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ORIGINAL ARTICLE

Hearing characteristics and otoradiological abnormalities in three patients with novel pathogenic variants of *KMT2D*-related Kabuki syndrome

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Abstract

Background: Kabuki syndrome 1 (KS1; OMIM:147920), which is characterized by distinctive dysmorphic facial features (such as arched eyebrows, long palpebral fissures with eversion of the lower lid, and large protuberant ears), intellectual disability, short stature, and dermatoglyphic and skeletal abnormalities, is brought on by pathogenic variants in *KMT2D* (OMIM:602113). In this work, three individuals with novel pathogenic *KMT2D* gene variants had their longitudinal audiological manifestations and ear structural characteristics outlined.

Methods: The longitudinal audiological data from neonatal hearing screening and a battery of several hearing tests were evaluated. The battery of hearing tests included tympanometry, distortion product otoacoustic emission (DPOAE), click-evoked air-conduction auditory brain-stem response (AC-ABR), click-evoked bone-conduction auditory brain-stem response (BC-ABR), narrow band CE-chirp auditory steady-state response (NB CE-chirp ASSR), and pure-tone audiometry (PTA). Phenotype identification and whole exome sequencing (WES) were performed on recruited individuals.

Results: All three patients (two females and on male; last evaluations at 14 months, 11 months, and 5.7 years, respectively) failed the newborn hearing screening, and the audiological follow-up data revealed mild to profound fluctuating hearing loss, which was directly influenced by the incidence and severity of otitis media with effusion (OME). When OME occurred, the AC-ABR thresholds increased from 30–75 dBnHL to 45–90 dBnHL. The threshold for the BC-ABR and BC-PTA was between 25 and 50 dBnHL, indicating mild to moderate sensorineural hearing loss (SNHL). The high-resolution computed tomography (HRCT) pictures indicated that all three patients had middle and inner ear abnormalities. Middle ear anomalies showed as diminished mastoid gasification and ossicle dysplasia. Cochlear dysplasia, a dilated vestibule, fusion of the vestibule with the horizontal semicircular canals, and a short and thick horizontal semicircular canal were

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visible on images of the inner ear. This study recruited three individuals with three novel pathogenic variants (c.5104C>T, c.10205delA, and c.12840delC) of *KMT2D* who were identified at ages 27 days, 2 months, and 5.5 years.

Conclusions: Hearing characteristics of three individuals with three novel pathogenic variants of *KMT2D* range from mild to profound fluctuating hearing loss with mild to moderate SNHL. HRCT scans showed that all three individuals had anatomical middle and inner ear abnormalities. KS 1 patients must get clinical therapy for OME, frequent auditory monitoring, and prompt intervention.

KEYWORDS

computed tomography, genotype–phenotype correlation, hearing loss, inner ear, Kabuki syndrome, *KMT2D*, middle ear

1 | INTRODUCTION

Kabuki syndrome was initially identified by Niikawa et al. (1981) and Kuroki et al. (1981) and was called for the similarities between the facial characteristics of patients and the traditional Japanese Kabuki makeup. Kabuki syndrome 1 (KS1; OMIM 147920) is caused by pathogenic variants in the *KMT2D* gene (NM 003482.3; also known as *MLL2*, *MLL4*, or *ALR*), which produces a histone H3 lysine 4 specific methyltransferase required for H3K4 di- and trimethylation (Ng et al., 2010). KS patients (55%–80%) exhibit mutations in *KMT2D* and are characterized by dysmorphic facial characteristics (including arched eyebrows, long palpebral fissures with eversion of the lower lid, and large protuberant ears), intellectual disability, short stature, and dermatoglyphic and skeletal abnormalities.

In 2013, a phenotypic scoring system was established to determine whether people with KS-associated characteristics were more likely to carry a heterozygous pathogenic variation in *KMT2D* (Makrythanasis et al., 2013). In 2018, clinical and molecular genetic specialists on KS developed the worldwide consensus diagnostic criteria for KS (Adam et al., 2019). However, the phenotypic scoring system did not elaborately discuss the audiological features of the KS, and the consensus diagnostic criteria only classify progressive SNHL as one of the supportive features, despite the fact that 82% of the reported cases in the medical literature indicated that hearing loss was a common finding in patients with KS (Barozzi et al., 2009). In addition, it was observed that 40% of patients with KS had hearing loss and 30% had middle ear dysfunction, which was mostly caused by immunodeficiency, resulting in vulnerability to upper airway infections that can easily develop to otitis media (Qiu & Yuan, 2019). The purpose of this study was to elucidate the hearing characteristics of three novel pathogenic variants of *KMT2D* by examining the data of audiological evaluation from birth to each audiological follow-up over short periods of time.

The various reasons of hearing loss in KS 1 individuals have not been thoroughly documented and were commonly attributed to recurrent middle ear infection; nonetheless, a number of studies have revealed instances of ossicular malformations, inner ear abnormalities (Baldridge et al., 2020; Shangguan et al., 2019; Tekin et al., 2006; Toutain et al., 1997). Therefore, HRCT was also conducted to detect the congenital anomalies of the middle and inner ear and to determine the kind of hearing impairment.

2 | MATERIALS AND METHODS

2.1 | Participants

This study enrolled three patients identified with heterozygous pathogenic *KMT2D* variants between 2021 and 2022 (two females and one male; ages 14 months, 11 months, and 5.7 years, respectively, at the last evaluation). Han Chinese was the ethnicity of every individual. At Ningbo Women and Children's Hospital, the WES and audiological follow-up were done. The parental DNA was extracted and examined to identify the haplotype phasing of each variation. Before genetic testing, the parents of every individual gave informed consent. The Ningbo Women and Children's Hospital Research Ethics Committee adopted the study protocols, and the ethics committee approved the study (No. EC2023-009).

2.2 | Audiological assessments

All audiological evaluations were conducted by seasoned audiologists. Later than 48 h after birth, DPOAE was

utilized for newborn hearing screening. Before each hearing test, otoscopy was conducted to inspect the ear canal and tympanic membrane for abnormalities or excess wax. Tympanometry, DPOAE, AC-ABR, BC-ABR, NB CE-chirp ASSR, and PTA were conducted (depending on the patient's age or cognitive status) to determine the type and severity of hearing loss.

2.2.1 | Tympanometry

Tympanometric measures were obtained using Interacoustic AT235 which was calibrated annually. One thousand Hertz tympanometry was conducted to infants under the age of 6 months, combined 226 and 1000 Hz tympanometry were applied to infants between the age of 7 months and 12 months, and 226 Hz tympanometry was performed to infants older than 12 months. Middle ear pathology was defined based on the following: type B of 226 Hz tympanometry or flat curve of 1000 Hz tympanometry.

2.2.2 | DPOAE

DPOAE was performed using an otoacoustic emission system (Maico, Germany). The f2 test frequencies were measured at 2000, 3000, 4000, and 5000 Hz. The primary tone stimulus intensities of L1 equaled to 65 dB SPL and L2 equaled to 55 dB SPL, with f2/f1 frequency ratio of 1.2. The amplitudes of distortion products at each analyzing frequency were obtained. The pass criteria included a signal-to-noise ratio of at least 7 dB and an absolute DPOAE signal level of at least −5 dB SPL, for at least three out of the four tested frequencies.

2.2.3 | Click-evoke ABRs and NB CE-chirp ASSR

Click-evoked AC-, BC-ABRs, and NB CE-chirp ASSR were conducted using Interacoustic Eclipse EP25 when patients were natural asleep or in a state of sedation. Surface recording electrodes were placed at the hairline of the forehead (positive), mastoid (negative), and the forehead (ground). Interelectrode impedance was maintained below 3 kΩ. For the click-evoked ABRs, clicks were presented in alternating polarity at a rate of 21.1/s through insert earphones (EAR-3A) and at a rate of 45.1/s through bone vibrator (Radioear B71). The artifact rejection was set at 40 μV and digital filtering from 100 to 1500 Hz. The stop criteria were set as follows: recording number of 2000, residual noise target of under 40 μV. The recording window was set from 0 to 16 ms relative to stimulus onset. The chirp stimuli were designed to compensate for the cochlear delay and to produce a bigger response (Dau et al., 2000). NB CE-chirp signals centered at 500, 1000, 2000, and 4000 Hz were used as the test stimuli and the modulation frequencies was 90 Hz. The stimulus level was calibrated in dB nHL and the estimated threshold (dB eHL) for this study was corrected threshold, correction factors were obtained from Interacoustics and were based on data from Rodrigues and Lewis (2014).

2.2.4 | PTA

Audiometric thresholds were obtained for each ear at 500, 1000, 2000, and 4000 Hz using calibrated Interacoustic AD 229b audiometer with sound-excluding HDA-300 headphones.

The hearing levels were determined by the thresholds of AC-ABR, NB CE-chirp ASSR, or PTA. Further, according to the *World Report on Hearing* in 2021, the grades of hearing loss were classified as mild (20 to <35 dB), moderate (35 to <50 dB), moderately severe (50 to <65 dB), severe (65 to <80 dB), or profound (80 to <95 dB), complete or total (>95 dB). Audiological data with transient middle ear pathology was defined by the presence of average airbone gap >10 dB at ABR or PTA.

2.2.5 | HRCT

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, 0.35, and 0.34 mm and increment of 0.4, 0.18, and 0.17 mm. Filtered-back projection reconstruction was used. CT scans were conducted in sedation to facilitate diagnostic image quality and to avoid the necessity to repeat an acquisition.

3 | RESULTS

3.1 | Genetic diagnoses and phenotypic feature of the patients

The study involved three patients (two females and one boy; mean age = 2.5 years, standard deviation = 2.6 years) from three unrelated Chinese families. WES verified that all three individuals were heterozygous for three novel *KMT2D* variants [NM_003482(hg19)], including one nonsense variation (c.5104C>T) and two frameshift variants

(c.10205delA, c.12840delC) (Table 1). In addition, none of these three variations are included in the ExAC, 1K genome project, nomAD, or nomAD-EAS databases. According to the American College of Medical Genetics and Genomics (ACMG) guideline, all the three variants were classified as likely pathogenic. In addition, the blood samples of the parents were analyzed by Sanger sequencing, and no variants were found, suggesting that all three novel variants were de novo.

In 2018, the Kabuki Syndrome international consensus diagnostic criteria (Adam et al., 2019) proposed that a definitive diagnosis could be made in any individual with a history of infantile hypotonia, developmental delay and/or intellectual disability, and one or both of the following major criteria: (1) a pathogenic or likely pathogenic variant in *KMT2D* or *KDM6A*; and (2) typical dysmorphic features at some point in life. Taking notice of the fact that patients 1, 2, and 3 all have palpebral fissure length measures that are above the mean value of +2SD for age and sex, we may say that all the patients have "long palpebral fissures." Based on the phenotype features of the three subjects, which were classified as infantile hypotonia, developmental delay and/or intellectual disability, typical dysmorphic features, and other supportive clinical features (Table 2), we can confirm that these three subjects were definitive pathogenic variants of Kabuki syndrome.

3.2 | Audiological features

All three patients failed the newborn hearing screening, and their first diagnostic hearing tests revealed moderate to severe hearing loss (50–75 dBnHL) with click-evoked AC-ABR testing. Tympanometry, DPOAE, click-evoked AC-ABR, click-evoked BC-ABR, and NB CE-chirp ASSR were done during the duration of audiological follow-up. Findings from Table 3 demonstrated that all three patients had recurrent OME, as determined by tympanometry and the air-bone gap in the ABR. Consequently, changing AC-ABR thresholds were detected. The BC-ABR thresholds were between 25 and 50dBnHL. In patient 1, the NB CE-chirp ASSR revealed an audiogram configuration in which the right ear had a high frequency sloping audiogram and the left ear had an ascending audiogram. Due to intellectual disability, patient 3 was unable to participate with PTA until she was 66 months old. Recent 6-time follow-up PTA audiogram revealed moderate SNHL in the left ear and mild SNHL in the right ear (Table 4).

3.3 | HRCT findings of the middle and inner ear

The images obtained from the HRCT showed a wide spectrum of malformations of the middle and inner ear (Table 5). All three patients showed incus dysplasia, and patient 1 also had a weak connection between the stapes and incus. Cochlear dysplasia (only the bottom and middle circles were visible in the left ears of patients 1 and 3), bone hardening of the oval windows in patient 2, and malformations of the vestibule and horizontal semicircular canal in patients 1 and 3 were noted as anatomical abnormalities of the inner ear (Figures 1–3).

4 | DISCUSSION

The incidence of KS is estimated to be one in 32,000 newborns in Japan (Niikawa et al., 1981), 1 in 86,000 in Australia and New Zealand, but the exact prevalence in the United States and Europe is unclear (Carcione et al., 1991). Several prior studies found that the phenotypic of KS takes longer to develop, making it difficult to diagnose KS in newborns; the average age of diagnosis was 2 years (Vaux et al., 2005). In this work, patients 1 and 2 were identified at a relatively early age of 27 days and 2 months, respectively, using phenotypic identification followed by WES confirmation of KS gene, mutation type or other syndrome overlap with KS. However, case 3 was identified at the age of 5.5 years despite presenting with evident phenotypical traits from birth and having been under medical surveillance for the entirety of her life, showing that a lack of understanding regarding the clinical diagnosis of KS was the primary cause. While molecular

TABLE 1 General information and details of the genotype, phenotype in the three patients.

	Patient 1	Patient 2	Patient 3
Sex	Male	Female	Female
Age of genomic diagnosis	27 days	2 months	5.5 years
Age of the last evaluation	2 years	2 years	6 years
Exon	21	35	40
cDNA change	c.5104C>T	c.10205delA	c.12840delC
Protein change	p. Arg1702*	p. Leu3402Argfs*31	p. Pro4281Leufs*103
Mutation type	Nonsense	Frameshift	Frameshift

TABLE 2 Details of the phenotypic features in the three patients.

	Patient 1	Patient 2	Patient 3
Infantile hypotonia	+	+	+
Developmental delay and/or intellectual disability	+	+	+
Typical dysmorphic features			
Long palpebral fissures with eversion of the lateral third of the lower eyelid	+	+	+
Left palpebral fissure (cm)	3.23	3.08	3.31
Right palpebral fissure (cm)	3.15	3.14	3.32
Comparison with the mean children (palpebral fissure)	+3.2SD	+3.1SD	+2.1SD
Arched eyebrows, sparse lateral one third	+	+	+
Short columella with depressed nasal tip	+	+	+
Large, prominent, or cupped ears	+	+	+
Persistent fingertip pads	+	+	+
Other supportive clinical feature			
High or cleft palate	+	+	+
Lip pits	+	+	+
Preauricular fistula	+	+	+
Congenital heart defects, excluding a patent ductus arteriosus	+	+	+
Short stature	+	+	+
Feeding difficulties	+	+	+
Renal anomalies	−	−	+
Joint laxity	−	−	+
Hypogammaglobulinemia or low serum IgA	+	+	+
Frequent infections	+	+	+
Dental abnormalities	Unknown	Unknown	+
Premature thelarche in females	Male	+	−
Hypospadias in males	+	Female	Female
Hyperinsulinemic hypoglycemia in infancy	+	−	−

genetic confirmation is considered the gold standard for diagnosis, not all pathogenic variants that lead to a phenotype will be detected with current Sanger sequencing, next-generation sequencing, and/or gene-targeted deletion/duplication analysis (Adam et al., 2019), genotypes analysis manifested great importance to the KS clinical diagnostic. Regarding the audiological characteristics of KS 1 patients, 23%–40% were considered to suffer hearing loss (Chen, 2006; Qiu & Yuan, 2019). Shangguan et al. (2019) hypothesized that Chinese KS patients were more likely to have hearing impairment (34% vs. 25%) than non-Chinese KS patients. An analysis of 81 published studies revealed that 110 patients with KS experienced hearing loss, including 20 instances of conductive hearing loss (CHL), three cases of mixed hearing loss, and 12 cases of SNHL. Of the remaining 35 cases of hearing loss, there were 20 cases of CHL, three cases of mixed hearing loss, and 12 cases of SNHL (Liu et al., 2015). Numerous factors contributed to the high incidence of unclear hearing loss type in previous studies; however, we hypothesized that KS patients were unable to collaborate with PTA due to intellectual retardation, and that a full accurate prediction of hearing loss through multiple objective audiology sessions did not occur as expected for a variety of reasons. For instance, a limited number of clinics have expertise in auditory electrophysiology for children and wait times can be lengthy; auditory assessments with electrophysiology containing frequency-specific thresholds and bone conductive thresholds require a very quiet patient, and the amount of naptime available for testing decreases as the child's age increases (Sininger et al., 2018); the conductive component of hearing loss could be underestimated using low-frequency tympanometry. In addition, although HRCT is the most effective imaging technique for determining the morphology of the middle and inner ear, it also necessitates close collaboration between radiologists and otologists. In low degree malformations, an inexperienced radiologist is more likely to ignore modest symptoms of dysplastic ossicles and deem them normal (Kösling et al., 2009). In addition, KS patients had additional significant clinical symptoms, including as eating problems, congenital heart malformations, and kidney anomalies; therefore, parents were more likely to overlook the hearing issue. Consequently, follow-up delay and even loss-to-follow-up are also significant factors. Patient 1 in this research underwent abdominal surgery for chylous ascites at 11 days of age and surgery for tethered cord syndrome at 5 months of age. Patient 2 was hospitalized for pneumonia twice. At ages 3 months, 2 years, and 3 years, Patient 3 underwent operations for renal abnormality and hip laxity. Nonetheless, due to the significant development of the neonatal hearing screening program, both patient 1 and patient 2 got six objective audiological evaluations around the age of 1 year. Patient 3 underwent eight objective hearing tests and six PTAs before reaching the age of 5.7 years.

In this investigation, patients from birth to 6 months of age were exposed to a probe tone of 1000 Hz, patients from birth to 6 months of age were subjected to combined 226 and 1000 Hz probe tone tympanometry, and children

TABLE 3 The outcomes of tympanometry, click-evoked AC- and BC-ABRs, and NB CE-chirp ASSR in the first diagnostic test and the follow-ups.

							NB CE-chirp ASSR (dB eHL)				
	Age (MOA)	Ear	Tympanometry	DPOAE	AC-ABR (dBnHL)	BC-ABR (dBnHL)	500 Hz	1000 Hz	2000 Hz	4000 Hz	The average threshold
Patient 1	3	L	Flat	Refer	55						
		R	Flat	Refer	75						
	5	L	Flat	Refer	55						
		R	Flat	Refer	75						
	7	L	B&Flat	Refer	55	30	65	55	45	50	53.75
		R	B&Flat	Refer	85	45	60	75	90	95	80
	9	L	B&Flat	Refer							
		R	B&Flat	Refer							
	11	L	B&Flat	Refer	65						
		R	B&Flat	Refer	90						
	14	L	B	Refer	70	35	85	80	70	70	76.25
		R	A	Refer	75	50	35	40	60	70	51.25
Patient 2	1	L	Flat	Refer	50						
		R	Flat	Refer	60						
	3	L	Positive peak	Refer	30	25					
		R	Positive peak	Refer	40	40					
	5	L	Flat	Refer	45						
		R	Positive peak	Refer	45						
	6	L	Flat	Refer							
		R	Positive peak	Refer							
	7	L	A and positive peak	Refer	30		20	15	25	25	21.25
		R	A and positive peak	Refer	45		40	35	35	45	38.75
	11	L	B&Flat	Refer							
		R	B&Flat	Refer							
Patient 3	3	L	Flat	Refer	50						
		R	Flat	Refer	60						
	7	L	B&Flat	Refer	65						
		R	B&Flat	Refer	60						
	10	L	B&Flat	Refer	65						
		R	B&Flat	Refer	65						
	17	L	C	Refer	45	35					
		R	B	Refer	60						
	30	L	C	Refer	50						
		R	C	Refer	40						
	54	L	B	Refer							
		R	B	Refer							
	57	L	A	Refer							
		R	A	Refer							
	66	L	C	Refer	50		25	35	35	40	33.75
		R	B	Refer	45		20	20	25	30	23.75

TABLE 4 The outcomes of PTA of patient 3.

Birthday	Date of test	Ear	Tympanometry	Pure-tone audiometry (dB HL)										
				DPOAE	Air conduction					The average threshold	Bone conduction				The average threshold
					500 Hz	1000 Hz	2000 Hz	4000 Hz			500 Hz	1000 Hz	2000 Hz	4000 Hz	
2016-11-11	2022-6-2	L	C	Refer	55	50	45	50	50		30	40	40	45	38.75
		R	B	Refer	50	40	40	35	43.75		15	20	25	20	20
	2022-6-12	L	C	Refer	50	45	45	45	46.25		35	40	45	40	40
		R	B	Refer	45	40	30	35	37.5		15	20	20	25	20
	2022-6-26	L	C	Refer	50	45	40	50	46.25		30	40	40	45	38.75
		R	C	Refer	45	35	25	30	33.75		20	25	25	25	23.75
	2022-7-24	L	A	Refer	45	45	40	50	45		35	40	40	45	40
		R	C	Refer	45	35	25	30	33.75		25	20	20	25	22.5
	2022-8-28	L	A	Refer	40	45	45	50	45		35	35	40	45	38.75
		R	A	Refer	30	25	30	35	30		25	25	25	30	26.25
	2022-9-7	L	A	Refer	40	45	45	50	45		35	40	40	40	41.25
		R	A	Refer	30	25	30	25	27.5		20	20	25	25	22.5

older than 1 year were subjected to 226 Hz probe tone tympanometry. Due to the anatomy and physiology of the outer and middle ear, which influence how acoustic energy is transmitted from the outer to the middle ear, 226-Hz tympanometry had poor sensitivity for detecting middle ear dysfunction in infants up to 6 months of age; therefore, high frequency tympanometry (HFT) with a 1000 Hz probe tone is recommended for infants from birth to 6 months of age. In addition, there is compelling evidence that HFT with a probe tone of 1000 Hz may identify conductive situations in newborns and early babies up to 6 months of age with good accuracy (Baldwin, 2006). Hoffmann suggested the use of a 1000 Hz probe tone in newborns up to 9 months of age and in older children with craniofacial deformities and reduced ear canal volumes (Hoffmann et al., 2013).

Due to the fact that each electrophysiological audiometric has its own benefits and drawbacks, numerous audiological tests should be recommended to confirm the results and arrive at an accurate prediction. ABR is the preferred measure for predicting the audiogram in babies and toddlers (Busa et al., 2007); however, the test periods for frequency-specific AC-ABR and BC-ABR were excessively long and typically exceeded the infants' sleep time. In addition, due to their other symptoms, such as feeding problems and upper respiratory infections, KS patients typically slept less than other children. Therefore, it is essential to select an appropriate and effective test battery for KS 1 patients with short sleep time. As stated earlier, NB CE-chirp ASSR was shown to yield lower (better) thresholds in significantly less time and was deemed a suitable option for electrophysiological audiometric testing (Sininger et al., 2018). In this work, a whole audiogram was predicted using frequency-specific stimuli (500, 1000, 2000, and 4000 Hz). Two experienced radiologists reviewed the HRCT pictures in collaboration with otologists and confirmed the presence of minor middle and inner ear abnormalities. Using AC- and BC-ABRs elicited by a click, the amount and type of hearing loss were determined.

Consequently, all three patients presented with recurrent OME, as determined by tympanometry, the air-bone gap of the ABR, and HRCT. OME recurrence in KS 1 patients was likely due to both the increased prevalence of upper respiratory infections and Eustachian tube dysfunction (Barozzi et al., 2009). At the patient's latest assessment at the age of 14 months, only the right ear had healed from OME. Similarly, among the six and eight rounds of audiological tests with electrophysiology for patients 2 and 3, respectively, four and six rounds had OME. OME enhanced the threshold range of click-evoked AC-ABR from 30–75 dBnHL to 45–90 dBnHL; the air-bone gap of ABR and PTA was between 17.5 and

TABLE 5 The radiological features of the middle and inner ear on HRCT images.

	Ear	Patient 1	Patient 2	Patient 3
Middle ear				
Ossicular chain	L	1. Dysplasia of the incus 2. Poor connection of the stapes and the incus	Irregular morphology of the long feet of the incus	Dysplasia of the incus
	R	1. Dysplasia of the incus 2. Poor connection of the stapes and the incus	Irregular morphology of the long feet of the incus	No abnormalities
Mastoid		Reduced pneumatization mastoids on both sides	Reduced pneumatization mastoids on both sides	Reduced pneumatization mastoids on both sides
Inner ear	L	Cochlear dysplasia, only the bottom and middle circles were visible	Bone hardening of the oval windows	1. Cochlear dysplasia, only the bottom and middle circles were visible 2. Fusion of the vestibule with the horizontal semicircular canal
	R	1. Dilated vestibule 2. Thick and short horizontal semicircular canal	Bone hardening of the oval windows	Short horizontal semicircular canal

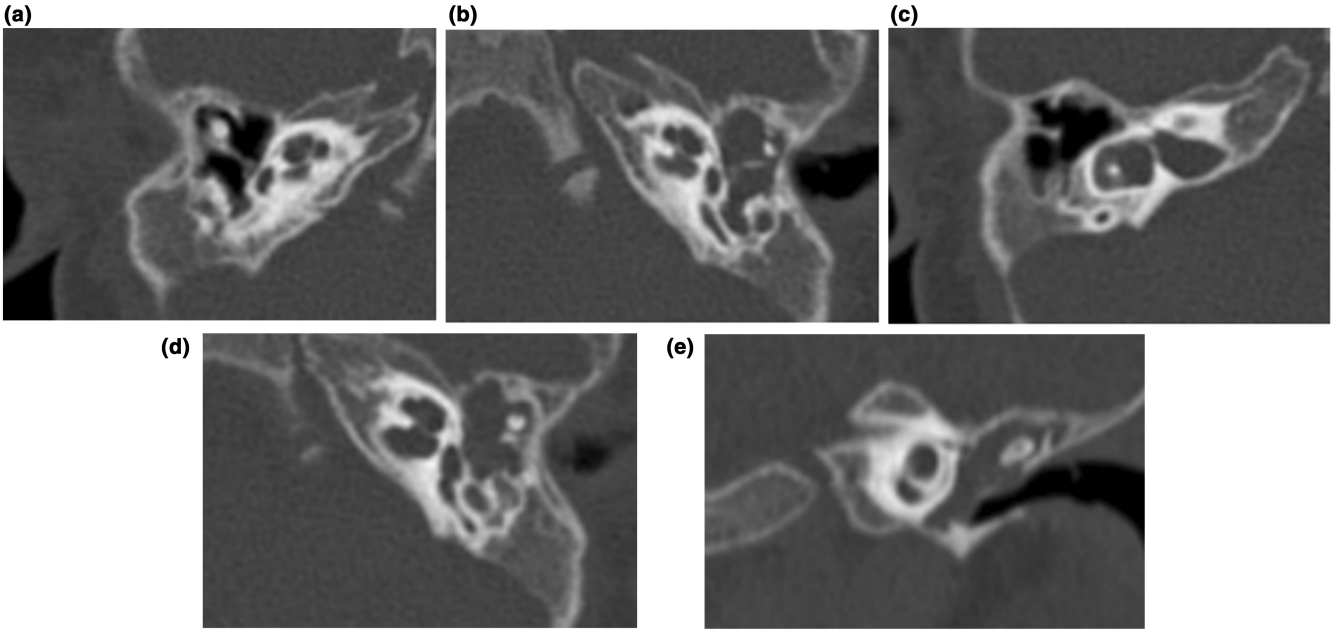

FIGURE 1 Axial and coronal images of HRCT of patient 1: (a) Right ear: dysplasia of the incus, poor connection of the stapes and the incus. (b) Left ear: the tympanic cavity is filled with effusion; poor connection of the stapes and the incus. (c) Right ear: enlarged vestibule, thick and short horizontal semicircular canal. (d, e) Left ear: the tympanic cavity is filled with effusion; cochlear dysplasia, only the bottom and middle circles were visible.

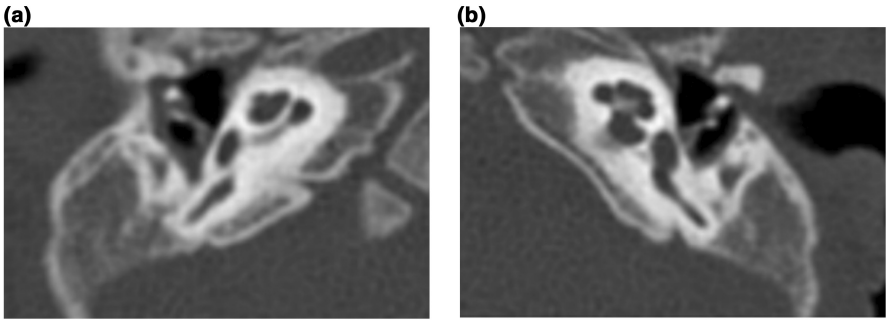

FIGURE 2 Axial images of HRCT of patient 2: (a) Right ear: irregular morphology of the long feet of the incus; bone hardening of the oval windows. (b) Left ear: irregular morphology of the long feet of the incus; bone hardening of the oval windows.

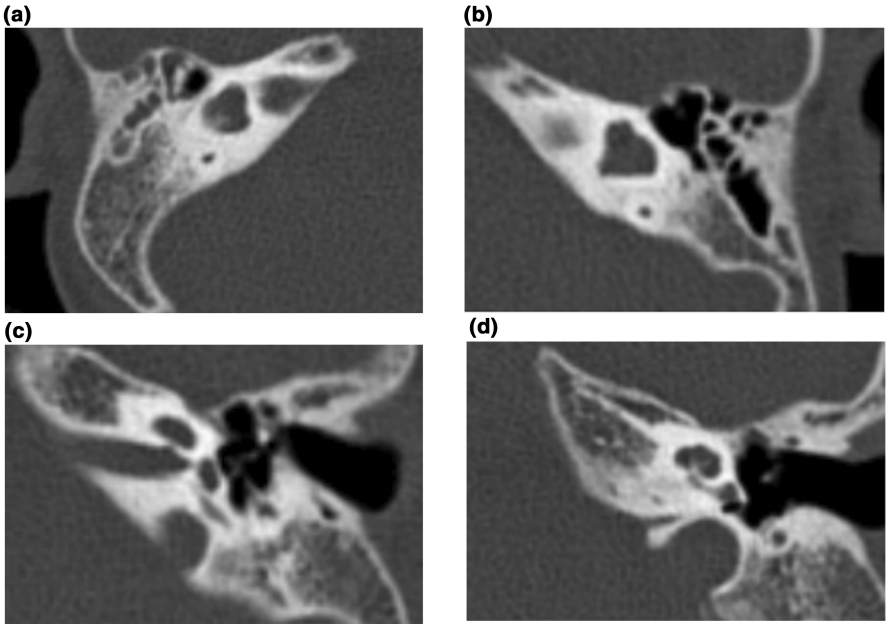

FIGURE 3 Axial images of HRCT of patient 3: (a) Right ear: short horizontal semicircular canal. (b) Left ear: fusion of the horizontal semicircular canal and vestibule. (c) Left ear: irregular morphology of the long feet of the incus. (d) Left ear: cochlear dysplasia, only the bottom and middle circles were visible.

40 dB. As seen by HRCT, all three patients exhibited decreased pneumatization of the mastoid on both sides, which Chen categorized as a less common characteristic in KS patients (Chen, 2006). As regards to the anatomical abnormality of the middle ear, there was limited research; however, in this study, all three patients had dysplasia of the incus, and patient 1 also had a poor connection between the incus and the stapes, which could partially explain the CHL despite the normal configuration of tympanometry and the absence of fluid in the right ear. However, the exact conductive component of hearing loss caused by ossicular malformation should be examined by a PTA when the patient is able to cooperate. Knowledge of embryology and anatomy suggested that the incus developed from the first branchial arch, which is important in the face's development (Senggen et al., 2011). We believed that the deformity of the incus was produced by the interruption of the development of the first branchial arch, which also caused other facial abnormalities in KS 1 patients, including large ears, high cleft, preauricular fistula, and lip pits. However, the slight abnormality of the ossicles in patients 2 and 3 did not result in CHL.

As related to SNHL, all the three patients exhibited with mild to moderate SNHL (click-evoked BC-ABR: 25–50 dBnHL) and structural abnormalities of the inner ear were also seen in HRCT. Patients 1 and 3 had cochlear dysplasia (only the bottom and middle circles were visible) in their left ear canals, as well as malformations of the vestibule and horizontal semicircular canal; patient 2 exhibited ossification of the oval windows. Filipponi E reported that 95% of the investigated ears had normal vestibular function, but KS patients were advised to have vestibular testing (Barozzi et al., 2009). Given the abnormality of the vestibule and horizontal semicircular canal in patients 1 and 3, it was thought that the vestibular function was affected. However, examination of the vestibular system is difficult in this study due to the young ages of patients 1 and 2, as well as patient 3's refusal to cooperate. Furthermore, international consensus diagnostic criteria identified increasing SNHL as one supporting clinical aspect (Adam et al., 2019). In this investigation, the longitude audiological data did not indicate significant hearing progression, maybe because to the young age of the subjects; nonetheless, this should be examined in future studies.

Due to the occurrence of mild to moderate SNHL in individuals with KS, OME must be treated clinically and timely or else speech and language retardation could happen. Although patient 1 had long-term OME, the parents did not consent to grommet insertion because patient 1 had previously undergone two operations, thus bone conduction hearing aids were placed at 7 months of age to compensate for the hearing loss. Patient 2 exhibited moderate SNHL with 30–45 dB nHL in the absence of OME at ages 3 and 7 months. The parents considered there was no necessity of the fitting of amplification but they agreed with attentive auditory monitoring. Patient 3 was diagnosed with recurrent OME from birth, and grommet placement was performed on both ears at the age of 2 years. In addition, between the ages of 30 and 66 months, the hearing issue received little treatment because of the severe symptoms of renal abnormality and hip laxity. At the age of 66 months, she was also diagnosed with OME and utilized a middle ear pressure equalization device for 1 month until her tympanometry results turned to be normal.

In 2010, it was found that heterozygous pathogenic variants in *KMT2D* were the cause of KS (Ng et al., 2010). More than 80% of known variations are nonsense, splice

site, or frameshift alleles that induce truncating alterations; these are the most prevalent mutations in *KMT2D* (Baldridge et al., 2020). In this investigation, the nonsense variant (exon 21, c.5104C>T, p. Arg1702*) de novo in patient 1 and the frameshift variants de novo in patients 2 (exon 35, c.10205delA, p. Leu3402Argfs*31) and 3 (exon 40, c.12840delC, p. Pro4281Leufs*103) resulted in truncated proteins which were expected to cause typical phenotypic features (Table 1). The three individuals were Han Chinese, and their facial characteristics included long palpebral fissures and the eversion of the lateral third of the lower eyelids. However, when the phenotypic and genotypic spectra of 43 Chinese with *KMT2D* variants were summarized and compared to a cohort of 86 non-Chinese KS patients, it was found that Chinese patients had a significantly lower frequency of the long palpebral fissures and the eversion of the lateral third of the lower eyelids (38.2% for both features) than non-Chinese KS patients (99% and 87%, respectively) (Makrythanasis et al., 2013). The author hypothesized that a lack of clinical acuity among Chinese practitioners in detecting these characteristics might explain for certain disparities (Shangguan et al., 2019).

Longitudinal audiological data from neonatal hearing screening and a series of hearing tests including DPOAE, AC-ABR, BC-ABR, NB CE-chirp ASSR, and PTA over short periods of time were evaluated in this study, and recruited individuals were phenotyped and subjected to WES. In this study, a total of three children were found to carry novel pathogenic *KMT2D* gene mutations. It provides new insights to enrich the understanding of the clinical phenotype and the preliminary conjecture of the mechanism of *KMT2D*-related Kabuki syndrome.

5 | CONCLUSIONS

Identifying the type and severity of hearing loss in KS 1 patients as early as possible is crucial. Early in life, many audiological diagnostic tests should be administered. Due to the partial SNHL and high recurrence of OME, therapeutic therapy, frequent monitoring, and prompt intervention are essential.

AUTHOR CONTRIBUTIONS

The conceptualization and supervision were provided by Haibo Li and Zhoushu Zheng. Lu Ding, Meihong Wang, Junhua Wu, and Yinghui Zhang designed and performed the experiments, collected data and informed consent. Zhoushu Zheng, Ming Tang, Jun Xu, and Haibo Li have analyzed and interpreted the results and wrote the article. Zhoushu Zheng, Liangjiong Wang, Junhua Wu, and Haibo Li have edited and corrected the article. All authors approved the final article.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding authors.

ETHICS STATEMENT

The study was approved by Ethics Committee of Ningbo Women and Children's Hospital (No. EC2023-009), in compliance with The Code of Ethics of the World Medical Association (Declaration of Helsinki). As the subjects of the research were all minors, written informed consents for mutation analyses were obtained from next of kin, caretakers, or guardians on behalf of the children enrolled in this study.

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