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ORIGINAL ARTICLE

Clinical characteristics of newborns with the deafness-associated GJB2 variant p.V37I in Changzhi, China

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ABSTRACT

Introduction: Hearing loss affects over 430 million people globally, with genetic factors accounting for at least 50% of congenital cases. The GJB2 gene mutations are the most common cause of non-syndromic hearing impairment (NSHI) worldwide, but the clinical characteristics of the GJB2 gene missense mutation c.109G>A, a variant that causes the protein-level amino acid substitution p.V37I, remain indistinct. The objective of this study is to evaluate the prevalence and clinical impact of the c.109G>A mutation in the GJB2 gene among neonates through audiological and genetic tests. **Methodology**: A cohort of 848 neonates was enrolled between February 2022 and February 2024. All subjects underwent hearing screening and deafness genetic screening for 7 hotspot variants in the GJB2 gene (c.109G>A, c.35delG, c.176_191del16, c.235delC, c.299_300delAT, c.79G>A, and c.341A>G). The c.109G>A mutation, its compound heterozygotes, and its respective hearing phenotypes were assessed. **Results**: The c.109G>A mutation was identified in 24 individuals (2.83%), including 11 heterozygotes, 12 compound heterozygotes, and one homozygote. Audiological test failure rates were significantly higher in c.109G>A carriers compared to non-carriers (18.2% vs. 0.7%, $P < 0.001$). The homozygous proband exhibited confirmed bilateral hearing loss, and a significant association was observed between genotype and hearing outcomes overall ($P = 0.003$). **Conclusion**: This study assessed the prevalence of GJB2 mutations and hearing screening outcomes in a Changzhi neonatal cohort. The results demonstrate a significant

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association between the c.109G>A variant and an increased risk of hearing loss, particularly in homozygous and compound heterozygous carriers. These findings demonstrate that GJB2 variant c.109G>A is significantly associated with neonatal hearing loss, underscoring its importance for inclusion in genetic screening programs to facilitate early intervention.

1. INTRODUCTION

According to the World Health Organization, over 5% of the global population—approximately 430 million people, including 34 million children—suffers from disabling hearing loss, a number projected to exceed 700 million by 2050 [1]. The causes of hearing loss are diverse, involving anatomical, infectious, and environmental factors [2]. However, genetic factors account for at least 50% of congenital or childhood hearing loss, with non-syndromic forms making up 70% of these cases, of which 80% follow an autosomal recessive inheritance pattern [3, 4].

Among the known genetic contributors, the GJB2 gene, which encodes connexin 26 protein for direct cell-to-cell communication, is the most frequently implicated in hereditary deafness worldwide. Mutations in GJB2, particularly in exon 2, disrupt gap junction function and are responsible for a significant proportion of autosomal recessive non-syndromic hearing loss [5].

Although over 50 GJB2 variants have been studied functionally, the impact of many remains unclear or disputed [6]. The c.109G>A (p.V37I) variant exhibits variable clinical expressivity [7]. It can cause bilateral or unilateral hearing loss ranging from mild to severe, sometimes even in individuals with the same genotype [8]. This mutation has also been linked to sudden-onset and progressive hearing loss [9].

Therefore, the present study aims to investigate the mutation spectrum of the GJB2 gene in our cohort and the pathogenic characteristics of the c.109G>A mutation. We focus on hotspot variants in the GJB2 gene and perform subgroup analyses based on gene sequencing and audiological evaluation techniques, with an emphasis on direct gene sequencing as the most accurate method for mutation identification. Elucidating the population distribution and pathogenicity of c.109G>A is critical for facilitating early diagnosis and guiding intervention strategies [10].

2. MATERIALS AND METHODS

2.1 Subjects

This study enrolled 848 neonates in Changzhi City, China, between February 2022 and February 2024. Following informed parental consent, heel-prick blood samples were collected 2–3 days after birth for concurrent genetic sequencing and audiological testing. Among the participants, 423 were identified as carriers of one or more of six GJB2 gene hotspot variants (c.35delG, c.176_191del16, c.235delC, c.299_300delAT, c.79G>A, and c.341A>G), while 425 were non-carriers.

Inclusion criteria comprised: (i) birth in Changzhi City; (ii) good general health; and (iii) parental residency in Changzhi. Exclusion criteria were: (i) samples that failed to meet national technical standards; (ii) poor infant health precluding testing; and (iii) non-resident parents.

2.2 Ethical approval

The genetic analysis was conducted at the Molecular Genetics Laboratory of Changzhi Maternal and Child Health Hospital. The study protocol was approved by the Institutional Ethics Committees of Changzhi Maternal and Child Health Hospital (Approval No.: CZSFYLL2025002) and Universiti Teknologi MARA (REC/05/2025[PG/MR/259]).

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2.3 DNA extraction and Sanger sequencing

Genomic DNA was isolated from dried blood spots with the TIANGEN Nucleic Acid Purification Kit (Beijing, China). Multiplex PCR was used to amplify target regions of the GJB2 gene encompassing seven loci (c.235delC, c.299delAT, c.176del16, c.109G>A, c.79G>A, c.341A>G, and c.35delG), employing the forward primer 5'-GTGTAAGAGTTGGTGTTTGCT-3′ and the reverse primer 5'-GAGCAGTCCACAGTGTTGG-3′. The amplified products were subsequently sequenced with the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (ABI, USA). Sequence chromatograms were analysed using Chromas 7.10 and DNAMAN 6.0.40 software (Fig S1).

2.4 Audiological evaluation and follow-up

Audiological screening was conducted between 2–3 days after birth using otoacoustic emissions (OAE) and/or automated auditory brainstem response (AABR). Hearing loss was defined as a threshold ≥25 dB in both ears. Infants who failed the initial and one-month re-screening underwent diagnostic audiological assessments to confirm hearing loss. Positive cases were followed up 6 months later to monitor auditory development.

2.5 Data and statistical analysis

Data analysis was performed using SPSS Statistics (IBM Corp, released 2011) and R software (version 4.3.3) in the R Studio environment. Descriptive statistics were used to summarize demographic and clinical characteristics. The association between groups was assessed using Fisher's exact test. A $P< 0.05$ was considered statistically significant.

3. RESULTS

Among the 848 neonates analysed, 24 individuals (2.83%) carried the GJB2 c.109G>A mutation. The mutation was slightly more prevalent in females (2.97%) than in males (2.68%). By genotype, 11 were single heterozygotes (1.29%), 12 were compound heterozygotes (1.41%), and 1 individual was homozygous (0.12%) (Table 1). Compound heterozygosity, most frequently with c.235delC, was the predominant form, suggesting its clinical relevance in this population.

Table 1. Distribution of GJB2 mutation in 848 individuals

Category	c.109G>A mutation carriers	Total cases	c.109G>A mutation frequency (%)
Gender			
Male	11	410	2.68
Female	13	438	2.97
Genotype			
Single heterozygous	11	58	1.29
Compound heterozygous	12	359	1.41
Homozygous	1	3	0.12
Non- carriers	0	428	0

Source: Ruijie Feng et al. (2026)

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3.1 Genotype-phenotype associations carrying the c.109G >A variant

Audiological outcomes were assessed in all carriers of the c.109G>A variant. Of the 11 heterozygous individuals, 2 (18.2%) failed the initial hearing screening. Among 12 compound heterozygotes, one (c.235delC/c.109G>A) failed the test. However, no confirmed cases of hearing impairment or follow-up data were available for these groups. The single homozygous case exhibited bilateral hearing loss and was confirmed as a case of hearing impairment (Table 2). These findings suggest that both heterozygous and compound heterozygous genotypes involving c.109G>A may contribute to hearing loss.

Table 2. Genotype and phenotype association of the participants with c.109G>A variation

Genotype	Type	Total	Failed in Audiological Test	Confirmed cases	Follow up
c.109G>A	Heter	11	2	–	–
c.35delG/c.109G>A/c.79G>A	CP	1	–	–	–
c.176-191del16/c.109G>A	CP	1	–	–	–
c.235delC/c.109G>A	CP	8	1	–	–
c.235delC/c.109G>A/c.79G>A	CP	1	–	–	–
c.235delC/c.109G>A/c.341A>G	CP	1	–	–	–
c.109G>A/c.109G>A	Homo	1	1	1	1

Notes: – means no event observed; Heter, heterozygote; Homo, homozygote; CP, compound heterozygote in a single gene.

Source: Ruijie Feng et al. (2026)

3.2 Analysis of hearing screening outcomes stratified by c.109G>A genotype

A Fisher's exact test revealed a significant association between participant genotype and audiological screening outcome ($P = 0.003$; Table 3). The hearing screening failure rate was highest among c.109G>A heterogeneous at 18.2% (2/11), compared to 0.7% in non-carriers (3/428), 1.5% in heterogeneous without the c.109G>A variant (6/395), and 7.7% in compound heterogeneous that included the c.109G>A variant (1/13). Relative risk analysis quantified this association, showing that c.109G>A heterogeneous had a 31.56-fold increased risk of failing the hearing test compared to non-carriers (95% CI: 3.82–214.65, $P < 0.001$). Compound heterogeneous with c.109G>A also had a significantly elevated risk (RR = 11.83; 95% CI: 0.563–100.49; $P = 0.038$). As shown in Figure 1, the bar chart compares the proportion of newborns who failed the hearing screening across four genotype groups. The c.109G>A heterozygous group shows the highest failure rate, indicating a significant association between this genotype and hearing impairment.

Table 3. Distribution of c.109G>A mutation in 848 individuals

Group	Audiological test		P value
	Passed	Failed	
Genotype of Participants			0.003
Negative	425	3	
c.109G>A heterozygous	9	2	
Heterozygous without c.109G>A	389	6	
Compound heterozygous with c.109G>A	12	1	

Source: Ruijie Feng et al. (2026)

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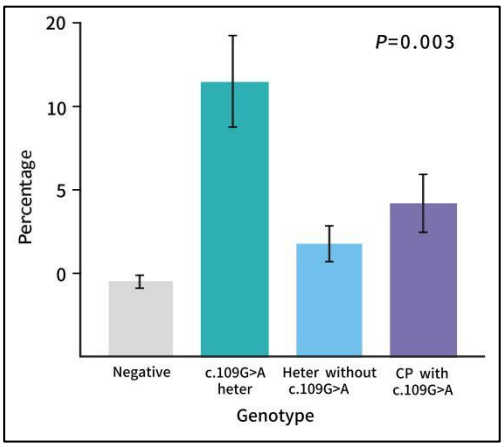

Fig. 1. Audiological Test Failures Among Neonatal Genotypes

Source: Ruijie Feng et al. (2026)

4. DISCUSSION

In this cohort of 848 neonates, the c.109G>A variant was detected in 24 individuals, yielding a carrier frequency of 2.83%. The genotypic distribution comprised 11 heterozygotes, 12 compound heterozygotes—most commonly paired with the c.235delC variant—and one homozygous case. A modestly higher carrier frequency was observed among females (2.97%) relative to males (2.68%). Audiological screening identified hearing test failures in 18.2% (2/11) of heterozygous carriers and 7.7% (1/13) of compound heterozygotes, while the homozygous individual presented with bilateral hearing loss.

A significantly elevated risk of hearing impairment was observed among c.109G>A heterozygotes compared to non-carriers (18.2% vs. 0.7%; $p < 0.001$), a finding corroborated by a significant Fisher's exact test ($p = 0.003$). Although compound heterozygotes also exhibited increased risk (RR = 11.83), the broad confidence interval underscores the need for confirmation in larger studies. Notably, the higher failure rate among heterozygotes compared to compound heterozygotes suggests possible independent pathogenicity of the c.109G>A variant. Nevertheless, these results warrant cautious interpretation due to the limited number of carriers and require validation in expanded cohorts.

The c.109G>A mutation is predominantly observed in Chinese populations, where it demonstrates a moderate overall prevalence of 7.7% [11, 12]. Notably, certain eastern regional cohorts within China report even higher allele frequencies, such as those in Zhaoqing (9.28%) and Xiamen (9.03%) [13, 14]. In contrast, the mutation is reported infrequently in non-Chinese populations. The allele frequency identified in this cohort is lower than those reported in other Chinese studies, suggesting that the distribution of the c.109G>A mutation may vary across different regions and ethnic groups. Moreover, the pathogenicity of c.109G>A remains controversial due to its variable expressivity. Initially considered a benign polymorphism, accumulating evidence indicates a higher prevalence among hearing loss patients, and functional studies suggest that the substitution of valine with isoleucine may disrupt connexin structure and impair gap junction function, supporting a pathogenic role [15, 16].

Furthermore, c.109G>A has been associated with delayed-onset and progressive hearing loss. Longitudinal studies indicate that homozygous and compound heterozygous individuals may experience gradual hearing deterioration, with some cases emerging later in childhood or adolescence. For example, Chai et al. (2015) [17] reported that 35% of homozygous individuals developed hearing loss after the neonatal period. The observed decrease in allele frequency with age in normal-hearing populations may

reflect undiagnosed mild or late-onset cases. Taken together, these findings not only expand the understanding of the pathogenic spectrum of c.109G>A but also provide the first evidence from Northern China, a region previously underrepresented in genetic epidemiology studies. From a public health perspective, identifying population-specific mutation frequencies is essential for tailoring neonatal screening strategies and ensuring that genetic counselling recommendations are contextually relevant and equitable across different Chinese regions.

5. LIMITATIONS

This study is limited by the small number of c.109G>A carriers, especially homozygous and compound heterozygous cases, which may affect statistical robustness. Audiological outcomes were based on initial screenings without long-term follow-up, limiting assessment of delayed-onset hearing loss. Additionally, potential confounding factors, such as other genetic variants or environmental influences, were not considered. Functional validation was also not performed within this cohort.

6. CONCLUSION

In conclusion, our findings demonstrate a significant association between the c.109G>A variant in the GJB2 gene and neonatal hearing loss, particularly among heterozygous and homozygous carriers. Although the overall allele frequency was relatively low in the Changzhi cohort compared with other Chinese populations, the variant showed a clear impact on hearing outcomes, suggesting pathogenic potential. This study contributes region-specific data from Northern China, highlighting the importance of incorporating c.109G>A into neonatal genetic screening programs to enable timely identification, intervention, and genetic counselling. Future studies with larger cohorts, long-term follow-up, and functional validation are warranted to clarify the mechanisms underlying c.109G>A–associated hearing loss and to refine its clinical interpretation across diverse populations.

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8. GENERATIVE A.I DISCLOSURE STATEMENT

No generative AI tools were utilized during the manuscript drafting, language polishing and figure creation process.

9. DATA AVAILABILITY

The data not published within this article are available from the corresponding author on reasonable request.

10. FUNDING

This research did not receive any external funding.

11. CONFLICT OF INTEREST

Authors declare none.

12. AUTHORS' CONTRIBUTIONS

Ruijie Feng, Mohd Nazri Abu, Xiaoze Li participated in the conception and design of the study and reviewed and edited the manuscript. Ernest Mangantig drafted the initial manuscript and prepared the primary tables and figures. Ruijie Feng and Zhipeng Hu performed data collection and analysis. Ernest Mangantig, and Wan Shahriman Yushdie Wan Yusoff assisted with figure preparation and summary statistics. All authors contributed to the study's conception and design, provided feedback on earlier versions of the manuscript, and reviewed and approved the final version.

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