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ORIGINAL ARTICLE

Distal RTA with nerve deafness: clinical spectrum and mutational analysis in five children

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Abstract Distal renal tubular acidosis (RTA) with nerve deafness is caused by mutations in the *ATP6V1B1* gene causing defective function of the H^+-ATPase proton pump. We report five acidotic children (four males) from four unrelated families: blood pH 7.21–7.33, serum bicarbonate 10.8–14.7 mEq/l, minimum urinary pH 6.5–7.1 and fractional excretion of bicarbonate in the presence of normal bicarbonatemia 1.1–5.7%. Growth retardation and nephrocalcinosis, but not hypercalciuria, were common presenting manifestations. Hearing was normally preserved in one of the patients whose sister was severely deaf. One child was homozygous for a known mutation in exon 1: C>T (R31X). Three children were homozygous for a splicing mutation, intron 6+1G>A. The other patient was a compound heterozygote, having this mutation and a previously unreported mutation in exon 10: G>A (E330K). Our report shows that hearing loss is not always present in the syndrome of distal renal tubular acidosis with nerve deafness and the absence of hypercalciuria at diagnosis and describes a new mutation responsible for the disease in the *ATP6V1B1* gene.

Keywords Metabolic acidosis · Distal renal tubular acidosis · Nephrocalcinosis · Gene mutation · *ATP6V1B1* gene · Hypercalciuria

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Introduction

Primary distal renal tubular acidosis is caused by a failure of the α-intercalated cells of the cortical collecting duct to secrete acid into the tubular lumen. Biochemical diagnostic criteria of distal RTA include sustained hyperchloremic metabolic acidosis with an inability to maximally acidify the urine below a pH of 5.5 and the absence of massive bicarbonaturia revealed by normal fractional excretion of bicarbonate in the presence of normal bicarbonatemia. Hypokalemia, hypercalciuria and hypocitraturia are common manifestations of distal RTA. Clinically, the disease is characterized by failure to thrive, nephrocalcinosis, polyuria and urolithiasis. In untreated cases, the progression of nephrocalcinosis may lead to chronic renal failure [1–3].

Most cases of primary distal RTA in children result from defective function of the vacuolar H^+-ATPase located at the apical surface of the α-intercalated cells. The H^+-ATPase is a proton pump formed by several subunits. Mutations in the *ATP6V0A4* gene, which encodes the a4 subunit, cause autosomal recessive distal RTA (OMIM #602722) [4]. Mutations in the *ATP6V1B1* gene, encoding the B1 subunit, result in autosomal recessive distal RTA associated with nerve deafness (OMIM #267300) [5]. ATP6V1B1 is

Table 1 Primer sequences used for PCR and sequencing

Primer pair	Forward	Reverse	Size of the PCR fragment (bp)
Exon 2	GATGCCTCTGTGTGTGAGCAG	CCTGCAGGGCAGGGGAGAG	192
Exons 3–4	CGGAGGAGGAGAAGGGACTTT	GGGTTTGGTCAGAGAAGCTGG	517
Exon 5	GGGTAGACAGTAGTGAGGGACACA	GGGCAGAACTTCCAAGAGTGG	218
Exon 6	CGAGGAGAGCAGGGAAGGGT	AGCAGCCCCAGCCTCTGCT	262
Exon 7	CCATGAGCCAGTGGTGCTCA	GGAGACAGGCTGCCTGCTCA	238
Exon 8	AGGGGCCAGGCCTTGCCC	CGGGGTCTGCCCACCTGAG	209
Exon 9	GGCTATGTGGTGCATTAGCCCA	GGTGTCAGGCTTAGGGAGGGGTA	242
Exons 10–11	CCCTTCCTAGCTTCAGCCTCTCA	TGCCTGGGGGCAGTGAAACAT	631

expressed in the cochlea and the endolymphatic sac, where the H^+-ATPase pump likely plays an important physiological role to maintain the endolymph pH at 7.4.

We report five patients with distal RTA having underlying mutations in the *ATP6V1B1* gene and discuss the association with hearing loss and the clinical spectrum of this entity.

Patients and methods

Patients

Five Spanish Caucasian children (four males) diagnosed with primary distal RTA according to the clinical and biochemical findings were studied. Hearing was assessed by pure-tone audiometry and/or auditory evoked responses. The data of patient I-1 have been partially reported [5, 6]. The children were members of four non-related families living in the same geographical region of Spain (Asturias). Their parents did not claim consanguinity.

Genetic analysis of *ATP6V1B1* gene

DNA was extracted by using the salting-out method [7] from blood samples from the patients and their parents, except for patient IV-1's father, who was not available. DNA from 100 healthy individuals was used as the control. Informed consent was obtained from all the participants in the study.

The 15 exons of *ATP6V1B1* were amplified by polymerase chain reaction (PCR) using the primers shown in Table 1. The amplified fragments were purified and sequenced on an automated ABI310 system, using BigDye chemistry (Applied Biosystems-Applera Corporaton, Drive Foster City, CA).

Results

Clinical and biochemical data

Tables 2 and 3 summarize the clinical and biochemical manifestations leading to the diagnosis of distal RTA in each patient.

Table 2 Clinical features at diagnosis of the five patients with distal RTA

Patients	Age	Sex	BW (g)	Presenting manifestations	Weight (g) [SDS]	Height or length (cm) [SDS]	Nerve deafness	NC or UL
I-1	3 months	Male	1,810	Growth retardation	2,700 [−5.30]	45 [−7.28]	Yes	Yes
II-1	4 months	Male	2,650	Vomiting and dehydration	4,280 [−3.54]	60 [−1.19]	Yes	Yes
III-1	5 years 7 months	Female	3,730	Abdominal pain	15,200 [−1.50]	100.8 [−2.45]	Yes	Yes
III-2	1 month	Male	3,330	Family study Failure to thrive	3,100 [−2.53]	52 [−0.80]	No	Yes
IV-1	5 months	Male	2,000	Growth retardation	3,710 [−4.80]	56 [−4.07]	Yes	Yes

The Roman numerals indicate the family to which each patient belonged; BW: birth weight; NC: nephrocalcinosis; UL: urolithiasis; SDS: standard deviation score.

Table 3 Biochemical features at diagnosis of the five patients with distal RTA

Patients	S Cr (mg/dl)	Blood venous pH	S HCO_3^- (mg/dl)	S K (mEq/l)	S Cl (mEq/l)	UpH	FE HCO_3^- (%)	U Ca/Cr (mg/mg)*
I-1	0.41	7.26	13.0	2.2	123	6.9	1.6	0.37
II-1	0.40	7.21	13.0	3.2	118	7.0	–	0.41
III-1	0.62	7.29	10.8	3.0	111	7.1	2.5	0.09
III-2	0.30	7.29	11.0	4.3	106	7.0	1.1	0.82
IV-1	0.46	7.33	14.7	3.7	107	6.5	5.7	0.70

S: serum; Cr: creatinine; UpH: minimum urinary pH with spontaneous metabolic acidosis; FE HCO_3^- : fractional excretion of bicarbonate with normal bicarbonatemia achieved after alkali treatment; U: urine. Ca/Cr: calcium to creatinine ratio. * The 95th percentile of normal reference values for U Ca/Cr was 0.81 mg/mg for infants aged 1/12-1 year (patients I-1, II-1, III-2 and IV-1) and 0.30 mg/mg for children aged 5–7 years (patient III-1) [8]. Isolated urine samples for Ca/Cr measurements were collected in the presence of metabolic acidosis at random between 8 and 12 a.m. in infants and the second morning sample in fasting state in patient III-1

ATP6V1B1 analysis

Four children were carriers of a previously reported splicing mutation: intron 6+1G>A [5]. Patients II-1, III-1 and III-2 were homozygous for this mutation. Patient IV-1 was a compound heterozygote who had this mutation and a previously unreported mutation in exon 10: G>A (E330K). Genotyping of this mutation by restriction enzyme (*Taq* I) digestion showed its absence in the 100 controls. Patient I-1 was homozygous for a known mutation in exon 1: C>T (R31X) [5]. The parents were mutation carriers.

Discussion

Our patients had three different *ATP6V1B1* gene mutations. The intron 6+1 G>A mutation was found homozygously in three patients from two families and in another who was a compound heterozygote. This child also had a mutation E330K, which had not been previously described. Although a functional analysis of the changes induced by this mutation was not performed, replacement of an acidic amino acid, glutamic acid, by a basic one, lysine, likely brings about important conformational alterations in the structure of the protein. Moreover, glutamic acid is an amino acid highly conserved in the composition of this protein among species, which suggests an important biological role. The presence of the splice-site mutation intron 6+1 g>a in four of the patients, homozygous in three, from families apparently unrelated might suggest a Spanish founder effect. Table 4 shows *ATP6V1B1* gene mutations reported in patients with distal RTA associated with nerve deafness [5, 9–11].

The five patients reported here provide interesting data on the syndrome of distal RTA associated with hearing loss. The two acidotic siblings of family III have the same mutation, but whereas the oldest sister (III-1) was severely deaf, her brother (III-2) hears normally. As this brother was born after his sister had been diagnosed, early and repeated postnatal explorations were performed, revealing metabolic acidosis in the 1st weeks of life and unimpaired hearing. Subsequently, he acquired language at a normal age. To our knowledge, systematic newborn hearing screening in patients later diagnosed with distal RTA with nerve deafness has not been reported, and the hearing status in the early postnatal period is unknown for these patients. We have previously reported patient I-1 and his dizygotic

Table 4 Mutations in the *ATP6V1B1* gene found in patients having DRTA with nerve deafness

First author (reference)	DNA mutation	Protein change
Karet [2]	91c>t	R31X
	1-bp del:497c	T166RfsX174
	1-bp del:1152c	P385PfsX395
	1-bp ins:1152c	P385PfsX441
	Intron 6+1 g>a	Splicing
	Intron 7+1 g>t	Splicing
	Intron 8+1 g>a	Splicing
	242t>c	L81P
	370c>t	R124W
	421t>g	M174R
	823a>c	T275P
	947 g>a	G316E
	Intron 9-2a>t	Splicing
	1937c>g	P346R
	1090 g>a	G364S
Stover [8]	469c>t	R157C
	368 g>t	G123V
	1-bp ins:1158c	I386fsX441
Hahn [9]	228 g>t	R76S
	368 g>t	G123V
Vargas-Poussou [10]	Intron 2+1 g>c	splicing
	1181 g>a	R394Q
Gil (present report)	988 g>a	E330K

Mutations are named following the *ATP6V1B1* sequence provided by http://www.ensembl.org

twin, who was deaf, but not acidotic [6]. The patients from these two families illustrate the clinical heterogeneity associated with *ATP6V1B1* mutations, ranging from severe deafness to normal audition, at least in the early stages. Patient III-2 had been treated since the 1st weeks of life with well-controlled bicarbonate supplementation. Sustained correction of the acidosis may have had a protective effect on the development and progression of deafness in this patient. Although it has been suggested that alkali treatment does not prevent the progression of hearing impairment [12], the late development of hearing loss in some patients with distal RTA linked to *ATP6V0A4* mutations might be related with inappropriate sustained control of acidosis.

Metabolic acidosis with reduced excretion of fixed acids and positive hydrogen ion balance leads to hypercalciuria. It has been proposed that in patients with primary distal RTA there is a significantly negative correlation between the serum bicarbonate concentration and urine calcium elimination, whereby normal values of urinary excretion of calcium may be used as a marker of well-controlled acidosis [13]. In this respect, it is interesting to note that our patients were normocalciuric at the time of diagnosis, in spite of the presence of simultaneous metabolic acidosis (Table 3). Levels of urinary calcium elimination are normally high in infants and decrease progressively as the child becomes older. Therefore, age must be strictly considered at the time of evaluating calcium excretion in the urine. In addition, the development of nephrocalcinosis and urolithiasis in the absence of hypercalciuria supports the important pathogenic role of hypocitraturia in the origin of these complications.

After a follow-up from 5 to 29 years, the five patients reported here had maintained normal glomerular filtration rates, persistent nephrocalcinosis, the need for a hearing prosthesis in the four cases with hearing impairment and growth improvement following alkali supplementation. This confirms the good prognosis of distal RTA when metabolic acidosis has been well controlled since an early age.

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