing loss in one or both ears were referred for further assessment and rehabilitation. The relationship between microRNAs (MiRNAs) and congenital hearing loss was confirmed through differential expression levels in newborns.

Results: Of the 158 infants, 8 showed abnormal AABR results. Univariable analysis identified 5 potential risk factors associated with hearing deterioration, with multivariable analysis pinpointing the number of maternal embryos, cytomegalovirus, and miR-431 as independently associated with hearing loss at 6 months ($P = 0.004$).

Conclusion: Early detection of hearing loss is vital for child development. Given the high incidence of hearing impairment in the study population, universal newborn hearing screening is essential. Assessment of miRNAs expression levels, maternal embryo count, and prenatal infections should be integrated into screening protocols for infants admitted for over 24 hours to prevent oversight of neural hearing loss cases.

Keywords: automated auditory brainstem response, neonatal screening, factors affecting hearing disorders

Introduction

The early identification and management of hearing disorders in neonates is a critical area of concern in paediatric health.[1] This is underscored by the profound impact that hearing impairments can have on the developmental trajectory of newborns. Newborns with hearing loss, whether bilateral or unilateral and particularly those with impairments above 1000 hz, face significant long-term consequences in their speech and language development.[2,3] Reduced auditory input during critical developmental phases can adversely affect the growth of the auditory nervous system and impede progress in social, emotional, behavioural, and cognitive domains. Furthermore, academic achievement, vocational opportunities, and economic self-sufficiency in later life can also be affected.[4,5]

The need for prompt identification is crucial due to the negative impact of late diagnosis on language growth. Such delays can impede timely communication development, missing the crucial 0–3 year period vital for speech and language.[6] Early hearing loss detection sets a benchmark for future assessments and informs treatment plans, including medical or surgical options for conductive loss. It also helps in embracing the condition and preparing for family-focused rehabilitation.[7,8]

Received: 15 November 2024
Accepted: 12 February 2025
Published: 8 May 2025

Even with newborn hearing screening programs in place globally, disparities in access and effectiveness are notable, particularly in developing regions.[9,10] The absence of national early detection initiatives for hearing loss in these areas results in many undiagnosed cases, with a reported prevalence of severe to profound hearing loss affecting about 4 per 1000 neonates.[11] In addition to environmental and prenatal risk factors, genetic factors are a major cause of congenital hearing loss, contributing to more than half of severe hearing loss cases in neonates.[12] Among these, mutations in the GJB2 (Connexin 26) and SLC26A4 (Pendrin) genes are particularly prevalent in the Chinese population. Studies have shown that GJB2 mutations account for approximately 17.34% of cases, while SLC26A4 mutations contribute to 15.83% of congenital hearing loss in northwest China.[13] In broader Chinese populations, the GJB2 gene is responsible for about 18.31% of cases of nonsyndromic hearing loss, and SLC26A4 mutations account for approximately 13.73%.[14] These findings underline the significance of screening for these genetic mutations in populations at risk, as their detection can facilitate early diagnosis and intervention, ultimately improving outcomes for affected neonates.

Hearing screening during the neonatal period is crucial for the timely detection and intervention of hearing loss. However, traditional screening methods primarily rely on physiological indicators, which may not fully identify individuals at potential risk of hearing impairment. In recent years, miRNAs, a class of small non-coding RNAs, have demonstrated significant roles in gene expression regulation and cellular differentiation, particularly in the development of complex organs such as the inner ear. miRNAs can influence the structural and functional development of the inner ear by regulating gene expression, and abnormal miRNAs expression may be associated with congenital hearing loss.[15] MiRNAs have diverse mechanisms, including binding to the 3' untranslated regions of target mRNAs, which can lead to mRNA degradation or translation prevention. In ear development, certain miRNAs are implicated in the structural formation and maturation of the ear, and they also play a role in the differentiation and upkeep of inner ear cells, modulating ion channels and signal transduction pathways related to hearing.[16] The abnormal expression of miRNAs in congenital hearing impairment can result in structural and functional disruptions of the inner ear.[17] Specific miRNAs, such as MiR-96, miR-183, and miR-431, are associated with hearing loss and may impact hair cell function and auditory transduction. miR-431, in particular, is of interest due to its connection with atypical inner ear development and its detectability as a biomarker in umbilical cord blood.

Our study aims to explore the current status of neonatal hearing screening and to investigate the factors influencing hearing disorders. Specifically, we hypothesize that certain miRNAs, particularly miR-431, exhibit differential expression in newborns with hearing loss compared to those with normal hearing, and that these miRNAs could serve as early biomarkers for hearing impairment. To test this hypothesis, we examine the expression levels of miRNAs such as miR-431, miR-183, and miR-96 in umbilical cord blood, alongside other factors including ABR thresholds, genetic mutations linked to hereditary deafness, and maternal factors such as embryonic quantity, birth weight, gestational age, and prenatal infections. By analyzing these elements, we aim to provide new insights into potential early intervention strategies that could enhance auditory health outcomes for newborns and inform future screening protocols.

Methods

Study Design

We conducted a prospective observational longitudinal study, which received approval from the institutional research board of the college. A total of 190 neonates were born in our hospital. The screening and data collection for this study were conducted from April 2023 to April 2024. This study received approval from the hospital's ethics review committee prior to commencement (Ethics Approval Number: [HCHLL-2024-301]) and strictly adhered to ethical review requirements. Before collecting miRNAs samples from the newborns, we thoroughly explained the study's purpose, methodology, potential risks, and benefits to the parents, ensuring their full understanding. miRNAs sample collection was only conducted after obtaining written informed consent from the parents to ensure that the research process complied with ethical standards.

Participants

We elected to enroll 158 neonates in the study. To select the participants, all neonates born during the study period were initially categorised into two groups: those with and without risk factors, based on the predetermined criteria outlined in the Joint Committee on Infant Hearing (JCIH) screening. Subsequently, each neonate was assigned a sequential number

and enrolled in the study using a random number generated computer method. Inclusion criteria: All newborn babies born in our hospital. Exclusion criteria: Fail to get parental consent.

Clinical Management

During the study period, neonates who exhibited signs of suspected sepsis or clinical sepsis received cefotaxime and amikacin as the initial empirical antibiotic therapy. In cases of newborns with asphyxial encephalopathy and concomitant sepsis, nephrotoxic drugs were avoided in the treatment regimen. If cultures yielded sterile results, antibiotic therapy was discontinued within 48 hours. As a second-line approach, piperacillin/tazobactam or cefepime was administered, and antibiotic selection was adjusted, based on the sensitivity pattern of the microorganism. In neonates with renal failure, drug doses were adjusted as necessary to ensure safe and effective treatment.

Risk Factors for Hearing Loss

Among the total of 190 newborns included in the study, 158 had no identified risk factors for hearing loss, while 32 newborns were found to have 1 or more risk factors as per the JCIH criteria. The assessed risk factors included the following: 1. non-syndromic deafness 2. syndromic deafness 3. intrauterine infections during pregnancy, especially TORCH (toxoplasma, rubella, cytomegalovirus, herpes) 4. ototoxic medication (eg aminoglycosides, loop diuretics, quinine derivatives). 5. alcohol and/or drug intake during pregnancy 6. prematurity (gestational age under 34 weeks, birth weight under 1,500 g) 7. severe perinatal hypoxia 8. infections (particularly Pneumococcus, Haemophilus influenzae meningitis, and encephalitis). 9. hyperbilirubinemia (blood bilirubin level >20 mg/dl). 10. ototoxic drugs (aminoglycosides and furosemide administration for more than 5 days without serum level monitoring) 11. neonatal pulmonary hypertension – mechanical ventilation for 5 days or longer (extracorporeal membrane oxygenation) 12. traumatic delivery 13. severe intracranial haemorrhage 14. neonatal convulsion 15. noise-induced hearing loss (preterm occurrence). Children who demonstrated normal (AABR) results were considered biologically sound, and no further evaluation was recommended for these children.

AABR Testing

In this study, we did not perform tympanometry prior to the AABR testing. While tympanometry is useful for evaluating the middle ear status, it was not deemed necessary in this study as the AABR system is primarily used for early screening of auditory responses in newborns. AABR is effective in detecting hearing loss regardless of mild middle ear issues that may not significantly affect auditory brainstem responses. Therefore, we proceeded directly with AABR testing to efficiently identify potential hearing impairments in the infants.

The AABR testing in our study was conducted under natural sleep conditions using an Evomatic 4000 evoked potential unit (Medtronic, Minneapolis, MN, USA). Standard Ag/AgCl electrodes were applied to the forehead and each mastoid, and paediatric insert earphones were connected to Etymotic ER3A stimulators (Etymotic Research, Elk Grove, IL, USA). This system is suitable for neonatal hearing screening. Unlike diagnostic ABR, AABR is primarily used for early screening of auditory responses in newborns to quickly identify infants who may have hearing loss.

The AABR evaluations were administered by a trained audiologist and were provided free of charge. The stimuli used included 100-millisecond rarefaction clicks and tone pips, presented at a rate of 25 per second, for a minimum of 1,000 presentations, with alternating triggering to enable the simultaneous examination of both ears.

In our study, all AABR tests were conducted under natural sleep conditions using a Medtronic Evomatic 4000 evoked potential system, with standard Ag/AgCl electrodes on the forehead and mastoid, and Etymotic ER3A insert earphones. Tests were performed by trained audiologists free of charge. Stimuli included 100-millisecond clicks and tone bursts at 25 per second, with a minimum of 1000 presentations. Filter settings were 100–3000 hz, with contralateral masking to prevent cross-hearing. The cutoff values for normal responses were 23–26 dB for clicks and 30 dB NHL for tone pips. Infants with confirmed significant hearing loss were referred to an ENT specialist for further evaluation and rehabilitation.

MiRNAs Collection

Within 24 hours after birth, samples were collected from umbilical cord blood to assess miRNAs expression levels using qPCR. The collected blood samples were immediately processed and frozen to ensure sample integrity and the accuracy of analysis results. All sample collection and processing were conducted by trained research personnel following standardized operating procedures to ensure data quality and experimental reproducibility.

Statistical Analysis

Patient details were logged in Microsoft Excel. We used SPSS software for analysis, applying Student's *t*-test and chi-square test for stats. At the same time, potential confounding factors such as maternal age were controlled through multivariable risk regression analysis to examine the independent association between miR-431 and hearing loss. Results with a p-value under 0.05 were seen as significant, suggesting the findings were not random but meaningful to the study.

Results

Screening Results

In the current study, we conducted initial screenings of 190 newborns using AABR at the age of 6 months. Among the screened infants, the AABR results indicated significant hearing loss for 15 infants. Among the 15 newborns with abnormal AABR results, 7 were in the group with identified risk factors, while the remaining 8 were in the group without any identified risk factors.

AABR Testing

In our study, a preliminary screening of newborns' hearing was conducted, along with click and tone-burst ABR (auditory brainstem response) tests. The click ABR test used rarefaction click stimuli to observe the emergence of wave V, establishing a normal response threshold of 23 to 26 dB. The tone-burst ABR test utilized tone stimuli, with a normal response threshold of 30 dB NHL (normal hearing level).[18] In this research, all tests were performed under natural sleep conditions and were provided free of charge by professional audiologists. Although we did not detail these specific results in the report, all newborns identified with significant hearing loss have been referred to otolaryngology specialists for further comprehensive assessment and rehabilitation.

Study Population

The demographic data of the study population, including factors such as gestational age, and the expression levels of MiRNAs, including miR-431, miR-183, and miR-96 in the groups without identified risk factors, are summarised in Tables 1 and 2.

Risk Factors for Hearing Loss

Univariable risk regression of the risk factors of hearing loss in infants (aged 6 months) were conducted. The risk factors of these infants were the number of maternal embryos (RR 0.039, 95% confidence interval [CI] 0.013–0.121), cytomegalovirus (CMV) (RR 0.135, 95% CI 0.023–0.785), miR-431 (RR 20.283, 95% CI 0–57.73), miR-183 (RR −2.257, 95% CI 0–12.59), and miR-96 (RR −3.409, 95% CI 0–25.74) as shown in Table 3.

Multivariable Risk Regression Analysis

The multivariable risk regression results (controlling for confounding factors) of the risk factors of hearing loss in infants (aged 6 months) were analysed. The risk factors at 6 months were the number of maternal embryos (RR 1.000, 95% CI 0–1.977), CMV (RR 1.000, 95% CI 0–1.978), and miR-431 (RR 11.988, 95% CI 0–11.92) as shown in Table 4.

MiRNAs Expression and Hearing Loss

We present the data from this study in the form of a flowchart, as shown in Figure 1. This is consistent with previous studies, the elevated expression of miR-431 in the hearing loss group is related to auditory developmental abnormalities, indicating its potential as a biomarker for hearing loss. In contrast, miR-183 and miR-96 are expressed at higher levels in the normal hearing group, suggesting their role in maintaining normal auditory function.

Table 1 Demographic Characteristics and Risk Factors of Study Subjects

Characteristics	Hearing Loss (n=8)	Normal (n=150)	*P*-value
Number of Maternal Embryos			<0.000
1	4 (50%)	148 (98.7%)	
2	4 (50%)	2 (1.3%)	
Toxoplasma			0.054
Positive	0 (0%)	1 (0.7%)	
Negative	8 (100%)	149 (99.3%)	
Rubella Virus			/
Positive	0 (0%)	0 (0%)	
Negative	8 (100%)	150 (100%)	
Cytomegalovirus			0.084
Positive	1 (12.5%)	2 (1.3%)	
Negative	7 (87.5%)	148 (98.7%)	
Herpes Simplex Virus			0.054
Positive	0 (0%)	1 (0.7%)	
Negative	8 (100%)	149 (99.3%)	
Syphilis			0.108
Positive	0 (0%)	2 (1.3%)	
Negative	8 (100%)	148 (98.7%)	

Table 2 Maternal Gestational Age and miRNA Expression Levels

Characteristics	Hearing Loss (n=8)	Normal (n=150)	*P*-value
Maternal Gestational Age	38.5 ± 2.027	39.2 ± 1.225	<0.000
miRNA-431	83.3 ± 7.538	49.9 ± 9.029	<0.000
miRNA-183	98.4 ± 4.838	135.6 ± 6.518	<0.000
miRNA-96	51.6 ± 3.897	77.3 ± 4.693	<0.000

Table 3 Univariable Risk Regression of Risk Factors of the Hearing Loss of Infants

Characteristics	Risk ratio	95% Confidence Interval		*P*-value
		Lower	Upper	
Number of maternal embryos	0.039	0.013	0.121	<0.000
Cytomegalovirus	0.135	0.023	0.785	0.024
Maternal gestational age	0.716	0.473	1.084	0.114
MirRNA-431	20.283	0.000	57.73	0.004
MirRNA-183	−2.257	0.000	12.59	<0.000
MirRNA-96	−3.409	0.000	25.74	<0.000

Table 4 Multivariable Risk Regression of Risk Factors of the Hearing Loss of Infants

Characteristics	Risk Ratio	95% Confidence Interval		*P*-value
		Lower	Upper	
Number of maternal embryos	1.000	0.000	1.977	<0.000
Cytomegalovirus	1.000	0.000	1.978	<0.000
MiRNA-431	11.988	0.000	11.92	0.004

https://doi.org/10.2147/JMDH.S506757

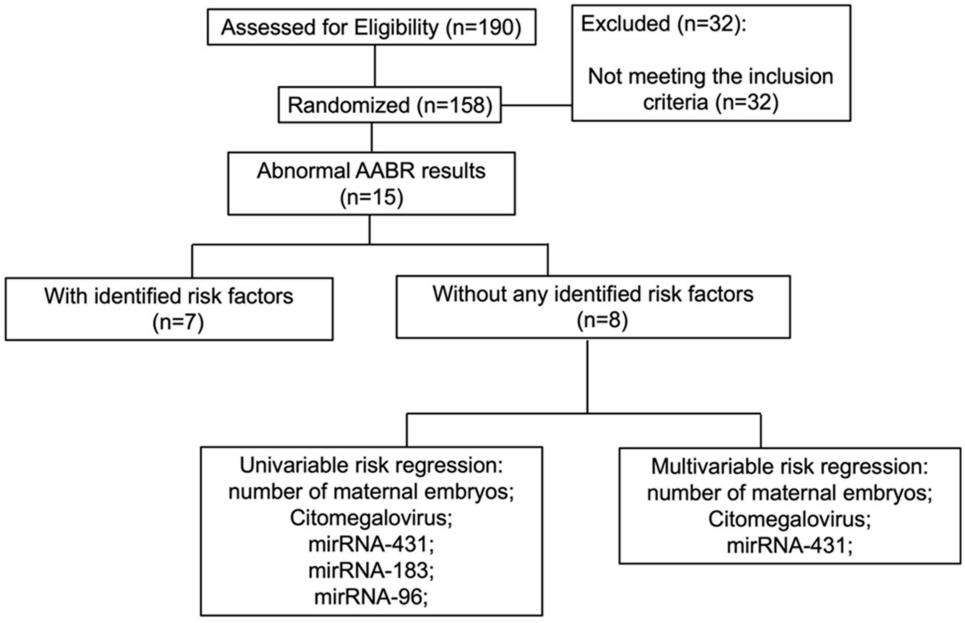

Figure 1 Flow Diagram for Participant Enrollment and Analysis in Clinical Study.

Discussion

Hearing loss is a complex condition that can be attributed to a variety of genetic and environmental factors. Genetic causes of congenital hearing loss are particularly important, as these genetic factors may involve mutations in many genes that affect the development and function of the auditory system.[19] In addition to the GJB2 and SLC26A4 genes, mutations in the GJB3 gene have also been found to be associated with hearing loss in some Asian populations, although the exact mechanisms are not yet fully understood.[20] Furthermore, mutations in the MT-RNR1 gene, particularly the m.1555A>G mutation, are associated with mitochondrial hereditary hearing loss, and the risk is further increased in the presence of noise exposure or the use of certain drugs (such as ototoxic drugs).[13,20] Another gene associated with hearing loss is the SLC26A5 gene, whose mutations are linked to congenital hearing loss and inner ear malformations (such as enlarged vestibular aqueduct), especially in patients with Pendred syndrome and non-syndromic hearing loss.[21]

In addition to these recognized genetic factors, recent studies have begun to emphasize the role of miRNAs in the development and maturation of the auditory system. MiRNAs are crucial for the development and maturation of the auditory system in mammals, especially within the inner ear. Studies have identified numerous miRNAs involved in auditory function, though the roles of many remain unclear.[22] The miR-183 family, including miR-96, miR-182, and miR-183, is essential for auditory development; deletion of this cluster in mice leads to severe hearing defects, such as progressive hearing loss and malformed stereocilia.[23] Mutations in miR-96 specifically have been linked to hereditary deafness in both mice and humans.[24,25] Similarly, miR-431 is associated with hearing loss and impacts inner ear maturation, with overexpression leading to reduced spiral ganglion neuron density by targeting genes like Eya4.[26,27] miR-124 also regulates pathways, such as the WNT pathway, essential for hair cell differentiation and stereocilia orientation.[28,29] These findings highlight the significant role of specific miRNAs in congenital hearing loss, providing insight into molecular mechanisms and potential therapeutic targets for intervention.

In this study, initial screenings using AABR of 190 newborns at the age of 6 months revealed that 15 infants had significant hearing loss. Seven of these infants had identifiable risk factors, while the remaining 8 exhibited no known risks. This result underscores the importance of universal screening and challenges the notion that only infants with known risk factors should be tested. The observed high incidence of hearing loss in our study is a significant finding that warrants in-depth discussion. It suggests that the prevalence of hearing impairment may be more widespread than previously estimated, particularly within our specific study population. This could be attributed to a variety of factors, including genetic predispositions, environmental influences, and prenatal or perinatal risk factors that were more

prevalent in our cohort. The high incidence underscores the importance of universal neonatal hearing screening and the need for further research to identify and address modifiable risk factors. It also highlights the potential role of miRNAs as emerging biomarkers for early detection and intervention in hearing loss.

The demographic data, including gestational age, gender, and the expression levels of miRNAs (miR-431, miR-183, and miR-96) provide crucial insights. Although the sample size was relatively small, the results highlight the intricate relationship between genetics and perinatal factors. The results of the present study also showed significant differences in the expression levels of miR-431, miR-183, and miR-96 detected in the umbilical cord blood of newborns in the hearing loss and normal groups. Expression levels of miR-431, in particular, are significantly associated with hearing impairment, underscoring their potential as biomarkers for hearing loss.

During the study period, neonates showing signs of suspected or clinical sepsis were treated with cefotaxime and amikacin as an initial empirical antibiotic therapy. For newborns with asphyxial encephalopathy and concurrent sepsis, nephrotoxic drugs were avoided to prevent additional complications. As a second-line approach, piperacillin/tazobactam or cefepime was used, adjusting the choice according to microbial sensitivity patterns.[30] This strategy minimised adverse effects and ensured optimal recovery.

Univariable risk regression analysis identified several significant risk factors, including the number of maternal embryos (RR 0.039, 95% CI 0.013–0.121) and CMV infection (RR 0.135, 95% CI 0.023–0.785). The miR-431 expression level (RR 20.283, 95% CI 0–57.73) was notably associated with hearing loss, aligning with previous studies linking microRNAs to auditory dysfunction.

Multivariable risk regression analysis confirmed maternal embryos (RR 1.000, 95% CI 0–1.977), CMV infection (RR 1.000, 95% CI 0–1.978), and miR-431 (RR 11.988, 95% CI 0–11.92) as significant risk factors. The overlapping confidence intervals indicated variability in their impacts across different populations, warranting further investigation.

The finding that maternal embryo number is a significant risk factor aligns with prior research. In our analysis, the number of maternal embryos (RR 0.039, 95% CI 0.013–0.121) was inversely related to the risk of hearing loss, which contrasts with other risk factors, such as CMV infection and miRNAs expression levels. The relationship between maternal embryo number and neonatal hearing loss has also been explored in other studies. For example, Umranikar et al found that infants from multiple-embryo pregnancies, such as twins or triplets, had a higher risk of preterm birth and subsequent hearing impairments due to complications like low birth weight.[31] Twin pregnancy itself may not be considered a direct cause of hearing loss, but it has been linked to a number of conditions that can lead to hearing loss, such as: Preterm labour: Twin pregnancies are more likely to lead to preterm labour, and preterm babies are more likely to develop hearing loss due to incomplete development. Low birth weight: newborns in twin pregnancies may have a low birth weight and low birth weight babies are at a higher risk of hearing loss. Risk of hypoxia: In twin pregnancies, the foetus may be at higher risk of hypoxia, which can lead to hearing loss. Infections: Twin pregnancies may increase the risk of infections in the mother. Certain infections, such as rubella and cytomegalovirus, may cause damage to the foetus' hearing. Medical interventions: Twin pregnancies may require additional medical interventions, such as the use of certain medications or surgery, which may be associated with hearing loss. Problems with the placenta and umbilical cord: Twin pregnancies have an increased risk of problems with the placenta and umbilical cord, which may affect the foetus's blood supply and oxygenation, which in turn may affect hearing development.

In this study, CMV infection was identified as a significant risk factor for neonatal hearing loss. Our findings indicate that CMV (RR 0.135, 95% CI 0.023–0.785) contributes notably to auditory impairment, reinforcing results from the existing literature. Congenital CMV infection is well-documented as one of the leading non-genetic causes of hearing loss in children.[32,33] Hilditch et al previously established CMV's strong correlation with sensorineural hearing loss (SNHL), highlighting that infants with CMV are at a higher risk than their uninfected peers.[33]

In terms of, miRNAs profiling, our study's identification of significant associations between miR-431 and miR-96 expression levels and hearing impairment is consistent with previous studies reporting microRNAs as potential biomarkers for auditory dysfunction.[34,35] However, while these studies focused on profiling using animal models, our research extends these findings to a clinical neonatal population, highlighting their relevance for real-world applications.

Overall, the findings of this study highlight the need for comprehensive neonatal screening programmes and further exploration into the genetic and environmental factors influencing hearing loss. The integration of miRNAs profiling with traditional screening methods could help to enhance early identification and enable targeted interventions to improve outcomes. Future studies should focus on expanding sample sizes and investigating long-term outcomes in infants diagnosed with hearing loss. The integration of miRNAs profiling with traditional screening methods could enhance early identification of hearing loss and enable more targeted interventions to improve outcomes. In particular, miRNAs biomarkers hold potential application value in regions with limited resources, where comprehensive screening programs may be challenging to implement. The use of miRNAs biomarkers as a supplementary or alternative screening tool could improve screening feasibility and efficiency in such settings, allowing for early detection even when access to standard screening equipment is restricted. However, the sample size in this study is relatively small, including only 158 newborns, of whom only 8 had hearing loss. The small sample size limits the power of statistical analysis and may affect the representativeness of the study results. Therefore, the conclusions of this study need to be validated with a larger sample size. We recommend that future studies use larger cohorts to further confirm the differential expression of miR-431 and its association with hearing loss. Additionally, a larger sample size would help identify potential confounding factors, thereby enhancing the robustness of the analysis and supporting the utility of miR-431 in hearing loss screening.

Conclusion

This study highlights the importance of universal neonatal hearing screening, particularly through AABR, and the value of identifying risk factors early in infancy. Our research emphasizes the role of miRNAs profiling, particularly the expression levels of miR-431, miR-183, and miR-96 in umbilical cord blood, which are significantly associated with congenital hearing loss. While miR-431 and miR-96 show potential as biomarkers for hearing loss, we acknowledge that these findings should complement, rather than replace, traditional screening methods such as ABR and genetic screening, which are widely accepted in China. The identification and management of risk factors, such as CMV infection and maternal embryo count, remain essential for reducing the incidence of hearing loss. Future studies should focus on larger sample sizes to validate these findings and further explore preventive strategies for managing prenatal infections and genetic predispositions.

Ethics Statement

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of Hunan Children's Hospital. The legal guardians provided their written informed consent to participate in this study.

Funding

Hunan Provincial Department of Science and Technology (Project No. 2019Sk1015); Hunan Provincial Health Commission (Project No. B2016037); Hunan Provincial Department of Science and Technology (Project No. 2018ZK4073); Hunan Provincial Department of Science and Technology (Project No. 2017SK50703).

Disclosure

The authors declare that they have no competing interests in this work.

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