Novel *OCRL* mutations in Chinese children with Lowe syndrome

Yan-Qin Zhang, Fang Wang, Jie Ding, Hui Yan, Yan-Ling Yang
Beijing, China

Background: **Lowe syndrome is a rare X-linked recessive hereditary disease caused by mutations of the *OCRL* gene, which encodes an inositol polyphosphate-5-phosphatase. The disease is clinically characterized by congenital cataracts, psychomotor retardation, and proximal tubulopathy.**

Methods: **We retrospectively reviewed three unrelated Chinese patients with Lowe syndrome, clinically diagnosed by the abnormalities of eyes, nervous system, and kidneys. Genetic analysis of the *OCRL* gene was done for the three patients as well as their family members.**

Results: **Three *OCRL* gene mutations were detected in our study. Two of the mutations, g.1897delT in exon 18 (patient 1) and g.1470delG in exon 15 (patient 2), were novel. A missense mutation (p.Y513C) in exon 15, which had been reported previously, was found in patient 3. The mothers of all patients were heterozygous carriers of the respective mutations.**

Conclusions: **Three Chinese children were diagnosed with Lowe syndrome through clinical and genetic analyses. And two novel mutations in the *OCRL* gene were identified.**

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Author Affiliations: Department of Pediatrics, Peking University First Hospital, Beijing, China (Zhang YQ, Wang F, Ding J, Yan H, Yang YL)

Corresponding Author: Ding J, MD, PhD, Department of Pediatrics, Peking University First Hospital, 100034 Beijing, China (Tel: 86-010-83572168; Fax: 86-010-66530532; Email: djnc_5855@126.com)

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Introduction

Lowe syndrome (oculo-cerebro-renal syndrome) is a rare X-linked genetic disease seen in approximately 1/200 000-1/500 000 births.[1] The clinical characteristics of Lowe syndrome were eye anomalies (congenital cataracts, infantile glaucoma) resulting in impaired vision, neurological deficits (infantile hypotonia with subsequent mental impairment), and renal tubular dysfunction of the Fanconi type.[2] The hallmark of the disease is the presence of prenatally developed cataracts. There are other frequently noted findings in Lowe syndrome, including absent deep tendon reflexes, maladaptive behaviors, hypercalciuria with nephrocalcinosis and nephrolithiasis, pathologic fractures and bone demineralization.[3,4]

The *OCRL* gene located on chromosome Xq25-q26 was identified responsible for Lowe syndrome, which encodes a phosphatidylinositol 4, 5-bisphosphate-5-phosphatase.[5,6] The protein is deficient in the fibroblasts of patients with Lowe syndrome.[6] Approximately 200 *OCRL* mutations have been identified so far, and 90% of them are located in 2 hot spots (exons 10-18 and 19-23) in the *OCRL* gene. However, correlation of the genotype with phenotype of Lowe syndrome has not been established.

We describe here three unrelated Chinese children with Lowe syndrome and report detection of two novel mutations in the *OCRL* gene.

Methods

Patients

We retrospectively reviewed three unrelated Chinese patients diagnosed with Lowe syndrome in our hospital in 2011. Lowe syndrome was diagnosed according to clinical manifestations including congenital cataracts, neurological deficits (infantile hypotonia and mental retardation) and kidney anomalies.[2,4,7] The Ethical Committee of Peking University First Hospital approved the project, and informed consents were obtained from the parents.

Mutation analysis

Blood samples were obtained from the three unrelated

Chinese patients and their mothers; genomic DNA was isolated using the BloodZol kit (TransGen Biotech, China). The 24 coding exons of *OCRL* and their flanking intronic sequences were amplified with primers designed using Primer3 (http://frodo.wi.mit.edu/primer3/). The sequence of the primers used for amplification and sequencing of the *OCRL* exons are shown in Table 1. PCR reactions were performed in a volume of 25 μL consisting of 12.5 μL 2×Taq PCR Master Mix (Tiangen Biotech, China), 1 μL 5 μmol/L forward primer and reverse primer, respectively, and 1 μL 100 ng/μL genomic DNA. The amplification of exon 1, 2 was carried out with the condition of 94°C for 5 minutes, followed by 38 cycles of 94°C for 30 seconds, an annealing step of 65°C for 45 seconds, and 72°C for 45 seconds, then 72°C for 10 minutes. And the other exons were amplified using touchdown PCR under the condition of annealing temperature from 64°C to 58°C, descending 1°C every 2 cycles, annealing at 58°C for 26 cycles. Direct sequencing was performed with an ABI 3730XL automatic sequencer using standard methods (SinoGenoMax, China).

Results

Clinical manifestations

All the three patients had bilateral congenital cataracts, and were diagnosed at the age of 9 months (patient 1) and 5 months (patients 2, 3) after birth, respectively. Both patients 2 and 3 had received surgical treatment of cataracts at 5 months of age. Their clinical manifestations are summarized in Table 2. The three

Table 1. Primer sequences used to analyze the exons of the *OCRL* gene

Exons	Forward primers	Reverse primers	Product size (bp)
1	GGAGCTGTTCCTCAAACGAC	CCTCCCCTCTCCCTTCTCT	183
2	CCGAAGGAGACCCTTGACTA	ACCTGGACCTGAACCTGTTG	233
3	CATGATGCTCAGATCCAGG	TTCGATATAACCCTTCAGCATTC	401
4	TTGCTCATAGTCTTTAGAACCCAG	CAGTGCTAATTTGTATGGCTGC	384
5-6	CCTTAGGGTTCCCTCTCTGC	GGGCCTGGACTTGATAAAAC	965
7-8	GCTTCTTCGAACTCCAATCC	GGCCAGCATAGAGACAGGAG	678
9-10	ATAAGCCCACTGCCTTTGTG	ATCAATCTGCCCTATCCCAG	1066
11-12	TACTCATTGTCCCTCCCAGC	CCCAATCCCTCACTGGTAATC	751
13	CCCCTTGTGAGATAGTGGTG	TTATCTTCCCTCCTCCCATC	432
14	TAGATGCTGTGGGAATGCTG	TGAGGCACTGAGCCATTAGG	431
15	ACAGGGGTTGTTAGGGAAGG	ATACGGACAGCATTTGGGAG	450
16	GGATGTTGTTTGCACCACAG	GCTACAACTGGAATGGAGGC	377
17-18	GTCTATGGCATTTGCACACC	TCACAACAAGAGCCAACTGC	985
18a	CTTTTCCTGCTCCGCTCTC	GGCAGGACTTTAGGGGAGTC	179
19	TCCCCAGTGAGAAGAAATGG	GTGCCACTGTGTGCAGGTAG	378
20	TGTTATTTTCACACCTCCATTTG	GGAAAGAGGTCAAAAGTTCCTG	410
21	CCATTAGGATCAATGTGCCC	CTCAATGTTGGTGTCCTGCC	399
22-23	ACAGATGAAATGGGTCCTGC	TATGGTAACTTTGGCTTGGC	759

Table 2. Clinical findings and laboratory data of three patients with Lowe syndrome

Variables	Patient 1	Patient 2	Patient 3	Normal range
Gender	Male	Male	Male	
Age at diagnosis (y)	0.8	2.2	4.5	
Clinical manifestations				
Congenital cataract	Yes	Yes	Yes	
Psychomotor retardation	Yes	Yes	Yes	
Rickets	Yes	Yes	Yes	
Urinalysis				
Proteinuria qualitative detection	+++	+	++-+++	
24 h urine protein (g/24 h)	ND	0.99	0.70-0.88	<0.15
Urine total protein-to-creatinine ratio (mg/mg)	7.7	ND	ND	<0.2
Percentage of LMWP (<67 kDa), %	41.0	48.9	40.0-50.6	
24 h calciuria (mmol/24h/kg)	ND	0.138	0.137	<0.1
Calcium/creatinine ratio (g/g)	ND	0.6	0.1-0.7	<0.2
Blood biochemistry				
Serum creatinine (umol/L)	28	26	32	44-133
Blood urea nitrogen (mmol/L)	1.3	3.0	4.6	1.8-7.1
Creatinine kinase (IU/L)	317	ND	173	25-195
Lactate dehydrogenase (IU/L)	394	ND	346	50-240
Aspartate aminotransferase (IU/L)	103	135	75-93	0-45

LMWP: low-molecular-weight protein; ND: not determined.

patients had hypotonia and severe motor and language development delay. Patient 1 could not hold his head when he was 9 months of age. Patient 2 achieved unsupportive sitting at 1 year of age and independent standing at 2 years of age. Patient 3 could not walk at 4.5 years of age. Patient 1 had feeding difficulties in infancy. All the patients had rickets, and low molecular-weight (LMW) proteinuria was detected in all three patients; patients 2 and 3 had hypercalciuria, and calciuria of patient 1 was not measured. Furthermore, urinary system ultrasonography revealed tiny hyperechogenic foci in the kidneys of the three patients. Elevated lactate dehydrogenase (LDH) was detected in both patients 1 and 3. Elevated creatinine kinase (CK) was detected in patient 1. Besides, all three patients had high aspartate aminotransferase (AST).

Mutation analysis of the *OCRL* gene

The mutations of the *OCRL* gene were analyzed in order to confirm the clinical diagnosis of Lowe syndrome. The results of this analysis are summarized in Table 3. Patient 1 had a novel 1-bp deletion mutation (g.1897delT) in exon 18 of the *OCRL* gene (Fig). Patient 2 had a novel 1-bp deletion mutation (g.1470delG) in exon 15 of the *OCRL* gene (Fig). Both of the two mutations were novel, and were predicted to result in premature terminations. Patient 3 had a missense mutation (p.Y513C) (Fig), which had been reported previously.[8] The mothers of the three patients were all heterozyous carriers of the respective mutations.

Discussion

Lowe syndrome is an X-linked multisystemic disease characterized by severe phenotype including bilateral congenital cataract, mental retardation, and proximal tubulopathy. Anomalies of eyes are the hallmark of Lowe syndrome, since dense congenital cataracts could be found in all affected boys and infantile glaucoma in approximately 50%.[4] In this study, the age at diagnosis of three patients was nine months, 2.2 and 4.5 years, respectively. The three patients had congenital cataracts resulting in impaired vision, and infantile hypotonia with subsequent psychomotor retardation, low molecular-weight proteinuria and nephrocalcinosis. All patients had rickets, and none of them had pathological fractures, which are one of the frequently noted findings of Lowe syndrome. Maia and colleagues[9] reported five patients with Lowe syndrome, and their mean age at first consultation was 76.5 months, about 6.4 years. And two of the five patients had a history of pathological fractures. Ke et al[10] reported three patients from two Chinese families with Lowe syndrome, and the

Table 3. *OCRL* mutations identified in this study

Patients	Exons	Mutation type	Nucleotide change*	Predicted effect on translation	Reference
1	18	1-bp deletion	g.1897delT	p.Ser633fs	Novel
2	15	1-bp deletion	g.1470delG	p. Gly490fs	Novel
3	15	Missense	g.1538A>G	p.Y513C	8

*: according to GenBank entry uc004eur.2.

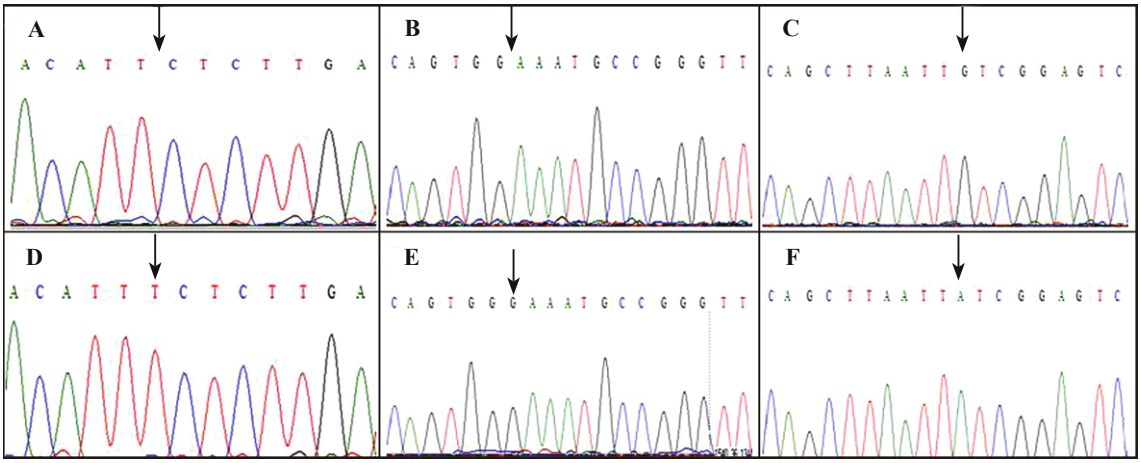

Fig. Genetic analysis of *OCRL* in patients and controls. **A:** arrow indicates the site of a novel 1-bp deletion mutation (g.1897delT, exon 18) in patient 1; **B:** arrow indicates the site of a novel 1-bp deletion mutation (g.1470delG, exon 15) in patient 2; **C:** arrow indicates the site of missense mutation (g.1538A>G, exon 15) in patient 3; **D-F:** the normal sequences in controls corresponding to the mutation sites of patients 1-3, respectively.

diagnostic age was 9, 26 and 32 years, respectively; all the patients had multiple fractures. Thus, pathological fractures should be considered during the follow-up of patients with Lowe syndrome.

Lowe syndrome is caused by mutations in the *OCRL* gene, which locates on chromosome Xq25-q26 and encodes a phosphatidylinositol 4, 5-bisphosphate-5-phosphatase. The protein is localized predominantly to the Golgi complex. It is hypothesized that deficiency of this enzyme results in dysregulation of Golgi vesicular transport, causing cataracts and abnormal neurological and renal function.[6] There are two major conserved domains in the *OCRL* protein: a phosphoinositide 5-phosphatase domain and a C-terminal RhoGAP domain.[11] Faucherre and colleagues[12] investigated the RhoGAP domain of *OCRL*, and found that loss of *OCRL* may contribute to mental retardation in patients with Lowe syndrome. Besides its PIP2 5-phosphatase activity, *OCRL* possesses the ability to bind to Rac GTPase, which is involved in neuronal morphogenesis and development of the central nervous system.[13,14] Coon et al[15] found that the cells of patients with Lowe syndrome have defects in the assembly of primary cilia, and this phenotype was reproduced in the cell lines by knock-down of the *OCRL* gene. The above results showed that there may be a potential mechanism for phenotypic characteristics of Lowe syndrome. Patients with Lowe syndrome have renal tubulopathy as seen in several ciliopathies, but do not develop renal cysts, which remains to be explained. This study provided new avenues for further investigation of pathomechanisms and therapeutic strategies in patients with Lowe syndrome.

Approximately 200 *OCRL* mutations have been identified so far. In the present study, we identified two novel mutations in the *OCRL* gene (g.1897delT in exon 18 and g.1470delG in exon 15), which were predicted to truncate the *OCRL* protein prematurely during translation, and a missense mutation (p.Y513C) in exon 15 reported previously. There were no special clinical characteristics of the two patients with novel mutations, and there was no apparent phenotypic difference between the patients with truncating mutation and missense mutation in our study.

Hoopes and colleagues[16] detected *OCRL* gene mutations in 13 male patients, who met strict criteria for Dent disease but lacked mutations in CLCN5. They found *OCRL* mutations in 5 patients, and none of the five patients had eye anomalies, which exist in typical Lowe syndrome. In addition, three of the five patients had mild mental retardation. Dent-2 disease was first reported by Bökenkamp et al.[17] They found that patients with Dent-2 disease showed an intermediate phenotype, including mild peripheral cataract and some degree of mental retardation. Why mutations of the *OCRL* gene are associated with both Lowe syndrome and Dent-2 disease? Hichri et al[18] reported that the specific mapping of the frameshift and nonsense mutations in exons 1-7 were identified for Dent disease, while the mutations in exons 8-23 were identified for Lowe syndrome. Therefore, the potential mechanism for different clinical expression is alternative initiation codons in the *OCRL* gene. Recently, Lozanovski and colleagues[19] reported a boy with Dent disease, with mild mental retardation, but without cataracts, had a 4-base deletion mutation (del259-262 TGTT) in exon 5 of the *OCRL* gene. In our study, the three patients had typical clinical features of Lowe syndrome and mutations in exon 15 and exon 18.

In conclusion, clinical and genetic analysis is valuable for diagnosis of Lowe syndrome as well as for genetic counseling to avoid reoccurrence within the same family.

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Competing interest: The Ethical Committee of Peking University First Hospital approved the project, and informed consent was obtained from the patients and their family members.

Contributors: Zhang YQ and Wang F contributed equally to this work. Ding J, Wang F, and Zhang YQ designed the study. All authors collected and analyzed the data, wrote the paper and approved the final version of the paper.

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