Original article

Novel *OCRL1* gene mutations in six Chinese families with Lowe syndrome

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Background: **Lowe syndrome, an X-linked, inheritable disease with clinical symptoms of congenital cataracts, incomplete Fanconi syndrome, and mental retardation, has an approximate incidence of 1 in 500 000. Nearly 200 *OCRL* mutations related to Lowe syndrome have been found worldwide, with only ten mutations among the Chinese population. Since more mutations may exist in Chinese patients, we sequenced and analyzed the *OCRL* genes of six children with Lowe syndrome in a medical center in China.**

Methods: **Peripheral blood was collected from six children with Lowe syndrome and their relatives, and ten healthy adults. Genomic DNA was extracted from the blood and applied to amplify the twenty-four exons and flanking introns of the *OCRL* gene. The mutations were identified by sequencing.**

Results: **Five mutations (c.1528C>T, c.2187insG, c.1366C>T, c.1499G>A, and c.2581G>A) of the *OCRL* gene were found in five families; c.2187insG and c.1366C>T were novel mutations. None of the five mutations were detected in 20 normal chromosomes. No mutation was found in the sixth family.**

Conclusion: **Two novel mutations of the *OCRL* gene, c.2187insG and c.1366C>T, were found in Chinese patients with Lowe syndrome, which will provide new clues for the etiology of Lowe syndrome and could be beneficial to genetic diagnosis of the condition.**

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Key words: **Lowe syndrome; mutation; *OCRL* gene**

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Introduction

Lowe syndrome, also known as oculocerebrorenal syndrome of Lowe (OCRL), is an X-linked, recessive, and inheritable disease with clinical symptoms of congenital cataracts, incomplete Fanconi syndrome, and mental retardation. The disease is very rare with an approximate incidence of 1 in 500 000.[1] The causative gene *OCRL* contains 5152 base pairs (bp) of nucleotides. It is located in chromosome Xq26, and contains 24 exons which encode a 105-KD protein, inositolpolyphosphate-5 phosphatase.[2-4] Failure to detect *OCRL* gene mRNA or its phosphatase activity in patients with Lowe syndrome suggests that loss of *OCRL* function causes Lowe syndrome.[5-7] A 24-bp fragment of exon 18a encodes eight amino acids, and is mainly expressed in the nervous system.[2,8] Nearly 200 *OCRL* mutations of Lowe syndrome have been reported worldwide,[5,8,9] but among the Chinese population only ten mutations have been documented. The following *OCRL* gene mutations have been identified in the Chinese population: c.1528C>T is a nonsense mutation in the exon 15,[10] c.880G>T is a nonsense mutation in the exon 10,[11] c.2626dupA is in the exon 24,[11] and c.2032C>T is a nonsense mutation in the exon 18.[12] c.2367insA (p. Ala813X) was seen in exon 22,[13] a missense mutation (1736 A→G) in exon 15,[14] a c.1499G>A (p.R500Q) mutation in exon 15,[15] g.1897delT in exon 18 (patient 1), g.1470delG in exon 15 (patient 2), and a missense mutation (p.Y513C) in exon 15.[16] In recent years, six patients have been diagnosed with symptoms of Lowe syndrome in our hospital. But whether these patients can be diagnosed with *OCRL* gene mutations or which *OCRL* gene mutation(s) might exist in these patients remains obscure. Given the large Chinese population, we postulated that more mutations might exist in the six patients with Lowe syndrome. Therefore, we sequenced and analyzed the *OCRL* genes of the six patients. Besides the four reported mutations, we identified two novel mutations among five families with Lowe syndrome, which have not been reported worldwide so far. We have expanded our knowledge of *OCRL* gene mutations and provided further clues to genetic diagnosis of Lowe syndrome.

Methods

OCRL gene amplification

The study was approved by the Ethics Committee of our medical center and all parents/guardians of the patients were informed of the study.

Genomic DNA was extracted from 3 mL of peripheral blood using Blood DNA miniprep kit (BIOMIGA, San Diego, CA, USA). Primers for exons 1 and 2 were designed using Primer 3 software according to the sequence of *OCRL* (NT_011786.16, NCBI). The primer sequences were as follows: exon 1 forward: 5'-TTCCTTGCTGGACCTGGTAG-3', exon 1 reverse: 5'-CTCTCTGCTCGGCCTCTG-3'; exon 2 forward: 5'-GGGTGGAAGACCCCCTTC-3', exon 2 reverse: 5'-GTTTGGGGTTGGGAGGTAAT-3'. The primers for the other 22 exons were selected according to a previous study.[13] The polymerase chain reaction (PCR) was performed using 2.5 μL of 10× TransTaq buffer, 2 μL of dNTP mixture (2.5 mmol/L of each), 0.3 μL (2.5 U/μL) of TransTaq (TaKaRa, Japan), 2 μL of each primer, and 50 ng of DNA template.

Sequencing and bioinformatics

The PCR products were sequenced using a sequencing analyzer 3730XL (Applied Biosystems, USA), analyzed using Chromas software (Technelysium Pty, South Brisbane QLD, Australia), and compared with the sequences in Genbank. The names of mutations were given according to the standards of Human DNA Sequence Variations Naming Suggestions.

Results

Clinical symptoms were examined in six children with Lowe syndrome

The six children with Lowe syndrome were all male. All of them exhibited congenital cataracts, psychomotor retardation, hypotonia, mental retardation, tendon reflexes, incomplete Fanconi syndrome, and renal tubular acidosis (Table).

OCRL mutations identified in five children with Lowe syndrome

OCRL mutations[5,8,14-16] were not detected in the ten healthy adults (Table). Mutation c.1528C>T at amino acid 510 glutamine (Fig. 1), found in patient 1, was a nonsense mutation that coded for a premature stop codon (p.Q510X) that was not detected in the patient's mother or brother. Mutation c.2187insG was found in

Table 1. Clinical findings and laboratory data of the six patients with Lowe syndrome

Variables	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Normal value
Age (y)	0.9	5	5	0.6	0.2	2	
Congenital cataracts	Yes	Yes	Yes	Yes	Yes	Yes	-
Growth retardation	Yes	Yes	Yes	Yes	Yes	Yes	-
Psychomotor retardation	Yes	Yes	Yes	Yes	Yes	Yes	110-147
Rickets	Yes	Yes	Yes	-	-	-	-
Proteinuria qualitative detection	++	++	+++	++	+	-	-
24 h urine protein (g/24 h)	0.86	1.66	2.09	1.13	0.69	-	-
Urinary β2-microglobulin (μg/L)	>500	>500	>500	>500	>500		<300
Amino acids in urine	A lot	No	No	No	No	No	No
Urea nitrogen (mmol/L)	2.85	3.87	3.78	2.37	1.48	3.12	2.90-8.90
Serum creatinine (μmol/L)	32	30	20	17	23	24	18-62
Sodium (mmol/L)	139	136	140	139	138	139	136-144
Potassium (mmol/L)	3.63	3.50	3.10	3.85	4.80	3.32	3.60-5.10
Chlorine (mmol/L)	106	115	106	105	105	103	96-108
Calcium (mmol/L)	2.05	2.10	2.26	2.40	2.49	1.07	2.19-2.69
Phosphorus (mmol/L)	1.03	0.75	1.05	1.72	1.83	1.32	1.29-1.94
Ammonia (μmol/L)	64.1	41.3	76.9	11.8	34.6	25.6	10.0-47.0
Lactate dehydrogenase (IU/L)	235	400	456	482	488	276	159-322
ALP (U/L)	213	1047	589	204	447	358	118-390
Blood pH	7.27	7.33	7.34	7.31	7.29	7.29	7.34-7.45
HCO_3^- (mmol/L)	17.9	17.1	16.5	20.7	25.2	20.1	18.5-24.5
BE (mmol/L)	-8.4	-7.9	-8.3	-5.2	-1.3	-6.2	-3.0-3.0
TSH (mU/L)	6.29	2.30	4.06	3.54	10.19	5.36	0.20-6.00
FT_3 (pmol/L)	11.60	7.91	8.62	5.92	2.04	3.74	2.30-6.30
Mothers of patients	No mutation	Carrier	No mutation	N/A	No mutation	-	-
Mutations	c.1528C>T	c.2187insG	c.1366C>T	c.1499G>A	c.2581G>A	-	-
In exon	Exon 15	Exon 19	Exon 14	Exon 15	Exon 22	-	-
Type of mutation	Nonsense	Insertion	Nonsense	Missense	Splicing	-	-
Change in protien	p.Q510X	p.Glu729fsX41	p.Q456X	p.R500Q	p.del exon 22	-	-
Known mutations	14	Novel	Novel	7,15,16	8,15	8	-

ALP: alkaline phosphatase; BE: base excess; TSH: thyroid stimulating hormone; FT_3: free triiodothyronine; "-": none; N/A: not applicable.

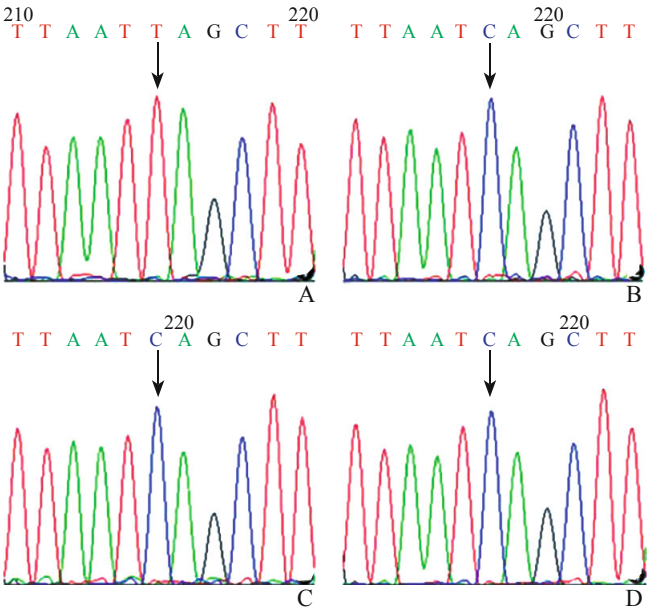

Fig. 1. Partial sequence of exon 15 of the *OCRL* gene from patient 1 (c.1528C>T) (**A**), his mother (**B**), his brother (**C**), and the control group (**D**). Arrows indicate the mutation that changed from C to T in patient 1.

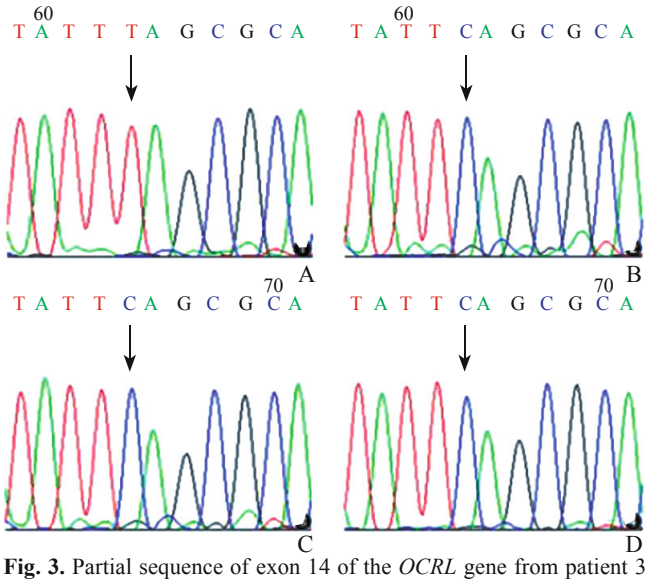

Fig. 3. Partial sequence of exon 14 of the *OCRL* gene from patient 3 (c.1366C>T) (**A**), his mother (**B**), his sister (**C**), and the control group (**D**). Arrows indicate the mutation that changed from C to T in patient 3.

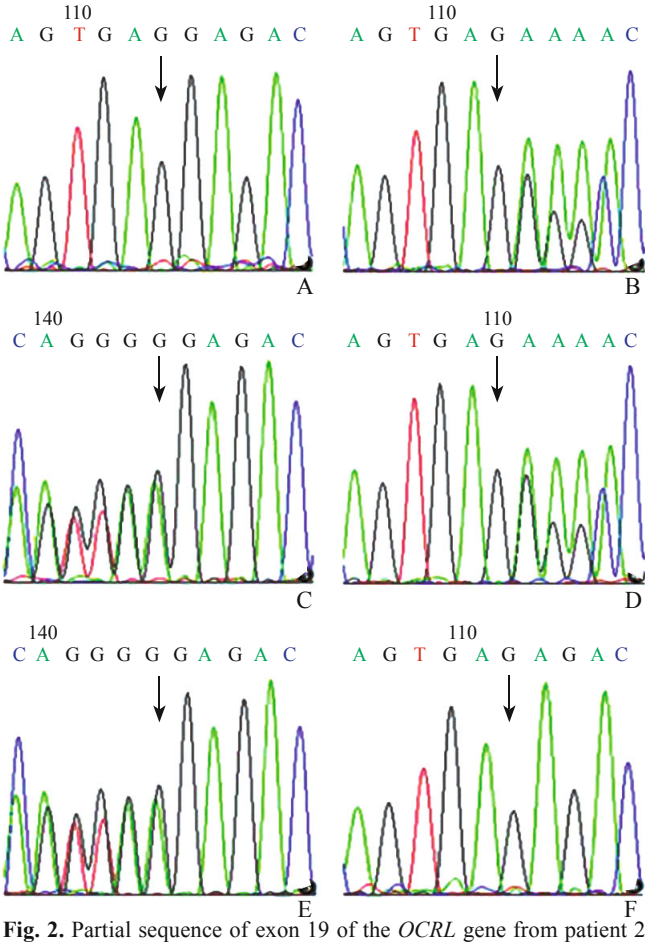

Fig. 2. Partial sequence of exon 19 of the *OCRL* gene from patient 2 (c.2187insG) (**A**), the direct sequences of his mother (**B**), the reverse sequences of his mother (**C**), the direct sequences of his grandmother (**D**), the reverse sequences of his grandmother (**E**), and the control group (**F**). Arrows indicate the mutation where G was inserted in patient 2.

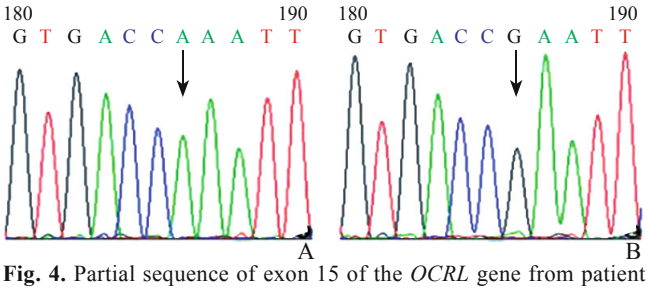

Fig. 4. Partial sequence of exon 15 of the *OCRL* gene from patient 4 (c.1499G>A) (**A**) and the control group (**B**). Arrows indicate the mutation that changed from G to A in patient 4.

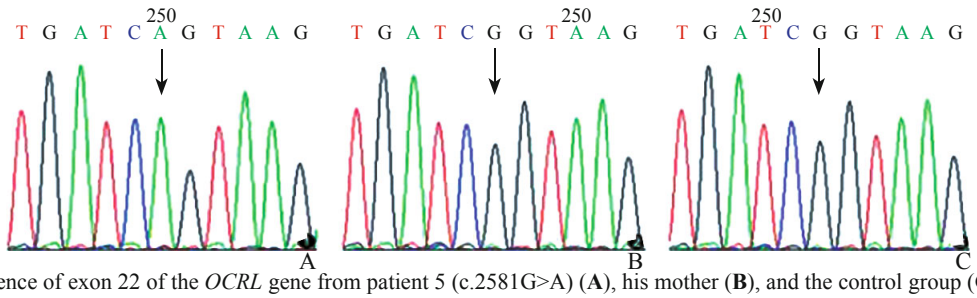

Fig. 5. Partial sequence of exon 22 of the *OCRL* gene from patient 5 (c.2581G>A) (**A**), his mother (**B**), and the control group (**C**). Arrows indicate the mutation that changed from G to A in patient 5.

patient 2 (Fig. 2); the patient's mother and grandmother were heterozygous carriers. The mutation was a homozygous insertion (p.Glu729fsX41) of one G after the first adenine (A) of nucleotide 2186, causing the termination of protein synthesis at amino acid 770 and generating a new peptide with 769 amino acids. Mutation c.1366C>T, occurring at amino acid 456 glutamine found in patient 3 (Fig. 3), was a nonsense mutation with a premature stop codon (p.Q456X), but was not detected in the patient's mother or brother. Mutation c.1499G>A found in patient 4 was a missense mutation (Fig. 4), causing the amino acid 500 arginine to become glutamine; the carrier of the mutation was unclear because the patient's mother did not agree to do the genetic test. Mutation c.2581G>A, found in patient 5, was a splicing mutation that was not detected in the patient's mother (Fig. 5). No *OCRL* gene mutation was found in patient 6, although the patient displayed clinical symptoms of Lowe syndrome.

Discussion

To determine which *OCRL* gene mutation(s) existed in the six patients with Lowe syndrome in our hospital, we sequenced and analyzed their *OCRL* genes. We identified two novel mutations within five families of patients with Lowe syndrome, c.2187insG and c.1366C>T, which have not yet been reported around the world.[5,7,9]

Among previously reported *OCRL* mutations, 93% occur in exons 10-23, particularly in exon 15,[8,9] and 76% cluster in the phosphatidyli-nositol bisphospbate 5-phosphatase region of exons 11-15.[4,5] Missense mutations reported to date domain spanning exons 9-15. Frameshift mutations or nonsense mutations are predominant in all exons but exons 2 and 3.[9]

In this study, the five mutations were found in exons 10-23, and three of them in exons 11-15, suggesting the common mutation spots in the *OCRL* gene.[5] Thus, exons 10-23 especially 11-15 should be first considered in a genetic analysis of Lowe syndrome.

OCRL gene mutations were not detected in the mothers of patient 1, 3, or 5, suggesting that the mutations from these three families might be also novel mutations that were not inherited from their mothers or their mothers were mosaic in the *OCRL* gene. In fact, 37.6% of mutations were novel mutations that were not inherited,[9] and approximately 4.5% of patients were somatic and/or germline mosaic.[8]

Three out of five mutations were nonsense mutations. That proportion was consistent with a previous study where 64% of *OCRL* mutations were nonsense mutations or frame-shift mutations that led to a premature stop codon.[7] Two mutations identified in this study were novel mutations. However, the mechanism of how these mutations cause Lowe syndrome remains to be investigated.

Although one patient in this study exhibited such symptoms as congenital cataract, growth retardation, unresponsive hypotonia, and renal tubule acidosis, which highly suggest that the patient had Lowe syndrome, and no mutation was identified in the *OCRL* gene. However, we could not exclude the presence of a mutation even though the mutation was not able to be detected by genomic sequencing. Further genomic sequencing might help to identify mutations in this patient. In addition, further phosphatidylinositol 4,5-bisphosphate phosphatase activity test also contributed to exclusion of Lowe syndrome.[4,5]

The symptoms of Lowe syndrome vary among patients, although congenital cataract is an important clue for diagnosis. Routine urine analysis, blood gas, and electrolyte analysis are generally carried out to examine metabolic acidosis, alkaline urine, low specific gravity, high phosphate in urine, proteinuria Fanconi syndrome, growth retardation, hypotonia and kidney damage. Analysis of *OCRL* mutation is necessary for final diagnosis of Lowe syndrome. In this study, the symptoms of Lowe syndrome and *OCRL* mutations were congruent. Therefore, the two novel mutations, c.2187insG and c.1366C>T, might be helpful for the diagnosis of Lowe syndrome.

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Contributors: Gao Y contributed to the concept and design. Jiang F contributed to the sequence and analysis of the genes. Jiang F and Ou ZY contributed to the analysis and interpretation of data. Jiang F drafted the article. Gao Y and Jiang F approved the final version.

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