Case report

OCRL1 mutation in a boy with Dent disease, mild mental retardation, but without cataracts

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Background: **Oculocerebrorenal (Lowe) syndrome is an X-linked multisystem disease characterized by renal proximal tubulopathy, mental retardation, and congenital cataracts. We present a 19-year-old boy who was found to have low molecular weight proteinuria, hypercalciuria, mild generalized hyperaminoaciduria and intermittent microscopic hematuria at the age of 3.**

Methods: **Standard clinical and biochemical examinations and mutational analysis of the *CLNC5* and *OCRL1* gene were performed for the patient.**

Results: **The patient fulfilled diagnostic criteria for Dent disease, but lacked mutation in *CLCN5*. Sequencing of candidate genes revealed a mutation in his *OCRL1* gene, which encodes for enzyme PIP2 5-phosphatase. The enzyme was not detected by western blot analysis, and decreased activity of the enzyme PIP2 5-phosphatase was observed in cultured skin fibroblasts. The boy had only mild mental retardation, mildly elevated muscle enzymes, but no neurological deficit or congenital cataracts, which are typical for Lowe syndrome.**

Conclusions: **Children with Dent phenotype who lack *CLCN5* mutation should be tested for *OCRL1* mutation. *OCRL1* mutations may present with mild clinical features and are not necessarily associated with congenital cataracts.**

World J Pediatr 2011;7(3):280-283

Key words: **cataracts;**
Dent disease;
Lowe syndrome;
***OCRL1*;**
CLCN5

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doi: 10.1007/s12519-011-0312-6
This paper was presented on the Annual Meeting of the European Society for Pediatric Nephrology, Istanbul, Turkey, September 10-13, 2005.

Introduction

Dent disease is an X-linked renal tubular disorder that is characterized by low molecular weight (LMW) proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and in some cases progression to renal failure.[1] Three other phenotypic variants are well described: X-linked recessive nephrolithiasis, X-linked hypophosphatemic rickets, and familial idiopathic LMW proteinuria. In most of Dent patients the disease is caused by inactivation of the *CLCN5* gene on the X chromosome (Xp11.22) encoding the chloride channel and chloride/proton antiporter ClC-5 which is expressed predominantly in the kidney.[2] Recently mutations in another gene (*OCRL1*) were identified in patients with Dent disease.[3-7] In this paper we present a pediatric patient who fulfilled the criteria for Dent disease. Since mutation in the *CLCN5* was not found, he underwent extensive genetic investigation along with four other similar cases, which unexpectedly demonstrated mutations in the *OCRL1* gene in all 5 patients.[3,8] He was among the first diagnosed Dent patients with *OCRL1* mutation (Dent 2 disease) and therefore we present detailed clinical and biochemical data of our patient, which are not in concert with the expected phenotypic characteristics of an individual with *OCRL1* gene mutation.

Case report

A three-year-old healthy boy was investigated in 1993 for mild proteinuria detected by chance. Physical examination did not show any abnormality. His renal function and complement studies were normal. At the age of ten he was admitted to the hospital due to increasing proteinuria (2-3 g/d). There was intermittent microscopic hematuria. He was normotensive, without edema or

abnormalities on physical examination. Renal biopsy showed normal histological and immunofluorescent findings. Analysis of urinary proteins with SDS-PAG electrophoresis showed a typical tubular pattern with the presence of LMW proteins (Fig. 1).

Serum biochemistry showed the following results: glucose 5.1 mmol/L, urea 3.9 mmol/L, creatinine 62 μmol/L, Na 140 mmol/L, K 4.7 mmol/L, Ca 2.37 mmol/L, Cl 105 mmol/L, HCO_3 23 mmol/L, Mg 1.0 mmol/L, P 1.5 mmol/L, and alkaline phosphatase 224 U/L. Urinalysis showed protein 2+, no glucose, and blood 1+. Esterase and nitrite tests were negative and pH was 6. Urinary excretion of β2 microglobulin was increased (5.58 mg/L, normal <0.15). Urinary calcium excretion was persistently increased with a maximal value of 14 mg/kg per day. Parathormon was 23 pg/mL. Evaluation of tubular function revealed normal urinary acidification after oral furosemide administration (the lowest urinary pH 5.41) and moderately reduced concentration capability after overnight fasting (urine specific gravity 1.018 g/cm^3). Tubular reabsorption of phosphate was normal (87%). There was mild generalized hyperaminoaciduria. Cystine content in white blood cells was normal (0.08 μmol/mg protein; normal <0.2) and excluded cystinosis. Ophthalmologic examination was also normal without evidence for deposits or cataracts. Ultrasound scan showed normal sized kidneys, with normal echogenicity of the parenchyma without deposits. Tc-99^{m}DMSA scan showed poor renal accumulation of radionuclide 3 hours after injection, but there was increased radioactivity over the bladder. The clinical diagnosis of Dent disease was based on LMW proteinuria, hypercalciuria, intermittent microscopic hematuria, and abnormal Tc-99^{m}DMSA scan. Since inactivating mutations of the chloride channel gene (*CLCN5*) were found in a majority of patients with Dent disease, the coding sequences and the promoter regions of the *CLCN5* gene was analyzed, but mutation was not detected.[8] Along with other 13 patients who presented phenotypically with Dent disease but lacked *CLCN5* mutations, candidate genes were extensively investigated and unexpectedly in five of them mutations in the *OCRL1* gene were detected.[3] In our patient, mutation was located in the 5th exon [4-base deletion; del259-262 (TGTT)]. This gene encodes synthesis of the enzyme PIP2 5-phosphatase whose activity was markedly reduced in skin fibroblasts (0.49 nmol/min/mg; normal 4.71). Western blotting showed protein expression was absent (Fig. 2). The boy was reevaluated to detect stigmata of oculocerebrorenal syndrome (Lowe syndrome) which is associated with *OCRL1* mutations, but abnormal physical or neurological deficit was not observed. Slit-lamp examination did not demonstrate the presence of cataracts. He had been attending regular school, but intelligence testing detected mild mental retardation that had not been previously recognized (VIQ=72, MIQ=57, GIQ=62 according to Wechsler Individual Test of Intelligence for Macedonian population). Reevaluation of the patient's files found that the level of creatinine phosphokinase (CPK) was mildly increased (326 U/L, normal 30-135) as well as that of transaminases (AST 44 U/L, normal 14-36 U/L; ALT 36 U/L, normal 14-36); these findings had been ignored at the initial patient's work-up. The levels of CPK (213 U/L) and transaminases (AST 51 U/L, ALT 39 U/L) were checked again when *OCRL1* mutation was detected, but the patient did not exhibit any sign of neuromuscular dysfunction.

After establishing the diagnosis, the patient was lost to follow up and did not receive any treatment. At the age of 19, he was located and invited for standard clinical and biochemical check-up. His renal function was normal (creatinine clearance 123 ml/min per 1.73 m^2). He had significant proteinuria (3.1 g/d) and hypercalciuria (9.3 mg/kg per day). His ultrasound scan demonstrated normal sized kidneys with normal echogenicity of the parenchyma without evidence for nephrocalcinosis or nephrolithiasis.

Case report

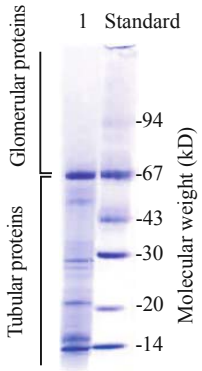

Fig. 1. Electropherogram showing a typical tubular pattern with the presence of low molecular weight proteins.

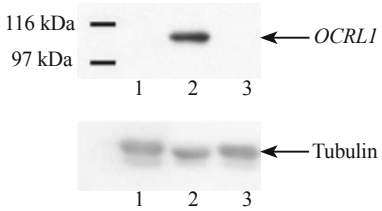

Fig. 2. Western blot analysis of *OCRL1* in control and patient's fibroblasts. Lane 1: a Lowe syndrome patient known not to express *OCRL1*; lane 2: an unaffected individual (control fibroblasts); lane 3: our patient. The positions and sizes of broad range protein markers (Bio-Rad) are shown on the left. Adequate protein loading in all lanes is demonstrated in the lower panel by western analysis of the same blot, using antibody against beta-tubulin as control.

Discussion

Oculocerebrorenal syndrome (Lowe syndrome) is an X-linked recessive multisystem disorder characterized by congenital cataracts, mental retardation and selective proximal tubulopathy with gradual progression to terminal renal failure. The disease presents a variety of neurological signs such as hypotonia, areflexia, seizures, neuroimaging abnormalities, or behavioral abnormalities.[9] A few patients may have a mild clinical phenotype,[10] but congenital cataracts is an obligatory sign, which if left untreated early in the life may lead to blindness. Female carriers may be detected by demonstration of typical lenticular opacities.[11,12] Proximal tubular dysfunction is characterized by LMW proteinuria, aminoaciduria, phosphaturia, acidosis and glucosuria. Failure to thrive, recurrent infections, fractures, joint problems and renal failure may threaten the lives of the patients and significantly influence their life expectancy.[13-15]

Our patient was completely asymptomatic when his proteinuria was detected by chance at the age of 3. He was reevaluated at the age of 10, and no abnormality was found. As LMW proteinuria, hypercalciuria and intermittent hematuria appeared he was diagnosed with Dent disease. Lack of mutation in the *CLCN5* gene was not surprising, but detection of *OCRL1* mutation was extremely unexpected and incompatible at that time with the expected phenotypic characteristics of an individual with *OCRL1* gene mutation.[16-18] Although the boy was considered to be quite normal, formal intelligence testing revealed that he had mild mental retardation that had not been recognized previously by the parents and teachers. Repeated ophthalmologic examination did not reveal the presence of cataracts or any other physical abnormality associated with Lowe syndrome. Reevaluation of data and subsequent testing showed that the boy had mildly increased values of CPK and transaminases. Elevated concentration of muscle enzymes has already been described in patients with Lowe syndrome suggesting muscle involvement.[13] Our patient did not show any signs of muscular weakness, hypotonia and neurological deficit.

Bökenkamp et al[19] summarized the clinical and biochemical data of 28 patients who had Dent phenotype associated with *OCRL1* mutations (Dent 2 disease) and compared with those having *CLCN5* mutations (Dent 1 disease) and Lowe syndrome. In general, Dent 2 patients lacked neurological deficits but a few of them had mild mental retardation. Two Dent 2 patients had mild peripheral cataracts which did not affect visual acuity. All Dent 2 patients had increased serum levels of CPK, LDH or both while only 10 of 28 Dent 1 patients had increased levels of one or both enzymes.

Shrimpton et al[7] identified 6 novel *OCRL1* mutations in 6 boys. Three patients had mild mental retardation and one had incipient cataracts on slit-lamp examination but normal vision. None of the patients had metabolic acidosis. Tosetto et al[20] studied 31 patients with Dent phenotype for *OCRL1* and *TMEM27* mutations. In 11 patients with classical features of Dent disease, 5 novel *OCRL1* mutations were detected (L88X, P161HfsX167, F270S, D506N and E720D). Two patients who had ocular involvement on slit-lamp examination were reported to have mild bilateral cortical lens opacity. Two patients had cognitive/behavioral impairement. In three patients there was mild elevation of creatinine phosphokinase levels ranging from 238 U/L to 368 U/L.

Although Dent 2 patients had significantly lower mean glomerular filtration rate compared with Dent 1 patients,[19] it is very speculative to predict the long-term renal outcome since the number of Dent 2 patients is still too small. The natural course of the disease is not known in addition to the factors which may predict the renal outcome. Interestingly, our patient, who had had significant proteinuria and hypercalciuria but had not received any treatment for 16 years, did not show any functional deterioration at occurrence of nephrolithiasis/nephrocalcinosis.

Why our patient with proven *OCRL1* mutation and lack of enzyme activity did not have cataracts and other serious multiorgan impairment as seen in patients with "classic" Lowe syndrome? In knockout mouse models, disruption of the *Ocrl1* gene and lack of its expression did not result in development of cataracts, neurological or renal dysfunction. The lethal phenotype was obtained by simultaneous deficiency of both *Ocrl1* and another phosphatase gene that is highly homologous to *Ocrl1* (*Inpp5b*). This indicates that *Inpp5b* phosphatase can compensate for *Ocrl1* deficiency.[21] If this compensation works in humans too and if there is variability among tissues and individuals in the expression of the compensating enzyme, one could explain the mild phenotypic expression, such as the absence of cataracts and the normal urinary acidifying ability, as in our patient with *OCRL1* mutation.

In conclusion, children with Dent phenotype who lack *CLCN5* mutation should be tested for *OCRL1* mutation. *OCRL1* mutations may present with mild clinical features and are not necessarily associated with congenital cataracts.

Acknowledgements

We would like to express our gratitude to Prof. S. Scheinman and Dr. R. Hoopes for mutational analysis and suggestions on manuscript preparation.

Funding: None.
Ethical approval: Not needed.
Competing interest: None declared.
Contributors: Lozanovski VJ contributed to data collection, biochemical analysis, interpretation of the results, and writing of the paper. Ristoska-Bojkovska N contributed to data collection and analysis and writing of the paper. Korneti P contributed to biochemical analysis, interpretation of the results, and writing of the paper. Gucev Z contributed to data collection, interpretation and analysis, and writing of the paper. Tasic V contributed to data collection and analysis, and writing of the paper.

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Received August 17, 2009
Accepted after revision January 11, 2010