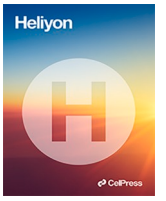
Heliyon 10 (2024) e24850

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon



Research article

Carrier frequencies, trends, and geographical distribution of hearing loss variants in China: The pooled analysis of 2,161,984 newborns

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ARTICLE INFO

Keywords:
China
Newborns
Hearing loss
Genetic screening
Meta-analysis

ABSTRACT

Objective: The aim of this study is to comprehensively investigate the prevalence and distribution patterns of three common genetic variants associated with hearing loss (HL) in Chinese neonatal population. **Methods:** Prior to June 30, 2023, an extensive search and screening process was conducted across multiple literature databases. R software was utilized for conducting meta-analyses, cartography, and correlation analyses. **Results:** Firstly, our study identified a total of 99 studies meeting the inclusion criteria. Notably, provinces such as Qinghai, Tibet, Jilin, and Heilongjiang lack large-scale genetic screening data for neonatal deafness. Secondly, in Chinese newborns, the carrier frequencies of GJB2 variants (c.235delC, c.299_300delAT) were 1.63 % (95 %CI 1.52 %–1.76 %) and 0.33 % (95 %CI 0.30 %–0.37 %); While SLC26A4 variants (c.919-2A > G, c.2168A > G) exhibited carrier rates of 0.95 % (95 %CI 0.86 %–1.04 %) and 0.17 % (95 %CI 0.15 %–0.19 %); Additionally, Mt 12S rRNA m.1555 A > G variant was found at a rate of 0.24 % (95 % CI 0.22 %–0.26 %). Thirdly, the mutation rate of GJB2 c.235delC was higher in the east of the Heihe-Tengchong line, whereas the mutation rate of Mt 12S rRNA m.1555 A > G variant exhibited the opposite pattern. Forthly, no significant correlation exhibited the opposite pattern of GJB2 variants, but there was a notable correlation among SLC26A4 variants. Lastly, strong regional distribution correlations were evident between mutation sites from different genes, particularly between SLC26A4 (c.919-2A > G and c.2168A > G) and GJB c.299_300delAT. **Conclusions:** The most prevalent deafness genes among Chinese neonates were GJB2 c.235delC variant, followed by SLC26A4 c.919-2A > G variant. These gene mutation rates exhibit significant regional distribution characteristics. Consequently, it is imperative to enhance genetic screening efforts to reduce the incidence of deafness in high-risk areas.

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https://doi.org/10.1016/j.heliyon.2024.e24850
Received 8 August 2023; Received in revised form 7 December 2023; Accepted 16 January 2024
Available online 28 January 2024

1. Introduction

Hearing loss (HL) remains a dominant congenital disorder, with a striking prevalence affecting between one to three newborns out of every 1000 live births. According to the most recent data from WHO, an overwhelming 430 million individuals globally are afflicted with debilitating HL, with children accounting for a disturbing 35 million [1]. In China, while universal neonatal hearing screenings detect approximately 30,000 newborns with HL annually, a significant number remain unregistered by the authorities, indicating a potential underestimation of the actual scale of this health challenge [2]. It is estimated that at least two-thirds of hearing loss in childhood are caused by genetic factors. Hearing's fundamental role in facilitating language acquisition and cognitive development, underscores the importance of timely and accurate detection of hearing impairments in newborns.

There are two monogenic forms of HL, including syndromic and non-syndromic HL (NSHL) [3]. At least 50 % of congenital HL was attributed to genetic factors, while 70 % showed NSHL [4]. However, HL is influenced not only by genetic factors involving one or multiple complex mutations but also by environmental factors such as trauma, drugs and infections. The cross-effects of both factors can also result in deafness, emphasizing the multifaceted nature of HL and underscoring the need for an all-encompassing preventive strategy. Extensive epidemiological studies in the Chinese population have identified GJB2, SLC26A4 and mitochondrial 12S rRNA as the primary pathogenic genes in patients with NSHL [5]. The GJB2 gene (Connexin 26) stands out as the leading contributor to autosomal recessive NSHL worldwide [6]. In East Asian populations, GJB2 c.235delC is notably prevalent [5]. GJB2 biallelic variants have been found in approximately 25 % of infants diagnosed with HL [7,8]. Conversely, infants who successfully passed the newborn hearing screenings were 11.8 times less likely to possess GJB2 variants [7,9]. Further insights reveal mutations in the SLC26A4 gene linked to Pendred syndrome (PDS) and enlarged vestibular aqueduct (EVA) observed in neonates or during early childhood [10,11]. The mutation c.919-2A > G in SLC26A4, common in the Chinese demographic, has a carrier frequency reaching 12.5 %. SLC26A4 mutation is associated with 3 % of newborn NSHL cases, a figure that sees a marked increase as years progress [5,12]. The mitochondrial 12S rRNA mutations are responsible for drug-causative HL [13]. Especially, the variant m.1555A > G, though infrequent in the general NSHL population [10], is the most common allele associated with aminoglycoside-induced deafness and NSHL in several ethnic groups [5,14].

The genetic landscape of HL in the Chinese population, while diverse, points to specific genes that can be targeted for early detection and intervention. Research indicates the progressive nature of NSHL, with its prevalence increasing by approximately 50 % during childhood, and doubling during adolescence. This rise is attributed to the delayed detection of congenital HL, late-onset of HL, and aminoglycoside-induced HL [10,15]. It's pivotal to recognize that neonates with HL experience improved outcomes when their condition is identified and addressed within the initial six months post-birth [16,17]. This finding accentuates the need for robust early diagnostic measures. Building on this, Morton et al. introduced the concept of "genetic screening for deafness" [10], a proposal later reinforced in 2007 by Chinese researchers, Wang et al. They suggested a comprehensive neonate hearing and deafness gene screening protocol, combined with regular follow-up and monitoring [18]. Despite the initiative, genetic screening for newborn deafness has been implemented in over 20 cities in China. However, large-scale studies are still limited, leaving the frequency of common HL mutations in the Chinese population and their distribution characteristics in different populations or regions unclear.

This study delves into a comprehensive examination of carrier frequencies and distribution patterns of prevalent HL gene variants in Chinese newborns. Harnessing the synergies of molecular screening and geographic information systems, our research aims to establish a robust scientific foundation. The overarching objective is to facilitate the development of enduring, region-specific strategies for preventing and controlling HL in China. Essentially, this research seeks to bridge the existing gap in genetic databases, paving the way for the formulation of targeted and effective policies in neonatal HL screening and management. The analysis of rare variants associated with complex phenotypes has been made possible through large-scale high-throughput data [19,20]. It is anticipated that future advancements in combined screening for HL genes will provide enhanced capabilities.

2. METHODS

2.1. Literature search strategy

We developed a protocol for the systematic review and meta-analysis, and followed the principles of the PRISMA statement [21]. Relevant studies were searched from PubMed, Web of Science, MEDLINE®, Cochrane library and the three most authoritative Chinese publication databases (CNKI, Wanfang and CQVIP) between 2012 and 2022. Our search task was to retrieve all the literature on the neonatal deafness genetic screening. We adopted a wide-ranging search strategy using a uniform predefined general search string. This strategy had been finalized to minimize the probability of excluding relevant papers from the present study. Our searches were based on combinations of the following index terms: ("newborn" or "neonate" or "infant") and ("hearing loss" or "hearing impairment" or "hearing impair" or "hearing impair" or "deafness" or "deaf mutism") and ("gene" or "genetic" or "genetic screening" or "genetic testing") and ("Chinese" or "China"). We also reviewed the reference lists of retrieved studies and review articles.

2.2. Inclusion and exclusion criteria

We utilized Endnote® (version X9) bibliographic software to create an electronic library, which collected all the retrieved studies from literature databases. After deleting the duplicated studies by the software identification, two independent authors (Jia Feng and Sijian Luo) performed title/abstract screening and full text screening in turn. When any disagreements happened, two independent

authors (Binguan Zhang and Jinbo Liu) reached agreement by discussion.

The studies would be included if they met following criteria: (1) the articles published as of June 30, 2023 in Chinese and English; (2) the study population was newborns born in China; (3) the studies detected the carrier frequencies of deafness gene variants; (4) the studies included three detection loci of GJB2 c.235delC, SLC26A4 c.919-2A > G, and m.1555 A > G of Mt 12S rRNA at the same time and clear detection methods (such as microarray, MALDI-TOF-MS, and sequencing); (5) The samples were derived from neonatal heel blood or peripheral blood.

The following exclusion criteria were applied: (1) Reviews, meta-analyses, reports, commentaries, indirect comparisons, conference abstracts, unpublished studies, basic experiment or mechanism, duplicated studies and other articles lacking original or incomplete data were excluded; (2) Pooled analyses of overlapping screening regions, screening times, screening institutions, and research teams were also excluded after careful discussion; (3) Newborn screening from special populations or institutions was excluded; (4) Studies with a total sample size of 1000 participants or less; (5) Rule out fetal cord blood.

2.3. Quality assessment and data extraction

The methodological quality and validity of the included studies were assessed using the tool used in Zhu et al.'s study [22], which was modified to assess the risk of bias in prevalence studies. Ten aspects were used to evaluate the quality of the included studies. The included studies were assessed with a score of 0 (high risk) or 1 (low risk) in each aspect. Study quality scores ranged from 0 to 10. A study with a score of 8–10 is considered "high quality", a study with a score of 4–7 is considered "medium quality", or else it is considered "low quality". The entire literature studies were extracted, and the quality system was evaluated by two independent researchers (Jia Feng and Sijian Luo). Only when two reviewers agreed, the study was included.

2.4. Statistical analysis

All of the analysis was performed using R software (R version 4.0.4). "meta" R package was used to conduct meta-analysis. Pooled rate and 95 % confidence intervals (CIs) were calculated to estimate the carrier frequencies of HL gene variants in newborns of China. A Chi2-based Q statistics test and I^2 test were used to assess the heterogeneity of the studies. If a significant Q-statistic ($P < 0.10$) or I^2 statistic ($I^2 > 50$ %), then a random-effects model was used for the meta-analysis. Otherwise, a fixed-effects model was used. In order to fully reflect the distribution of the carrier frequencies of HL gene variants in newborns of China, we performed subgroup analysis by research geographic location and genetic screening method. "maps", "geojsonsf", "sf" and "rgdal" R packages were applied to make the map of China. Publication bias was visualized via funnel plots and tested by Egger's linear regression. Sensitivity analysis was used to assess the stability of the meta-analysis. When multiple statistical tests are conducted or comparisons are made across different effect sizes or subgroups, P-values should undergo FDR correction. All *P* values are presented as two-tailed, and $P < 0.05$ were considered statistically significant.

3. RESULTS

3.1. Characteristics of included studies

A total of 2511 potential studies were identified from various databases (PubMed: 439, Cochrane library: 5, Web of Science: 78, Wanfang: 908, CNKI: 565 and CQVIP: 516). After a thorough screening of titles, abstracts, and full texts, 99 studies (8 in English and 91 in Chinese) met our criteria **(Supplementary Data S1)**. The selection process is detailed in Fig. 1. Here, one study from Taiwan was

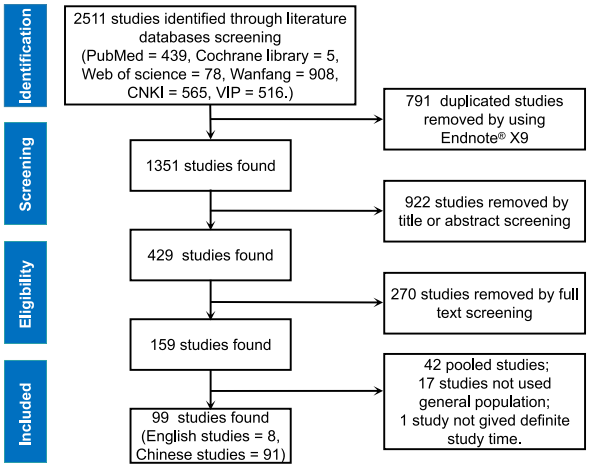

Fig. 1. Flow-chart describing the literature search and study selection processes.

included in this analysis, although only three variants were detected simultaneously (GJB2 c.235delC, SLC26A4 c.919-2A > G and Mt 12S rRNA m.1555A > G). From the studies analyzed, we found there were 27 provinces which all reported the carrying rate of GJB2 (c.235delC & c.299_300delAT), SLC26A4 (c.919-2A > G & c.2168A > G) and Mt 12S rRNA m.1555A > G mutation in general population. However, four provinces, namely Heilongjiang, Jilin, Qinghai, and Tibet, have not conducted large-scale testing for these mutation sites among newborns (Screening population >1000). Comprehensive details of each study can be found in Table S1. Cumulatively, the total sample size from the 99 studies was 2,161,984, with individual provincial samples ranging between 1024 and 471,994 participants. These studies spanned from 2006 to 2022. Regarding quality assessment, only 7 studies were deemed of medium quality, with the remainder classified as high quality.

3.2. Carrying rate and distribution patterns of common deafness gene variants in Chinese newborns

We conducted a meta-analysis encompassing 99 studies involving 2,161,984 neonates, focusing on 5-mutation sites. Table 1 presents the results of overall meta-analysis for each site. Notably, Heterogeneity was observed in pooled analysis. Therefore, we used the random effects model to calculate the related indexes of the heterogeneity test between studies. The overall meta-analysis showed that among Chinese newborns, the carrier frequencies of GJB2 variants (c.235delC, c.299_300delAT) were 1.63 % (95 %CI 1.52 %–1.76 %) and 0.33 % (95 %CI 0.30 %–0.37 %); SLC26A4 variants (c.919-2A > G, c.2168A > G) were 0.95 % (95 %CI 0.86 %–1.04 %) and 0.17 % (95 %CI 0.15 %–0.19 %); Mt 12S rRNA m.1555 A > G variants was 0.24 % (95 % CI 0.22 %–0.26 %) (Figs. S1–5). Notably, the data indicated high carrier frequencies of GJB2 c.235delC variants in Chinese general newborns, followed by SLC26A4 c.919-2A > G variants, which aligns with previous research findings [3,23]. Subsequent sensitivity analysis confirmed the stability of the meta-analysis results, with no significant alterations observed as a result of variations in the number of included studies. This crucial discovery underscores the reliability and robustness of our meta-analysis results (Figs. S6–10).

Previous studies have underscored the highly heterogeneous nature of HL gene mutation frequencies, influenced by factors such as region, population and race [3,23]. To delve deeper into this heterogeneity, we conducted detailed subgroup meta-analyses by province and by seven regions. According to the traditional geographical regions of China, we have divided China into seven regions, which included Central China, East China, North China, Northeast China, Northwest China, South China, and Southwest China, the carrier frequencies of each mutation site was shown in Table 2 (Figs. S11–15). Despite this division, substantial heterogeneity persisted in subgroup meta-analysis by seven regions, suggesting that the carrying rate is influenced by multiple factors. The number of studies reported in East and South China was 31 and 29, respectively, and the total number of studies reported in the other five regions was 39, especially the northeast China region only one. It is worth mentioning that the carrying rate of GJB2 c.299_300delAT and SLC26A4 c.2168A > G showed a consistent trend of gradually decreasing from the northeast region to the southwest region (Fig. S16).

Subsequently, a province-based subgroup meta-analysis by province was performed (Table 2; Figs. S17–21). Following this, we created a geographical map illustrating the distribution of common mutation loci in Chinese newborns by province (Fig. 2A–E). The map vividly highlights the absence of large-scale genetic screening for neonatal deafness in Tibet, Qinghai, Jilin and Heilongjiang provinces. In contrast, Guangdong and Zhejiang provinces have been the focus of numerous studies, with 24 and 11 articles, respectively. The province with the highest frequency of GJB2 variants (c.235delC, c.299_300delAT) was Hubei and Shandong, while SLC26A4 variants (c.919-2A > G, c.2168A > G) were more prevalent in Shanxi and Ningxia/Shandong. The presence of Mt 12S rRNA m.1555 A > G variants was notably pronounced in Gansu. Among the screened newborns, the majority of the GJB2 c.235delC mutation carriers were found to reside to the east of the Heihe-Tengchong line (an imaginary line that divides the area of China into two roughly equal parts with contrasting population densities; west of the line: 57 % of the area, but only 6 % of the population; east of the line: 43 % of the area, but 94 % of the population). Interestingly, the prevalence of Mt 12S rRNA m.1555A > G was relatively high in the western regions, on the other side of the Heihe-Tengchong line.

Furthermore, we described the distribution of the most common deafness genes, namely GJB2 c.235delC and SLC26A4 c.919-2A > G, among school children with hearing impairment based on research data provided by Dai et al. [24,25]. Our analysis aimed to identify commonalities and disparities in the distribution of these two mutation sites between newborns and school-aged children. Despite the absence of genetic screening of deaf children in certain areas, our findings revealed distinct distribution patterns of deafness-related genes in children compared to newborns (Table S2, Fig. 3A–B). Specifically, the school enrichment effect of GJB2 C.235delC was the most pronounced in Inner Mongolia and Jiangsu provinces, while the enrichment effect of SLC26A4 c.919-2A > G was the most evident in Central China, particularly in Henan Province. These observations suggest that the establishment of schools for the deaf in these regions, including their number and location, has been strategically sound.

Table 1
Meta-analysis of the related indexes of common deaf genes among newborns in 2,161,984 of 99 studies in China.

Gene	Event number	I^2 (%)	*P* value	Estimated proportion (%)	95 %CI (%)	Model
GJB2 c.235delC	36116	94.35	0	1.63	1.52 1.76	Random
GJB2 c.299_300delAT	7955	90.94	0	0.33	0.30–0.37	Random
SLC26A4 c.919-2A > G	21984	96.00	0	0.95	0.86–1.04	Random
SLC26A4 c.2168A > G	3534	90.31	<0.0001	0.17	0.15–0.19	Random
Mt 12S rRNA m.1555A > G	5254	77.35	<0.0001	0.24	0.22–0.26	Random

Table 2
Subgroup analyses for the pooled proportion of overall common deafness genes variation in Chinese newborns.

	Study, n	Sample, n	GJB2 c.235delC			GJB2 c.299_300delAT			SLC26A4 c.919-2A > G			SLC26A4 c.2168A > G			Mt 12S rRNA m.1555A > G		
			Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)	Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)	Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)	Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)	Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)
Region																	
Central China	12	149682	2980	71.19 (<0.01)	2.05 (1.91–2.21)	653	76.95 (<0.01)	0.39 (0.32–0.48)	1641	88.47 (<0.01)	0.92 (0.77–1.10)	339	88.09 (<0.01)	0.21 (0.14–0.30)	393	75.24 (<0.01)	0.25 (0.20–0.32)
East China	31*	357229*	6797	85.62 (<0.01)	1.99 (1.81–2.19)	1337	87.07 (<0.01)	0.38 (0.32–0.46)	3779	88.33 (<0.01)	1.09 (0.92–1.29)	609	84.39 (<0.01)	0.20 (0.17–0.25)	887	61.54 (<0.01)	0.25 (0.21–0.28)
North China	15	607413	10647	92.5 (<0.01)	1.69 (1.52–1.88)	3065	62.91 (<0.01)	0.50 (046–0.54)	8426	92.24 (<0.01)	1.34 (1.20–1.50)	1532	60.55 (<0.01)	0.25 (0.22–0.27)	1396	70.26 (<0.01)	0.23 (0.21–0.26)
Northeast China	1	1272	30	/	2.36 (1.60–3.35)	4	/	0.31 (0.09–0.80)	7	/	0.55 (0.22–1.13)	2	/	0.16 (0.02–0.57)	2	/	0.16 (0.02–0.57)
Northwest China	6	35119	353	93.47 (<0.01)	1.15 (0.76–1.76)	154	0 (0.78)	0.44 (0.38–0.52)	418	13.60 (0.33)	1.20 (1.08–1.34)	64	67.49 (<0.01)	0.21 (0.13–0.33)	129	63.5 (0.02)	0.33 (0.23–0.47)
South China	29	417655	5321	95.36 (<0.01)	1.30 (1.12–1.50)	856	84.48 (<0.01)	0.21 (0.17–0.25)	3022	94.71 (<0.01)	0.70 (0.58–0.85)	450	81.54 (<0.01)	0.11 (0.09–0.15)	822	72.97 (<0.01)	0.21 (0.18–0.25)
Southwest China	5	593614	9988	95.86 (<0.01)	1.39 (1.15–1.68)	1886	90.16 (<0.01)	0.29 (0.22–0.36)	4691	96.02 (<0.01)	0.70 (0.54–0.90)	538	85.9 (<0.01)	0.08 (0.05–0.12)	1625	93.34 (<0.01)	0.26 (0.18–0.36)
Province																	
Anhui	2	4897	116	0 (0.58)	2.36 (1.94–2.79)	27	0 (0.71)	0.55 (0.34–0.76)	72	35.28 (0.21)	1.45 (1.03–1.87)	11	0 (0.68)	0.22 (0.11–0.38)	15	27.03 (0.24)	0.29 (0.11–0.46)
Beijing	5	287053	5521	67.6 (0.01)	1.87 (1.72–2.02)	1432	0 (0.69)	0.50 (0.47–0.52)	3809	0 (0.80)	1.33 (1.28–1.37)	783	0 (0.75)	0.27 (0.25–0.29)	660	51.19 (0.08)	0.24 (0.20–0.28)
Chongqing	1	1583	18	/	1.14 (0.68–1.79)	4	/	0.25 (0.07–0.65)	4	/	0.25 (0.0–0.65)	1	/	0.06 (0.00–0.35)	4	/	0.25 (0.07–0.65)
Fujian	1	11684	177	/	1.51 (1.30–1.75)	29	/	0.25 (0.17–0.36)	111	/	0.95 (0.78–1.14)	15	/	0.13 (0.07–0.21)	31	/	0.27 (0.18–0.038)
Gansu	1	7004	61	/	0.87 (0.67–1.12)	29	/	0.41 (0.28–0.59)	79	/	1.13 (0.89–1.40)	14	/	0.20 (0.11–0.34)	34	/	0.49 (0.34–0.68)
Guangdong	24	332724	4429	95.43 (<0.01)	1.33 (1.12–1.57)	710	90.27 (<0.01)	0.22 (0.18–0.27)	2616	94.02 (<0.01)	0.75 (0.60–0.93)	395	78.94 (<0.01)	0.12 (0.09–0.16)	690	71.26 (<0.01)	0.22 (0.18–0.26)
Guangxi	4	77807	795	89.47 (<0.01)	1.123 (0.86–1.49)	134	0 (0.57)	0.17 (0.14–0.20)	344	0 (0.62)	0.44 (0.40–0.49)	40	0 (0.61)	0.05 (0.04–0.07)	119	76.81 (<0.01)	0.19 (0.13–0.29)
Guizhou	1	99582	1357	/	1.36 (1.29–1.44)	213	/	0.21 (0.19–0.24)	539	/	0.54 (0.05–0.59)	43	/	0.04 (0.03–0.06)	158	/	0.16 (0.13–0.19)
Hainan	1	7124	97	/	1.36 (1.11–1.66)	12	/	0.17 (0.09–0.29)	62	/	0.87 (0.67–1.11)	15	/	0.21 (0.12–0.35)	13	/	0.18 (0.10–0.31)
Hebei	3	59729	1078	49.3 (0.14)	1.80 (1.64–1.96)	292	0 (0.65)	0.49 (0.43–0.54)	798	73.06 (0.002)	1.33 (1.15–1.51)	144	0 (0.66)	0.24 (0.20–0.28)	126	9.13 (0.33)	0.21 (0.17–0.25)
Henan	7	112371	2156	35.61 (0.16)	1.94 (1.82–2.07)	546	84.26 (0.01)	0.46 (0.33–0.58)	1383	76.61 (<0.01)	1.12 (0.95–1.29)	266	90.56 (<0.01)	0.22 (0.12–0.36)	253	65.89 (<0.01)	0.19 (0.13–0.25)
Hubei	1	2450	62	/	2.53 (1.95–3.23)	8	/	0.33 (0.14–0.64)	13	/	0.53 (0.28–0.91)	4	/	0.16 (0.04–0.42)	4	/	0.16 (0.04–0.42)

(*continued on next page*)

Table 2 (*continued*)

	Study, n	Sample, n	GJB2 c.235delC			GJB2 c.299_300delAT			SLC26A4 c.919-2A > G			SLC26A4 c.2168A > G			Mt 12S rRNA m.1555A > G		
			Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)	Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)	Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)	Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)	Event, n	I^2 (%) (*P*)	Rate (%) (95 % CI)
Hunan	4	34861	762	80.44 (<0.01)	2.06 (1.69–2.42)	99	5.19 (0.37)	0.28 (0.21–0.36)	245	0 (0.73)	0.70 (0.61–0.79)	69	87.72 (<0.01)	0.20 (0.07–0.40)	136	71.9 (0.01)	0.34 (0.22–0.46)
Inner Mongolia	3	35674	361	79.19 (<0.01)	1.16 (0.73–1.58)	123	64.46 (0.006)	0.42 (0.22–0.61)	306	84.05 (<0.01)	0.90 (0.40–1.41)	56	70.25 (0.03)	0.19 (0.08–0.35)	65	29.76 (0.24)	0.20 (0.11–0.28)
Jiangsu	8	156536	3146	81.95 (<0.01)	2.29 (2.06–2.54)	557	38.8 (0.12)	0.42 (0.33–0.51)	1788	82.08 (<0.01)	1.30 (1.11–1.51)	226	92.95 (<0.01)	0.23 (0.15–0.35)	458	55.2 (0.03)	0.28 (0.22–0.35)
Jiangxi	2	31306	589	63.76 (0.10)	1.93 (1.63–2.24)	105	33.94 (0.22)	0.34 (0.25–0.43)	289	65.58 (0.09)	0.88 (0.68–1.08)	53	48.35 (0.16)	0.18 (0.11–0.26)	74	76.11 (0.04)	0.27 (0.11–0.42)
Liaoning	1	1272	30	/	2.36 (1.60–3.35)	4	/	0.31 (0.09–0.80)	7	/	0.55 (0.22–1.13)	2	/	0.16 (0.02–0.57)	2	/	0.16 (0.02–0.57)
Ningxia	2	10847	168	0 % (0.84)	1.55 (1.32–1.78)	49	23.53 (0.25)	0.45 (0.29–0.61)	140	0 (0.71)	1.29 (1.08–1.50)	31	0 (0.32)	0.28 (0.19–0.39)	26	0 (0.82)	0.24 (0.15–0.33)
Shaanxi	2	14146	83	93.41 (<0.01)	1.14 (0.00–2.56)	65	0 (0.81)	0.46 (0.35–0.57)	161	66.1 (0.09)	1.31 (0.69–1.94)	14	78.69 (0.03)	0.15 (0.00–0.52)	64	0 (0.46)	0.45 (0.34–0.56)
Shandong	4	63958	1194	83.3 (<0.01)	1.91 (1.43–2.39)	428	75.81 (<0.01)	0.67 (0.48–0.86)	787	58.64 (0.06)	1.29 (1.06–1.52)	193	24.81 (0.26)	0.28 (0.21–0.35)	165	24.81 (0.26)	0.25 (0.19–0.31)
Shanghai	2	4922	96	16.98 (0.27)	1.92 (1.50–2.35)	18	0 (0.51)	0.36 (0.19–0.52)	59	0 (0.61)	1.19 (0.89–1.50)	13	0 (0.67)	0.26 (0.13–0.43)	9	0 (0.82)	0.18 (0.06–0.30)
Shanxi	2	84631	1327	86.6 (<0.01)	1.64 (1.35–1.92)	483	36.51 (0.21)	0.56 (0.49–0.63)	1439	86.62 (<0.01)	1.77 (1.47–2.07)	175	0 (0.75)	0.21 (0.18–0.24)	260	0 (0.37)	0.31 (0.27–0.34)
Sichuan	2	488994	8580	86.98 (<0.01)	1.65 (1.39–1.90)	1659	0 (0.82)	0.34 (0.32–0.63)	4117	14.42 (0.28)	0.84 (0.79–0.88)	490	0 (0.92)	0.10 (0.09–0.11)	1448	41.45 (0.19)	0.29 (0.24–0.33)
Taiwan	1	5173	50	/	0.97 (0.72–1.27)	/	/	/	60	/	1.16 (0.89–1.49)	/	/	/	4	/	0.08 (0.02–0.20)
Tianjin	2	140326	2660	50.72 (0.15)	1.90 (1.80–2.00)	735	0 (0.93)	0.52 (0.49–0.56)	2074	76.08 (0.04)	1.49 (1.35–1.52)	374	49.16 (0.16)	0.27 (0.23–0.31)	285	0 (0.46)	0.20 (0.18–0.23)
Xinjiang	1	3122	41	/	1.31 (0.94–1.78)	11	/	0.35 (0.18–0.63)	38	/	1.22 (0.86–1.67)	5	/	0.16 (0.05–0.37)	5	/	0.16 (0.05–0.37)
Yunnan	1	3455	33	/	0.96 (0.66–1.34)	10	/	0.29 (0.14–0.53)	31	/	0.90 (0.61–1.27)	4	/	0.12 (0.03–0.30)	15	/	0.43 (0.24–0.72)
Zhejiang	11	78753	1429	89.87 (<0.01)	1.91 (1.56–2.23)	173	93.03 (<0.01)	0.29 (0.19–0.38)	613	94.3 (<0.01)	0.86 (0.54–1.37)	98	74.11 (<0.01)	0.14 (0.09–0.23)	131	72.28 (<0.01)	0.21 (0.14–0.31)

Note : "/": The subgroup analysis included only one study and could not calculate heterogeneity; "*": Due to the lack of gene detection at GJB2 c.299_300delAT and SLC26A4 c.2168A > G loci in Taiwan, 28 studies were included and 352,056 newborns were screened in meta-analysis.

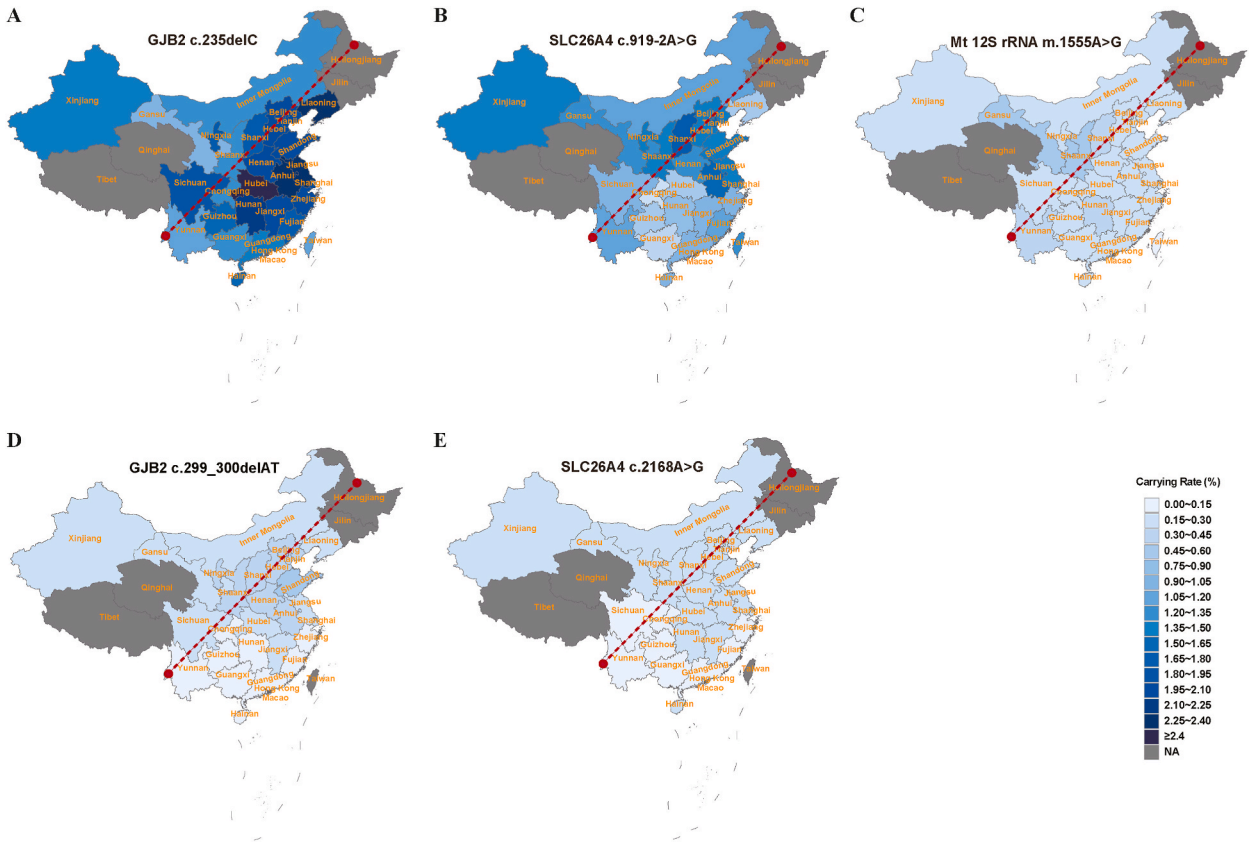

Fig. 2. The carrying rate of the 5-mutation sites in the newborns in each province of China (provincial data obtained by subgroup analysis). A. GJB2 c.235delC; B. SLC26A4 c.919_2A > G; C. Mt 12S rRNA m.1555A > G; D. GJB2 c.299_300delAT; E. SLC26A4 c.2168A > G. Note: Of the screened newborns, 95 % cases (except part of Ningxia and Sichuan province) were located to the east of the Heihe-Tengchong line (an imaginary line that divides the area of China into two roughly equal parts with contrasting population densities; west of the line: 57 % of the area, but only 6 % of the population; east of the line: 43 % of the area, but 94 % of the population).

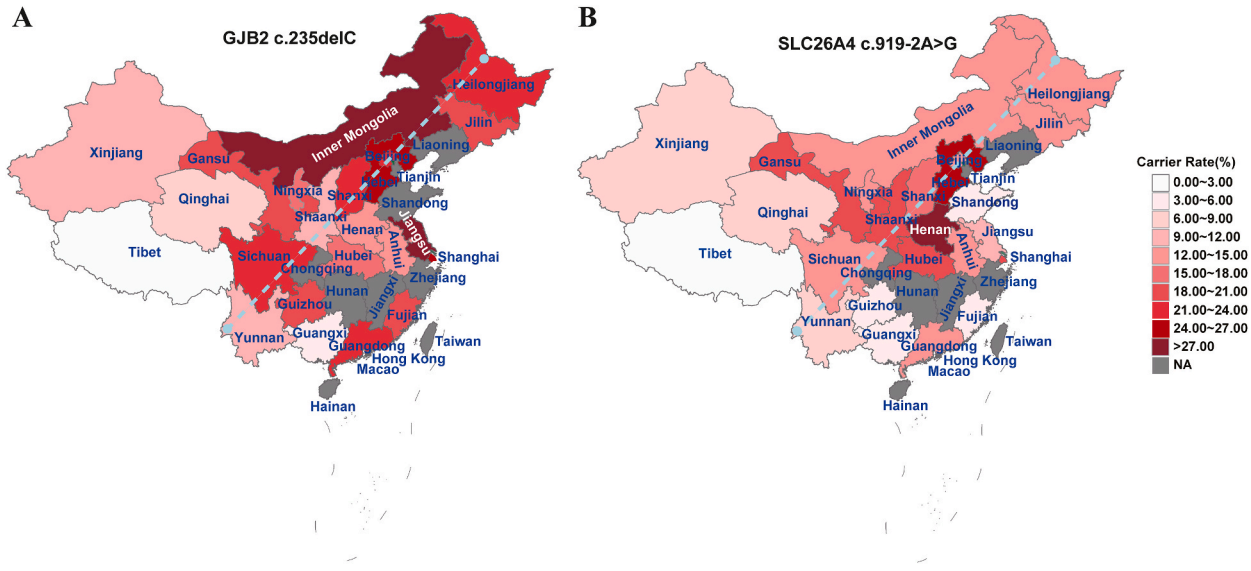

Fig. 3. The carrying rate of GJB2 c.235delC and SLC26A4 c.919-2A > G in the deaf school child in each province of China. A. GJB2 c.235delC; B. SLC26A4 c.919_2A > G.

3.3. The relationship between genetic variants in deafness

We conducted an analysis to examine the relationship between genetic variants associated with deafness. To assess the distribution correlation among five mutation sites, we created a trend histogram of carry-rate distribution and a correlation scatter plot. The pairwise carrying rates exhibited a discernible distribution trend among these sites. Only correlations with a significance level of $P < 0.05$ are presented in Fig. 4A–D. Upon careful analysis, we observed that there was no significant regional mutation correlation between GJB2 c.235delC and c.299_300delAT. However, SLC26A4 c.919-2A > G and c.2168A > G showed a strong regional mutation correlation. Remarkably, two mutation sites from different genes demonstrated a robust regional distribution correlation, particularly between SLC26A4 (c.919-2A > G and c.2168A > G) and GJB2 c.299_300delAT. This led us to speculate that even mutations in different genes can exhibit a synergistic effect on their prevalence distribution patterns due to population migration. Not surprisingly, mitochondrial gene mutation site (Mt 12S rRNA m.1555A > G) exhibited no significant or weak correlation with the carrying rate of the four-mutation locus in autosomal gene, suggesting that the mutation characteristics of the autosomal genes differed from those of mitochondrial genes.

Subsequently, we analyzed the relative carrier ratios of different loci for GJB2 and SLC266A4, respectively. We found that the relative ratio changes of different mutation sites on GJB2 and SLC266A4 had obvious regional characteristics, and the ratio distribution between these two genes was significantly different ($P < 0.05$) (Fig. 5). In addition, the ratio of c.299_300delAT to c.235delC was significantly higher than the ratio of c.2168A > G to c.919-2A > G, particularly in the northern regions, north of the Heihe-Tengchong line. In summary, the mutation landscape of deafness genes displays regional characteristics. Moreover, different loci within the same gene exhibit varying distribution characteristics across different provinces.

3.4. Publication bias

To assess the potential presence of publication bias, we conducted both a funnel plot analysis and Egger's test (Fig. 6A–E). Notably, for the GJB2 gene c.235delC variant, statistical evidence from Egger's test indicates the absence of publication bias. However, several other genetic variants exhibited evidence of publication bias ($P > 0.05$).

4. Discussion

With the expansion of molecular biology research techniques, more relationships between genetic HL and genetic abnormalities have been revealed [26]. Building upon the pioneering initiative of Beijing in conducting deafness gene screening for newborns in 2020, numerous provinces and cities across the country have incorporated this project into the people's livelihood project, and have successively carried out free newborn deafness gene screening [27]. After more than a decade of clinical practice and accumulation,

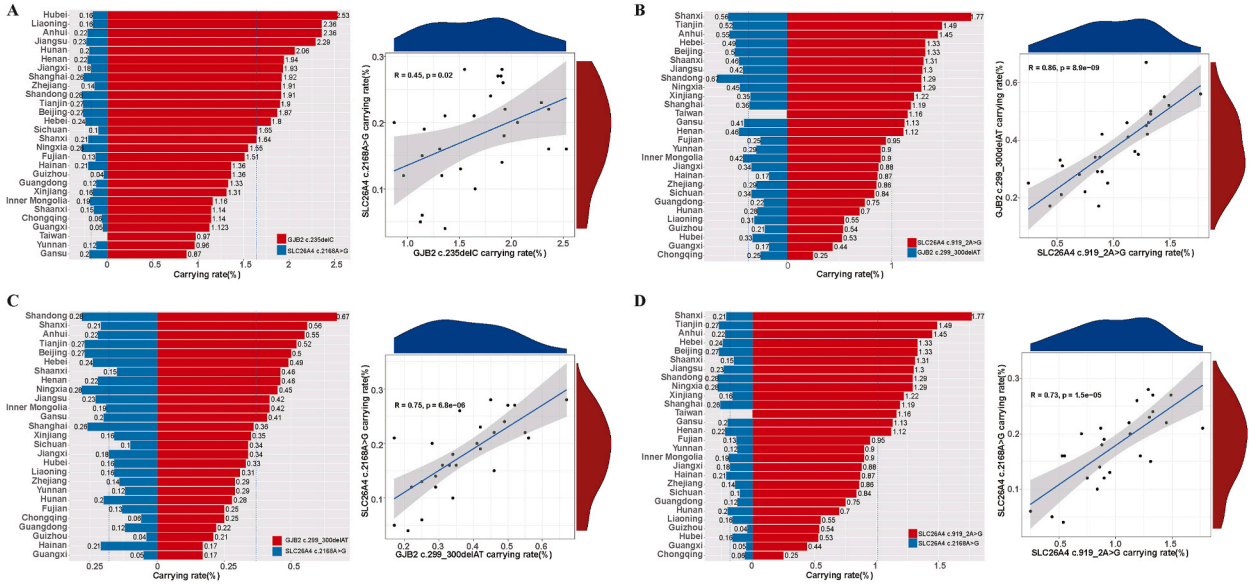

Fig. 4. The dual-sided bar chart and correlation analysis describe the distribution trends and correlations of carrier rates between mutation sites in 28 provinces. A. GJB2 c.235delC and SLC26A4 c.2168A > G; B. SLC26A4 c.919_2A > G and GJB2 c.299_300delAT; C. GJB2 c.299_300delAT and SLC26A4 c.2168A > G; D. SLC26A4 c.919_2A > G and SLC26A4 c.2168A > G. Note: The vertical axis of dual-sided bar chart represents provinces, while the horizontal axis depicts the carrier rates for the genetic mutation sites. The red (Descending order; Reference) and blue sub-bar charts respectively illustrate the distribution of carrier rates for two genetic mutation sites across different regions. The blue dashed line represents the mean of mutation site (provincial data obtained by subgroup analysis).

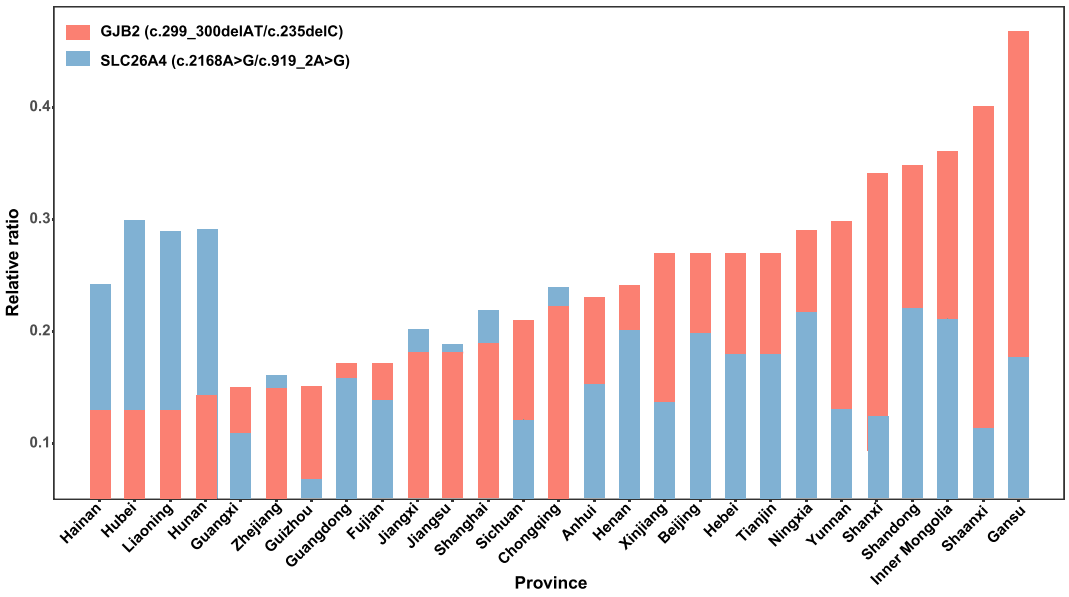

Fig. 5. Bar chart of the relative ratio of carrying rate of different loci of the same gene in different provinces. Note: Using the GJB2 (c.299_300delAT/c.235delC) ratio as a reference, this ratio gradually increases from left to right.

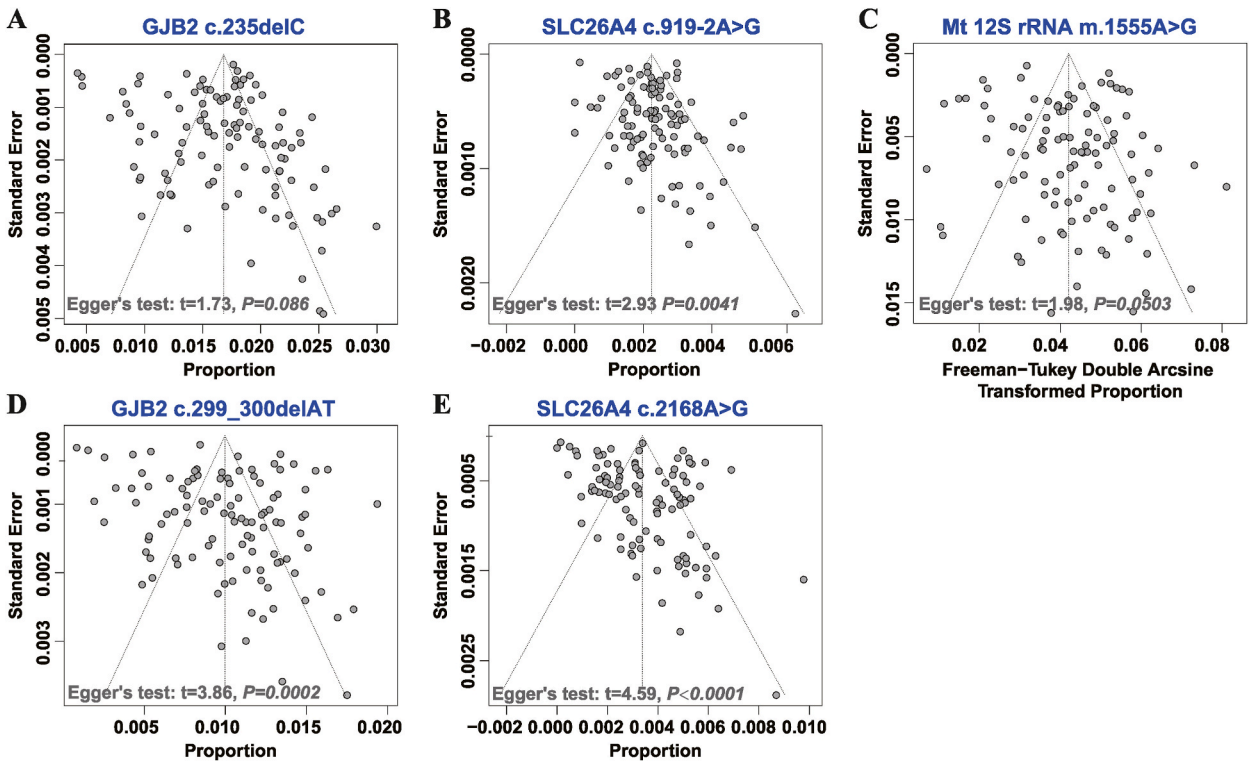

Fig. 6. Funnel plot of the risk of publication bias for the carrying proportion of 5-mutation sites. A. GJB2 c.235delC; B. SLC26A4 c.919_2A > G; C. Mt 12S rRNA m.1555A > G; D. GJB2 c.299_300delAT; E. SLC26A4 c.2168A > G.

domestic newborn genetic screening has entered a phase of rapid development. Owing to variances in economic and technological development across different regions of the country, the progress of newborn deafness genetic screening exhibits some discrepancies. This study presents an overview of the current status of neonatal deafness gene screening in diverse regions of Chin, highlighting the distribution characteristics of common variants (GJB2, SLC26A4 and Mt 12S rRNA genes) within each region. It offers valuable

insights to facilitate the broader adoption and application of deafness gene screening. Our analysis encompasses 99 studies focusing on the carrier rates of three common deafness genes (5-mutation sites) in Chinese newborns. This dataset comprises approximately 2,161, 984 newborns, making it the most comprehensive and systematic analysis of deafness genes in Chinese newborns.

There exist four types of primary screening detection methods for deafness genes, such as hybridization, mass spectrometry, sequencing and PCR analysis. Based on these methods, we first performed a subgroup meta-analysis (Table S3, Figs. S22–26). However, it is essential to note that substantial heterogeneity persisted among the related studies for each method. This underscores that the rate of gene variation is influenced by multiple contributing factors. In addition, our investigation revealed no significant disparity in the carrier rates of variation sites in autosomal deafness genes, such as GJB2 and SLC26A4, among the four methods. Consequently, this study leveraged different methods to obtain positive cases for combined analysis, which proved to be a viable approach. Nonetheless, it's worth highlighting that the proportion of Mt 12S rRNA gene variation exhibited notable differences among detection methods, potentially attributed to the mitochondrial gene's low mutation rate, limited sample size, distinctive structural attributes and notable margin of error in detection.

Although we conducted multiple subgroup meta-analyses, there was still considerable heterogeneity among studies. This is consistent with a large number of previous studies [3,28]. Despite the substantial degree of heterogeneity encountered in our analysis, the amalgamated findings remain valuable for summarizing the implementation of genetic screening programs for neonatal deafness in China. It is worth noting that the wealth of data generated from neonatal deafness gene screening holds the potential to facilitate an in-depth analysis of the origins of this heterogeneity in the future.

Genetic screening for deafness plays a pivotal role in the early detection, diagnosis and treatment of hearing-impaired children. Our findings illuminate significant disparities in the implementation of genetic screening programs for deafness across distinct regions. Notably, the adoption rate of genetic screening for deafness east of the Heihe-Tengchong line surpasses that in western regions. Specifically, Guangdong and Zhejiang provinces have taken substantial strides in the widespread genetic screening of newborns (**Table 2**). Nevertheless, owing to disparities in medical infrastructure, particularly the underdeveloped healthcare facilities in western regions situated west of the Heihe-Tengchong line, there may be instances of missed screening and erroneous assessments. For instance, large-scale genetic screening for deafness remains unimplemented in Tibet and Qinghai. Additionally, the screening scope in select provinces and cities remains limited, which may not accurately represent the regional prevalence of deafness gene carriers. These screening gaps pose challenges to guiding the prevention and treatment of hearing impairment in these regions. Our study underscores the potential relationship between the implementation of neonatal deafness genetic screening and regional economic development. It further underscores the need to enhance support and investment in central and western regions while striving to minimize healthcare disparities between regions.

Normally, most cases of HHL are classic single-gene genetic diseases. Among more than 120 well-known sensorineural HL-related genes (Hereditary Hearing Loss Homepage: http://hereditaryhearingloss.org), GJB2 and SLC26A4 are the most common chromosome genes, and Mt 12S rRNA m.1555A > G is the most common mitochondrial gene [29]. In this study, we confirmed that the most common variant in inherited HL is attributed to GJB2 in the Chinese population, followed by SLC26A4 [3,23]. Our results were basically consistent with Fu et al.'s research and the carrier frequency of c.235delC was 1 %–2 % in Chinese newborns [3]. A meta-analysis by Wan et al. showed that c.919-2A > G (OR 13.44, 95 % CI 8.58 to 21.06, z = 11.34, $P < 0.00001$) and 2168A > G (OR 11.21, 95 % CI 4.69 to 26.78, z = 5.44, $P < 0.00001$), which reflected that the risk of HL among people carrying 919-2A > G variant is greatly increased [30].

The geographical distribution of GJB2 c.235delC mutation carriers primarily concentrated east of the Heihe-Tengchong line, encompassing the majority of China's population. The presence of multiple factors, such as a relatively developed economy, intricate population structures, substantial migration, and high mobility, likely contributes to the widespread prevalence of these genes in this region. Therefore, there is an imperative need to enhance public awareness of genetic screening and encourage greater participation in genetic screening initiatives. Conversely, carriers of mt.1555A > G mutations were notably clustered in the northwest of the Hihe-Tengchong line, particularly in Ningxia and Shaanxi. It's essential to emphasize that all mitochondrial mtDNA variants are maternally inherited, meaning the variant can be passed to offspring through the mother. Populations carrying Mt 12S rRNA variants exhibit heightened susceptibility to aminoglycosides, even minute quantities of which can result in aminoglycoside antibiotic-induced deafness [31]. Thus, factors such as high endogamy, limited population mobility or migration, and potentially inappropriate antibiotic usage may contribute to the observed prevalence in the Northwest. Consequently, a concentrated focus on ototoxicity gene screening is imperative in these regions.

Notably, we found no strong consistency in the mutation distribution patterns of common variants between newborns and deaf school students, warranting further comprehensive studies for confirmation. The spatiotemporal enrichment effect of deafness schools is believed to contribute to a higher mutation rate of deafness genes among children with deafness compared to newborns. Intriguingly, we observed that the enrichment effect of the c.235delC mutation site was most pronounced in Inner Mongolia and Jiangsu. However, it's important to highlight that this mutation site did not hold the highest prevalence in these regions, particularly in Inner Mongolia, where the newborn carrying frequency is relatively low. This suggests that the deafness protection measures, including the number and geographical distribution of deafness schools, appear to be relatively well-planned in these areas. Similarly, the c.919-2A > G gene exhibited the most robust enrichment effect in Henan, despite a relatively low carrier rate among newborns. These findings underscore that economically developed regions do not necessarily possess the most effective deafness protection measures. On the contrary, some economically disadvantaged areas have demonstrated notable success in safeguarding against deafness. Therefore, economically developed regions possess the capacity to further optimize their deafness patient care initiatives to better serve this population.

Through a comprehensive analysis of carrier rate distribution patterns among gene variants, we conducted the first-ever analysis of

the correlation between the distribution characteristics of common deafness gene variants among Chinese newborns. Our analysis confirmed that deafness gene variants exhibit significant regional distribution characteristics. Loci within the same gene share similarities in regional distribution patterns (e.g., SLC26A4 c.919-2A > G and c.2168A > G). However, they may also manifest different regional distribution patterns (e.g., GJB2 c.235delC and c.299_300delAT). Additionally, we noted the potential for a synergistic effect of population migration among different gene variants, particularly between GJB2 c.299_300delAT and SLC26A4 (c.919-2A > G and c.2168A > G). However, this hypothesis necessitates further substantiation through extensive research. Consequently, it is imperative to conduct multi-gene and multi-locus combined screening in regions exhibiting a high carrier rate of deafness gene variants. Nonetheless, the need for expanded combination genetic screening warrants through reliable analytical tools and further corroborative studies [32].

5. Conclusion

To carry out genetic screening of newborn deafness in some areas of China is of positive significance for exploring the prevalence of deafness gene mutations and exploring the genetic causes of deafness. This study analyzed the status of neonatal deafness gene screening in different regions of China, and analyzed the programs and data of neonatal deafness gene screening in many institutions, which can enrich the database of neonatal deafness gene screening in China and provide reference for further application. However, we also realize that there are still some problems with deafness gene screening at present. Especially, the insufficient screening coverage in China, which will guide our future work in deafness genes neonatal screening. In addition, only 15.1 % of the included studies were from midwifery institutions at all levels (including rural and urban), which has significant regional representation. 2.0 % studies included clearly indicated that the study population had ethnic classification information, so the population diversity in multiple regions was underrepresented. Finally, this study lacks the data on the degree of HL at each gene locus, which makes it impossible to combine molecular characteristics with phenotypic information for analysis. In conclusion, this analysis used a combination of molecular screening and geographic information system to analyze the data, identify the types and frequency of common deafness gene mutations in Chinese newborns, and provide a basis for early observation and prevention measures for deaf patients.

Ethics declarations

Review and/or approval by an ethics committee was not needed for this study because our research is based on a secondary analysis of previously published articles.

FUNDING

This work was supported by the Sichuan Science and Technology Program, China (2023YFQ0096, 2022YFS0312), Industry-University-Research Cooperation Cultivation Project of Luzhou-Medical University (2020LZXNYD02), Scientific Research Program of Southwest Medical University, China (2022QN071).

CRediT authorship contribution statement

Jia Feng: Writing – review & editing, Writing – original draft, Data curation, Conceptualization. **Zhangrui Zeng:** Data curation. **Sijian Luo:** Visualization, Data curation. **Xuexue Liu:** Data curation. **Qing Luo:** Data curation. **Kui Yang:** Funding acquisition. **Guanbin Zhang:** Writing – review & editing, Validation. **Jinbo Liu:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e24850.

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