Short Communication

Dent 2 Disease—Clinical and Laboratory Features

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Abstract

**Background.** Dent 2 disease is recently recognized entity which is characterized by Dent-like phenotype and presence of \( OCRL1 \) mutation. In this work we present clinical and laboratory features of two Macedonian children who fulfilled criteria for Dent 2 disease.

**Methods.** A systematic screening for low molecular proteinuria by SDS-PAG electrophoresis was performed to all male patients who were referred because of persistent proteinuria between 2000-2005.

**Results.** The first patient was a 12 year-old boy who was found to have low molecular proteinuria, hypercalciuria, mild hyperaminoaciduria and intermittent microscopic hematuria. He lacked mutation in \( CLCN5 \) gene, but was found to have a novel mutation in his \( OCRL1 \) gene. Although this mutation is associated with oculocerebrorenal syndrome of Lowe the boy had only mild mental retardation but no neurological deficit or congenital cataracts, which are typical for Lowe syndrome. The second patient was a 10 year-old boy who presented with low molecular proteinuria and hypercalciuria. He was also found to have a novel mutation in \( OCRL1 \) gene. He had normal intelligence, neurological status and lack of cataracts. The both patients were found to have mildly elevated muscle enzymes and CPK without clinical consequence.

**Conclusion:** SDS-PAGE is a useful screening tool for low molecular proteinuria. Patients with Dent like phenotype who lack \( CLCN5 \) mutation should be tested for \( OCRL1 \) mutations, which are not necessarily associated with congenital cataracts and neurological deficits.

**Keywords:** Dent 2 disease, low molecular weight proteinuria, SDS-PAGE, oculocerebrorenal syndrome, \( OCRL1 \), cataracts,

Introduction

Dent’s disease is a proximal tubulopathy which is characterized by low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, rickets, and progression to renal failure in some patients [1,2]. The disease is caused by mutations in the \( CLCN5 \) gene that encodes the voltage-gated chloride channel CIC-5 [2-5]. The data obtained from the studies in CIC-5 knockout mice showed that loss of CIC-5 function results in an endosomal acidification defect and reduced proximal tubular protein reabsorption, which explain the symptoms of Dent’s disease. Recently it has been shown that mutations in the \( OCRL1 \) gene, may lead to clinical and laboratory features consistent with Dent’s phenotype - Dent 2 disease [3,4]. In this work we present clinical and laboratory features of two Macedonian children who fulfilled criteria for Dent 2 disease.

Patients and methods

Screening for low molecular proteinuria was performed using standard stress tolerance test [6]. Urine samples were obtained from the patients at various levels of physical activity: at rest, regular physical activity and immediately after strenuous activity. Determination of total protein was performed by Muelman’s method. Qualitative analysis of urinary proteins was performed using horizontal gradient 4-22% SDS-PAG electrophoresis. Separated fractions were identified with standards with known molecular weight. Low molecular weight proteins were quantified in relative terms as percentage to total proteins after laser densitometry. Calcium excretion was assessed from 24 hour urine samples (normal values less than 4 mg/kg/day). Clinical evaluation included: anthropometry (body weight and height), thorough ophthalmological and audiological examination and neurodevelopmental assessment. Intelligence testing was performed using Wechsler scale for Macedonian children. Imaging studies included kidney ultrasound scan, Tc99mDMSA scintigraphy. Bone mineral density was assessed by dual energy X-ray absorptiometry. Genetic analysis was performed at referent laboratories and included testing for mutation in all coding regions of \( CLCN5 \) and \( OCRL1 \) gene [3,4].

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Results

During the period 2000-2005 two children with identified with the diagnosis of Dent’s disease. Analysis of urinary proteins in both showed the predominant presence of low molecular weight proteinuria with fractions having molecular weight less than 40 KD and these included \( \beta_2 \) microglobulin and retinol binding protein (Figure 1). The ratio tubular/glomerular proteins was greater than 1.0 in both.

Clinical features

The first patient was a 12 year-old boy who had two admissions at the hospital due to asymptomatic proteinuria. At the second admission renal biopsy was performed but normal histology and immunofluorescence did not lead to correct diagnosis. After performing standardized stress tolerance and SDS-PAGE of urinary proteins he was found to have low molecular proteinuria. Additional laboratory studies confirmed that he had hypercalciuria (11 mg/kg/d), mild hyperaminoaciduria and intermittent microscopic hematuria. His kidney ultrasound scan was normal and there were no deposits. His neurological assessment did not reveal any deficit. Formal intelligence testing showed that he had mild mental retardation (IQ 72). Repeated ophthalmological investigations confirmed that he had excellent visus and absence of cataracts. Serum and urine biochemistry was completely normal, except that he had mild increase of creatine phosphokinase (CPK), lactico dehydrogenase (LDH) and AST (aspartate aminotransferase). The family history was negative for nephrolithiasis, nephrocalcinosis or renal failure.

The second patient was a 10 year-old boy who was found to have proteinuria at school screening which was believed to be orthostatic in origin. The boy underwent stress tolerance test and analysis of urinary proteins by SDS-PAGE. He was found to have persistent proteinuria (including samples taken at rest) with predominant low molecular fractions. He was also found to have hypercalciuria. He had normal kidney ultrasound scan. He had normal intelligence, neurological status and lack of cataracts. Family history was negative for nephrolithiasis, nephrocalcinosis or renal failure.

Mutational analysis: Mutational analysis of the \( CLCN5 \) gene was performed firstly, and since mutation was not found in both patients, \( OCRL1 \) gene was tested at reference laboratories [3,4]. The both patients revealed novel mutations that arose de novo.

Discussion

In this work we presented two patients from Macedonia who presented clinically as Dent’s disease, but both showed mutation in the \( OCRL1 \) gene. Hoopes et al. found in their series of 32 families with Dent’s disease mutations in \( CLCN5 \) gene in 19 (60%), while in 5 families (16%) mutations in \( OCRL1 \) gene were found [3]. The patients who had \( OCRL1 \) gene mutation underwent detailed clinical, neurological and ophthalmological investigations but none of these patients had clinical stigmata of Lowe syndrome. The most importantly none of \( OCRL1 \) patients had congenital cataracts which is a cardinal feature of Lowe syndrome [7]. Lowe syndrome is a multisystem disorder which besides congenital cataracts manifests severe psychomotor retardation and renal Fancony syndrome [7,8]. The genetic basis of the disease is mutation in the \( OCRL1 \) gene which encodes enzyme PIP\(_2\) 5-phosphatase responsible for intracellular trafficking [9-12]. The ultimate prognosis is poor due to the progressive renal dysfunction [13-16]. In contrast the patients with Dent disease present relatively mild renal affection including low molecular weight proteinuria, hypercalciuria and nephrocalcinosis. Acidosis is rarely present. Recently Utsch et al. [4] confirmed the previous findings from the Hoopes’ report [3]. In a multicentric study 18 male patients from 16 families with Dent phenotype but without \( CLCN5 \) mutation were investigated for defects in the \( OCRL1 \) gene. Four novel \( OCRL1 \) mutations comprising either frameshifts (Q70RfsX88, T121NfsI22) or missense mutations (I257T, R476W) were detected. None of the patients manifested mental retardation neither cataracts. When analysing their group of patients with Dent phenotype mutations in \( CLCN5 \) gene were found in 54.3%, \( OCRL1 \) mutation in 11.4%, whereas one third of patients did not have any mutation suggesting genetic heterogeneity leading to Dent phenotype [4,5]. It remains to identify so far unknown genes. The authors speculated that such contrast between Lowe syndrome and Dent 2 disease could be attributed to tissue specific alternative \( OCRL1 \) tran-
scripts, but their investigations did not provide such evidence. In animal models where expression of OCRL1 gene is prevented by targeted disruption of this gene there is no evidence of cataracts, neurological abnormalities, or renal Fanconi syndrome. If another gene INPP5B is simultaneously disrupted, this leads to lethal phenotype. This gene is highly homologous to OCRL1 gene and its gene product Inpp5b phosphatase can compensate for absence of the OCRL1 enzyme deficiency [17]. One can speculate that the expression of the compensating enzyme may vary between the tissues and individuals and this may explain such diversity in phenotypic manifestation of OCRL1 mutations (from isolated renal phenotype in Dent 2 disease to multisystem disorder in Lowe syndrome).

In conclusion, our patients were detected owing to the widespread use of SDS-PAGE electrophoresis of urinary proteins in our clinical practice. This method can easily detect low molecular proteinuria, which is a hallmark of proteins in our clinical practice. This method can easily detect low molecular proteinuria, which is a hallmark of Dent disease. The patients who lack CLCN5 mutation should be tested for OCRL1 mutation. This new entity Dent 2 disease represents a part of the phenotypic spectrum caused by the mutations in OCRL1 gene.

Conflict of interest statement. None declared.

References