

MUTATION IN BRIEF

OCRL Mutation Analysis in Italian Patients With Lowe Syndrome

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The oculocerebrorenal syndrome of Lowe (OCRL, also called OCRL1) is a rare X-linked disorder characterized by major abnormalities of eyes, nervous system, and kidneys. The gene responsible for OCRL was identified by positional cloning and encodes an inositol polyphosphate-5-phosphatase. We performed the molecular analysis in 9 Italian patients and 26 relatives and we detected the mutations in all the examined patients. Eight mutations out of nine had never been described and consisted of truncating mutations (frameshift, nonsense, splice site and genomic deletion), and missense mutations. The mutations were distributed in the second half of the gene as previously described in other populations. In three cases the mutations were absent in the mothers confirming the occurrence of novel mutations in this disorder. Our results on the Italian population are similar to the data previously obtained in other populations. © 2004 Wiley-Liss, Inc.

KEY WORDS: Lowe syndrome; Oculocerebrorenal syndrome; *OCRL*; mutation analysis

INTRODUCTION

Lowe oculocerebrorenal syndrome (OCRL, also called OCRL1; MIM# 309000) is a rare X-linked disorder characterized by congenital cataracts, muscular hypotonia, mental retardation, renal tubular dysfunction (Lowe et al 1952). The *OCRL* gene is located on chromosome Xq25-26 and its genomic structure has been partly elucidated (Attree et al., 1992; Nussbaum et al., 1997). The gene contains 24 exons, the coding region including exons 2-24. It encodes a 105 kDa phosphatidylinositol 4,5-biphosphate 5-phosphatase which is characterized by two consensus motifs, domain I and domain II, involved in substrate binding and catalysis (Jefferson et al., 1996). The enzyme is localized to the Golgi apparatus (Suchy et al., 1995). The protein may control cellular levels of phosphatidyl inositol 4,5-biphosphate. The majority of patients with Lowe syndrome do not show detectable levels of transcripts or of phosphatase activity (Attree et al., 1992). According to these data most of the mutations so far described produce truncated proteins and the missense mutations occur in the conserved catalytic domains (Lin et al., 1997; Lin et al., 1998; Kawano et al., 1998; Kubota et al., 1998; Satre et al., 1999; Monnier et al., 2000; Roschinger et al., 2000).

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According to Haldane (1935), Lowe syndrome, as X-linked recessive disease, is characterized by frequent occurrence of spontaneous mutations and therefore different mutations are present in independent families. We report here the genetic study of nine Italian patients and their relatives. The search for the mutations was performed on the DNA of nine unrelated male patients and their relatives.

MATERIALS AND METHODS

Patients

Blood samples were obtained from nine patients with Lowe syndrome and 26 relatives. All the probands are of Italian origin. The diagnosis was done on the basis of classically recognized criteria including mental retardation, bilateral congenital cataract, congenital hypotonia and tubulopathy. Our patients vary between 4 and 21 years of age. The diagnosis was done at the age of 5 months old for most of the patients, but two were diagnosed at 8 and 9 months of age, respectively. Ocular examination was performed also in the mothers and in females at risk belonging to the families.

Mutation analysis

DNA was extracted by standard methods. The 23 coding exons and their flanking intronic sequences of *OCRL* gene were amplified from genomic DNA using forward and reverse primers designed according to nucleotide sequences of *OCRL* (Table 1).

Table 1: Primers Used to Amplify *OCRL* Exons*

Exon	Forward primer	Reverse primer
2	F2: TGCCCCAGAGCCGAGCAGAGA	R2: GGTGACCCGTCGCGGTTTG
3	F3: CCTAGAGCAATATCTAGCTGTCA	R3: TACATAATGCAGTAATGACCGAG
4	F4: TGAGGAGTTCCATTTGGTTAC	R4: TTCTTAGGCTTAGCCTACATG
5	F5: GTGTGCTTCCTATATTAGAGAT	R5: ATCAGCTATCAGGGCTTAAG
6	F6: CTGTATCCAAAAATGGTCTGG	R6: AGGAGAATGTGTCTGACATCAG
7	F7: ACCACTGATCAAATTGTGATC	R7: ACTATAATTCTCCTACAAGGC
8	F8: GCCTTGTAGGAGAATTATAGT	R8: CTTAAATATCAACAGGCCACT
9	F9: CATACCTTTGTATGGAAGCG	R9: TACAGTAGGTTTTACCAACAGT
10	F10: GTGAACAGAGCAGTTCCTATAA	R10: GATAATGGAATAACTCCCCTG
11	F11: ACTAGTATATCATCTTGTATGGA	R11: GCAACTGAACTTTACATGAAC
12	F12: GTTCATGTAAAGTTTCAGTTGC	R12: ATTTAATCTCTACACTATCCA
13	F13: AGTGGTGAGTGAGCCCTTAT	R13: CAGTAAGACGTTTCCATCACTCC
14	F14: ATAGGAACAGTGGCTTATCAAC	R14: CAATATGGAGGGTCAGAAAT
15	F15: TAAACAAGAGAGCCTAACCCCT	R15: CCTCTAGTAATTGATACTTAACAC
16	F16: GTTAGATAATCTCCAAGGGAG	R16: TTCTGTGCTAACCACAGTGAG
17	F17: ATCCTCTATGGAATAATCCAAC	R17: TCATGACATCACCAGCAG
18	F18: GGTTCCTTATACTCTTT	R18: AGGACGTCACTTAAGTATTGAG
19	F19: GCATGACCAGAATTTGAAGGA	R19: GAGGTGTTGTGATTTCCATATAG
20	F20: GGTAATCATCATAACCTCAG	R20: TATAGGTGCGAAGAAGAGT
21	F21: AGCTTGAGGAAAGGAGCTC	R21: CCTATCTGGCTCTGTAATAACT
22	F22: TACAGCAGCTGTAAGTTCC	R22: CTGCATATGCAGTAAGGT
23	F23: ACCTTACTGCATATGCAG	R23: AACTTGAAATTTCTGCAATAGACC

*Based on the sequence of *OCRL* (UCSC Genome Bioinformatics: <http://genome.ucsc.edu>, +1 is A of initiation ATG, based on the sequence GenBank# NM_000276.3).

Reactions were performed in a 25 μ l mix containing 100 ng of genomic DNA, 200 μ M of each dNTP, 1 μ M each of forward and reverse primers, 0.5 U of Amplitaq Gold polymerase (Applied Biosystems) and 2.5 μ l of 10X standard buffer. A 95° denaturation step of 10 min was followed by 30 amplification cycles with the following parameters: 94°C for 30 s, an annealing step for 30 s and 72°C for 1 min and a final step at 72° for 7 min. PCR amplified fragments were purified by QIAquick DNA purification kit and sequenced on an ABI 3100 automated sequencer (Applied Biosystems). The mutations were all confirmed by sequencing the reverse strand and by a

second reaction. The missense mutations have been confirmed by the study of 100 normal chromosomes by Amplification Refractory Mutation System (ARMS).

In one patient total RNA was extracted from lymphocytes by the use of Rneasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and 0.2 µg of total RNA was reverse transcribed using the multi scribe RT-PCR system TaqMan (Applied Biosystem). 5 µl of cDNA was amplified according to the above described PCR conditions using a forward primer located in exon 17 (5' GTGAAGTTTCGGCAACTA 3') and a reverse primer located in exon 18 (5' CGGATTGGTCTTTTCATACG3'). PCR products were purified and directly sequenced on an ABI 3100 apparatus. The mutations were named according to the cDNA sequence described in National Human Genome Research Institute (NHGRI) <http://research.nhgri.nih.gov/>.

RESULTS AND DISCUSSION

The 23 coding exons were individually examined by direct sequencing. The analysis revealed a sequence mutation in all the DNA samples of the male patients (Table 2). Eight out of nine were new mutations, the c.2032C>T (p.Arg678X) in exon 18 identified in patient SL-08 has already been described in two unrelated patients (Lin et al., 1998; Monnier et al., 2000). Two mutations, in exons 14 (c.1380T>G, p.Tyr460X) and 18 (c.2032C>T, p.Arg678X), were nonsense mutations. Both the nonsense mutations (in patients SL-04 and SL-08) create stop codons, which are predicted to result in premature truncation of the protein. Two affected males (SL-01 and SL-02) carried 1 bp deletion in exon 8 (c.559delA, p.Asn187fsX45), where mutations had never been detected, and in exon 20 (c.2246delA, p.Gln749fsX58), respectively, both leading to frameshift and premature termination codons.

Table 2. Mutations of the OCRL Gene Identified in Nine Patients with Lowe Syndrome

Patient	exon	mutation type	cDNA Number ^a	predicted effect	status of the mother
SL-01	8	1 bp deletion	c.559delA	p.Asn187fsX45	Carrier
SL-02	9	Missense	c.690G>T	p.Trp230Cys	Carrier
SL-03	12	Splice site	c.1193+1G>C	r.spl? p:?	Non carrier
SL-04	14	Nonsense	c.1380T>G	p.Tyr460X	Non carrier
SL-05	15	Missense	c.1529T>A	p.Val510Asp	Carrier
SL-06	17	Missense	c.1679T>A	p.Val560Glu	Carrier
SL-07	18	17 bp deletion	c.1829-5_1829-21del	(r.1828_1829 ins1828+218_1828+233 ins1828+251_1828+255) p.Asn610fsX16	Carrier
SL-08	18	Nonsense	c.2032C>T ^b	p.Arg678X	Non carrier
SL-09	20	1 bp deletion	c.2246delA	p.Gln749fsX58	Carrier

^a base number is according to NHGRI <http://research.nhgri.nih.gov/>

^b Recurrent mutation (Lin et al., 1998; Monnier et al., 2000).

Three missense mutations were identified in exons 9 (c.690G>T, p.Trp230Cys), 15 (c.1529T>A, p.Val510Asp), 17 (c.1679T>A, p.Val560Glu). Two of them, in exons 9 and 15 (patients SL-02 and SL-05) are located in the

phosphoinositide phosphatase domain where the majority of missense mutations has been described. The patient SL-05 carrying the mutation in exon 15 died at 2 years of age. The functional expression of the missense mutations are not easily proved but our mutations meet some of the criteria that have been defined (Cotton and Scriver 1998), such as the absence of the mutation in the normal population and the segregation of the mutation with the trait in the families. In fact all the cases here reported were familiar and the mutations were also identified in the mothers and grand-mothers and moreover the mutations have not been detected in one hundred normal males.

One patient (SL-03) carried a splice site mutation: the c.1193 +1G>C (r.spl? p.?) transversion in the donor site in the intron 12, which could not be proved by RT-PCR analysis because the RNA was not available. One patient (SL-07) showed a 17-bp deletion in intron 17 (c.1829-5_1829-21del; r.1828_1829 ins1828+218_1828+233 ins1828+251_1828+255; p.Asn610fsX16). The same mutation was detected at the heterozygous state in his mother. The RT-PCR analysis of the exons 17-18 region was performed using total RNA extracted from lymphocytes of the patient and his mother. The patient showed one fragment 20 bp longer than normal, which was present also in the mother. By sequencing analysis the longer fragment showed that it derived by the use of an alternative acceptor splice site upstream the deletion in intron 17, which leads to a premature stop codon in exon 18.

All the mutations identified in our patients either predict to result in a truncated protein or they are missense mutations localized in one of the conserved domains. The only exception is the missense mutation identified in patient SL-06 which is located in none of the conserved regions. A better knowledge of the protein structure could give insight about its effect on the protein function and will be helpful to tempt a genotype-phenotype correlation, which was not clear in our study.

Although the number of families reported in this study is small, the data are similar to those previously described (Lin et al., 1998; Satre et al., 1999; Monnier et al., 2000). In fact the distribution of the mutations is not uniform, one third are sporadic cases and the majority of the mutations predict truncated proteins as already reported. It was not possible to demonstrate the occurrence of germinal mosaicism in the sporadic cases, but it has since been proved in several cases it must be taken in account during the genetic counseling (Satre et al., 1999). Considering the severity of the disease and the lack of definitive therapy, the prevention through carrier identification and prenatal diagnosis, is the only real choice for the families.

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