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A Novel *OCRL1* Mutation in a Patient with the Mild Phenotype of Lowe Syndrome

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Oculocerebrorenal syndrome of Lowe (OCRL, OMIM 309000), also known as Lowe syndrome, is a rare X-linked multisystem disorder characterized by congenital cataracts, mental retardation, and Fanconi syndrome of the kidney proximal tubules. Lowe syndrome is caused by mutations in the gene encoding a member of the inositol polyphosphate-5-phosphatase protein family (OCRL1) on chromosome Xq26.1. *OCRL1* contains 24 exons and encodes a 105-kDa phosphatidylinositol (4,5) bisphosphate 5-phosphatase. An OCRL1 isoform generated by alternative splicing is predominantly expressed in brain, and localizes to the trans-Golgi network, lysosomes, and endosomes. Impaired inositol polyphosphate-5-phosphatase activity elevates phosphatidylinositol (4,5) bisphosphate levels that are required for vesicle trafficking within the Golgi apparatus, actin cytoskeleton remodeling closely associated with Golgi, and endosomal membrane trafficking. Accordingly, abnormalities in the actin cytoskeleton may influence the function of renal epithelial cells in patients with Lowe syndrome. *OCRL1* mutations exist in about 95% of patients with Lowe syndrome, and new mutations occur in 32% affected males. We here describe a Japanese male with the mild phenotype of Lowe syndrome. Physical examination revealed mild congenital bilateral cataracts, mild mental disability, and short stature. Proteinuria was also mild with a high β2-microglobulinuria level. Nucleotide sequence analysis identified a hemizygous mutation (T-to-C transition) at nucleotide 2039 in exon 18 that substitutes Ser (TCT) for Phe (TTT) at amino acid position 680. This missense mutation is located outside the known catalytic domain that is encoded by exons 4 through 15. The present patient carries a novel *OCRL1* mutation that is helpful for genetic counseling.

Keywords: clinical phenotype; Dent-2; Lowe syndrome; novel mutation; Oculocerebrorenal syndrome of Lowe
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Introduction

Oculocerebrorenal syndrome of Lowe (OCRL, OMIM 309000), also known as Lowe syndrome, is a rare X-linked multisystem disorder characterized by congenital cataracts, muscular hypotonia, mental retardation, maladaptive behavior, renal tubular dysfunction, vitamin-D-resistant rickets, and scoliosis (Lowe et al. 1952). Fanconi syndrome of the renal tubule and intellectual impairment are common. Patients suffer from progressive kidney wasting and end-stage renal disease, and the severity of clinical symptoms and the age of onset vary (Charnas and Nussbaum 1995).

Lowe syndrome is caused by mutations in the gene that encodes OCRL1, a member of the inositol-5-phosphatase protein family. *OCRL1* maps to chromosome Xq26.1 (Attree et al. 1992) and contains 24 exons with exon 1 being a non-coding exon. The coding region includes exons 2-23. An alternately spliced transcript containing exon 18a is expressed in neurological tissue (Nussbaum et al. 1997). *OCRL1* encodes a 105-kDa phosphatidylinositol (4,5) bisphosphate [PI(4,5)P2] 5-phosphatase, which is lost or expressed at decreased levels in affected males (Zhang et al. 1995; Suchy et al. 1995). OCRL1 comprises a central 5-phosphtase domain with substrate preference for PI(4,5) P2 and PI(3,4,5)P3 (Schmid et al. 2004; Astle et al. 2006). Phosphoinositides are phosphorylated metabolites of phosphatidylinositol and play key roles in cell physiology, including signaling, cytoskeletal regulation, and membrane trafficking (Di Paolo and De Camilli 2006).

Dent disease is an X-linked proximal renal tubulopathy, characterized by low molecular weight proteinuria (LMWP), hypercalciuria, and progressive renal insufficiency (Dent-1, OMIM 300009) associated with mutations in *CLCN5* that encodes a chloride channel (CLC-5). CLC-5 localizes primarily to the subapical endosomes of epithelial kidney cells, contributes to the acidification of intraendosomal compartments, and participates in membrane recycling in the proximal tubule (Piwon et al. 2000). Although

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Dent disease caused by *OCRL1* mutations is classified as Dent disease 2 (Dent-2, OMIM 300555), Dent-1 disease affects only the kidney. The spectrum of symptoms of Dent-2 disease ranges from apparently exclusive kidney manifestations to involvement of other organs, notably brain and muscle, which overlap with those of Lowe syndrome. The distinction between the two diagnoses rests mainly on eye abnormalities such as congenital cataract and glaucoma; however, mild peripheral cataracts are present in some patients with Dent-2 disease. There are individual differences in clinical phenotypes, ranging from severe Lowe syndrome with typical ocular, neurological, and renal characteristics, to Dent-2 which is characterized by renal impairment and embrace atypical forms of Lowe syndrome presenting with incomplete eye symptoms such as peripheral cataract or moderate neurological problems (Bökenkamp et al. 2009).

OCRL1 mutations are detected in approximately 95% of patients with Lowe syndrome. Most mutations are deletions, frame-shifts and translation terminators. Missense mutations and splice-site mutations are less frequent. New mutations are present in 32% of males with Lowe syndrome (Lewis et al. 2012). We here identified a novel *OCRL1* missense mutation in exon 18. Identification of mutations associated with Lowe syndrome in Japanese patients will be useful for clinical diagnosis and genetic counseling.

Case Report

The Japanese male was aged 18 years and was born after 40 weeks of gestation following an uncomplicated pregnancy and vaginal delivery. At birth, he weighed 3,400 g and was 50 cm long. No history of fetal or neonatal asphyxia was recorded. The patient lifted his head at 3 months, sat at 7 months, walked unassisted at 13 months, and his physical activity was normal thereafter. No family history of renal or eye disease was documented. Signs of proteinuria were first noticed at 3 years of age, and at age 5 years, he was admitted for an examination for renal disease. Physical examination revealed mild congenital bilateral cataracts, mild mental disability, and short stature (−2.0 S.D.). A mild degree of proteinuria (0.3 g/day) and a high β2-microglobulinuria level (55,448 μg/day) were noted at admission. Renal size and shape were normal according to the findings of an ultrasound examination. Histological examination of a renal biopsy revealed mild mesangial proliferative glomerulonephritis, mild focal mononuclear cell infiltration, and interstitial focal fibrosis. Tubular injury, including atrophy of tubular cells, and necrosis were not detected. Creatinine clearance was normal (120.5 ml/min/1.73 m^2) with no apparent abnormal findings upon examination of his blood, including electrolytes, serum creatinine, and blood urea nitrogen. Aspartate aminotransferase and alanine aminotransferase activities were also within their normal ranges. Lactate dehydrogenase activity was mildly elevated (405 IU/l), and creatinine kinase activity was normal. Metabolic acidosis was not detected. No treatment was required. On follow-up, his IQ was 82 and he attends a school for children with special needs.

Materials and Methods

Genomic DNA (289 ng/μl) was prepared from peripheral blood leukocytes and used as template for polymerase chain reaction (PCR) amplification of *OCRL1* exons 5-22 (GeneBank locus NM_000276.3). The sample was obtained after receiving initial informed consent for the clinical diagnosis of OCRL. The nucleotide sequences of the amplicons were determined using dye terminator methods. Single nucleotide polymorphisms were detected using Phred/Phrap/PolyPhred software. The Ethics Committee of the Kinki University School of Medicine approved this study.

Results

We determined the nucleotide sequence of the entire coding-region of *OCRL1* in the patient using primers described. We thus detected a T-to-C transition at nucleotide 2039 (2039T>C) in exon 18, which is predicted to substitute a phenylalanine residue for a serine residue at codon 680 (Ser680Phe). Details of the strategy for amplification and sequencing are summarized in Fig. 1. Unfortunately, we were unable to obtain the genetic history of the patient's family.

Discussion

Reduced activity or absence of inositol polyphosphate-5-phosphatase (INPP5B) leads to elevated intracellular levels of its substrate, PI(4,5)P2. The loss of INPP5B causes a defect in intracellular protein trafficking and actin dynamics. Because actin cytoskeleton remodeling is closely connected to both Golgi and endosomal membrane trafficking, abnormalities in the actin cytoskeleton may act on a number of cellular processes in the renal epithelium of patients with

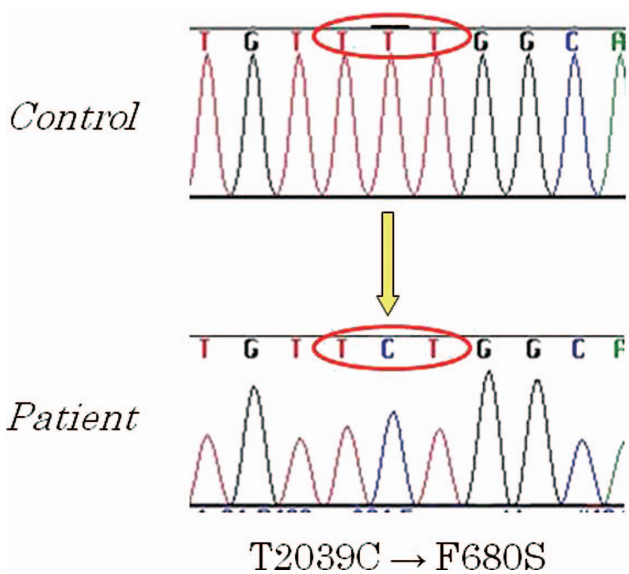

Fig. 1. Nucleotide sequence analysis of *OCRL1*.
The T-to-C transition at nucleotide 2039 converts TTT (Phe) to TCT (Ser) in exon 18.

Lowe syndrome. Although *OCRL1* is widely expressed in different tissues, defects occur in only a few specific tissues such as the eyes, kidney, and central nervous system. Disease may be asymptomatic or exhibit unusual clinical features (Olivos-Glander et al. 1995), and congenital bilateral cataracts are present at birth in all patients. There is no evidence for or apparent relationship among tissue dysfunction, clinical phenotype, and abnormality in *OCRL1* expression. Further, there are no published studies that establish the relationship among the dysfunction of a specific organ, *OCRL1* mutation, and levels of PI(4,5)P2 5-phosphatase activity.

We here describe, for the first time to our knowledge, a patient harboring an *OCRL1* mutation in exon 18. Based on the known phenotypic heterogeneity of OCRL patients, we believe that understanding the correlation between distinct mutations and different clinical phenotypes is desirable for genetic counseling and diagnosis.

Renal tubular acidosis is a prominent feature of Lowe syndrome, usually requiring alkali therapy to ameliorate growth retardation, although phenotypic variability of renal and neurological phenotypes is a well-known feature. The spectrum of proximal tubulopathy is not associated with specific *OCRL1* mutations. Hichri et al. (2011) reported three patients with Lowe syndrome harboring mutations in exon 18, including a nonsense mutation; however, these patients did not exhibit Fanconi syndrome and renal failure. Similarly, the present patient's disease was not complicated by these symptoms and may represent a type of kidney complication caused by the expression of OCRL1 with a substitution at amino acid residue 680 that resides outside the enzyme's main functional domain. Although some patients harbor mutations in adjacent codons of exon 18, detailed clinical examinations were not performed and prevented us from comparing their phenotypes with the phenotype of our patient.

OCRL1 mutations occur in approximately 25% of families with Dent disease without *CLCN5* mutations. A recent study showed that *OCRL1* mutations lead to a Dent-like phenotype, Dent-2 disease, which manifests mild developmental delays and visually insignificant peripheral cataracts (Hoopes et al. 2005: Bökenkamp et al. 2009). Kenworthy et al. (1993) reported that 47 males with Lowe syndrome had a mean IQ of 45-54, characteristic of moderate mental impairment, and that the IQs of 25% were within the normal range (>70). Further, >80% exhibited maladaptive behavior, particularly stubbornness, temper tantrums, and other stereotypic behaviors. Three patients, who exhibited remarkable differences in phenotypes with respect to neurological functions, harbored two missense mutations in exon 15 and one splice-site mutation in exon 22 in *OCRL1* (Kawano et al. 1998). Among three other patients with *OCRL1* nonsense mutations, two suffered from severe mental retardation and one exhibited a significant developmental delay but was able to work at a sheltered workshop (Leahey et al. 1993).

There are few published reports on the correlation between clinical severity of neurological findings and *OCRL1* mutations. Most patients show behavioral disturbances, irritability, outbursts of anger, and stereotypic behavior (Kenworthy et al. 1993). Therefore, the severity of the developmental disorder may remain mild in patients with the missense mutations. However, the association between IQ in the present patient and the phenotype associated with exon 18 is unknown, because this information does not exist for other patients with exon 18 mutations.

Most *OCRL1* mutations in patients with Dent-2 disease occur in exons 4 through 15, and all missense mutations are located within the phosphatidylinositol 5-phosphate domain that primarily affects OCRL1 function in the kidney (Hoopes et al. 2005). Shrimpton et al. (2009) propose that an OCRL1 isoform including exons 8 through at least 15 is normally expressed and is functional in the brain and eye tissues of patients with Dent-2 disease. Recently, mutations in exons 19 and 21 near the C terminus were identified in patients with Dent-2 disease that overlaps with the extrarenal phenotype of Lowe syndrome.

Dent-2 disease is distinguished from classical Lowe syndrome according to the presence or absence of clinically evident extrarenal symptoms such as congenital cataracts, severe mental retardation, or characteristic behavioral abnormalities (Kenworthy and Charnas 1995). Our present patient had peripheral but not dense, cataracts. He did not exhibit renal tubular acidosis, hypophosphatemia, mental retardation and motor neuron disability, which are considered classical symptoms of Lowe syndrome. The absence of cataracts does not exclude the possibility of *OCRL1* mutations (Pasternack et al. 2013). Furthermore, the existence of cataracts does not exclude Dent disease as previously reported for patients with cataracts diagnosed with Dent-2 disease.

Recently, a mouse model for Lowe syndrome and Dent-2 disease tubulopathy was established by expressing human *INPP5B* in *Ocrl*$^{-/-}$ mice (Bothwell et al. 2010), which exhibited decreased postnatal growth, LMWP, and aminoaciduria, suggesting that the human *INPP5B* genotype may influence the clinical manifestations of Lowe syndrome/Dent-2 disease. To expand the range of clinical phenotype, even the existence of cataracts, seems to be a diagnostic opportunity to reconsider as patients with Dent-2 disease who have been diagnosed initially with Lowe syndrome (Shrimptom et al. 2009). Moreover, the correlation of genotype with phenotype is not established. Further research may define the correlation between genotypes and clinical phenotypes.

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Conflict of Interest

The authors declare no conflict of interest.

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