The genetic and clinical spectrum of a large cohort of patients with distal renal tubular acidosis

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Primary distal renal tubular acidosis is a rare genetic disease. Mutations in *SLC4A1*, *ATP6V0A4,* and *ATP6V1B1* genes have been described as the cause of the disease, transmitted as either an autosomal dominant or recessive trait. Particular clinical features, such as sensorineural hearing loss, have been mainly described in association with mutations in one gene instead of the others. Nevertheless, the diagnosis of distal renal tubular acidosis is essentially based on clinical and laboratory findings, and the series of patients described so far are usually represented by small cohorts. Therefore, a strict genotype-phenotype correlation is still lacking, and questions about whether clinical and laboratory data should direct the genetic analysis remain open. Here, we applied next-generation sequencing in 89 patients with a clinical diagnosis of distal renal tubular acidosis, analyzing the prevalence of genetic defects in *SLC4A1*, *ATP6V0A4,* and *ATP6V1B1* genes and the clinical phenotype. A genetic cause was determined in 71.9% of cases. In our group of sporadic cases, clinical features, including sensorineural hearing loss, are not specific indicators of the causal underlying gene. Mutations in the *ATP6V0A4* gene are quite as frequent as mutations in *ATP6V1B1* in patients with recessive disease. Chronic kidney disease was frequent in patients with a long history of the disease. Thus, our results suggest that when distal renal tubular acidosis is suspected, complete genetic testing could be considered, irrespective of the clinical phenotype of the patient.

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Renal tubular acidosis (RTA) is characterized by persistent, normal serum anion gap metabolic acidosis. Different types of RTA can be distinguished on the basis of clinical, pathophysiologic, and molecular criteria. Primary forms result from specific genetic defects in transporters/enzymes involved in renal bicarbonate (HCO_3^-) reabsorption or hydrogen (H^+) secretion and usually become clinically evident during infancy or early childhood.[1]

Distal RTA (dRTA) is a rare genetic disorder in which the main clinical features are vomiting, diarrhea and/or constipation, loss of appetite, polydipsia and polyuria, nephrocalcinosis, nephrolithiasis, osteomalacia, and rickets. Most

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pediatric cases are primary, and patients are often affected by growth retardation caused by chronic metabolic acidosis unless alkali therapy is initiated early in life. In this disorder, the α-intercalated cells in the collecting duct are unable to secrete H^+ and to properly acidify urine or to reabsorb HCO_3^-.[1,2]

dRTA can be transmitted as either an autosomal dominant (AD) or autosomal recessive (AR) trait.[3] AD forms typically become clinically manifest in adolescence or adulthood and are usually caused by mutations in the *SLC4A1* gene, encoding the basolateral Cl^-/HCO_3^- exchanger.[1,4,5] This gene has different promoter regions and can undergo alternative splicing, thus regulating the expression and sequence characteristics of the kidney and erythrocyte isoforms. Therefore, mutations in the *SLC4A1* gene can cause dRTA and/or hemolytic anemia with red cell morphology anomalies, also in a recessive manner.[6,7]

AR dRTA is associated with mutations in *ATP6V0A4* and *ATP6V1B1* genes encoding for the A4 and B1 subunits of the apical H+ATPase pump, respectively. Patients can present with early or absent/late sensorineural hearing loss (SNHL). Subjects without hearing defects usually carry mutations in the *ATP6V0A4* gene (except for some rare variants), whereas those with deafness have mutations in the *ATP6V1B1* gene.[8–18]

The diagnosis of dRTA is based on clinical and laboratory features. However, a molecular diagnosis is of great importance in order to provide the patient and the family with adequate genetic counseling to better assess the patient's prognosis and to define genotype-phenotype correlations. Nevertheless, only a small series of patients with dRTA has been studied and genetically characterized so far.[4–26] For this reason, a strict and reliable genotype-phenotype correlation is still lacking.

In this study, we applied a next-generation sequencing approach to 89 patients with a clinical diagnosis of dRTA. The aim of this work was to analyze the prevalence of genetic defects in *SLC4A1*, *ATP6V0A4*, and *ATP6V1B1* genes and to assess the clinical phenotype of patients that are eventually typical of the different genetic forms of the disease.

RESULTS

Genetic analysis

A total of 89 patients with a reported clinical diagnosis of dRTA were analyzed (Supplementary Tables S1 and S2). Consanguinity was noted only in 4 families (cases 19, 30, 60, and 64). All the patients underwent genetic testing for *SLC4A1*, *ATP6V1B1*, and *ATP6V0A4* genes. Among 89 patients, 64 showed causative mutations (71.9%) in 1 of the 3 genes (Tables 1–3, Figure 1a). Mutations were distributed as follows (Figure 1a): 9 patients presented pathogenic variants in the *SLC4A1* gene (10.1%) (Table 1, cases 1–9), 30 patients had causative mutations in the *ATP6V0A4* gene (33.7%) (Table 2, cases 10–39), and 25 patients had mutations in the *ATP6V1B1* gene (28.0%) (Table 3, cases 42–66).

Among patients carrying pathogenic variants in the *SLC4A1* gene, 7 (6.7%) satisfied criteria for a molecular diagnosis of AD dRTA (Table 1, cases 1–7), whereas 2 (2.2%) were homozygous (Table 1, cases 8 and 9). All the mutations were missense, and 6 of them were *de novo*. All of these variants were already reported as pathogenic.[5,7,27–29]

In the *ATP6V0A4* gene, we found 11 missense, 6 nonsense, 5 splicing, and 5 frameshift mutations and 1 nonstop change and 1 intragenic deletion (Table 2). In addition, 3 cases showed a triplet exon deletion. Mutations were detected in 30 cases, 17 of them in homozygosis and 14 in compound heterozygosis. In 20 cases, it was possible to assess the pattern of inheritance through the analysis of both parents, whereas in the remaining cases, blood samples from the parents were not available. In 5 cases (cases 13, 20, 30, 32, and 35), we found homozygous pathogenic variants not reported in the literature yet; 7 cases (cases 14, 17, 21, 25, 26,

Table 1 | Cases with mutations in the *SLC4A1* gene

Case	Pathogenic mutations	Mother	Father	Relatives	Ref.
1	c.[1765C>T]+[=] p.[Arg589Cys]+[=]	WT	WT	WT	Bruce *et al.*[5]
2	c.[1937G>A]+[=] p.[Arg646Gln]+[=]	NA	NA	NA	Zelinski *et al.*[27]
3	c.[1766G>A]+[=] p.[Arg589Hys]+[=]	WT	WT	WT	Bruce *et al.*[5]
4	c.[1765G>A]+[=] p.[Arg589Cys]+[=]	WT	WT	NA	Bruce *et al.*[5]
5	c.[1825G>A]+[=] p.[Gly609Arg]+[=]	WT	WT	NA	Rungroj *et al.*[28]
6	c.[1765C>T]+[=] p.[Arg589Cys]+[=]	WT	WT	WT	Bruce *et al.*[5]
7	c.[1766G>A]+[=] p.[Arg589His]+[=]	WT	WT	WT	Bruce *et al.*[5]
8	c.[388G>A]+[388G>A] p.[Gly130Arg]+[Gly130Arg]	c.[388G>A]+[=] p.[Gly130Arg]+[=]	c.[388G>A]+[=] p.[Gly130Arg]+[=]	NA	Inoue *et al.*[29]
9	c.[2102G>A]+[2102G>A] p.[Gly701Asp]+[Gly701Asp]	c.[2102G>A]+[=] p.[Gly701Asp]+[=]	c.[2102G>A]+[=] p.[Gly701Asp]+[=]	NA	Tanphaichitr *et al.*[7]

NA, not available; WT, wild type; [=], represents the wild-type allele, consistent with Human Genome Variation Society (HGVS) nomenclature.