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Comparison of Genetic, Auditory Features, and Systemic Clinical Phenotype in 14 Families with Syndromic Hearing Loss

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Introduction: Syndromic hearing loss (SHL) is characterized by distinctive clinical phenotypes as well as genetic and phenotypic heterogeneity. More than 400 species of SHL have been described, the majority of which are autosomal dominant.

Methods: 11 forms of SHL were obtained from 14 unrelated families with probands ranging in age from 5 to 78 months. The results of whole exome sequencing(WES), audiological characteristics, middle and inner ear radiological findings, and additional clinical phenotype characteristics were retrospectively analyzed.

Results: Fourteen people with SHL were found. Two of them had Waardenburg syndrome, two had Branchio-Oto-Renal syndrome, two had CHARGE syndrome, and one had Treacher Collins syndrome, Kleefstra syndrome, Muenke syndrome, Osteopathia Striata with Cranial Sclerosis, Ayme-Gripp syndrome, Tatton-Brown-Rahman syndrome, Stickler syndrome, or Stapes Ankylosis with Broad Thumbs and Toes. In this investigation, ten variants were first reported.

Discussion: The combination of a neonatal hearing screening and WES can diagnose syndrome-type hearing loss in infancy and childhood, according to our findings, expansion of the gene variant spectrum and phenotype for various age groups of SHL is essential and can provide valuable guidelines for clinical intervention decisions. It is imperative for medical practitioners to conduct diligent and prolonged patient monitoring due to the inherent variability in both the auditory impairment and the comprehensive clinical manifestation of SHL.

Keywords: syndromic hearing loss, whole exome sequencing, neonatal hearing screening, phenotype-genotype correlations

Introduction

Hereditary deafness can be classified as syndromic hearing loss (SHL) or non-syndromic hearing loss (NSHL) based on whether it is combined with other system dysfunctions. SHL affects roughly 3 out of every 10,000 live births[1], and there are over 400 reported SHL species, most of which have autosomal dominant inheritance. According to previous researches, syndromic deafness mainly included Pendred syndrome, Alport syndrome, Usher syndromes, Waardenburg syndrome, Branchio-oto-renal syndrome, CHARGE syndrome, etc.[2] In China, the percentage of Usher syndromes in East Asian populations was presumed to be higher than that in European populations.[3] SHL can cause unique clinical phenotypes and a lot of genetic heterogeneity and phenotypic diversity. This is shown by the fact that different variations in the same gene or even at the same locus can cause different phenotypic traits. Additionally, some other pathogenic genes have not been identified, greatly increasing the difficulty of clinical diagnosis and treatment. There are differences in the incidence data for SHL among countries. Roughly 15% of all congenital hearing loss is syndromic in the United

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States,[1,4] while Chinese scholars reported that SHL accounts for 30%[5]. The differences in these data are related to the selection bias of the study objectives, such as different races, familial or sporadic cases, and also to different molecular diagnostic therapies. This study retrospectively examined the genetic variants identified using WES as well as the hearing and other clinical features of 11 types of SHL in 14 han Chinese families who are not related to each other. The goal was to help us learn more about the different types of SHL.

Materials and Methods

Participants

The study subjects were 14 patients who were referred to the neonatal hearing screening and underwent audiological diagnosis at Ningbo Women and Children's Hospital from September 2020 to September 2023, including 3 males and 11 females, aged from 5 to 78 months old, with a median age of 11 months old. The present study received ethical approval from the Medical Ethics Committee at Ningbo Women and Children's Hospital, assigned Approval Number EC2023-009. Additionally, appropriate informed consent was secured from the parents or legal guardians of the patients, granting permission for the disclosure of case-specific information and accompanying visual materials in this publication.

Whole Exome Sequencing (WES)

Peripheral blood specimens were collected from the proband and his parents. Genomic DNA was extracted using a QIAamp DNA kit (QIAGEN, Germany), according to the manufacturer's instructions. DNA libraries were constructed using the WHS Exome Library Prep Kit customized by Twist and sequenced on the MGlSEQ-T7 platform (BGl Shenzhen, Guangdong Province, china). The quality of raw data was checked by FASTOC. After removing the low-quality reads, the adaptor reads were mapped to the reference genome GRch38/hg38, with the Burrows-Wheeler Aligner. Indels, copy number variants (CNVs), and single-nucleotide variants (SNVs) were identified using the GATK Haplotypecaller instrument. GATK variant filtration was used to filter the variants. Each variant was compared with public databases, including the single-nucleotide polymorphism database (dbSNP, https://www.ncbi.nlm.nih.gov/snp/, 1000 genomes project (https://www.internationalgenome.org/) exome aggregation consortium (ExAc, https://gnomad, broadinstitute.org/), and gnomAD (https://gnomad.broadinstitute.org/). Additionally, selected variants were forecasted using PolyPhen-2, SpliceAl, sorting intolerant from tolerant, human splicing finder, mutation taster, REVEL, and CADD. Variants were classified according to the variant interpretation guidelines of the American College of Medical Genetics andGenomics (ACMG).[6] CNVs were named using CLAMMS and XHMM.[7,8] Loss of heterozygosity (LOH) was determined using exomeAl and H3M2. The database of genomic variants (DGV) (http://dgv,tcag.ca/dgv/app/ home), Database of chromosomal lmbalance and Phenotypes in Humans using Ensembl Resources (DECIPHER; https://www.deciphergenomics.org/browser), and clinical Genome Resource (ClinGen; http://www.ncbi, nlm.nih.gov/projects/dbvar/clingen/) were used to determine the pathogenicity of candidate CNVs whose clinic significance was categorized using the 2020 ACMG CNV interpretation guidelines.[9]

Audiological Assessments and Radiological Examinations

Audiological tests included tympanometry, distortion product otoacoustic emissions (DPOAE), click-evoked air- and bone-conduction auditory brainstem response (ABR), NB CE-chirp auditory multifrequency steady-state response (ASSR), and pure tone audiometry (PTA), following the principle of cross validation. Hearing loss levels were classified according to the worldwide accepted criterion.[10] The average threshold at 0.5, 1, 2, and 4 kHz determined by PTA (mild: 26–40 dBHL, moderate: 41–55 dBHL, moderately severe: 56–70 dBHL, severe: 71–90 dBHL, profound: more than 91 dBHL) served as the definition of hearing loss. The stimulus level of ABR was calibrated in dB nHL, and the estimated threshold (dB eHL) of NB CE-chirp ASSR for this study was the corrected threshold, of which the correction factors were obtained from Interacoustics and were based on data from Rodrigues and Lewis[11]. Radiological examinations included HRCT of the temporal bone and MRI of the inner ear. HRCT of the temporal bone without contrast was performed on a 16-row multi-detector CT scanner (Brilliance 16, Philips Healthcare) at 100 kV and 120 mAs. Axial, coronal and parasagittal image series were reconstructed with slice thickness of 0.8 mm, 0.35 mm, 0.34 mm and

increment of 0.4 mm, 0.18 mm, 0.17 mm. Filtered-back projection reconstruction was used. MRI was performed on a Philips clinical scanner with a magnetic flux density of 1.5 Tesla. The examination protocol consisted of a heavily T2-weighted 3D-driven equilibrium (DRIVE) fast spin echo sequence with an echo time (TE) of 250 ms, a repetition time (TR) of 1500 ms. It was reconstructed in the imaging workstation, with a thickness of 3 mm and a layer spacing of 0 mm. Horizontal and vertical tomographic images of both auditory nerves were obtained.

Results

Genotype Characteristics

Out of the 11 different kinds of SHL observed, two instances were identified for each of the following syndromes: Waardenburg syndrome, Branchio-Oto-Renal syndrome, and Charge syndrome. The observed cases included one instance each of Treacher Collins syndrome, Kleefstra syndrome, Muenke syndrome, Ayme-Gripp syndrome, Tatton-Brown-Rahman syndrome, Stickler syndrome type 2, Stapes Ankylosis with Broad Thumbs and Toes, and Osteopathia Striata with Cranial Sclerosis. Based on the guidelines for assessing the pathogenicity of variants by ACMG and ClinGen, the observed data reveals the presence of two cases of pathogenic variations (P), nine cases of likely pathogenic variations (LP), and three cases of variants of uncertain significance (VUS). A comprehensive analysis revealed the presence of 12 mutant genes and 14 variant sites, which were found to be distributed among autosomes 2, 3, 4, 5, 6, 8, 9, 16, 17, 22, and X. Among these, 2 instances were inherited from the father and 2 cases from the mother. Notably, 10 of the variant sites identified in this study represent novel findings, as summarized in Table 1.

Audiological Features and Radiological Results

In this study, a total of 14 cases of deafness were observed, with 13 cases (92.9%) exhibiting bilateral deafness and 1 case presenting with unilateral deafness, specifically classified as Waardenburg syndrome type 4C. Among the sample population of 14 individuals, 10 cases (71.4%) were diagnosed with sensorineural hearing loss, while 4 cases (28.6%) exhibited mixed hearing loss. The severity of hearing loss varied among the participants' ears. Mild hearing loss was observed in 9 ears, accounting for 34.6% (9/26) of the sample. Moderate hearing loss was present in 4 ears, representing 15.4% (4/26) of the total. 1 ear exhibited moderately severe hearing loss, accounting for 3.8% (1/26) of the sample. 2 ears had severe hearing loss, which accounted for 7.7% (2/26) of the total. Profound hearing loss was observed in 9 ears, representing 34.6% (9/26) of the sample. Among the cases examined, case 11 exhibited a progressive mixed hearing loss. Air conduction click-evoked ABR measurements revealed a sensorineural hearing loss of 40dBnHL in the left ear and 35dBnHL in the right ear at 1 month of age. As reported, patients with syndromic hearing loss was prone to have upper respiratory infections. In this case, at 7 and 13 months of age, the type of tympanogram of both ears presented with type B, indicating otitis media with effusion, leading to an increase in the thresholds of the air conduction click-evoked ABR. At 7 months of age, the hearing threshold was 50dBnHL in both ears. Subsequently, at 13 months of age, the left ear exhibited a hearing threshold of 55dBnHL, while the right ear had a threshold of 60dBnHL. Additionally, bone conduction click-evoked ABR measurements indicated a hearing threshold of 35dBnHL in both ears. Radiological findings revealed abnormal results in 62.5% (5 out of 8) of the cases. Specifically, three cases exhibited cochlear/semicircular canal dysplasia, namely Waardenburg Syndrome Type 4C in case 3 and CHARGE syndrome in cases 6 and 7. Additionally, one case presented with bilateral ossicular chain malformation and cochlear dysplasia, associated with Branchio-Oto-Renal Syndrome Type 1 in case 4. Lastly, one case displayed auditory nerve aplasia and subvestibular nerve dysplasia, also linked to Branchio-Oto-Renal Syndrome Type 1 in case 5. Please refer to Table 2 and Figure 1.

Phenotypic Features Beyond Deafness

Among the 14 cases, 10 individuals exhibited special facial characteristics, accounting for 71.4% of the sample. These features included mandibulofacial hypoplasia in one case, blue irises in two cases, a distinctively flat face in one case, as well as other notable facial attributes such as low set ears, auricular deformities, short head deformities, protruding forehead, hypertelorism, microcephaly, depressed nasal bridge, preauricular fistula, and short nasal column (Figure 2). Three cases presented with cardiac anomalies, consisting of two cases of atrial septal defect and one case of combined atrial septal defect and ventricular

Table 1 Genotype Characteristics of Syndromic Hearing Loss in 14 Probands

Patient ID	Sex	Months of Age	Gene	Chromosomal Location (hg38)	Exome/ Introns	Transcript/cDNA/ Protein Change	gnomAD_ EAS	Assessment of the Pathogenicity of Genetic Variants	Variants Rating	Zygote Type	Inheritance	Syndrome (OMIM)	Origination	First Report
1	M	11	*TCOF1*	Chr5:150374180	exon8	NM_001371623.1:c.877del:p.Ala293Profs*34	—	PVS1 +PM2_Supporting	LP	Het	AD	Treacher Collins Syndrome Type 1 (154500)	De novo	Y
2	M	31	*MITF*	Chr3:69956469	exon8	NM_001354604.2:c.970A>G:p.Arg324Gly	—	PS3_Supporting +PS4_Supporting +PM5 +PM2_Supporting +PP3	LP	Het	AD	Waardenburg Syndrome Type 2A (193510)	De novo	N
3	F	12	*SOX10*	chr22:38369812–38369816	exon4	NM_006941.4: c.1087_1091delCCCCAinsGCCCCT (p.Pro363AlafsTer39)	—	PVS1+PS2 +PM2_Supporting	P	Het	AD	Waardenburg Syndrome Type 4C (613266)	De novo	Y
4	F	78	*EYA1*	chr8:72229887	exon7	NM_000503.6:c.456del:p.L152Ffs*89	—	PVS1 +PM2_Supporting	LP	Het	AD	Branchio-Oto-Renal Syndrome Type 1 (113650)	Father	Y
5	F	5	*EYA1*	chr8:71216776	exon14	NM_000503.6:c.1276G>A:p.G426S	0.0006	PM2_Supporting	VUS	Het	AD	Branchio-Oto-Renal Syndrome Type 1 (113650)	Father	N
6	F	10	*CHD7*	chr8:61693549	intron2	NM_017780.4:c.1666–10T>A	—	PS2 +PM2_Supporting	VUS	Het	AD	CHARGE Syndrome (214800)	De novo	Y
7	F	15	*CHD7*	chr8:60821813	exon10	NM_017780.4:c.2721G>C:p.Lys907Asn	—	PS2 +PM2_Supporting +PM1+PP3	LP	Het	AD	CHARGE Syndrome (214800)	De novo	Y
8	F	13	*NOG*	chr17:56594874	exon1	NM_005450.6:c.651G>A:p.Trp217*	—	PVS1 +PM2_Supporting +PP4	LP	Het	AD	Stapes Ankylosis with Broad Thumbs and Toes (184460)	Mother	Y
9	M	6	*EHMT1*	chr9:137813133	exon20	NM_024757.5:c.2995del:p.A999Pfs*11	—	PVS1+PS2 +PM2_Supporting	P	Het	AD	Kleefstra Syndrome Type 1 (610253)	De novo	Y
10	F	7	*FGFR3*	Chr4:1801844	exon7	NM_000142.5:c.749C>G:p.P250R	—	PM2_Supporting +PS3+PS4+PP4	P	Het	AD	Muenke Syndrome (602849)	Mother	N

11	F	21	*AMER1*	ChrX:64192316	exon2	NM_152424.4:c.971C>A:p.Ser324*	—	PVS1 Strong+PM6+ PM2 Supporting	LP	Het	XLD	Osteopathia Striata with Cranial Sclerosis (300373)	De novo	Y
12	F	17	*MAF*	Chr16:79599718	exon1	NM_005360.5:c.185C>G:p.Thr62Arg	—	PS2+PM2 Supporting+PP3	LP	Het	AD	Ayme-Gripp Syndrome (601088)	De novo	N
13	F	7	*DNMT3A*	chr2:25245279	exon13	NM_153759.5:c.1528G>A:p.V510I	0.000193	PM2 Supporting +PS2	VUS	Het	AD	Tatton-Brown-Rahman Syndrome (615879)	De novo	Y
14	F	5	*COL11A2*	chr6:33166808	exon59	NM_080680.3:c.4250G>T:p.G1417V	—	PS2 +PM2_Supporting +PP3	LP	Het	AD	Stickler Syndrome Type 2 (604841)	De novo	Y

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Table 2 The Audiological Characteristics, Radiological Features of Syndromic Hearing Loss in 14 Probands

Patient ID	Months of Age	Tympanometry Left/Right	DPOAE Left/ Right	Air Conducted Click-Evoked ABR		Bone Conducted Click-Evoked ABR		Air Conducted NB CE-chirp ASSR Left/Right				Type of Hearing Loss	Radiological Features of Middle and Inner Ear
				Left	Right	Left	Right	500Hz	1000Hz	2000Hz	4000Hz		
1	11	B/B	R/R	50	45	30	30	85/85	45/70	55/55	45/55	MHL	-
2	31	A/A	R/R	>100	>100	-	-	>100/ >100	>100/ >100	95/>100	>100/ >100	SNHL	Normal
3	12	A/A	R/R	100	20	-	-	>100/0	100/5	100/5	>100/10	SNHL, Unilateral deafness	Posterior semicircular canal dysplasia of both sides; enlarged vestibule in the right ear
5	5	Positive peak/ Positive peak	R/R	95	>100	-	-	>100/ 100	>100/ 100	100/90	90/90	SNHL	Bilateral auditory nerve and the left subvestibular nerve aplasia; the right subvestibular nerve dysplasia
6	10	A/A	R/R	90	95	-	-	60/85	95/95	95/90	90/90	SNHL	Bilateral cochlear malformations, only the bottom and the middle circle was observed; enlarged vestibule; bilateral semicircular canal malformations; bilateral auditory nerve dysplasia
7	15	A/A	R/R	70	30	>45	30	-	-	-	-	SNHL	Semicircular canal malformations
8	1	Positive peak / Positive peak	R/R	40	35	-	-	-	-	-	-	MHL, Progressive	-
	7	A/A	R/R	50	50	35	35	40/35	40/35	30/30	30/30		
	8	A/A	R/R	45	50	-	-	-	-	-	-		
	13	A/A	R/R	55	60	-	-	-	-	-	-		
9	6	A/A	R/R	50	30	-	-	-	-	-	-	SNHL	Normal
10	7	A/A	R/R	30	35	30	35	-	-	-	-	SNHL	-
11	21	A/A	R/R	30	25	-	-	30/20	25/20	20/20	30/25	SNHL	Normal
12	17	A/A	R/R	>100	>100	-	-	-	-	-	-	SNHL	-

13	7	A/A	R/R	55	75	30	30	50/80	60/55	55/65	60/70	MHL	-
14	5	Positive peak / Positive peak	R/R	40	40	-	-	25/25	30/30	40/30	40/40	SNHL	-
				Pure tone audiometry (Left/Right)									
				Air conducted threshold				Bone conducted threshold					
				500Hz	1000Hz	2000Hz	4000Hz	500Hz	1000Hz	2000Hz	4000Hz		
4	78	A/A	R/R	80/120	70/115	45/120	35/105	45/>65	55/>70	45/>75	45/>80	MHL	Bilateral ossicular chain malformation; bilateral cochlear dysplasia, only the bottom and half of the middle circle was observed on the left side, only the bottom circle was observed on the right side; bilateral auditory nerves dysplasia

Abbreviations: -, not tested; R, refered; SNHL, sensorineural hearing loss; MHL, mixed hearing loss.

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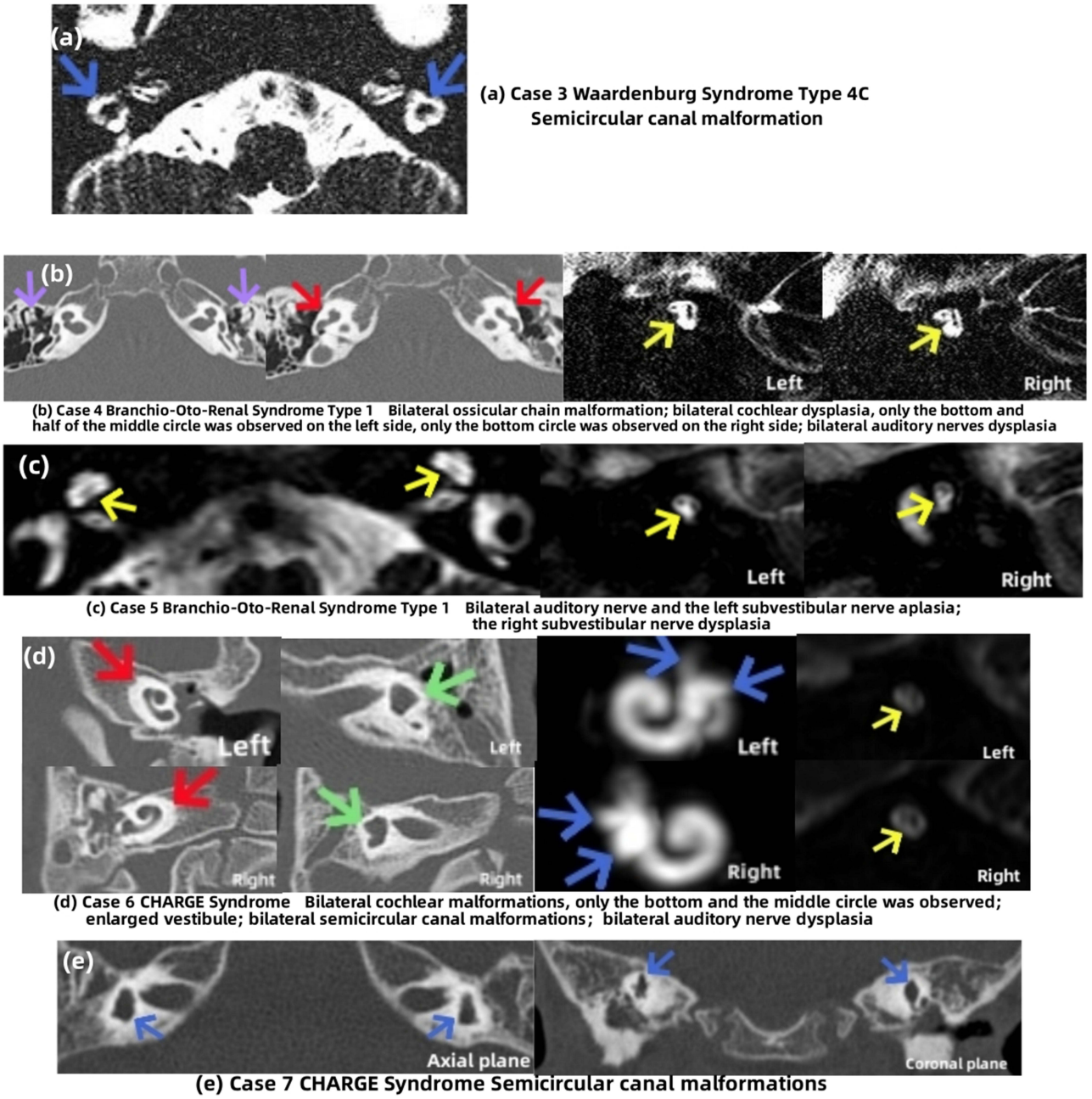

Figure 1 The present study examines the radiological imaging of five instances displaying abnormalities in the middle and inner ears. The cochlea is shown by the red arrow, the vestibule by the green arrow, the semicircular canal by the blue arrow, and the auditory and vestibular nerve by the yellow arrow.

septal defect. Abnormal brain development was observed, characterized by bilateral ventricular dilation, widening of the extracerebral space, brain hypoplasia, premature closure of the coronal suture, asymmetric bilateral ventricles, and a larger occipital cistern. Other phenotypic features observed in various syndromes are as follows: skin albinism and megacolon in Waardenburg syndrome, branchial fistula in Branchio-Oto-Renal syndrome, posterior nostril stenosis in CHARGE syndrome, wide thumb and toe in Stapes Ankylosis with Broad Thumbs and Toes. Additionally, these syndromes may present with additional features such as intestinal obstruction, pelvic signs, and irregular thoracic and rib morphology (Table 3).

Figure 2 Facial appearance of the 10 cases with rare facial characteristics.

Discussion

With the ongoing advancements in the newborn hearing screening, it has become possible to diagnose, intervene, and rehabilitate congenital hearing impairment in newborns at an early stage of life. Additionally, this system also facilitates early diagnosis of SHL. In addition, the rapid development of molecular research on hereditary hearing loss has facilitated the accurate diagnosis of the underlying causes of SHL. WES is a high-throughput sequencing technique utilized to capture and enrich the DNA sequences of the exon regions across the entire genome.[12] The method does not demonstrate bias in all genes and offers a more comprehensive approach to genetic detection. The utilization of genetic testing plays a crucial role in the identification of novel pathogenic genes, and the identification of previously unknown pathogenic sites within known pathogenic genes. Compared to targeted capture sequencing of deafness-related genes,

Table 3 Clinical Phenotype Characteristics of Syndromic Hearing Loss in 14 Probands

Patient ID	Sex	Facial Feature	Heart	Brain	Growth and Development	Other
1	M	Downward-slanting palpebral fissures; micrognathia; deformity of the auricles	Normal	Normal	Growth retardation	Not found
2	M	Blue iris of both eyes	Normal	Normal	Normal	Skin albinism
3	F	Blue iris of both eyes	Normal	Normal	Normal	Skin albinism, megacolon
4	F	Low set ears, deformity of the auricles	Normal	Normal	Normal	branchial fistula
5	F	Receding jaw	Atrial septal defect, ventricular septal defect	Normal	Growth retardation	Not found
6	F	None	Normal	Normal	Normal	Not found
7	F	None	Normal	Normal	Normal	Choanal stenosis
8	F	None	Normal	Normal	Normal	Bilateral wide thumbs and toes, proximal interphalangeal joint of the fifth fingers
9	M	Microcephaly; bilateral helix deformity; preauricular fistula of the right ear	Normal	Bilateral ventricular dilation, widening of extracerebral space, and possible brain hypoplasia	Normal	Lower intestinal obstruction
10	F	Protruding forehead, inverted eyes, brachycephaly	Normal	Premature closure of the coronal suture	Normal	Incomplete intestinal obstruction
11	F	Hypertelorism, depressed nose bridge, raised forehead, protruding upper lip	Atrial septal defect	Asymmetric bilateral ventricles, slightly smaller right ventricle shape, greater occipital cistern	Normal	Not found
12	F	Distinctive flat facial appearance; depressed nasal bridge, hypertelorism, sparse eyebrows low set ears; small and oblique eye fissures	Normal	-	Growth retardation	Irregular morphology of some thoracic vertebrae and bilateral ribs
13	F	Small eye fissures	Atrial septal defect	-	Normal	Not found
14	F	None	Normal	-	Normal	Pelvic dysplasia

WES had a higher rate of molecular diagnosis in both sporadic and familial cases of deafness.[13] The molecular basis of deafness has been successfully identified in 47.3% of patients with sporadic deafness,[14] and in 56% of patients with familial deafness.[15] This study employed a comprehensive approach, which included physical examination, phenotype analysis, detailed family history consultation, and WES, in order to investigate patients presenting with suspected SHL. Fourteen cases of eleven types of SHL were identified, with the exception of case 5 and case 13 in the gnomAD database.

The frequencies of these cases in the East Asian (EAS) population were 0.0006 and 0.000193, respectively. However, the remaining cases were not included in the ExAC, gnomAD, gnomAD_EAS, and the 1000 Genome Asian Population Database. The first report documented 10 variant sites. In addition, the median age of the participants was 11 months. Regarding the participants, the median age reported corresponds to the timing of their last hearing assessment. All participants underwent their initial hearing diagnosis within the first three months of life, and our intention was to track their subsequent hearing evaluations. However, a subset of participants did not undergo WES at an early age, leading to a delay in definitive diagnosis. We postulate that with increased research funding, the timing of genetic diagnosis for syndromic hearing loss could be significantly expedited.

This finding provides strong evidence for the efficacy and significance of integrating neonatal hearing screening system with WES in the early detection of Sensorineural Hearing Loss during infancy. However, the successful management of SHL necessitates clinical audiologists and otolaryngologists to possess a comprehensive comprehension and recognition capacity of the clinical phenotype of SHL. Additionally, the involvement of medical professionals from various relevant departments, including genetic counseling experts, molecular laboratory experts, physicians, cardiologists, ophthalmologists, and plastic surgeons, is crucial. This multidisciplinary consultation approach ensures the professionalism and accuracy of clinical data. On the contrary, the existing guidelines for interpreting variants in the context of genetic diagnosis of SHL still possess certain limitations. One such limitation is that a considerable number of variants are still classified as clinically insignificant, referred to as variants of VUS, during the genetic diagnostic process. In the present study, three cases exhibiting specific phenotypic characteristics were classified as variants of VUS. The aforementioned classification can be ascribed to the restricted understanding of specific SHL situations and the infrequency of the variant population under consideration. With the increasing availability of data from populations harboring the identical variant and advancements in bioinformatics technology, there is a need to update the guidelines in order to streamline the process of identifying variant characteristics.[13]

The study observed a total of 14 cases of SHL, predominantly characterized by bilateral sensorineural hearing impairment. Approximately one-third of the cases exhibited mild hearing loss. Case 10 presented with a mild sensorineural hearing loss associated with Muenke Syndrome, which was inherited maternally. And the mild hearing loss of the girl went unnoticed by her mother until she underwent a Pure Tone Audiometry (PTA) test. If not for the neonatal hearing screening, cases of mild hearing loss could be prone to misdiagnosis or mistaken for individuals with normal hearing abilities. In previous studies, it has been reported that 72.1% of individuals diagnosed with Treacher Collins syndrome, which is caused by variants in the *TCOF1* gene, exhibited external auditory canal atresia. Furthermore, these individuals commonly experienced moderate to severe hearing loss, as documented by Rosa et al[16] and Vincent and Geneviève.[17] In the present study, a case is described wherein the patient exhibited mild deformity of the auricles and bilateral moderate mixed hearing loss. This presentation was hypothesized to be a mild phenotype of Treacher Collins syndrome, caused by a variant in the *TCOF1* gene. Case 2 and case 3 were diagnosed with Waardenburg syndrome type 2A and 4C, respectively, which were caused by variants in the *MITF* and *SOX10* genes. Case 2 exhibited bilateral profound sensorineural hearing loss, while case 3 displayed unilateral profound sensorineural hearing loss in the left ear, which is a rare occurrence as reported in the literature. This is noteworthy because the probability of hearing loss resulting from the *SOX10* gene variant is 96.5%, and the majority of cases are typically bilateral, as stated by Song et al.[18] In addition, the frequent observation of hypoplasia of the semicircular canals and enlarged vestibules in the inner ear malformations findings of *SOX10* variant was highly penetrant when interpreted by a specialized radiologist.[19] Accordingly, case 3 exhibited dysplasia of the posterior semicircular canal (Figure 1a). Case 4 and case 5 were both attributed to genetic variants in the *EYA1* gene, leading to the manifestation of Branchio-Oto-Renal syndrome. Case 4 presented with malformations of the auricle and ossicular chain, leading to a mixed hearing loss characterized by moderately severe hearing loss in the left ear and complete deafness in the right ear. In contrast, case 5 did not exhibit auricle and middle ear malformations, but instead had bilateral auditory nerve aplasia and left vestibular nerve aplasia. Additionally, MRI at the age of 5 months revealed dysplasia of the right vestibular nerve, a rare finding that has been infrequently reported in the literature (Figure 1b and c). Case 6 and case 7 were diagnosed with CHARGE syndrome, which was attributed to the presence of the pathogenic gene *CHD7*. Both cases exhibited bilateral semicircular canal malformations, as depicted in Figure 1d and e. Additionally, case 6 presented with cochlear dysplasia, which aligns with the prevailing structural

malformation commonly observed in CHARGE syndrome, as reported in the literature.[20] However, the severity of hearing impairment in the right ear of Case 7 was classified as mild sensorineural hearing loss, indicating a condition that is presumed to have incomplete penetrance.

Besides deafness, other clinical phenotypes of sensorineural hearing loss also demonstrate significant phenotypic heterogeneity within the same type of SHL, or even among individuals with the same mutated gene. The individuals diagnosed with Treacher Collins, Ayme Gripp, Waardenburg, Muenke syndrome, and Osteopathia Striata with Cranial Sclerosis in this study exhibited distinctive facial characteristics that were readily identifiable (Figure 2). Both Case 2 and Case 3 presented instances of Waardenburg syndrome accompanied by skin albinism. However, Case 3 was diagnosed with Waardenburg syndrome 4C type, which is characterized by the presence of megacolon as a symptom. Multiple studies have indicated that the relationship between the genotype and phenotype of Branchio-Oto-Renal syndrome remains uncertain.[21,22] In the present study, it was observed that case 4 presented with branchial fistula, whereas case 5 exhibited atrial septal defect and growth retardation. However, no renal abnormalities were detected in either of these cases. In the two instances of CHARGE syndrome caused by a CHD7 variant, a cDNA alteration was observed in intron 2 in case 6. This alteration is presumed to impact the machinery responsible for mRNA splicing, subsequently leading to pathogenic effects on transcription, mRNA processing, and translation. Neither of them exhibited the characteristic phenotype of coloboma, heart malformations, atresia of the choanae, retardation of growth or development, genital anomalies, and ear malformations. However, case 7 did present with choanal stenosis, which could potentially be associated with incomplete penetration of CHARGE syndrome.[20] However, it should be noted that the two cases under study were of a very young age, with one being 10 months old and the other 15 months old. Given the delayed onset characteristics of the SHL phenotype, it is yet to be determined whether the observed symptoms will intensify in the later stages. Further investigation and medical follow-up will be necessary to address this question. Therefore, it is of utmost importance to conduct regular follow-up and specialized examinations for patients with SHL. Additionally, there is a need to continuously expand the database of phenotypic characteristics across different age groups. This will serve as a reliable foundation for clinical differentiation, accurate diagnosis, effective treatment plans, and the development of prognostic interventions.

The timely identification of SHL can provide valuable insights into the development of hearing impairment, as well as other associated systemic conditions, thereby facilitating appropriate medical interventions. Early detection and intervention of symptoms in infants and young children can be facilitated by this technology, enabling doctors and parents to effectively respond to the occurrence of serious clinical symptoms. Case 8 presented a condition known as Stapes Ankylosis with Broad Thumbs and Toes, which was attributed to a variant in the *NOG* gene located on chromosome 17. This condition could potentially be misdiagnosed as simple otosclerosis if WES is not performed.[23] The infant underwent neonatal hearing screening for both ears, and the initial diagnostic hearing test was performed at 45 days of age. The auditory thresholds of the left and right ear exhibited an increase of 15 and 30 dB, respectively, over the course of one year. The binaural hearing threshold of the bone conduction click-evoked ABR was measured at 35 dBnHL, suggesting the presence of progressive mixed hearing loss. Upon conducting a medical history inquiry, it was discovered that the child's mother, grandmother, and great-grandfather had also exhibited symptoms of hearing loss. Both the mother and grandmother exhibited symptoms of moderately severe mixed hearing loss. However, the precise degree of hearing loss in the great-grandfather remains uncertain as he was unable to undergo hearing tests due to his advanced age. Additionally, a physical examination indicated the presence of broad thumbs and toes on both sides in the baby, her mother, and her grandmother. The infant also exhibited symphalangism in the proximal interphalangeal joints of both fifth fingers (Figure 3). The presence of conductive hearing loss and congenital stapes sclerosis in the WES report carries significant clinical implications. This suggests that audiologists should formulate a comprehensive follow-up plan to assess the rate and severity of hearing loss. Regarding the strategy for improving hearing loss, stapes surgery has been demonstrated as an effective therapeutic option that can maintain satisfactory hearing outcomes in the long term.[24,25] This individual has previously received bone conduction hearing aids as an intervention. The decision to proceed with exploratory tympanotomy and stapes surgery will depend on the results of the follow-up hearing assessment and the individual's growth and development. In addition to this specific SHL, the phenotypic spectrum observed in other cases can also contribute to predicting the disease progression pattern. Early intervention upon the emergence of certain

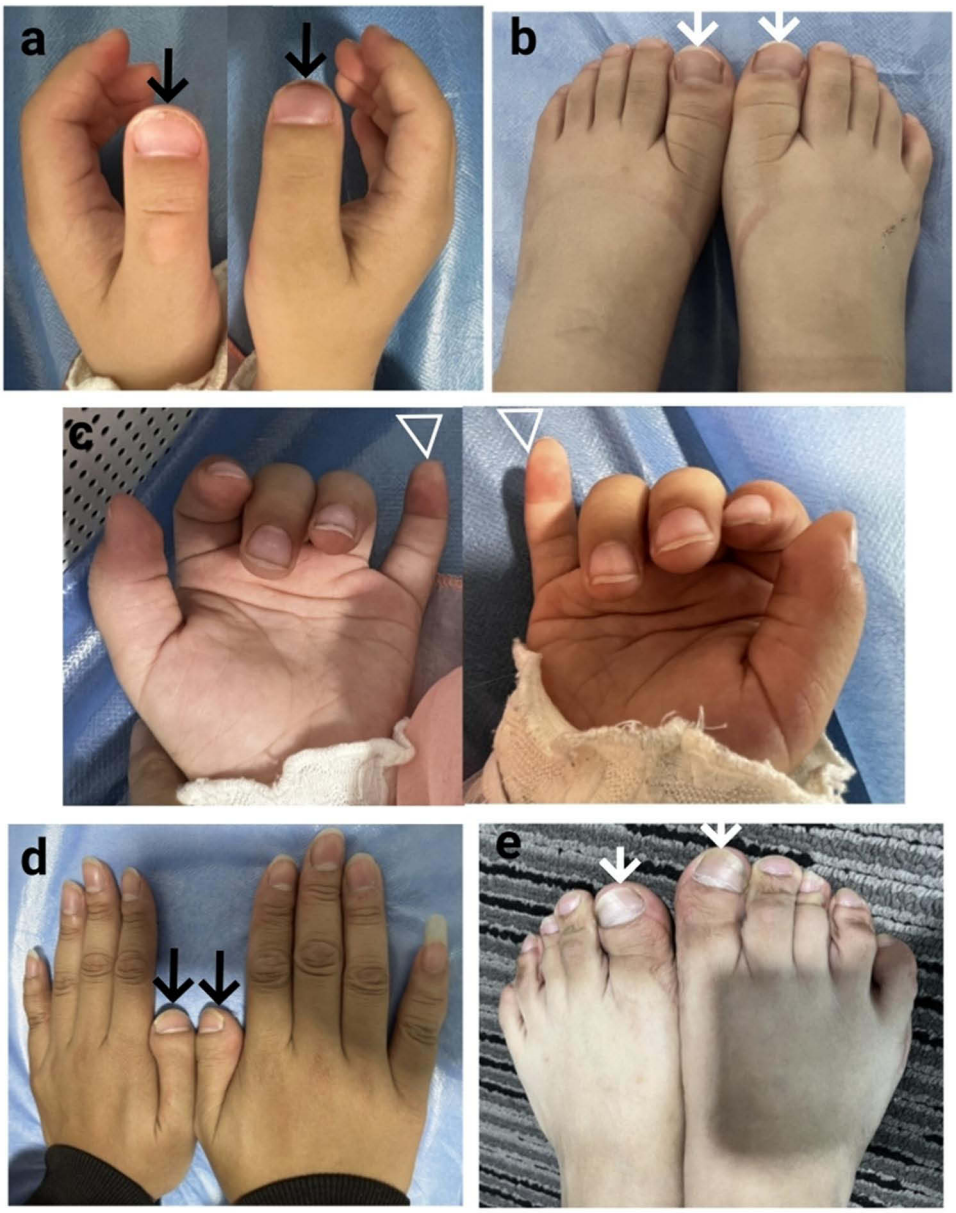

Figure 3 The present study examines the observations made on the hands and feet of case 8 and her mother, who both exhibit the syndrome of Stapes Ankylosis with Broad Thumbs and Toes. In the first scenario, denoted as (a, b, c), the value of 8 is considered. In the second situation, denoted as (d, e), the mother of the value 8 is being discussed. The presence of black arrows denotes the existence of wide thumbs, whereas white arrows signify the presence of broad toes. Additionally, white triangles are used to highlight the proximal interphalangeal joint of the fifth finger.

symptoms can help prevent the occurrence of severe diseases or mitigate the severity of symptoms. For instance, conditions like Muenke syndrome, Ayme Gripp syndrome, and Tatton-Brown-Rahman syndrome have been associated with a potential risk of epilepsy. As for Ayme Gripp syndrome, the literature to date has reported only 21 individuals from 19 families. One of the characteristic symptoms of this condition is the presence of congenital cataract, which has been reported in all cases except for one individual. However, it is worth noting that cataract was not observed in a seven-year-old Indian boy,[26] nor in our study at the age of one year. Whether this absence of cataract is an incomplete or delayed manifestation of the condition remains uncertain and warrants further comprehensive investigation in future studies. A recent study conducted by König et al[27] revealed that the presence of isolated cytokine-enriched pericardial effusion is a significant characteristic of Ayme Gripp syndrome. Additionally, it has been suggested that individuals with Tatton-Brown-Rahman syndrome may have a higher incidence rate of cancer compared to the general population,

warranting further attention.[28] Hence, it is suggested that the documentation of the WES report be retained for future utilization.

In addition, the field of in vitro fertilization has made significant advancements, leading to the increased maturity of its technology. As a result, preimplantation genetic testing for monogenic diseases has emerged as an effective strategy for preventing birth defects that are linked to hereditary disorders.[29] The study identified 14 cases of SHL, all of which exhibited dominant inheritance patterns. Notably, the observation of case 11 reveals an inheritance pattern consistent with X-linked dominance, indicating a 50% probability of transmitting the disease to children and its manifestation in both male and female individuals to varying degrees. The timely identification of SHLcan offer valuable insights for genetic counseling, marriage and fertility guidance, and prenatal intervention strategies for affected families. At the same time, Bi et al[30] argue that genetic counselors should exercise caution when offering advice, providing comprehensive information, and offering psychological support. This is particularly important due to the varying concepts and perspectives across different countries. The authors emphasize the need for genetic counselors to promote a wide range of informed choices for individuals seeking their services.

Conclusions

The clinical heterogeneity of syndromic deafness is significant. The combination of a neonatal hearing screening and WES can diagnose SHL in infancy and childhood, according to our findings, expansion of the gene variant spectrum and phenotype database for various age groups of SHL is essential and can provide valuable guidelines for clinical intervention decisions. It is imperative for medical practitioners to conduct diligent and prolonged patient monitoring due to the inherent variability in both the auditory impairment and the comprehensive clinical manifestation of SHL.

Data Sharing Statement

The original contributions presented in the study are included in the article or supplementary material, further inquiries can be directed to the corresponding authors.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflict of interest.

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