

# Novel mutation in *OCRL* leading to a severe form of Lowe syndrome

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## Abstract

• **AIM:** To investigate the phenotype and genotype of a family with X-linked recessive Lowe syndrome.

• **METHODS:** All the members in the Chinese pedigree underwent comprehensive ophthalmologic and systemic examinations. Genomic DNA was isolated from peripheral blood of the pedigree members and 100 unrelated healthy Chinese subjects. Direct sequencing was performed to screen the exons and intron boundaries of *OCRL*.

• **RESULTS:** The ophthalmological and systemic examinations suggested that the affected individual had Lowe syndrome. The phenotype in the pedigree is severe and consistent among all the affected individuals except for an individual who additionally suffered from congenital heart disease and laryngeal cartilage dysplasia. Directional Sanger sequencing identified a complex mutation c.(2368\_2368delG; c.2370A>C) in the Rho-GTPase activating protein domain. This complex mutation causes termination of protein synthesis at amino acid 824 and result in a new peptide with 823 amino acids (p.Ala790ProfsX34). This mutation was not detected in 100 unrelated healthy Chinese subjects.

• **CONCLUSION:** Our findings expand the phenotypic and genotypic spectrum of Lowe syndrome.

• **KEYWORDS:** Lowe syndrome; oculocerebrorenal syndrome; *OCRL*; congenital membranous cataract

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## INTRODUCTION

Lowe oculocerebrorenal syndrome<sup>[1]</sup> (OMIM #309000; Lowe syndrome) is a very rare X-linked recessive pathology which is characterized by multiple disorders involving the eyes, the central nervous system, and the kidneys<sup>[2]</sup>. The prevalence of this disease ranges between 1:500 000 to 1:1 000 000<sup>[3]</sup>. The causative gene *OCRL*, located on chromosome Xq26.1, encodes an inositol polyphosphate 5-phosphatase<sup>[4-6]</sup>. There are approximately 250 mutations of *OCRL* gene reported causing Lowe syndrome according to the Human Gene Mutation Database (HGMD)<sup>[7]</sup>. The most commonly mutated category was missense and nonsense mutations (49%), followed by small deletions (20%), splicing mutations (12%), small insertions (9%), complete gene deletion, and large insertion.

Herein, we present a Lowe syndrome pedigree with a complex mutation in *OCRL* gene. We expand the phenotype and genotype of Lowe syndrome.

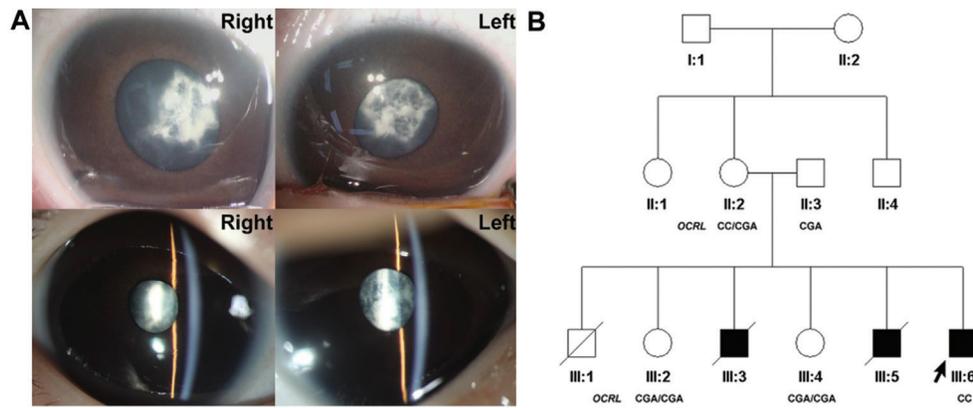
## SUBJECTS AND METHODS

**Ethical Approval** Informed written consent was obtained from each participant or legal guardian according to the tenets of the Declaration of Helsinki. The research protocol was approved by the Institutional Review Board/Ethics Committee of Sun Yat-sen University (Guangzhou, China).

A family with Lowe syndrome was recruited in the Zhongshan Ophthalmic Center, Sun Yat-sen University, Guangzhou, China. Complete disease history was taken. Comprehensive ophthalmic and systemic examinations were performed in the proband and his family members. Eight individuals from the family participated in the present study (only disease history was available for the three deceased individuals). One hundred unrelated Chinese subjects were recruited as controls. Genomic DNA was isolated from peripheral blood following manufacture's instruction (TIANGEN Biotech Co. Ltd., Beijing, China). Exons and intron boundaries of *OCRL* were screened by direct sequencing. The polymerase chain reaction (PCR) was carried out with condition as previously described<sup>[8]</sup>. The PCR products were sequenced using the BigDye Terminator Cycle sequencing kit (ABI Applied Biosystems; Sangon Co., Shanghai, China).

## RESULTS

We identified a family with X-linked congenital cataract (Figure 1A). The proband was an 8-month-old boy (III:6,



**Figure 1 Slit-lamp photographs of the proband and family pedigree** A: Slit-lamp photographs of the proband of the pedigree illustrating membranous cataracts; B: Pedigree drawing with the genotypes of *OCRL*. The proband is marked with an arrow. Square and circle indicate male and female respectively. Filled and blank symbols represent affected and unaffected individuals, respectively. Diagonal line through a symbol represent deceased individual.

Figure 1B) born to non-consanguineous Chinese parents. He was delivered after a full-term pregnancy. Muscular hypotonia, hypoxic-ischemic encephalopathy, and undescended testicle were diagnosed at birth, and congenital cataract was diagnosed at the age of 1mo. His development was markedly delayed. At the age of 8mo, he still could not raise his head, turn over and sit by himself. Apart from membranous cataract, a severe form of congenital cataract, the proband presented with small pupils which were difficult to be dilated. The largest pupil diameter was 5 mm in the right eye and 3.5 mm in the left eye after mydriasis using compound tropicamide. Abnormality in urine protein test (urine protein ++) and blood coagulation (thrombin time 22.9s) were noticed as a preoperative check before cataract extraction. Further examination detected a renal tubular dysfunction (beta-microglobulin 77.20 mg/L, beta-microglobulin/creatinine 63591.43  $\mu\text{g}/\text{mmol}$ , retinol-binding protein 3.26 mg/L, alpha-microglobulin 110.0 mg/L, N-acetyl- $\beta$ -D-glucosaminidase 30.7 U/L).

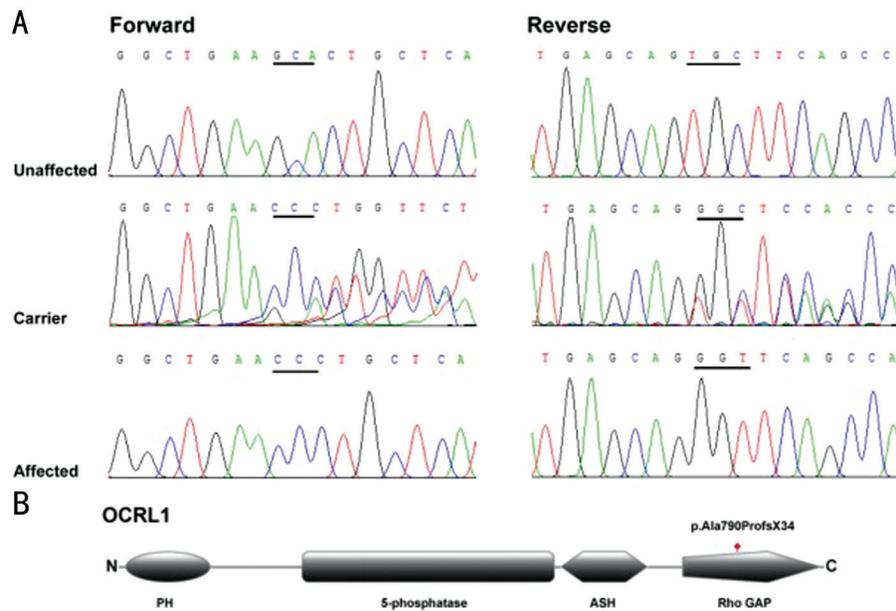
The parents (II:2 and II:3) and the sisters (III:2 and III:4) of the proband are healthy but all the brothers of the proband died in early age. The first child (III:1) of the family was a boy, and he was delivered after a full-term pregnancy. He died during delivery for unknown reason. Therefore, the diagnosis of Lowe is undetermined. The second boy (III:3) in the pedigree died of hypoxic-ischemic encephalopathy at 1.5-month old. He also suffered from congenital cataract and undescended testicle. The third boy (III:5) of this family died during the surgery of congenital heart disease at the age of 8mo. He also suffered from congenital cataract, hypoxic-ischemic encephalopathy, laryngeal cartilage dysplasia, undescended testicle, and developmental delay. No abnormality was detected in the hearing test of all the children in the pedigree. The ophthalmological and systemic evaluations, as well as the medical history of the family, were consistent with a diagnosis of Lowe syndrome.

By direct sequencing of the coding and flanking regions of *OCRL* (NM\_000276.3, MIM number 300535), a complex mutation c.(2368\_2368delG; c.2370A>C) was detected in the proband (III:6). The mutation (Figure 2A) is located in exon 21 of *OCRL*. Deletion of a single base pair at nucleotide position 2368 (c.2368\_2368delG) and a variant at nucleotide position 2370 (c.2370A>C) were located in the same allele causing the termination of protein synthesis at amino acid 824 and result in a new peptide with 823 amino acids (p.Ala790ProfsX34). The heterozygous variants were detected in the patient's mother. Both variants have not been detected in the patient's father (II:3), the patient's sisters (III:2 and III:4), the 100 healthy Chinese adults, the ExAC database, and the 1000 Genomes database. No previously report was found in the literature.

**DISCUSSION**

In the present study, we detected a complex mutation c.(2368\_2368delG; c.2370A>C) in one allele of *OCRL* in a Chinese family with Lowe syndrome. In this family, all the sons died in a very early age without sequence data except for the proband. Both daughters are healthy without any sign of Lowe syndrome. Although previous studies suggested the mutation in *OCRL* showed phenotypic heterogeneity<sup>[9-11]</sup>, the present pedigree had relatively similar phenotype among the affected and the suspected affected family members. III:6, III:3, and III:5 showed severe hypoxic-ischemic encephalopathy, severe muscular hypotonia, bilateral congenital cataract, and undescended testicle. Notably, III:5 was additionally diagnosed with congenital heart disease and laryngeal dysplasia.

Mutations in *OCRL* gene, encoding inositol polyphosphate 5-phosphatase, have been reported causing the Lowe syndrome. The mRNA transcript of full-length *OCRL* gene contains 24 exons, including an alternatively spliced 18a exon. Therefore, two isoforms are produced. Isoform A (*OCRL1* protein) encodes 8 more amino acids than isoform B, and is expressed ubiquitously. In contrast, isoform B is not expressed



**Figure 2 Genetic information** A: Sequence chromatograms of the c.2368\_2368delG and c.2370A>C (p.Ala790ProfsX34) variation identified; B: Domain structure of OCRL1 with the complex mutation. Graphic overview of the protein encoded by *OCRL*. Structural or functional domains are depicted, as well as the position of the mutation. PH: Pleckstrin homology domain; 5-phosphatase: 5-phosphatase catalytic domain; ASH: ASPM, SPD-2, Hydin domain; Rho GAP: Rho-GAPase activating protein domain.

in the brain<sup>[4,12]</sup>. Most *OCRL* mutations associated with Lowe syndrome are located in exons 8 to 23<sup>[3,7,13]</sup>. *OCRL1* contains 4 domains (Figure 2B). The N-terminal pleckstrin homology (PH) domain (encoded by exon 2-5) plays a part in recognizing phosphatidylinositol 4,5-bisphosphate [PI(4,5)P<sub>2</sub>] on the membrane<sup>[14]</sup>. The central 5-phosphatase catalytic domain (encoded by exon 9-15) shows catalytic properties<sup>[15-16]</sup>. The ASH (ASPM, SPD-2, Hydin) domain and the C-terminal noncatalytic Rho-GTPase activating protein (Rho GAP) domain (encoded by exon 16-22) mediate the interaction of *OCRL1* and its partners<sup>[17]</sup>.

Lowe syndrome is caused by partial or complete loss of PI(4,5)P<sub>2</sub>ase activity<sup>[18-19]</sup>. The missense mutations are likely to produce less *OCRL1* protein with decreased PI(4,5)P<sub>2</sub>ase activity<sup>[10]</sup>. The possible mechanism of the decrease amount of the *OCRL1* with missense mutation is that the deleterious *OCRL1* induced the activation of the endoplasmic reticulum-associated degradation or of the unfolded protein response<sup>[20]</sup>. For nonsense, frameshift, deletion, and splicing mutations, the mutations were associated with low mRNA content which was likely to be caused by the decreased transcription and nonsense mediated decay<sup>[7,10,21-22]</sup>. In the present pedigree, we identified a complex mutation. Although the c.2370A>C is a synonymous mutation, there was a deletion (c.2368\_2368delG) occurred two base pairs ahead of it. The combined effect of the two variants is the generation of a shorter new peptide (823 amino acids) than usual, and the amino acid 790 changes from alanine (reference) and histidine (c.2368\_2368delG only) to proline (c.2368\_2368delG and c.2370A>C). The

pathogenic mechanism involved in this complex mutation is probably nonsense mediated decay of the mRNA induced by the premature stop codon. Moreover, the present mutation resulting in shorter protein with incomplete Rho GAP domain might have an impact on the interaction between *OCRL1* and its partners. However, further study is needed to elucidate the actual pathogenic mechanism.

The types of mutations are unlikely to correlate with the severity of the disease. Even in the same family, the phenotypes of the affected individuals showed heterogeneity. The variability was explained by the difference in the patients' genetic background<sup>[9-10,23-24]</sup>. In contrast, the phenotype of the present family showed intrafamilial consistency. The three members (III:3, III:5, and III:6) shared similar phenotype (bilateral congenital cataract, severe hypoxic-ischemic encephalopathy, severe muscular hypotonia, and undescended testicle). However, III:5 suffered from congenital heart disease and laryngeal dysplasia which might indicate some inconsistency. Apart from genetic background difference, a potential explanation is that the patients (III:3 and III:6) were still young and hasn't shown the symptom yet.

Additionally, bilateral pupils difficult to be dilated, congenital heart disease, and laryngeal dysplasia are the symptom which were not reported in previous studies. Since *OCRL* expresses ubiquitously, including eye and heart, the occurrence of abnormality in iris and heart suggests Lowe syndrome might also have an impact on the uvea and cardiovascular system. This correlation needs to be validated by more Lowe syndrome pedigrees.

In summary, we identified a complex mutation c.(2368\_2368delG; c.2370A>C) of *OCRL* in a Chinese family. The phenotype in this pedigree includes severe hypoxic-ischemic encephalopathy, severe muscular hypotonia, congenital heart disease, laryngeal dysplasia, bilateral congenital membranous cataracts, bilateral pupils difficult to be dilated, and undescended testicle. Our findings expand the phenotypic and genotypic spectrum of Lowe syndrome.

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**Conflicts of Interest:** Zhou FQ, None; Wang QW, None; Liu ZZ, None; Zhang XL, None; Wang DN, None; Dongye MM, None; Lin HT, None; Chen WR, None.

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