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## Uso a longo prazo de enalapril e hidroclorotiazida em dois pacientes com novas mutações com doença de Dent tipo 1

The long-term use of enalapril and hydrochlorothiazide in two novel mutations patients with Dent's disease type 1

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### RESUMO

A doença de Dent é uma tubulopatia ligada ao X causada por mutações no gene que codifica o canal de cloro CLCN-5 e é caracterizada por proteinúria de baixo peso molecular, hipercaleiúria, nefrocalcinose e insuficiência renal. Vários casos têm sido descritos, nos quais o único sintoma na apresentação foi proteinúria assintomática e glomerulosclerose global ou segmentar. A insuficiência renal nesses pacientes pode ser causada pela hipercaleiúria e proteinúria persistente. Portanto, o inibidor da enzima de conversão da angiotensina e os tiazídicos poderiam ser úteis. O objetivo desta pesquisa é relatar os efeitos destas drogas em dois pacientes com doença de Dent tipo 1 com mutações novas. Neste relato não foram observadas correlações significativas entre dose de hidroclorotiazida e calcíúria e entre enalapril e proteinúria. Este achado é importante, pois, sendo pacientes poliúricos, o uso destas drogas poderia prejudicar a função renal

**Palavras-chave:** Enalapril. Proteinúria. Hipercaleiúria. Hidroclorotiazida. Doença de Dent.

### ABSTRACT

Dent's disease type 1 is an X-linked tubular disease caused by mutations in the renal chloride channel CLCN-5, and it is characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, and renal failure. Several cases have been described in which the only presenting symptoms were asymptomatic proteinuria, and focal segmental or global glomerulosclerosis. The renal failure in these patients may be caused by hypercalciuria and persistent proteinuria. Therefore, angiotensin convertase enzyme inhibitor and thiazides could be useful. Our aim is to report the effects of these drugs in two novel mutations patients with Dent's disease type 1. In this report, no significant correlations between dosage of hydrochlorothiazide and calciuria and no significant correlations between proteinuria and dosage of enalapril were detected. This is important since these are polyuric patients and these drugs could be dangerous to their renal function.

**Keywords:** Enalapril. Proteinuria. Hypercalciuria. Hydrochlorothiazide. Dent Disease.

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conflicts of interest.

### INTRODUCTION

Dent's disease type 1 is an X-linked tubular disease caused by mutations in the CLCN5 gene that encodes the electrogenic chloride/proton exchanger ClC-5.<sup>1</sup> This disease is characterized by low molecular weight (LMW) proteinuria, hypercalciuria, nephrocalcinosis, and renal failure. Patients may present other features of proximal tubular impairment.<sup>2</sup> The most consistent indication of affected males is increased excretion of LMW proteins.<sup>3</sup> Females are

considered as carriers, and they show milder LMW proteinuria.<sup>4</sup> Several cases have been described in which the only presenting symptoms were proteinuria and focal segmental or global glomerulosclerosis (FSGS).<sup>5</sup> The major consequence of Dent's disease is a progression to end-stage renal disease (ESRD), but the cause is unknown. Although hypercalciuria cannot directly account for the glomerular abnormalities, the interstitial injury caused by renal calcification may contribute to glomerular injury.<sup>6</sup> The persistent proteinuria can also

contribute to it. Therefore, angiotensin converting enzyme inhibitor and thiazides could be useful to these patients. Our aim is to report the effects of enalapril and hydrochlorothiazide (HCTZ) in two novel mutations in Dent's disease type 1 Brazilian patients. The molecular studies were made in Tokyo University. The parents signed the informed consent.

### CASE 1

An 11-year-old boy was referred for evaluation and Table 1 presents the tests performed. He had a history of failure to thrive and nephrotic proteinuria since he was six years-old. The patient was treated with immunosuppressive drugs without response. Family history was negative and the parents were non-consanguineous. His renal biopsy showed glomerular sclerosis (23/84 glomerulus), segmental mesangial proliferation and adherence to Bowman capsule, mild interstitial fibrosis, and tubular atrophy. Immunofluorescence showed IgM. The patient had nephrotic proteinuria, hypercalciuria, without hypercholesterolemia and hypoalbuminemia. The renal ultrasound showed bilateral nephrocalcinosis grade III. Potassium citrate (0.5 mEq/kg/day) and enalapril were introduced. Dent's disease was suspected, therefore an isolated urinary sample was collected to measure urinary RBP (uRBP) of the patient and his mother, which showed first uRBP mother's patient of 0.21 mg/L and the second ones was 0.63 mg/L; patient's uRBP was 55.9 mg/L (normal value is up to 0.4 mg/L). A molecular study was carried out and revealed a novel missense G329D (GGT to GAT) mutation in Exon 8 of the CLCN5. The mother was a carrier and the father had no mutations (Figure 1 – Case A). The patient was treated with enalapril (0.18 ± 0.06 mg/kg/day),

potassium citrate, and HCTZ (0.81 ± 0.17 mg/kg/day) for 41 months. Figures 2 and 3 show, respectively, the evolution of proteinuria and creatinine clearance estimated by stature according to Schwartz formula<sup>7</sup> parameters. The patient did not tolerate (hypotension symptoms) higher doses of these medications.

There were no significant correlations between dosage of enalapril and proteinuria (n = 12 measurements, r = -0.19 and p = 0.56) or between calciuria and dosage of HCTZ (n = 11 measurements, r = -0.15 and p = 0.65), with Pearson's correlation coefficient. The diuresis observed was of 2.0 ± 0.9 mL/kg/hour. After three months without enalapril and HCTZ, a decrease in serum creatinine level from 1.50 to 1.06 mg/dL was observed, and proteinuria and hypercalciuria were kept in the same range.

### CASE 2

A four-year-old male was referred to investigate hypercalciuria and proteinuria. Table 1 presents the tests that were performed in this patient. He presented failure to thrive and polydipsia when he was 1 year-old. The renal biopsy showed calcification in interstitium. His grandfather had nephrolithiasis and the parents were non-consanguineous. This patient had a nephrotic proteinuria without hypoalbuminemia or hypercholesterolemia, hypercalciuria and normocalcemia. Nephrocalcinosis grade I was detected. The uRBP mother's patient was of 0.9 mg/L and the patient presented uRBP of 62 mg/L. The molecular study showed a novel missense L278W (TTG to TGG) mutation in Exon 8 of the CLCN5. The mother was a carrier and the father had no mutations (Figure 1 – Case B). This patient was treated with potassium citrate (0.5 mEq/kg/day), HCTZ (1.90 ± 0.62 mg/kg/day) and enalapril (0.31 ± 0.05 mg/kg/day) for 48 months.

**Table 1** TESTS AT FIRST ATTENDANCE OF THE TWO DENT'S DISEASE PATIENTS WITH LONG-TERM FOLLOW-UP

Test	Case 1	Case 2	Normal values
Serum creatinine (mg/dL)	1.1	0.3	0.5 – 0.8
Serum urea (mg/dL)	37	30	13 – 36
Serum albumin (g/L)	5	4.5	3.8 – 5.6
Ionic calcium (mmol/L)	1.25	1.31	1.12 – 1.32
Serum sodium/serum potassium (mEq/L)	141 / 4.5	142 / 4.0	135 – 147 / 3.7 – 5.0
Estimated creatinine clearance (mL/min./1.73 m <sup>2</sup> )	66.5	172	≥ 90
Calciuria (mg/kg/day)	13.5	6.3	< 4
Proteinuria (mg/kg/day)	51	65	< 10
Total cholesterol (mg/dL)	186	147	< 170
Triglycerides (mg/dL)	36	46	< 100

**Figure 1.** DNA sequencing chromatograms of patients and parents. A: Case 1; B: Case 2.

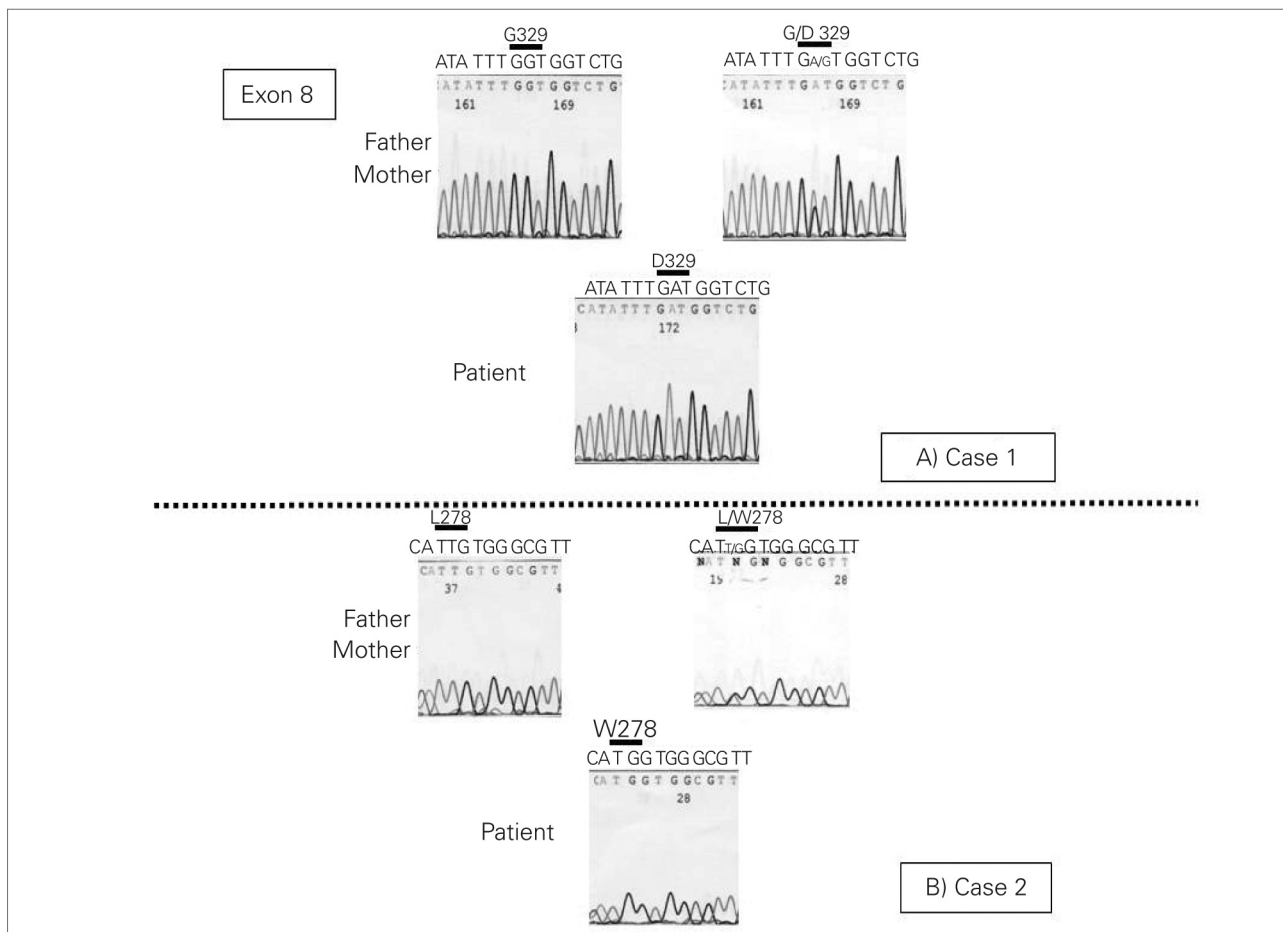
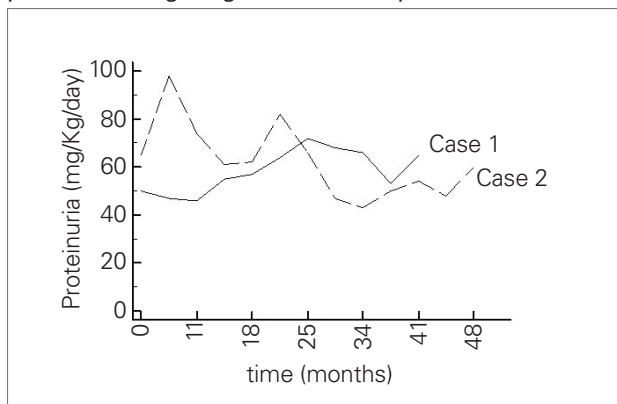
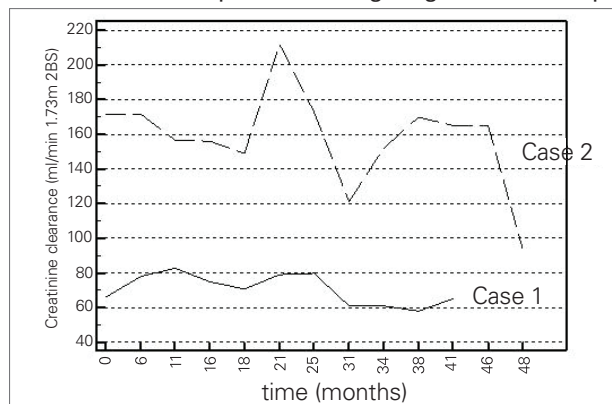


Figure available in color at the website: [www.jbn.org.br/](http://www.jbn.org.br/)

**Figure 2.** Proteinuria (mg/kg/day) in two Dent's disease patients during long-term follow-up.



**Figure 3.** Creatinine clearance estimated by stature in two Dent's disease patients during long-term follow-up.



Figures 2 and 3 show, respectively, the evolution of proteinuria and creatinine clearance estimated by stature according to Schwartz Formula.<sup>7</sup> Although he tolerated higher doses of the drugs than patient 1, no significant correlation between proteinuria and dosage of enalapril ( $n = 12$  measurements,  $r = 0.43$  and  $p = 0.16$ ) and between calciuria and dosage of HCTZ ( $n = 12$  measurements,

$r = -0.26$  and  $p = 0.4$ ) were detected (Pearson's correlation coefficient). Diuresis during this period was of  $3.2 \pm 2.0$  mL/kg/hour.

**METHOD OF DNA SEQUENCING**

Mutational analysis of the CLCN5 gene – leukocyte DNA was extracted and cDNA was purified. They

were used with CLCN5-specific primers for polymerase chain reaction (PCR) amplification, using the previously described conditions.<sup>8</sup> The DNA sequence of the PCR products was determined by the use of Taq polymerase cycle sequencing and a semi-automated detection system (ABI 373A sequencer; Perkin Elmer Japan Applied Biosystems Division, Japan).

## DISCUSSION

This study adopted three diagnostic criteria for Dent's disease: LMW proteinuria, hypercalciuria, and one of the following: nephrocalcinosis, kidney stones, hypophosphatemia, renal failure, aminoaciduria, rickets, or a positive family.<sup>9</sup> However, Copelovitch *et al.* suggested that the diagnosis should be considered in patients with nephrotic proteinuria without hypoalbuminemia or edema and FSGS.<sup>10</sup> This is important to avoid immunosuppressors as in Case 1. Dent's disease type 1 is caused by mutations in the CLCN5 gene. Over one fourth of the mutations is missense, and they eliminate or reduce ClC-5 currents.<sup>11</sup>

The current treatment is supportive. High doses of thiazides can reduce calciuria and the risk of nephrolithiasis; however, they are poorly tolerated for long periods in these polyuric patients, frequently associated with hypovolemia and hypokalemia.<sup>12,13</sup> In addition, calcium concentration in the urine can be halved just by doubling the water intake, avoiding the effects of thiazide. In addition to LMW proteinuria, the glomerular component may increase during follow-up, and enalapril could be useful. We were not able to detect a significant correlation between the dosage of HCTZ and calciuria, as well as, proteinuria and enalapril, and this drug can even decrease the glomerular filtration rate, which was demonstrated by the decrease in serum creatinine after enalapril withdrawn in Case 1. Finally, the two missense mutations identified are predicted at the very important sites of ClC-5 function. Ashida and Ymamoto are now analyzing the sites of missense mutations identified so far by computational three-dimensional modeling. According to their results, the two mutations are located at critical sites, which are crucial for substrate transport or dimer formation (personal communication with professors Ashida and Ymamoto).

Since this computational modeling theory is under preparation of the manuscript, the precise data could not be presented. Furthermore, during the analysis of more than 100 families with Dent's disease,

Sekine *et al.* have been sequencing all of the exons of CLCN5, and the two mutations above were not identified. These facts strongly indicated the two mutations in this paper are exactly responsible for the development of Dent's disease, and not SNPs.

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