A novel *CLCN5* mutation in a Chinese boy with Dent's disease

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Background: **Dent's disease is a rare X-linked recessive hereditary disease caused by mutations in either the *CLCN5* or *OCRL1* genes. This disease is characterized by manifestations of proximal renal tubule dysfunction associated with low molecular weight proteinuria (LMWP), hypercalciuria, nephrocalcinosis, nephrolithiasis, and progressive renal failure.**

Methods: **We report a Chinese boy with Dent's disease, clinically diagnosed by LMWP and hypercalciuria. Genetic analysis was made of the *CLCN5* and *OCRL1* genes. Related studies were also reviewed.**

Results: **A splice site mutation IVS6, +2T>C of the *CLCN5* gene was revealed in this case, and it was not reported previously.**

Conclusions: **Clinical and genetic analysis is valuable for the diagnosis of Dent's disease. A novel mutation in the *CLCN5* gene was identified in our patient.**

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Key words: ***CLCN5*;**
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Introduction

Dent's disease is a rare X-linked recessive hereditary disease characterized by low molecular weight proteinuria (LMWP), hypercalciuria, nephrocalcinosis, nephrolithiasis and progressive renal failure.[1] Patients may also present variable manifestations of proximal tubule dysfunctions such as aminoaciduria, phosphaturia, glycosuria, kaliuresis, and uricosuria, producing a partial Fanconi syndrome.[2] A minority of patients are likely to develop rickets or osteomalacia.[2] Recently, cases of Dent's disease characterized by features of Batter syndrome such as hypokalemic metabolic alkalosis have also been reported.[3] At least two genes, *CLCN5* and *OCRL1*, are associated with Dent's disease.[4,5]

Dent's disease was first described by Charles Enrique Dent and M. Friedman in 1964.[6] According to the literature of 2010, only 250 affected families were reported worldwide;[7,8] however the disease has been rarely seen in Chinese children.[9,10] Here we present a case of Dent's disease with identification of a novel mutation site on the *CLCN5* gene.

Case report

An 8-year-old Chinese boy presented with a two-month history of moderate proteinuria. But no edema, oliguria, microscopic hematuria or hypertension was identified during the course of the disease. The child has always been healthy up to the present. There was no significant family history of diseases.

Physical examination showed no abnormalities such as ophthalmic and hearing disorders. Laboratory test revealed proteinuria at level of 1-3+, with no microscopic hematuria. The urine protein/creatinine ratio was 1.6 mg/mg (normal, <0.2 mg/mg). Twenty-four hour urine protein was 0.83g/day (34.7 mg/kg per day). Urine protein electrophoresis showed 59.1% low molecular weight protein, 34.7% albumin, and 6.2% high molecular weight protein. Urine β2 microglobulin was14 040 μg/L (normal, <250 μg/L) which was compatible with tubular proteinuria. The urine calcium/creatinine ratio was 0.45 (normal, <0.21). Twenty-four hour urine calcium was 0.19 mmol/kg per day (normal, <0.1 mmol/kg). Biochemical analysis showed normal levels of sodium, potassium, calcium, alkaline phosphatase, acid-base balance, and normal creatinine clearance. Hepatitis A, B and C

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markers were all negative. Erythrocyte sedimentation rate, antistreptolysin O, complement C3 and C4 were normal. Antinuclear antibody and antineutrophil cytoplasmic antibodies were negative. Kidney ultrasound showed a normal urinary system without stone, nephrocalcinosis or nutcracker phenomenon.

Informed consent was obtained from the legal guardians of the patient before genetic testing. Blood sample was collected and genomic DNA extracted using a BloodGen Midi Kit (CWBIO, Beijing, China). Primers were designed to amplify the exons and exon-intron boundaries. Sequences were analyzed using DNASTAR. In this patient, there was a novel splice site mutation in the *CLCN5* gene IVS6, +2 T>C found (Fig.).

We extracted total RNA using TRI Reagent BD (SIGMA: T3809) to further verify the result. cDNA was generated using Superscript™ III Reverse Transcriptase (Invitrogen 18080-093) according to manufacturers' instructions. But our result showed that *CLCN5* coding sequence could not be amplified successfully no matter how we changed the conditions and redesigned the primers. We speculated that the splice site mutation in *CLCN5* gene IVS6, +2 T>C in this patient might result in the instability of mRNA, which is difficult to detect. Our result also indicated that the splice site mutation in the *CLCN5* gene might be the disease gene of this patient.

Discussion

Dent's disease is usually seen in childhood or early adulthood, particularly in male children. Because of inactivation of X chromosome, female patients with this disease are generally asymptomatic. Although a typical phenotype of Dent's disease often enables a clinical diagnosis, less severe sub-clinical cases may be under-diagnosed. Progression to end-stage renal failure occurs between the 3rd and 5th decades of life in 30%-80% of male patients.[1] A long-term follow-up is necessary for these patients.

Dent's disease may be caused by mutations in either the *CLCN5* or *OCRL1* gene. *CLCN5* encodes a 746 amino-acid electrogenic Cl-/ of H + exchanger (ClC-5), which belongs to the ClC family of Cl-channels/ transporters. The occurrence of the predominantly renal manifestations and their association with mutations in *CLCN5* is referred to as Dent's disease 1. *OCRL1* encodes a phosphatidylinositol bisphosphate 5- phosphatase and mutations are also associated with Lowe syndrome. Patients with Dent's disease in whom *OCRL1* mutations have been found may present extra-renal manifestations such as mild intellectual impairment, hypotonia and cataract. The occurrence of these extra-renal manifestations with mutations in *OCRL1* relating to Lowe syndrome is referred to as Dent disease 2. *CLCN5* mutations are present in approximately 60% of patients with Dent's disease, whereas *OCRL1* mutations are found in only 15% of the patients. The total number of reported *CLCN5* mutations is approximately 150, and these are scattered throughout the coding region, with no evidence for major mutational hot spots.[7] No genotype-phenotype correlation has been described so far, and there is considerable intra-familial variability in disease severity.[11-13]

The clinical diagnostic criteria for Dent's disease are as follows: (1) LMWP; (2) hypercalciuria; and (3) at least one of the following: nephrocalcinosis, kidney stones, hematuria, hypophosphatemia and renal insufficiency. The identification of mutation in either *CLCN5* or *OCRL1* confirms the diagnosis of Dent's disease. However, some patients with *CLCN5* gene mutations only showed LMWP or hypercalciuria alone.[14,15] Thus, in the presence of an identified *CLCN5* mutation, only

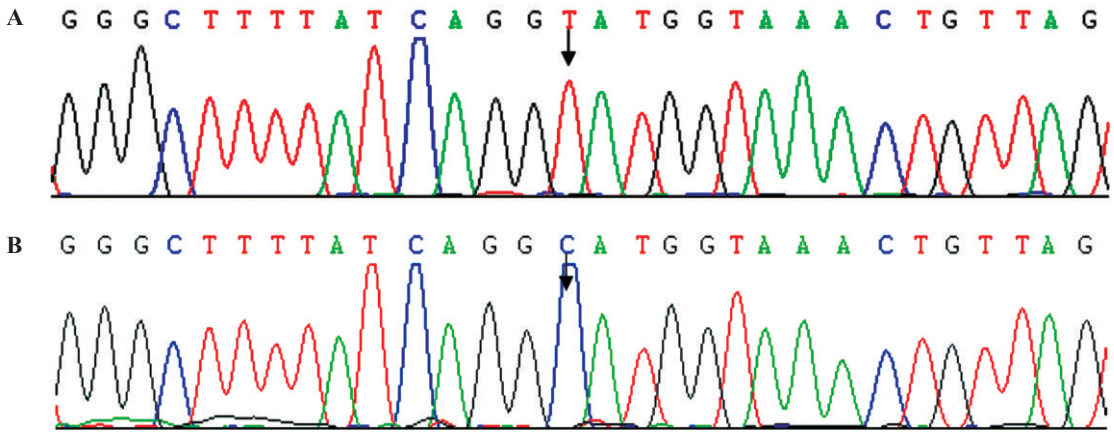

Fig. A splice site mutation in the *CLCN5* gene. **A:** *CLCN5* sequence of normal sample; **B:** *CLCN5* sequence of patient's sample, a new splice site mutation in the *CLCN5* gene IVS6, +2 T>C.

one of the above clinical criteria may be sufficient to establish an affected status in an individual.

Current treatments in patients with Dent's disease are mainly supportive, focusing on the treatment of hypercalciuria and the prevention of nephrolithiasis. Although therapy with thiazide diuretics can reduce urinary calcium excretion and consequently to decrease the risk of nephrolithiasis, great caution should be taken in children because of such side effects as extracellular dehydration and hypokalemia.[16] Furthermore, there is no clear correlation between hypercalciuria/nephrocalcinosis and renal failure. A hypercalciuric CIC-5 KO mouse model of Dent's disease has demonstrated that a high-citrate diet can preserve renal function and a slow progression of renal disease.[17] Further investigations are needed.

In our patient, we not only considered glomerular diseases, but also renal tubular disease screening. Urine protein analysis revealed that the urine protein of the patient was composed of low molecular weight protein with a higher level of β2-microglobulin. Further examination showed hypercalciuria, which is suggestive of Dent's disease. The subsequent *CLCN5* genetic testing demonstrated a novel splice site mutation IVS6, +2 T>C, which confirmed the diagnosis of Dent's disease. This patient had a normal renal function but no nephrocalcinosis or renal stone disease. An appropriate diagnosis could help the patient to avoid the toxic effects associated with immunosuppressive medications. A long-term follow-up is necessary for this patient.

In summary, LMWP and hypercalciuria are the main clinical features of Dent's disease. Clinical and genetic analysis is valuable for the diagnosis of Dent's disease.

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Competing interest: None declared.
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