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Hearing Loss Related to Gene Mutations in Distal Renal Tubular Acidosis

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Keywords
Distal renal tubular acidosis · Hearing loss · Gene mutations · Large vestibular aqueduct syndrome

Abstract

Introduction: Distal renal tubular acidosis (dRTA) is a disease that may develop either primarily or secondarily, resulting from urinary acidification defects in distal tubules. Hearing loss may accompany primary forms of dRTA. This study aims to determine the characteristics of hearing loss due to different gene mutations in patients with dRTA. ***Methods:*** Behavioral and electrophysiological audiological evaluations were performed after otolaryngology examination in 21 patients with clinically diagnosed dRTA. Radiological imaging of the inner ear (n = 9) was conducted and results of genetic analyses using next-generation sequencing method (n = 16) were included. ***Results:*** Twenty-one patients with dRTA from 20 unrelated families, aged between 8 months and 33 years (median = 12, interquartile range = 20), participated. All patients with *ATP6V1B1* mutations (n = 9) had different degrees of hearing loss. There was one patient with hearing loss in patients with *ATP6V0A4* mutations (n = 6). One patient with the *WDR72* mutation had normal hearing. Large vestibular aqueduct syndrome (LVAS) was detected in 6 (67%) of 9 patients whose radiological evaluation results were available. ***Conclusions:*** LVAS is common in patients with dRTA and may influence the type and severity of hearing loss in these patients. The possibility of both congenital and late-onset and progressive hearing loss should be considered in dRTA patients. A regular audiological follow-up is essential for the early detection of a possible late-onset or progressive hearing loss in these patients.

Introduction

The inner ear and kidney show various ultrastructural and physiological similarities based on clinical and experimental studies, even if they represent different systems in the human body [Quick et al., 1973; Arnold, 1984]. Since these structures share several common transporters/channels, various studies have supported the view that genetic disorders affecting tubules of the kidneys may be

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accompanied by hearing loss [Izzedine et al., 2004; Peters et al., 2004; Torban and Goodyer, 2009].

Distal renal tubular acidosis (dRTA), which could develop primarily or secondarily, is caused by a urinary acidification defect in distal tubules and characterized by hyperchloremic metabolic acidosis with a normal serum anion gap, impairment of growth, polyuria, hypokalemia, hypercalciuria, nephrocalcinosis, and urolithiasis. Hearing loss may also occur in some primary forms of this disorder [Rodríguez-Soriano, 2000; Soriano, 2002]. Primary dRTA may be inherited as autosomal dominant and autosomal recessive. While autosomal dominant dRTA is associated with *SLC4A1* mutations encoding the anion (Cl^-/HCO_3^-) exchanger 1 (AE1) in the intercalated cells of the kidney [Rodríguez-Soriano, 2000; Soriano, 2002], the autosomal recessive dRTA is associated with mutations in *ATP6V0B1* or *ATP6V0A4* genes, encoding the B1 and A4 subunits of the apical H^+-ATPase pump, respectively [Karet et al., 1999b; Stover et al., 2002; Ruf et al., 2003; Palazzo et al., 2017]. Recent studies have demonstrated mutations in *FOXI1, WDR72,* and *ATP6V1C2* genes associated with dRTA and thereby have expanded the genetic spectrum of the disease [Enerbäck et al., 2018; Rungroj et al., 2018; Jobst-Schwan et al., 2020].

The association between dRTA and hearing loss was first reported by Royer and Broyer in 1967 [Royer and Broyer, 1967]. Since then, hearing loss with different characteristics in *ATP6V0A4* and *ATP6V1B1* mutations has been reported in many studies [Gil et al., 2007; Aksakal et al., 2009; Gao et al., 2014; Zeinali et al., 2014]. More recent studies have also reported hearing loss in *FOXI1* mutations associated with dRTA [Enerbäck et al., 2018]. Although dRTA is mainly associated with progressive sensorineural hearing loss (SNHL), conductive hearing loss and mixed hearing loss (MHL) have also been described [Zakzouk et al., 1995; Yashima et al., 2010; Gao et al., 2014]. In several case studies, large vestibular aqueduct syndrome (LVAS) has been reported in patients with dRTA [Berrettini et al., 2002; Shinjo et al., 2005; Andreucci et al., 2009]. This study aims to determine clinical characteristics focusing on hearing loss in patients diagnosed with dRTA, to make genotype-phenotype correlations in terms of hearing loss, and to evaluate the effect of variable audiological features on follow-up and intervention processes.

Materials and Methods

Patients

A group of 21 patients from 20 unrelated families diagnosed with dRTA at the Division of Pediatric Nephrology of Hacettepe University were included in the study. The patients had no history of any other genetic or metabolic diseases, ototoxic drug use, exposure to noise, or middle ear surgery. Ear examination and audiological assessment were performed at the Department of Otorhinolaryngology and Department of Audiology of Hacettepe University.

Genetic Analysis

Genetic analyses were performed at the Hacettepe University Nephrogenetics Laboratory. DNA was extracted from peripheral blood using a commercial kit according to the manufacturer's recommendations (Invitrogen PureLink Genomic DNA Mini Kit). All patient samples were run on a gene panel containing 41 genes whose mutations have been associated with various tubular disorders (*ATP6V0A4, ATP6V1B1, SLC3A1, SLC5A2, NEK8, SLC12A3, IQCB1, BSND, WDR72, SLC4A4, CLCN5, SCNN1B, FXYD2, NPHP1, SLC4A2, CLDN16, OCRL1, GLIS2, KCNJ1, NPHP4, CEP290, GATA3, AGXT, INVS, SALL1, PAX2, SLC5A1, TMEM67, AVPR2, HNF1B, PKHD1, SLC12A1, NPHP3, UMOD, PKD2, BICC1, PKD1, ACE, DZIP1L, MAPKBP1, GRHPR*) via a next-generation sequencing method using the Ion S5 System® (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's recommendations. Data were analyzed using Ingenuity® Variant Analysis™ software (Qiagen, Redwood City, CA, USA).

The following reference sequences of the National Center for Biotechnology Information (NCBI) (corresponding Ensembl) were used: *ATP6V0A4*NM_020632.3 (ENST00000310018.7) and *ATP6V1B1*NM_001692.4 (ENST00000234396.10). All mutations were confirmed by direct sequencing using BigDye v3.1 chemistry and an ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Some mutations uncovered in the present study have already been described elsewhere and have been reported previously (Table 1) [Karet et al., 1999a; Karet et al., 1999b; Smith et al., 2000; Stover et al., 2002; Borthwick et al., 2003; Ruf et al., 2003; Li et al., 2012; Liu et al., 2018; Jobst-Schwan et al., 2020].

Audiological Evaluation

Pure tone air and bone conduction thresholds were measured between 125–8,000 Hz and 500–4,000 Hz, respectively, with age-appropriate behavioral tests. The degree of hearing loss was determined according to the pure tone average (PTA), which is the average of hearing thresholds at frequencies of 500, 1,000, and 2,000 Hz [Clark, 1981]. Pure tone hearing thresholds were tested using the GSI AudioStar Pro Clinical Audiometer (Grason-Stadler, MN, USA) in a double-walled sound booth (IAC Acoustics, IL, USA). The hearing loss configuration was evaluated visually.

Tympanometry and acoustic reflex thresholds measurement were performed using the Interacoustics AT235 Clinical Tympanometer (Interacoustics, Middelfart, Denmark). In the tympanometric evaluation, middle ear peak pressure, static admittance, and ear canal volumes were measured and the results were classified according to Jerger's tympanogram types [Jerger, 1970]. The ipsilateral acoustic reflex thresholds (IART) were determined at 500, 1,000, 2,000, and 4,000 Hz and evaluated as normal in the 70–100 dB sound pressure level [Wiley et al., 1987]. To assess the cochlear function, transient evoked atoacoustic emissions (TEOAEs) were recorded at 1,000–4,000 Hz using Otodynamics ILO292 USB II (Otodynamics, Hatfield, UK). The stimulus intensity level was at 80 ± 3 dB sound pressure level. Emission responses were accepted "present" when the signal-to-noise ratio of the emission amplitudes was 6 dB and above in at least 3 of the 5 frequencies measured.

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Table 1. Demographic and clinical features of the patients

Patient No.	Variation (predicted amino acid change)[a]	Sex	Current age	Consang.	Age at diagnosis (dRTA)	Serum electrolytes at the last visit			Reference
						Na^+, mEq/L	K^+, mEq/L	HCO_3^-, mEq/L	
1	ATP6V1B1: c.232G>A (p.Gly78Arg) (Hom)	F	12 y	Yes	5 m	139	3.7	23.5	(Borthwick et al., 2003)
2	ATP6V1B1: 546delC (p.Ile183Serfs*25) (Hom)	F	22 y	Yes	2 m	138	3.9	23	(Ruf et al., 2003)
3	ATP6V1B1: c.140C>T (p.Arg29*) (Hom)	M	33 y	Yes	7 m	139	3.7	22	(Ruf et al., 2003)
4	ATP6V1B1: c.687+1G>T (splice site) (Hom)	M	33 y	Yes	2 m	140	3.9	24	(Ruf et al., 2003)
5	ATP6V1B1: c.823A>C (p.Thr275Pro) (Hom)	F	5 y	Yes	1 m	140	3.8	22.5	(Karet et al., 1999b)
6[b]	ATP6V1B1: c.484_486delGAG (p.Glu162del) (Hom)	M	12 y	Yes	6 m	141	3.8	22	Novel
7	ATP6V1B1: c.1037C>G (p.Pro346Arg) (Hom)	M	8 m	Yes	2 m	138	4.6	22.4	(Karet et al., 1999b)
8	ATP6V1B1: c.497delC (p.Thr166Argfs*9) (Hom)	F	2 y	No	2.5 m	141	4.8	25	(Karet et al., 1999b)
9[b]	ATP6V1B1: c.484_486delGAG (p.Glu162del) (Hom)	F	7 y	Yes	2 m	136	3.6	24.9	Novel
10	ATP6V0A4: c.1030−2A>C (splice site) (Hom)	F	25 y	No	1 m	140	2.8	21	(Stover et al., 2002)
11	ATP6V0A4: c.2257−1G>A (Hom)	M	9 m	Yes	1 m	136	3.5	24	(Li et al., 2012)
12	ATP6V0A4: c.2446A>G (p.Lys816Glu) (Hom)	F	32 y	Yes	1 m	138	3.6	22	(Jobst-Schwan et al., 2020)
13	ATP6V0A4: c.2419C>T (p.Arg807*) (Hom)	F	13 y	Yes	1 m	139	4.0	24.3	(Liu et al., 2018)
14	ATP6V0A4: c.292−1G>A (splice site) (Hom)	F	8 y	Yes	2 m	140	4.0	23.9	(Smith et al., 2000)
15	ATP6V0A4: c.1840A>T (p.Ile614Phe) (Het); c.1831_1832delAGinsT (p.Ile612Serfs*37) (Het)	M	1 y	Yes	1 m	138	4.0	22.5	(Stover et al., 2002)
16	WDR72: c.477_485 dup (p.Ile159_Cys161dup) (Hom)	F	32 y	Yes	6 m	141	3.9	21.1	(Jobst-Schwan et al., 2020)
17	N/A	F	15 y	Yes	3 m	139	3.7	22.7	
18	N/A	F	26 y	Yes	1 m	139	2.9	22	
19	N/A	M	11 y	Yes	3 m	142	4.1	23.8	
20	N/A	M	3 y	Yes	2.5 m	142	4.4	18.2	
21	N/A	F	21 y	No	5 m	135	4.7	21.4	

*Stop codon. [a]Variations and predicted amino acid changes have been named according to the guidelines of the Human Genome Variation Society using Mutalyzer software (https://mutalyzer.nl) using the following National Center for Biotechnology Information (NCBI) transcript numbers ATP6V1B1 NM_001692.4; ATP6V0A4 NM_020632.3; and WDR72 NM_182758.4. F, female; M, male; Consang., consanguinity between parents; y, year; m, month; N/A, not available; Hom, homozygous; Het, heterozygous. [b]Siblings.

To evaluate the auditory pathway at the brainstem level, ABRs (auditory brainstem responses) were recorded in a Faraday cage-featured test room using the Vivosonic Integrity ABR system (Vivosonic, Toronto, Canada). The single-channel recording was made with disposable electrodes. The forehead for the non-inverting electrode, mastoid of the test ear for the inverting electrode, and contralateral ear (opposite to the test ear) for the ground electrode were used. The artifact rejection level was set at 20 μV. The analysis time window was 20 ms, and the band-pass filter was between 30 and 3,000 Hz. Electrode impedances were below 5 kOhm. Click stimuli correlated to behavioral hearing thresholds between 2,000 and 4,000 Hz [Gorga et al., 2006] and 500 Hz tone-burst stimuli were used instead of tonal stimuli with different frequencies to reveal the hearing loss configuration and shorten the test time. ABR stimuli were delivered with ER3A (Etymotic Research, IL, USA) insert earphones. The number of sweeps and the stimulation rate were set at 2,000 and 37.7/s, respectively. The wave V thresholds were determined. All patients were tested in a supine position, in natural sleep, or in a calm state.

Radiological Evaluation

High-resolution computed tomography and magnetic resonance imaging were recommended to all patients for evaluation of inner ear structures. Results of 9 patients who gave consent for radiological evaluation or had previous high-resolution computed tomography or MR results were included in the study (Table 2). The remaining patients or their parents did not provide consent for radiological examination due to their concern for the potential risks of radiation exposure and/or the need for sedation during the procedure. Axial computed tomography and T2-weighted images were used to evaluate the vestibular aqueduct.

Statistical Analysis

All statistical analysis of this study, which was designed as a within-group descriptive study, was performed with the SPSS 24 (IBM Corp., Armonk, NY, USA). The Kolmogorov-Smirnov test was used to determine whether the data conformed to the normal distribution. Since audiological data did not show a normal distribution, the median and interquartile range values were used for descriptive statistics. The Mann-Whitney U test was used to compare the PTA in *ATP6V1B1* and *ATP6V0A4* mutations. $p < 0.05$ was considered statistically significant.

Results

Demographic Data

Twenty-one patients with dRTA from 20 unrelated families were included in the study. The ages of the patients ranged from 8 months to 33 years (median 12 years, interquartile range 20 years): 13 (61.9%) were female and 8 (38.1%) were male. Parental consanguinity was present in 18 families (85.7%). More than one affected patients in nine families (42.9%) were present. Genetic analyses were performed in 16 patients and nine had *ATP6V1B1*, six had *ATP6V0A4*, and one had *WDR72* mutations (Table 1).

Audiological and Radiological Results

Audiological evaluations showed that 8 patients (38.1%) had normal hearing, whereas 13 patients (61.9%) had different degrees and types of hearing loss. Detailed audiological evaluation results including behavioral test methods, PTAs, ABR thresholds, TEOAEs, and LVAS findings are presented in Table 2. Radiological imaging examples of patients with LVAS are given in Figure 1.

Patients with ATP6V1B1 Mutations

All patients with biallelic *ATP6V1B1* mutation ($n = 9$) had various degrees of hearing loss from moderate to profound (Table 2/Section 1; Fig. 2a). The high-frequency hearing loss configuration was prominent on all audiograms. According to the results of the tympanometric evaluation, all patients had normal middle ear functions. The IART and TEOAEs were not obtained. In Click ABR, wave V was completely absent at the maximum intensity level (99 dB nHL) in six patients. In 500 Hz tone-burst ABR, wave V was completely absent at the maximum intensity level (105 dB nHL) in four patients. Bilateral LVAS was present in five (83%) of six patients with radiological imaging. Three patients had unilateral cochlear implants, one patient had bilateral cochlear implants, and five patients had bilateral hearing aids. One of the unilateral cochlear implant users had a hearing aid in her contralateral ear (patient 1). Cochlear implant was recommended to two patients who did not benefit from bilateral hearing aids (patients 2 and 4).

Patients with ATP6V0A4 Mutations

Behavioral audiological evaluation results of patients ($n = 6$) with biallelic *ATP6V0A4* mutations revealed moderate-to-severe SNHL in one patient (patient 10). The rest of the patients had normal hearing according to their PTAs (Table 2/Section 2; Fig. 2b). Middle ear functions were normal in all patients. IART and TEOAEs were absent in a patient with hearing loss. Click and 500 Hz tone-burst ABR results were normal in people with normal hearing, consistent with other audiological evaluation outcomes. The presence of LVAS could not be evaluated because the patient with hearing loss did not give consent for radiological imaging.

Patient with WDR72 Mutation

One patient with a pathogenic *WDR72* mutation was identified. In this patient, PTAs were within normal limits, but hearing thresholds deteriorated with increasing frequency (>4 kHz) in both ears (Table 2/Section

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Table 2. Behavioral and electrophysiological audiological evaluation results and radiological findings of patients

	Patient No.	Testing method	AC PTA (dB)		ABR threshold (dB)				TEOAE	Age at diagnosis (HL)	Current hearing status	HL type	Cochlear implants/hearing aids	LVAS
			R	L	click		500 Hz TB							
					R	L	R	L						
Section 1 (ATP6V1B1)	1	C.A.	108+	88	N/R	N/R	105	85	Absent	3 y	L: severe HL/R: profound HL	SNHL	Right CI/left HA	No
	2	C.A.	110+	102	N/R	N/R	100	100	Absent	6 m	Bilateral profound HL	SNHL	Bilateral HA	Yes
	3	C.A.	107	117	N/R	N/R	N/R	N/R	Absent	7 y	Bilateral profound HL	SNHL	Unilateral CI	Yes
	4	C.A.	97	95	N/R	N/R	N/R	N/R	Absent	1 y	Bilateral profound HL	SNHL	Bilateral HA	Yes
	5	C.P.A.	27	58	50	70	35	65	Absent	3 y	L: moderate-severe HL/R: mild HL	SNHL	Bilateral HA	N/A
	6	C.A.	92	90	N/R	N/R	N/R	N/R	Absent	3 y	Bilateral profound HL	SNHL	Unilateral CI	Yes
	7	B.O.A.	N/A	N/A	N/R	N/R	N/R	N/R	Absent	8 m	Bilateral profound HL	SNHL	Bilateral CI	N/A
	8	V.R.A.	68*	68*	60	50	60	60	Absent	1 m	Bilateral moderate-severe HL	SNHL	Bilateral HA	N/A
	9	C.A.	67	53	90	80	65	70	Absent	2 m	L: moderate-severe HL/R: severe HL	MHL	Bilateral HA	Yes
Section 2 (ATP6V0A4)	10	C.A.	87	65	N/A	N/A	N/A	N/A	Absent	3.5 y	L: moderate-severe HL/R: severe HL	SNHL	Bilateral HA	N/A
	11	B.O.A.	25*	25*	20	20	40	50	Present	–	Normal hearing	NH	–	N/A
	12	C.A.	12	10	20	20	50	40	Present	–	Normal hearing	NH	–	N/A
	13	C.A.	13	15	25	30	45	50	Present	–	Normal hearing	NH	–	N/A
	14	C.A.	13	10	20	20	50	45	Present	–	Normal hearing	NH	–	N/A
	15	V.R.A.	30*	30*	20	20	45	40	Present	–	Normal hearing	NH	–	N/A
Section 3 (WDR72)	16	C.A.	−2	−2	15	15	40	40	Present	–	Normal hearing	NH	–	N/A
Section 4 (unknown)	17	C.A.	90+	90+	N/R	N/R	90	90	Absent	1 m	Bilateral profound HL	SNHL	Unilateral CI	No
	18	C.A.	40	37	55	55	50	50	Absent	25 y	Bilateral mild HL	SNHL	–	No
	19	C.A.	53	45	55	60	50	55	Absent	1.5 m	Bilateral moderate	MHL	Bilateral HA	Yes
	20	B.O.A.	35*	35*	–	–	–	–	Present	–	Normal hearing	NH	–	N/A
	21	C.A.	5	3	20	20	30	30	Present	–	Normal hearing	NH	–	N/A

AC PTA, air-conducted pure tone average; ABR, auditory brainstem response; HL, hearing loss, LVAS, large vestibular aqueduct syndrome; L, left; R, right; C.A., conventional audiometry; C.P.A., conditioned play audiometry; V.R.A., visual reinforcement audiometry; B.O.A., behavioral observation audiometry; TB, tone burst; NH, normal hearing; SNHL, sensorineural hearing loss; MHL, mixed type hearing loss; y, year; m, month; N/R, no response; N/A, not available. *The PTAs in the free field were considered the same for both ears.

3; Fig. 2c). Middle ear functions were normal in both ears. IARTs and TEOAE responses were obtained at all frequencies. ABR thresholds for clicks and 500 Hz tone-burst stimuli were within normal limits.

Patients without Genetic Testing

We could not identify the genetic background in five patients. Pure tone audiometry results of this group ranged from normal hearing to MHL and SNHL (Table 2/Section 4; Fig. 2d), and middle ear functions were normal. Three of 5 patients had hearing loss. In one of these patients, IARTs were obtained except for 4,000 Hz, while in the other 2 patients they were not. TEOAEs were absent bilaterally in these three patients. ABR results were consistent with pure tone audiometry results. Bilateral LVAS was found in one patient with bilateral moderate MHL (patient 19). The patient with bilateral profound SNHL had a unilateral cochlear implant (patient 17), and the patient with bilateral moderate MHL had bilateral hearing aids (patient 19). Late-onset (25 years old), bilateral mild SNHL was detected in one patient and hearing aids were recommended to this patient (patient 18).

PTA Comparisons according to the Mutations

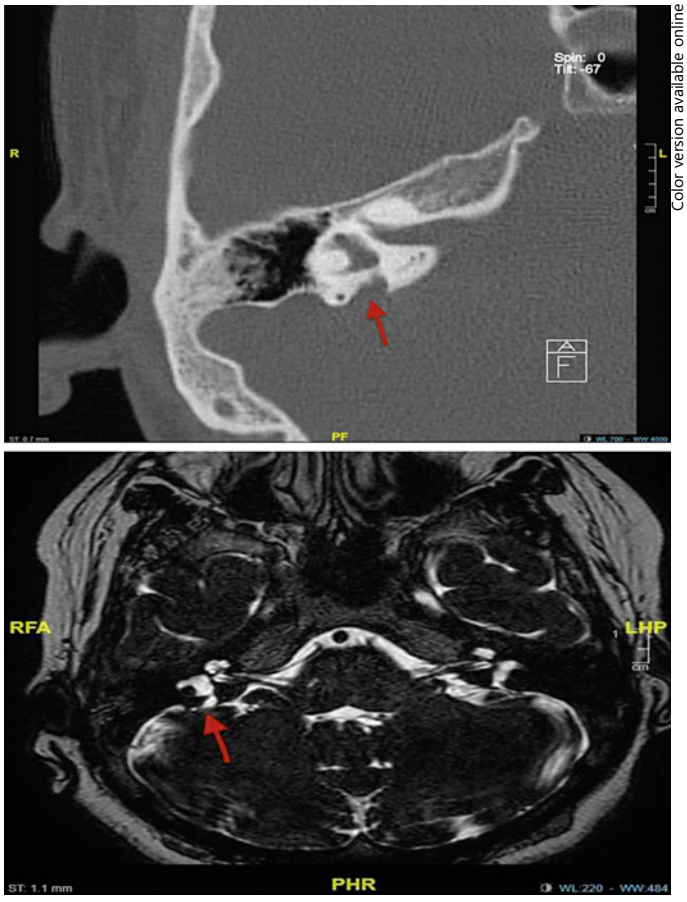
The PTAs were compared between *ATP6V1B1* and *ATP6V0A4* mutations. The mean of the PTAs in the *ATP6V1B1* mutations was statistically significantly higher than the mean of the PTAs in the *ATPV0A4* mutations ($p < 0.05$) (Table 3).

Discussion

dRTA is a genetic disease that develops because of the insufficiency in acid secretion by alpha intercalated cells in the distal tubule of the kidney. Primary dRTA is mainly associated with mutations in the *ATP6V1B1*, *ATP6V0A4*, and *SLC4A1* genes [Karet, 2002; Batlle and Haque, 2012]. The *WDR72* gene, related to amylogenesis imperfecta, a structural tooth disorder, may cause dRTA [Misgar et al., 2017; Rungroj et al., 2018; Zhang et al., 2019]. It has been reported that mutations in the *FOXI1*, which belongs to the Forkhead transcription factor family and is responsible for regulating the H^+-ATPase pump, may also cause dRTA [Vidarsson et al., 2009; Enerbäck et al., 2018]. Additionally, in a recent study in which the *ATP6V1C2*, *WDR72*, and *SLC4A2* genes were screened in individuals with dRTA, the *ATP6V1C2* was found to be a new candidate gene for autosomal recessive dRTA, and the relationship between the *WDR72* mutation and dRTA was confirmed, but no evidence supported the association of *SLC4A2* mutations with dRTA.

It is known that the coexistence of dRTA and hearing loss may be seen in mutations in genes encoding different subunits of the apical H^+-ATPase pump expressed in the kidney and inner ear [Stanković et al., 1997; Karet et al., 1999b; Stover et al., 2002; Norgett et al., 2012; Subasioglu Uzak et al., 2013; Lopez-Garcia et al., 2019]. Our study confirmed that mutations in two of these genes, *ATP6V1B1* and *ATP6V0A4*, are accompanied by different features of hearing loss. The prevalence of hearing loss was higher in the *ATP6V1B1* mutations, and patients with *ATP6V1B1* mutation had statistically significantly higher PTAs than patients with *ATP6V0A4* mutation. These findings are consistent with previous reports noted that hearing loss is more severe in *ATP6V1B1* mutations than in *ATP6V0A4* mutations [Karet et al., 1999a; Stover et al., 2002; Gao et al., 2014; Zeinali et al., 2014]. In previous reports, *ATP6V1B1* mutations were associated with early-onset hearing loss, whereas *ATP6V0A4* mutations were associated with late-onset hearing loss [Karet, 2002; Stover et al., 2002]. However, recent studies indicate that early-onset hearing loss can also occur in *ATP6V0A4* mutations [Vargas-Poussou et al., 2006; Batlle and Haque, 2012; Kose et al., 2014]. In our study, a patient with *ATPV0A4* mutation had severe hearing loss that was noticed in the early stages of life (3 years old). The possibility of congenital hearing loss cannot be excluded for this patient, as the patient's newborn hearing screening results are not available. Given these findings, our study supports the previous report, which noted that the onset age or severity of hearing loss in patients with dRTA is not a sufficient factor to distinguish underlying genetic abnormalities [Vargas-Poussou et al., 2006].

Pathogenic variations in the *WDR72* gene have been described as a cause of hereditary dRTA in the literature [Rungroj et al., 2018], but there are no currently published data demonstrating the expression of the *WDR72* gene in the ear or the relationship between mutations of this gene and hearing loss. In our study, the PTAs of the patient with *WDR72* mutations were within normal limits (−10/15 dB HL). However, the patient's hearing thresholds were approaching the 15 dB HL level, which is the upper limit for normal hearing, especially in the high-frequency region (4,000–8,000 Hz). Increased hearing thresholds at high frequencies were particularly evident in audiograms of patients with hearing loss. Regardless of the mutation type in these patients, hearing thresholds increased at high frequencies compared to middle and low frequencies. This audiogram configuration may be a warning for late-onset or progressive hearing loss; therefore, we recommend a periodic audiological follow-up for all dRTA patients, even if



Color version available online

Fig. 1. Radiological imaging examples of patients with LVAS (red arrows).

the initial evaluation results were normal. A regular audiological follow-up is also crucial for evaluating the efficiency of hearing aids fitting in patients with hearing loss. Cochlear implantation should be considered when hearing aid performance is insufficient due to progression of hearing loss. In our study, three patients who were determined not to benefit from hearing aids due to the progression of their hearing loss were referred for cochlear implantation.

LVAS, one of the most common inner ear malformations, may be seen in many different forms of syndromic and nonsyndromic hearing loss. LVAS may manifest with normal hearing or a sensorineural or mixed hearing loss ranging from mild to profound degree [Gopen et al., 2011; Griffith and Wangemann, 2011]. dRTA is one of the syndromic forms associated with LVAS, and several studies have reported the presence of LVAS in both *ATP6V1B1* and *ATP6V0A4* mutations [Joshua et al., 2008; Andreucci et al., 2009; Yashima et al., 2010; Nikki et al., 2012; Lorente-Cánovas et al., 2013]. In our study, we found that five of the six patients with LVAS had *ATP6V1B1* mutations, four of which had bilateral profound SNHL and one had bilateral severe MHL. In one patient with LVAS and bilateral moderate MHL, the underlying genetic abnormality was unknown. Although LVAS is not considered to be the direct cause of hearing loss in dRTA, it is thought that differences in the type, degree, and progression of hearing loss seen in patients with dRTA may be related to LVAS.

Despite normal middle ear findings, some patients with LVAS have air-bone gaps in their audiograms. This is thought to be a cochlear conductive component resulting from the "third window" effect of the LVAS on sound transmission within the labyrinth [Griffith and Wangemann, 2011]. In our study, middle ear findings were normal in 2 patients with MHL. Bilateral LVAS was present in the radiological evaluation findings of both patients. The presence of air-bone gaps on the audiograms of patients with dRTA that cannot be explained by middle ear findings may be considered as an indicator for the LVAS. Although LVAS is a congenital inner ear abnormality, hearing loss may become evident in time [Griffith and Wangemann, 2011]. Patients may manifest sudden hearing loss after a head trauma or barotrauma, or fluctuating and progressive hearing loss [Gopen et al., 2011; Griffith and Wangemann, 2011]. Therefore, regular monitoring of hearing status is essential in LVAS. Patients and/or their families should be counseled to avoid risk factors such as head trauma or barotrauma to preserve residual hearing in these patients. Given the possible late-onset of LVAS, radiological imaging may be recommended to evaluate the presence of LVAS in all patients with dRTA, even without hearing loss.

The exact pathophysiological mechanisms of LVAS and its relationship with mutations of the corresponding genes in patients with dRTA have remained to be determined. A study has shown that the lack of endocochlear potential and disruption of endolymph pH homeostasis due to the dysfunction of H^+-ATPases may play an important role in the occurrence of LVAS [Lorente-Cánovas et al., 2013]. Another study reported that a hydroelectric imbalance that may develop due to gene mutations in patients with dRTA may cause abnormal growth of the vestibular canal in fetal life and in the first 3–4 years after birth [Berrettini et al., 2002]. However, more studies are needed to better understand the relationship between gene mutations in dRTA, hearing loss, and LVAS.

Early diagnosis of hearing loss in patients with dRTA plays a critical role to improve the quality of life for these patients [Swayamprakasam et al., 2011]. Newborn hearing screening programs, which make it possible to detect hearing loss even in the first 3 days of life, are

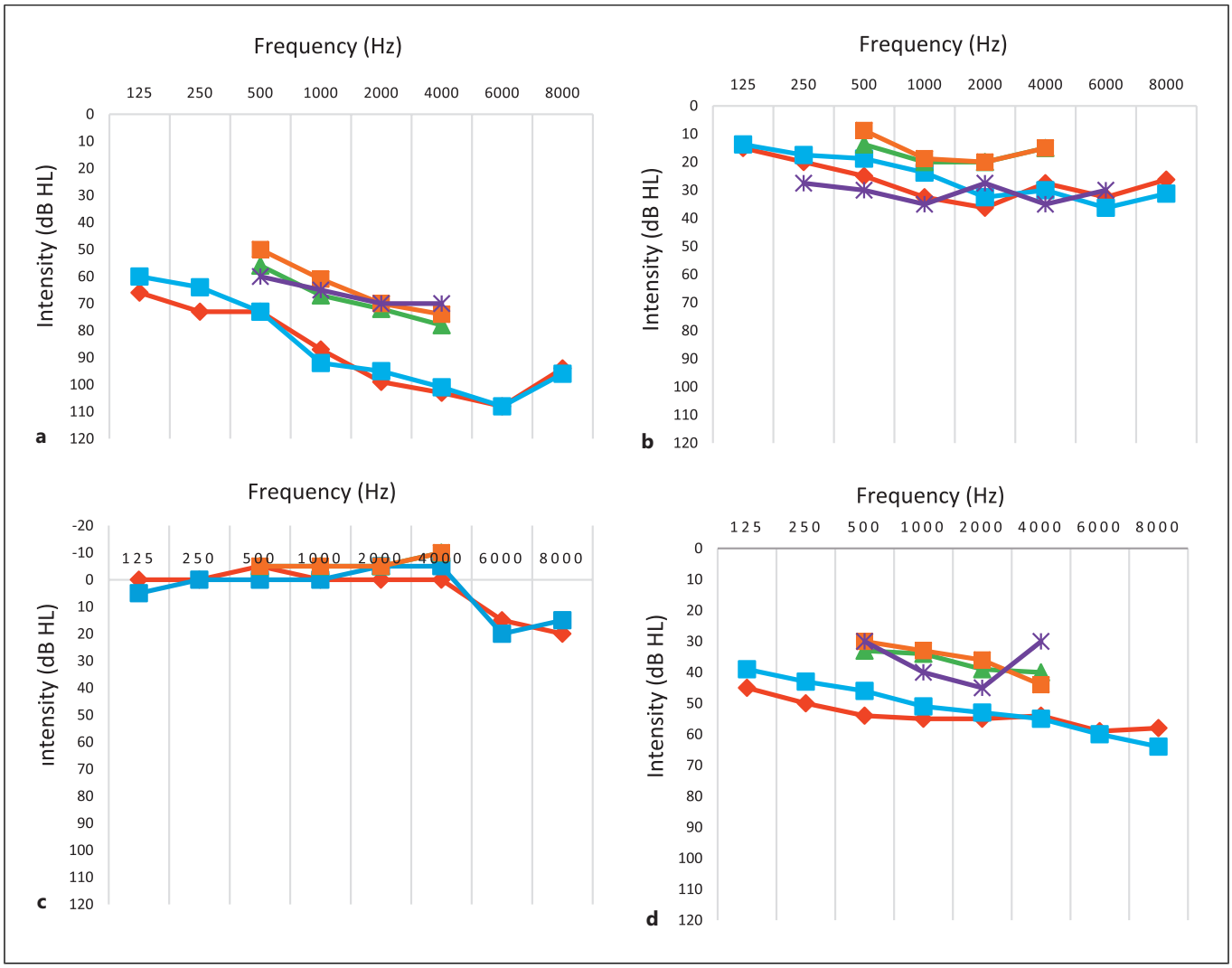


Fig. 2. Averages of the right and left ear air conduction and bone conduction hearing thresholds of patients according to gene groups. **a** *Atp6v1b1* mutations. **b** *Atp6v0a4* mutations. **c** *Wdr72* mutations. **d** Patients without genetic testing. Red line: right air conduction, blue line: left air conduction, green line: right bone conduction, orange line: left bone conduction, purple line: free field. ("No response" thresholds are marked based on the audiometer's maximum output level at the relevant frequency.)

Table 3. Comparison of pure tone average according to ATP6V1B1 and ATP6V0A4 mutations

	ATP6V1B1 (n = 9)		ATP6V0A4 (n = 6)		p value
	median	interquartile range (Q3-Q1)	median	interquartile range (Q3-Q1)	
Right AC PTA (dB)	92	107–67	19	29–13	0.02*
Left AC PTA (dB)	88	95–58	20	29–11	0.02*

PTA, pure tone average; AC, air conduction; BC, bone conduction. *Significant difference at 0.05.

extremely effective in the early diagnosis of hearing loss [Morton and Nance, 2006]. To eliminate the negative effects of auditory deprivation on speech and language development, congenital hearing loss should be detected in the first 3 months of life, which is the critical period for neural maturation, and patients should be provided with access to appropriate amplification and rehabilitation options in the first 6 months. In addition, in patients with hearing loss in dRTA, a regular otologic and audiological follow-up will provide early detection of a possible progression in hearing loss. Although patients with dRTA have normal hearing in newborn hearing screening, periodic otologic and audiological evaluations should be recommended in terms of late-onset hearing loss.

Conclusion

This is the first study to include a detailed assessment of audiological features in dRTA patients. However, the study has several limitations. One of these limitations is that the effects of different gene mutations causing dRTA on inner ear structures could not be compared because some patients did not have radiological imaging results. Another limitation is that it could not be determined whether the hearing loss is congenital in many patients diagnosed with hearing loss in the early stages of life since the newborn hearing screening results of these patients are not available. Longitudinal studies involving newborn hearing screening results can provide monitoring of the hearing status of patients with dRTA from birth to adulthood. The variety of underlying genetic factors in dRTA causes a genetic heterogeneity in hearing loss associated with this disease. Studies evaluating the effects of mutations in newly discovered genes associated with dRTA on hearing will improve our knowledge of the characteristics of hearing status in this disease.

Statement of Ethics

This study was approved by the Non-Interventional Clinical Research Ethics Board of Hacettepe University (March 6, 2018, protocol number: GO 18/154-31). Written informed consent was obtained from all participants over the age of 18 years and from parents/legal guardians for all participants under the age of 18 years.

Conflict of Interest Statement

The authors have no conflicts of interest.

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Author Contributions

Conceptualization and study design: Ezgi Ay, Emre Gurses, Rezan Topaloglu, and Gulsum Aydan Genc. Audiological evaluation: Ezgi Ay, Emre Gurses, Filiz Aslan, and Gulsum Aydan Genc. Otologic and radiological evaluation: Munir Demir Bajin and Levent Sennaroglu. Nephrological evaluation: Bora Gulhan, Ali Duzova, Rezan Topaloglu, and Fatih Ozaltin. Methodology: Ezgi Ay, Asuman Alniacik, and Gulsum Aydan Genc. Statistical analysis: Asuman Alniacik and Ezgi Ay. Genetic analyses: Fatih Ozaltın. Writing original draft: Ezgi AY, Emre Gurses, and Filiz Aslan. Editing of manuscript: Gulsum Aydan Genc, Rezan Topaloglu, Asuman Alniacik, Bora Gulhan, Fatih Ozaltın, Ali Duzova, Munir Demir Bajin, and Levent Sennaroglu. All authors read and approved the final manuscript.

Data Availability Statement

All data used during this study are included in this article. Further inquiries can be directed to the corresponding author.

References

Aksakal MZT, Nayir A, Karet FE. Distal renal tubuler asidozlu vakalarımızda sensorinöral işitme kaybı ve ATP6V1B1 gen mutasyonu ilişkisi. Çocuk Dergisi. 2009;9(4):172–5.

Andreucci E, Bianchi B, Carboni I, Lavoratti G, Mortilla M, Fonda C, et al. Inner ear abnormalities in four patients with dRTA and SNHL: clinical and genetic heterogeneity. Pediatr Nephrol. 2009;24(11):2147–53.

Arnold W. Inner ear and renal diseases. Ann Otol Rhinol Laryngol Suppl. 1984;112(4_suppl):119–24.

Batlle D, Haque SK. Genetic causes and mechanisms of distal renal tubular acidosis. Nephrol Dial Transpl. 2012;27(10):3691–704.

Berrettini S, Franceschini SS, Forli F, Ravecca F, Massimetti M, Neri E. Distal renal tubular acidosis associated with isolated large vestibular aqueduct and sensorineural hearing loss. Ann Otol Rhinol Laryngol. 2002;111(5 Pt 1):385–91.

Borthwick KJ, Kandemir N, Topaloglu R, Kornak U, Bakkaloglu A, Yordam N, et al. A phenocopy of CAII deficiency: a novel genetic explanation for inherited infantile osteopetrosis with distal renal tubular acidosis. J Med Genet. 2003;40(2):115–21.

Clark JG. Uses and abuses of hearing loss classification. Asha. 1981;23(7):493–500.

Enerbäck S, Nilsson D, Edwards N, Heglind M, Alkanderi S, Ashton E, et al. Acidosis and deafness in patients with recessive mutations in FOXI1. J Am Soc Nephrol. 2018;29(3):1041–8.

Gao Y, Xu Y, Li Q, Lang Y, Dong Q, Shao L. Mutation analysis and audiologic assessment in six Chinese children with primary distal renal tubular acidosis. Ren Fail. 2014;36(8):1226–32.

Gil H, Santos F, García E, Álvarez MV, Ordóñez FA, Málaga S, et al. Distal RTA with nerve deafness: clinical spectrum and mutational analysis in five children. Pediatr Nephrol. 2007;22(6):825–8.
Gopen Q, Zhou G, Whittemore K, Kenna M. Enlarged vestibular aqueduct: review of controversial aspects. Laryngoscope. 2011; 121(9):1971–8.
Gorga MP, Johnson TA, Kaminski JR, Beauchaine KL, Garner CA, Neely ST. Using a combination of click-and toneburst-evoked auditory brainstem response measurements to estimate pure-tone thresholds. Ear Hear. 2006;27(1):60–74.
Griffith AJ, Wangemann P. Hearing loss associated with enlargement of the vestibular aqueduct: mechanistic insights from clinical phenotypes, genotypes, and mouse models. Hear Res. 2011;281(1–2):11–7.
Izzedine H, Tankere F, Launay-Vacher V, Deray G. Ear and kidney syndromes: molecular versus clinical approach. Kidney Int. 2004; 65(2):369–85.
Jerger J. Clinical experience with impedance audiometry. Arch Otolaryngol. 1970;92(4): 311–24.
Jobst-Schwan T, Klämbt V, Tarsio M, Heneghan JF, Majmundar AJ, Shril S, et al. Whole exome sequencing identified ATP6V1C2 as a novel candidate gene for recessive distal renal tubular acidosis. Kidney Int. 2020;97(3):567–79.
Joshua B, Kaplan DM, Raveh E, Lotan D, Anikster Y. Audiometric and imaging characteristics of distal renal tubular acidosis and deafness. J Laryngol Otol. 2008;122(2):193–8.
Karet FE. Inherited distal renal tubular acidosis. J Am Soc Nephrol. 2002;13(8):2178–84.
Karet FE, Finberg KE, Nayir A, Bakkaloglu A, Ozen S, Hulton SA, et al. Localization of a gene for autosomal recessive distal renal tubular acidosis with normal hearing (rdRTA2) to 7q33-34. Am J Hum Genet. 1999a;65(6): 1656–65.
Karet FE, Finberg KE, Nelson RD, Nayir A, Mocan H, Sanjad SA, et al. Mutations in the gene encoding B1 subunit of H+-ATPase cause renal tubular acidosis with sensorineural deafness. Nat Genet. 1999b;21(1): 84–90.
Kose E, Sirin Kose S, Alparslan C, Kasap Demir B, Berdeli A, Mutlubas Ozsan F, et al. Val2Ala mutation in the Atp6v0a4 gene causes early-onset sensorineural hearing loss in children with recessive distal renal tubular acidosis: a case report. Ren Fail. 2014;36(5):808–10.
Li X, Chai Y, Tao Z, Li L, Huang Z, Li Y, et al. Novel mutations in ATP6V0A4 are associated with atypical progressive sensorineural hearing loss in a Chinese patient with distal renal tubular acidosis. Int J Pediatr Otorhinolaryngol. 2012; 76(1):152–4.
Liu J, Shen Q, Li G, Zhai Y, Fang X, Xu H. Clinical and genetic analysis of distal renal tubular acidosis in three Chinese children. Ren Fail. 2018;40(1):520–6.
Lopez-Garcia SC, Emma F, Walsh SB, Fila M, Hooman N, Zaniew M, et al. Treatment and long-term outcome in primary distal renal tubular acidosis. Nephrol Dial Transpl. 2019; 34(6):981–91.
Lorente-Cánovas B, Ingham N, Norgett EE, Golder ZJ, Karet Frankl FE, Steel KP. Mice deficient in H+-ATPase a4 subunit have severe hearing impairment associated with enlarged endolymphatic compartments within the inner ear. Dis Model Mech. 2013;6(2):434–42.
Misgar RA, Hassan Z, Wani AI, Bashir MI. Amelogenesis imperfecta with distal renal tubular acidosis: a novel syndrome? Indian J Nephrol. 2017;27(3):225–7.
Morton CC, Nance WE. Newborn hearing screening: a silent revolution. N Engl J Med. 2006;354(20):2151–64.
Nikki R, Martin B, Gus OG, Mato N, Elena T, Paul G. Endolymphatic sac enlargement in a girl with a novel mutation for distal renal tubular acidosis and severe deafness. Case Rep Pediatr. 2012;2012:605053.
Norgett EE, Golder ZJ, Lorente-Cánovas B, Ingham N, Steel KP, Karet Frankl FE. Atp6v0a4 knockout mouse is a model of distal renal tubular acidosis with hearing loss, with additional extrarenal phenotype. Proc Natl Acad Sci U S A. 2012;109(34):13775–80.
Palazzo V, Provenzano A, Becherucci F, Sansavini G, Mazzinghi B, Orlandini V, et al. The genetic and clinical spectrum of a large cohort of patients with distal renal tubular acidosis. Kidney Int. 2017;91(5):1243–55.
Peters TA, Monnens LAH, Cremers CWRJ, Curfs JHAJ. Genetic disorders of transporters/channels in the inner ear and their relation to the kidney. Pediatr Nephrol. 2004;19(11): 1194–201.
Quick CA, Fish A, Brown C. The relationship between cochlea and kidney. Laryngoscope. 1973;83(9):1469–82.
Rodríguez-Soriano J. New insights into the pathogenesis of renal tubular acidosis–from functional to molecular studies. Pediatr Nephrol. 2000;14(12):1121–36.
Royer P, Broyer M. Proceedings of Actualites Nephrologiques de l'Hopital Necker. Flammarion Paris; 1967. p. 73–92.
Ruf R, Rensing C, Topaloglu R, Guay-Woodford L, Klein C, Vollmer M, et al. Confirmation of the ATP6B1 gene as responsible for distal renal tubular acidosis. Pediatr Nephrol. 2003; 18(2):105–9.
Rungroj N, Nettuwakul C, Sawasdee N, Sangnual S, Deejai N, Misgar RA, et al. Distal renal tubular acidosis caused by tryptophan-aspartate repeat domain 72 (WDR72) mutations. Clin Genet. 2018;94(5):409–18.
Shinjo Y, Kaga K, Igarashi T. Distal renal tubular acidosis associated with large vestibular aqueduct and sensorineural hearing loss. Acta Otolaryngol. 2005;125(6):667–70.
Smith AN, Skaug J, Choate KA, Nayir A, Bakkaloglu A, Ozen S, et al. Mutations in ATP6N1B, encoding a new kidney vacuolar proton pump 116-kD subunit, cause recessive distal renal tubular acidosis with preserved hearing. Nat Genet. 2000;26(1):71–5.
Rodríguez Soriano J. Renal tubular acidosis: the clinical entity. J Am Soc Nephrol. 2002;13(8): 2160–70.
Stanković KM, Brown D, Alper SL, Adams JC. Localization of pH regulating proteins H+ ATPase and Cl– HCO3– exchanger in the Guinea pig inner ear. Hear Res. 1997;114(1-2):21–34.
Stover EH, Borthwick KJ, Bavalia C, Eady N, Fritz DM, Rungroj N, et al. Novel ATP6V1B1 and ATP6V0A4 mutations in autosomal recessive distal renal tubular acidosis with new evidence for hearing loss. J Med Genet. 2002; 39(11):796–803.
Subasioglu Uzak A, Cakar N, Comak E, Yalcinkaya F, Tekin M. ATP6V1B1 mutations in distal renal tubular acidosis and sensorineural hearing loss: clinical and genetic spectrum of five families. Ren Fail. 2013;35(9):1281–4.
Swayamprakasam AP, Stover E, Norgett E, Blake-Palmer KG, Cunningham MJ, Karet FE. Importance of early audiologic assessment in distal renal tubular acidosis. Int Med Case Rep J. 2011;4:7–11.
Torban E, Goodyer P. The kidney and ear: emerging parallel functions. Annu Rev Med. 2009;60:339–53.
Vargas-Poussou R, Houillier P, Le Pottier N, Strompf L, Loirat C, Baudouin V, et al. Genetic investigation of autosomal recessive distal renal tubular acidosis: evidence for early sensorineural hearing loss associated with mutations in the ATP6V0A4 gene. J Am Soc Nephrol. 2006;17(5):1437–43.
Vidarsson H, Westergren R, Heglind M, Blomqvist SR, Breton S, Enerbäck S. The forkhead transcription factor Foxi1 is a master regulator of vacuolar H-ATPase proton pump subunits in the inner ear, kidney and epididymis. PLoS One. 2009;4(2):e4471.
Wiley TL, Oviatt DL, Block MG. Acoustic-immittance measures in normal ears. J Speech Hear Res. 1987;30(2):161–70.
Yashima T, Noguchi Y, Kawashima Y, Rai T, Ito T, Kitamura K. Novel ATP6V1B1 mutations in distal renal tubular acidosis and hearing loss. Acta Otolaryngol. 2010;130(9):1002–8.
Zakzouk SM, Sobki SH, Mansour F, Al Anazy FH. Hearing impairment in association with distal renal tubular acidosis among Saudi children. J Laryngol Otol. 1995;109(10):930–4.
Zeinali F, Mohseni M, Fadaee M, Fattahi Z, Najmabadi H, Otukesh H, et al. Investigation of ATP6V1B1 and ATP6V0A4 genes causing hereditary hearing loss associated with distal renal tubular acidosis in Iranian families. J Laryngol Otol. 2014;128(12):1056–9.
Zhang H, Koruyucu M, Seymen F, Kasimoglu Y, Kim J-W, Tinawi S, et al. WDR72 mutations associated with amelogenesis imperfecta and acidosis. J Dent Res. 2019;98(5):541–8.