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Characterization of a Germline Mosaicism in Families with Lowe syndrome, and Identification of Seven Novel Mutations in the *OCRL1* Gene

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Summary

The oculocerebrorenal syndrome of Lowe (OCRL) is an X-linked disorder characterized by major abnormalities of eyes, nervous system, and kidneys. Mutations in the *OCRL1* gene have been associated with the disease. *OCRL1* encodes a phosphatidylinositol 4,5-biphosphate (PtdIns[4,5]P2) 5-phosphatase. We have examined the *OCRL1* gene in eight unrelated patients with OCRL and have found seven new mutations and one recurrent in-frame deletion. Among the new mutations, two nonsense mutations (R317X and E558X) and three other frameshift mutations caused premature termination of the protein. A missense mutation, R483G, was located in the highly conserved PtdIns(4,5)P2 5-phosphatase domain. Finally, one frameshift mutation, 2799delC, modifies the C-terminal part of OCRL1, with an extension of six amino acids. Altogether, 70% of missense mutations are located in exon 15, and 52% of all mutations cluster in exons 11–15. We also identified two new microsatellite markers for the *OCRL1* locus, and we detected a germline mosaicism in one family. This observation has direct implications for genetic counseling of Lowe syndrome families.

Introduction

The oculocerebrorenal syndrome of Lowe (OCRL [MIM 309000]) is a rare X-linked disorder characterized by a severe pleiotropic phenotype including mental retardation, congenital cataract and renal Fanconi syndrome (Lowe et al. 1952). The *OCRL1* gene has been mapped to chromosome Xq24.26 (Silver et al. 1987) and cloned (Attree et al. 1992). Its genomic structure has been recently described (Nussbaum et al. 1997). The coding region includes exons 2–23, with an alternative spliced exon (18a) expressed in neurological tissues (Nussbaum et al. 1997). The gene product is an inositol polyphosphate 5-phosphatase, belonging to the *inositol* 5-phosphatase gene family and characterized by two conserved amino acid domains involved in substrate binding and catalysis (Jefferson et al. 1996). Inositol 5-phosphatases have been characterized by their substrate specificity, leading to their classification as type I or type II phosphatases. The former act on the soluble head-group inositol polyphosphate, whereas type II phosphatases can also employ lipid substrates (Woscholski and Parker 1997). The OCRL1 protein was first localized in fibroblasts associated with the Golgi apparatus (Olivos-Glander et al. 1995; Suchy et al. 1995). The protein may control cellular levels of phosphatidyl inositol 4,5-biphosphate [PtdIns(4,5)P_2], a critical metabolite that is involved in Golgi vesicular transport. Recently, a study performed on kidney proximal tubule cells compared the properties of full-length OCRL1 with those of a truncated OCRL1 still encoding the region homologous to the 5-phosphatase II. It showed that only the normal protein is associated with lysosomal membranes and that OCRL1 may function by regulating the lysosomal pool of PtdIns(4,5)P_2.

The majority of OCRL patients have either no detectable OCRL1 mRNA (Attree et al. 1992) or no detectable 5-phosphatase activity (Lin et al. 1997), suggesting that a loss of OCRL1 function is the cause of Lowe syndrome. Although most of the reported mutations are nonsense and frameshift mutations leading to a truncated protein, missense mutations and short in-

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frame deletions have been also reported (Leahey et al. 1993; Lin et al. 1997; Kawano et al. 1998; Kubota et al. 1998; Lin et al. 1998). Only one case of a 1.2-kb genomic deletion leading to the loss of exon 14 has been described so far (Lin et al. 1997). This suggests that exon deletion is not a common mechanism of mutation in Lowe syndrome.

X-linked recessive diseases are characterized by the frequent occurrence of spontaneous mutations (Haldane 1935), and different mutations are to be expected in independent families. However, recurrences of nonsense and missense mutations have been described in unrelated patients (Leahey et al. 1993; Lin et al. 1997). We report here on the genetic study of eight families, seven European and one Lebanese. The search for mutations was performed on genomic DNA from the affected male patients. Eight mutations were identified, including seven newly identified mutations and one recurrent mutation previously described in a North American family. Germinal mosaicism is a major problem in risk estimation for an X-linked disease (Murphy et al. 1974). With the development of direct mutation detection, germinal mosaicism has been demonstrated in a majority of severe dominant diseases as well as in X-linked diseases. However, no germinal mosaicism had yet been reported in families affected by Lowe syndrome. We performed haplotyping of the families in order to determine the origin of the mutation in the different families. Using new closely linked flanking markers from Généthon, we found in one family a germline mosaicism in the grandmother. This finding confirms that mosaicism is a general phenomenon accompanying new mutations in X-linked recessive disorders and must now be taken into account in genetic counseling of families with Lowe syndrome.

Material and Methods

Samples from Patients

The study included eight families, originating from France (LS01FR, LS02FR, LS04FR, and LS07FR), Spain (LS03ES, LS05ES, and LS06LS), and Lebanon (LS08LI) (fig. 1). Five of these eight pedigrees had a family history of at least two male patients carrying a clinical diagnosis of Lowe syndrome on the basis of the classic triad of defects affecting lens, brain and kidney. In one family (LS06ES), the two affected sons were deceased. The search for mutations in OCRL1 in this family was performed on the DNA of the carrier mother, who herself presented ocular symptoms. Blood was collected from the affected patients, from their parents, and from relatives, with the informed consent of all. When necessary buccal swabs, hair roots, and urinary cells were collected, to investigate somatic mosaicisms.

DNA Extraction and Amplification

Genomic DNA was extracted from whole blood by a rapid guanidine method (Jeanpierre 1987). When hair-root, buccal-swab, and urinary cells were analyzed, genomic DNA was extracted by Chelex following the manufacturer's instructions. The 23 coding exons of *OCRL1* and their flanking intronic sequences were amplified using forward and reverse primers, as described by Nussbaum et al. (1997). Reactions were performed in a 25-μl mix containing 100 ng genomic DNA, 200 μM of each dNTP, 1 μM each of forward and reverse primers, 0.5 U *Taq* polymerase (Promega), and 1.5 mM $MgCl_2$, except for the amplification of exon 16, which was performed with 2.5 mM $MgCl_2$. A 95°C denaturation step of 5 min was followed by 30–32 amplification cycles with the following parameters: 94°C for 30 s, an annealing step for 30 s, and 72°C for 1 min.

Single Strand Conformation Analysis (SSCA)

Each exon was amplified as described above in the presence of 1 μCi -α-[^{32}P]-dCTP(10 Ci/mmol). PCR products were denatured in loading buffer (95% formamide, 0.01 M NaOH, 0.025% xylene cyanol, 0.025% bromophenol blue) at 95°C for 2 min, were cooled on ice and were separated on a nondenaturating DNA-sequencing gel prior to autoradiography. Two separating conditions were used for each sample: a run on a 6% acrylamide gel at 4°C and 40 W for 2 to 3 h, and a run on a 6% acrylamide/10% glycerol gel at room temperature and 4 W overnight.

Sequencing

PCR-amplified fragments obtained from genomic DNA were purified by use of the QIAquick DNA-purification system (Qiagen). To eliminate any sequence due to faulty polymerase activity, products from three independent amplifications were sequenced on an an ABI 373 apparatus, with the PCR primers used as sequencing primers.

Haplotyping and Data Analysis

DNA from family members was typed by two flanking microsatellite-repeat markers described by Nussbaum et al. (1997): DXS6854 and DXS6855. Additional CA-repeat markers from Généthon—DXS994, DXS1047, and DXS1206—were tested for linkage to the *OCRL1* locus. Primer sequences and allele sizes have been previously reported (Weissenbach et al. 1992).

For each primer pair, 0.4 pmol forward primer was 5' end-labeled with [^{32}P] and was added to a 25-μl PCR mix containing 50 ng genomic DNA, 200 μM each dNTP, 1 μM each of forward and reverse primers, and 0.25 U *Taq* polymerase (Appligène). Amplification was

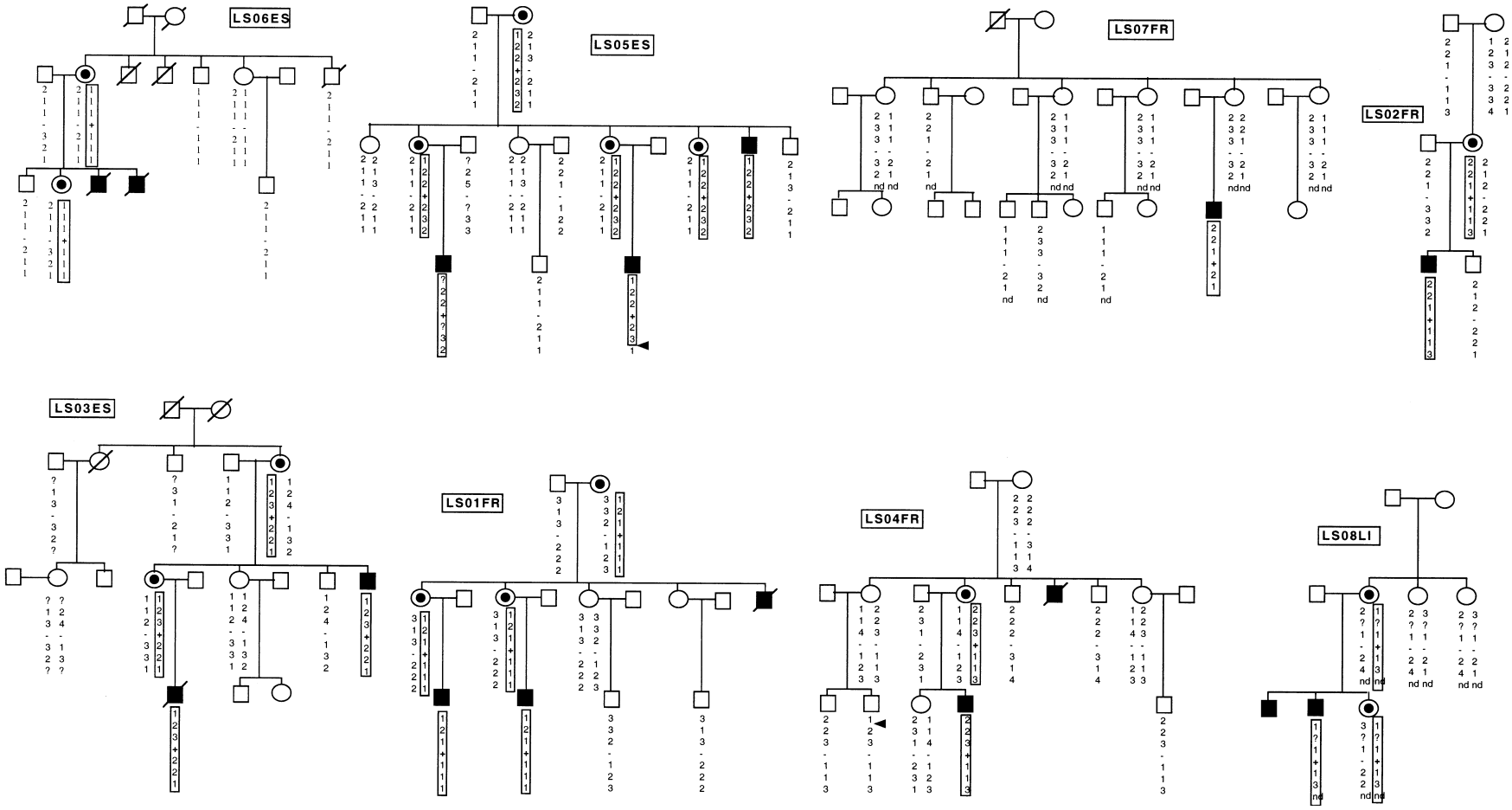


Figure 1 Segregation of mutations and of chromosome-X markers in families with Lowe syndrome. Patients with OCRL syndrome are indicated by blackened symbols. The presence of the *OCRL1* mutation is indicated by a plus sign (+) or a minus sign (−). Results of typing for the polymorphic microsatellite markers—DXS1206, DXS1047, DXS6854, *OCRL1* mutation, DXS6855, DXS994, and HPRT—are shown in order, from top to bottom. For clarity of presentation, alleles associated with the disease are boxed. Arrows indicate recombination events.

performed for 25–30 running cycles with the following parameters: 30 s at 94°C, 30 s at the annealing temperature (60°C for DXS994, DXS1047, and DXS1206) and 1 min at 72°C. After denaturation with formamide, amplified products were separated on a 8% polyacrylamide/8 M urea DNA-sequencing gel prior to autoradiography. Consecutive allele numbers were assigned, and the results were documented by Cyrillic 2.0 software (Cherwell Scientific Publishing). Two-point linkage analysis was performed by means of the LINKAGE 5.2 package (Lathrop and Lalouel 1984). Parameters were set as follows: the disease-allele frequency at 10^{-5}, the disease penetrance at 1.0, and the OCRL mutation rate at 10^{-6}.

Results

Identification of Mutations

The 23 coding exons and the exon-intron boundaries of the *OCRL1* gene were screened by SSCA. An aberrant migration of certain fragments was found in each family. Sequencing of the corresponding exons led to the identification of seven new mutations and of one recurrent mutation (table 1). This last mutation was an in-frame triple-base deletion that led to the deletion of a well-conserved threonine residue at position 350 or 351 (Lin et al. 1997). Four patients carried a short out-of-frame deletion or insertion—1152insA, 1954delG, 1856delCTTC, or 1859delCCTT—leading, for three patients, to a premature termination of the protein. Interestingly, the frameshift mutation 2799delC in exon 23 led to a change of the C-terminal domain of the protein: the last 25 amino acids MARQTPSDRQRAQFLLGFLLGSEED were lost and were replaced by the following 32 amino acids: WQDRLQVTASVLFSSFWAFCLGAKKTKAFTVL. This clearly introduced changes in the conformational and charge properties of the C-terminal domain. The last three acidic amino acids (EED) of the native protein were replaced in the mutated OCRL1 by four amino acids (KKTK), three of which were positively charged. Predictive structure analysis using the Protean software (DNASTAR) showed that both surface probability and hydrophilicity plots of the C-terminal part of the mutated protein differed from those of the normal OCRL1. Thus, the C-terminal domain of the mutated OCRL1 appeared to be less exposed at the surface of the protein. Two nonsense mutations, R317X and E558X, and one missense mutation, R483G, were also found in the three last families. To screen the family members for the identified mutations, we investigated a possible change in enzymatic restriction sites, introduced by the different mutations. Three changes created a new restriction site, and one abolished an existing site. This allowed us to propose a simple test for prenatal diagnosis after identification of all the carriers in the respective families. Two other mutations could be investigated by a rapid heteroduplex method (table 1). In all families tested, the identified *OCRL1* mutations were associated only with the disease phenotype or with a carrier status. An additional polymorphism in exon 17, leading to a E to G substitution at position 568, was found in families LSO5ES, LSO6ES, and LSO7FR. This position does not correspond to a conserved amino acid when one compares protein sequences of various inositol-5-phosphatases.

Neomutation and Germline Mosaicism

To determine the origin of the mutation in the different families, we performed haplotyping of the families (fig. 1). As a first step, and to obtain as much information as possible, new microsatellite markers from Généthon were characterized at the *OCRL1* locus. We identified two markers, DXS1047 and DXS994, which are closely linked to the *OCRL1* locus (maximum LOD scores of 18.8 and 14.5, respectively, at $\theta = 0$). However, the absence of recombinants between these markers and Lowe syndrome did not allow us to localize them as distal or proximal from *OCRL1*. Thus, the deduced genetic map of the *OCRL1* locus on chromosome Xq26.1 is as follows: cen–DXS1206–(8 cM)–(DXS6854–[DXS1047, DXS994, *OCRL1*]–DXS6855)–(4 cM)–HPRT–tel. These new markers, together with the already described flanking markers DXS6854 and DXS6855

Table 1

Mutations of the *OCRL1* Gene Identified in Eight Patients with Lowe Syndrome

Family	Exon	Mutation Type	DNA Change[a]	Predicted Protein Change	Screening Method[b]
LS01FR	11	1-bp insertion	1152insA	Frameshift and stop	*Ssp*I (+)
LS03ES	11	Nonsense	C1177T	R317X	*Taq*I (−)
LS06EX	12	3-bp deletion	1276delAAC	DT350 or 351[c]	Heteroduplex
LS04FR	15	Missense	C1738G	R483G	SSCA
LS07FR	16	4-bp deletion	1856delCTTC or 1859delCCTT	Frameshift and stop	Heteroduplex
LS05ES	17	1-bp deletion	1954 delG	Frameshift and stop	SSCA
LS08LI	17	Nonsense	G1900T	E558X	*Mse*I (+)
LS02FR	23	1-bp deletion	2799delC	Change of the C-terminus, starting at codon 860	*Bsl*I (+)

[a] Base number is according to GenBank entry U57627.

[b] Used to screen the mutation in the family members : (+) = creation of a restriction site; (−) = loss of a restriction site.

[c] Recurrent mutation (Lin et al. 1997)

(Nussbaum et al. 1997), helped us characterize neomutations. One neomutation arose in an affected child in family LS07FR. No evidence for a somatic mosaicism in her mother was found when other tissues (buccal-swab, hair-root, and urinary cells) were tested (data not shown). This result was in agreement with the absence of ocular symptoms when a slit-lamp exam was conducted on the mother. Two other neomutations arose in the mothers of affected children: one on the chromosome of grandpaternal origin (in family LS02FR) and one on the chromosome of grandmaternal origin (in family LS06ES). Interestingly, in family LS04FR, the *OCRL1* mutation was not present in the apparently obligate-carrier grandmother (individual I-2; fig. 1). Clinical misdiagnosis of the deceased affected boy (patient II-6) can be ruled out, since this patient has been extensively clinically documented as the first French patient with an OCRL syndrome (Habib et al. 1962). As demonstrated by analysis of the haplotypes, the grandmother has transmitted three types of X chromosome to her offspring. The first chromosome X (haplotype 2-2-3-*OCRL1*-1-1-3) is present in individuals with (patient II-4) and without (individuals II-2 and II-8) the c1738g mutation. The second chromosome X (haplotype 2-2-2-*OCRL1*-3-1-4) is present in unaffected individuals II-5 and II-7. Clinical examination of the grandmother did not reveal ocular symptoms. We therefore infer the presence of a germline mosaicism in the grandmother. To distinguish pure germline mosaicism from somatic/germline mosaicism, we tested three different tissues, besides blood cells, for the c1738g mutation. As shown in figure 2, presence of the OCRL1 mutation was evident in urinary cells. A conformer with the same electrophoretic comportment (fig. 2, arrows) was present in DNA samples obtained either from the affected child (lane 6), the carrier mother (lane 5), or the obligate carrier grandmother (lane 4). The weak signal obtained for the mutated conformer in urinary cells of the grandmother indicated that only a small fraction of these cells harbor the mutation. In the same conditions, no mutated conformer was detected in buccal swabs or hair roots of the grandmother (fig. 2, lanes 2 and 3). These results clearly indicate that, although the mosaicism within the grandmother is not generalized to all tissues, it is not strictly restricted to the germinal cell line.

Discussion

Seven new mutations and one recurrent mutation were identified in the *OCRL1* gene in one carrier mother and in seven affected patients. The screening strategy, based on SSCA followed by the sequencing of the 23 coding exons of the gene, appeared to be highly effective for this gene, probably because of the short size of the exons and because of their nucleotide content. Five of the eight mutations identified led to the production of truncated proteins. This is in good agreement with previously published data (Lin et al. 1997) showing that 64% of all mutations are nonsense or frameshift mutations leading to a premature termination of OCRL1. One of the mutations was a recurrent loss of a threonine at position 350 or 351 in one of the highly conserved domains of the protein (fig. 3). This mutation has been shown previously to significantly decrease the 5-phosphatase activity of *OCRL1* (Lin et al. 1997).

One missense mutation was identified in exon 15. The substitution changed the arginine residue at position 483 to a glycine. Two other reports have recently reported

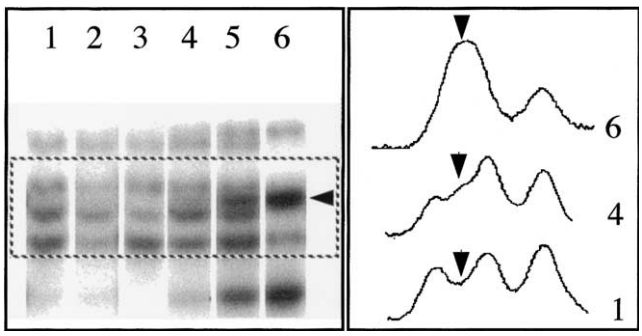


Figure 2 SSCA of c1738g mutation in different tissues of obligate-carrier grandmother in family LS04FR. SSCA was performed as described in Material and Methods. The left panel shows the SSCA profiles of exon 15, amplified from DNA samples extracted from different tissues (blood, hair roots, buccal swabs, and urinary cells) and from different individuals: I-2 (obligate-carrier grandmother), II-4 (carrier mother), and III-4 (affected child). Lane 1, Blood of the grandmother; Lane 2, Hair-root cells of grandmother; Lane 3, Buccal swab of grandmother; Lane 4, urinary cells of grandmother; Lane 5, Blood of the mother; Lane 6, Blood of the affected child. Scanned profiles of lanes 1, 4, and 6, corresponding to the framed area, are presented in the right panel. For the sake of clarity, the plots of lanes 2 and 3 (which were similar to that of lane 1) are not presented. Gel pictures were analyzed by use of NIH image software. Arrows indicate the position of the mutated conformer in the different samples.

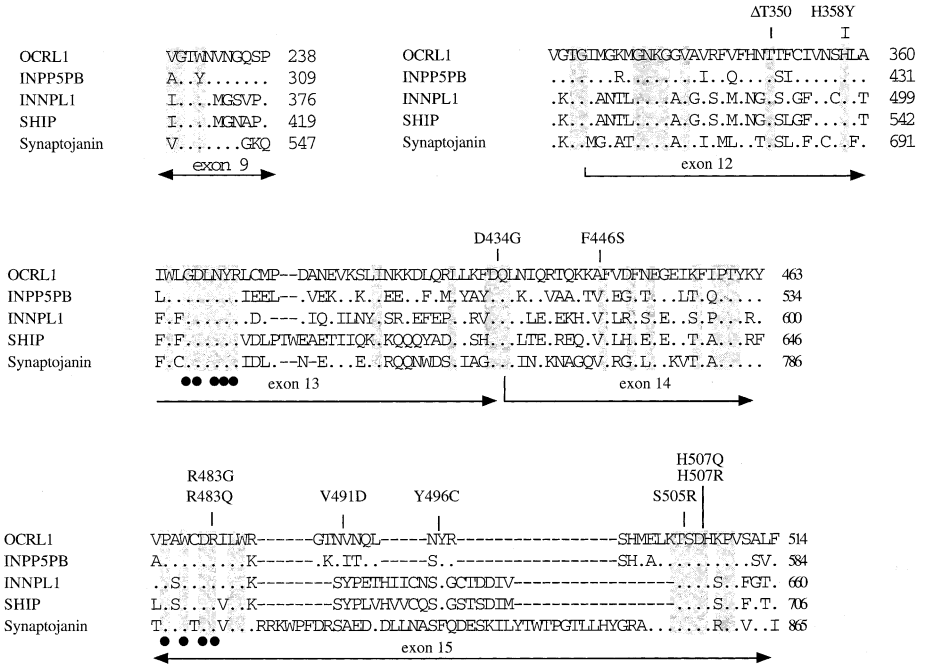


Figure 3 Blocks of highly conserved amino acid sequences shared by five human phosphoinositide and inositol polyphosphate 5-phosphatases. Numbers indicate the amino acid residue for each protein and are derived from their GenBank entries (OCRL1, U57627; INPP5B [inositol polyphosphate 5-phosphatase type II], M74161; INPPL1 [inositol polyphosphate-like protein 1], L36818; SHIP [SH2-containing inositol 5-phosphatase], U57650; and synaptojanin, AF009040). Amino acids that are conserved in all five proteins are shaded. Amino acid changes of OCRL1 that correspond to missense mutations or in-frame deletions associated with Lowe syndrome are indicated above the sequences. Exon position is indicated below the sequences, and amino acids participating in the 5-phosphatase active site are dotted. Protein alignments were performed by use of Megalign sofware (DNASTAR).

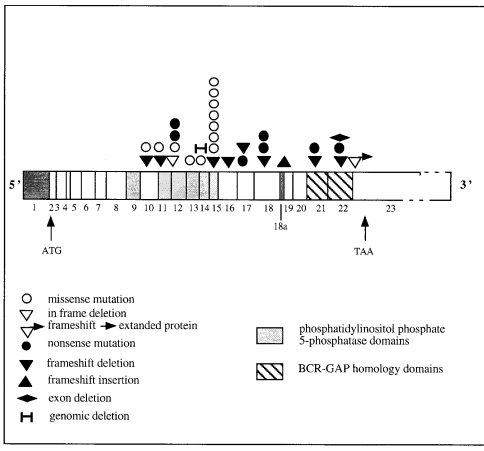
a change of this arginine residue into a glutamine in unrelated families (Lin et al.1998; Kawano et al. 1997). Owing to the use of a primal annotation of the *OCRL1* cDNA (Attree et al. 1992), this mutation was referred to as "R577Q" and was associated with a severe decrease in the amount of OCRL1 in the patient's fibroblasts (Kawano et al. 1997). Therefore the R483 residue appears critical in pathogenesis, since changes of this amino acid represent >10% of all mutations described so far in OCRL patients. Figure 3 presents sequence comparisons between various human phosphoinositide and inositol 5-phosphatases and shows that the highly conserved R483 residue is located in a sequence motif described as part of the active site of 5-phosphatases (Jefferson and Majerus 1995). This motif was shown to be involved directly in substrate binding in the 43-kD inositol 5-phosphatase (Drayer et al. 1996). Furthermore, site-directed mutagenesis of the conserved arginine in the type II inositol 5-phosphatase (R554) abolishes its enzymatic activity (Jefferson and Majerus 1996). On the basis of these data, the R483G mutation found in the present study is likely to play a pathogenic role in family LS04FR. Interestingly, 70% of the missense mutations—which represent 30% of all mutations described so far—are located in exon 15. This exon appears, therefore, to be a prime candidate when one is searching for new mutations in families with Lowe syndrome. It should be noted that all missense mutations described in exon 15 (except the V491D mutation) affect well-conserved amino acids (fig. 3).

The frameshift mutation, 2799delC, located in the last exon leads to a change of the last 26 C-terminal amino acids. This mutation is a neomutation that arose in the gametes of the grandfather (fig. 1). The C-terminal domain of *OCRL1* shares no homology with that of other inositol 5-phosphatases, and, to date, no specific function has been assigned to this region. However, it has been postulated that domains outside the type II 5-phosphatase region are not essential for catalytic activity but could influence regulation or localization of the protein (Woscholski and Parker 1997). On the basis of protein-sequence analysis using dedicated software, it was predicted that modification of the C-terminal domain, induced by the 2799delC mutation, could lead to the loss of potential phosphorylation sites and to the creation of a putative myristoylation site. This could be of interest if one considers that the C-terminal part of OCRL1 may be involved in the regulation of enzyme activity. Since

the carrier mother (II-2, family LS02FR) is heterozygous for the mutation and presents no clinical symptoms, one may also conclude that the mutated OCRL1 exhibits no deleterious effect. However, one cannot exclude the possibility that the modification of the C-terminus of OCRL1 leads to a defect in the processing of the protein, for instance in its localization or stability. Study of the cellular localization of the mutant protein would be therefore of interest to reveal a possible role played by the C-terminal domain in the subcellular localization of the protein and/or in the interaction with various organelles (Golgi complex, lysosomes). Unfortunately, fibroblasts from this family were not available at the time of this study.

Search for mutations in families with Lowe syndrome, based on SSCA prescreening and subsequent sequencing of the genomic DNA, is a simple and convenient method allowing, in most cases, both a direct diagnosis of carriers and prenatal diagnosis. When one considers the distribution of mutations associated with Lowe syndrome reported so far in the literature, including the seven new mutations described in this study, one can clearly see that mutations are not uniformly distributed throughout the *OCRL1* gene (fig. 4). All mutations are concentrated in only half of the 24 exons: exons 10–19 and 21–23. Interestingly, 52% of the *OCRL1* mutations cluster in five exons spanning the PtdIns(4,5)P_2 5-phosphatase domain: exons 11–15. This percentage has fallen from the 76% reported by Lin et al. (1998) but still indicates that this region is a hot spot for mutations in *OCRL1* and should be considered a prime target when one is looking for mutations.

Two new informative microsatellite markers closely linked to the *OCRL1* locus, DXS994 and DXS1047, have been identified. These new markers map to a distance of 0 cM from the Lowe syndrome in the families studied. Together with flanking DXS6854 and DXS6855 markers, these markers allow an efficient and informative haplotyping of the *OCRL1* locus and can therefore be used for genetic counseling in the absence of a characterized mutation. Haplotyping can also provide additional information, such as the origin of the transmitted chromosome in the case of a neomutation. Three neomutations have been identified in this study, either in affected children or in their mothers. The number of neomutations found is in agreement with the rate of spontaneous mutations expected for X-linked diseases. On the basis of data generated by haplotyping and by the identification of the *OCRL1* mutation, a germline mosaicism was suspected within the grandmother (patient I-2) of family LS04FR (fig. 1). As indicated in the results section, the grandmother transmitted three types of chromosome to her children, one of which bore the R483G mutation. An alternative explanation to the presence of a germline mosaicism is the occurrence of two independent neomutations in the affected boy (II-6) and his carrier sister (II-4). Considering the rarity of Lowe syndrome, this is unlikely. Furthermore, presence of a mosaicism within the grandmother was confirmed by analysis for the mutation in different tissues. As evidenced by results based on PCR analysis of urinary cells, the mosaicism within the grandmother was a somatic/germline mosaicism. Considering their embryogenic origin and the fact that they can be easily obtained, urinary cells may therefore be an interesting tissue to investigate to distinguish between pure germline mosaicism and so-



exon	DNA mutation	mutation type	predicted change	patients	references
10	1051 del g	1-bp deletion	frameshift / stop	1	b
10	c1060t	nonsense	Q278X	1	b
11	1152/1153 ins a	1-bp insertion	frameshift / stop	1	f
11	c1177t	nonsense	R317X	1	f
12	1276/1279 del acc	3-bp deletion	DT350 or T351	2	b,f
12	c1300t	missense	H358Y	1	c
12	c1339t	nonsense	Q371X	1	b
12	c1399t	nonsense	Q391X	1	e
13	a1529g	missense	D434G	1	b
14	genomic deletion	exon deletion	frameshift / stop	1	b
14	t1564c	missense	F446S	1	b
15	g1676a	missense	R483Q	3	c,d
15	c1675g	missense	R483G	1	f
15	1695/1696/1697del a	1-bp deletion	frameshift / stop	1	b
15	a1700t	missense	V491D	1	c
15	a1715g	missense	Y496C	1	c
15	c1743g	missense	S505R	1	e
15	a1748g	missense	H507R	1	b
15	c1749g	missense	H507Q	1	d
16	1856 delcttc or 1859/1863/1867 del cctt		frameshift / stop	1	f
17	g1900t	nonsense	E558X	1	f
17	1954/1955 del g	1-bp deletion	frameshift / stop	1	f
18	2100/2101/2102 del ct	2-bp deletion	frameshift / stop	1	c
18	c2164t	nonsense	R646X	1	b
18	c2260t	nonsense	R678X	1	c
19	2380 ins a	1-bp insertion	frameshift / stop	1	c
21	2535/2536/2537 del gt	2-bp deletion	frameshift : stop	3	b,c
21	c2644t	nonsense	Q806X	1	c
22	c2707t	nonsense	R827X	2	a
22	g2734a	exon deletion	frameshift / stop	1	d
22	?	exon deletion	frameshift / stop	1	a
23	2799/2800 del c	1-bp deletion	frameshift	1	f

Figure 4 Schematic representation of exonic location of known mutations along the human *OCRL1* gene. Exons are numbered 1–23, with an alternatively spliced exon shown as 18a, in accordance with the report by Nussbaum et al. (1997). "Number of patients" refers to the number of unrelated patients tested who had the same mutation. a = Leahey et al. 1993; b = Lin et al. 1997; c = Lin et al. 1998; d = Kawano et al. 1998; e =Kubota et al. 1998; f = present study.

matic/germline mosaicism. This first description of a mosaicism present in the germline in a family with Lowe syndrome has direct implications for genetic counseling. Germline mosaicism associated with new mutations has been well documented in X-linked diseases, especially in Duchenne muscular dystrophy (DMD) after the first reports by Bakker et al. (1987) and Darras et al. (1987). In DMD the recurrence risk for a proved new mutation was estimated to be 14%–18% when the haplotype at risk is known (Bakker et al. 1987; van Essen et al. 1992) and has been further refined by Passos-Bueno et al. (1992) with regard to the site of the deletion: 30% for a de novo proximal deletion and ~4% for a distal one. It is obvious that knowledge about the occurrence of germline mosaicism is important for genetic counseling and must be taken into account. In the case of OCRL syndrome, evaluation of the recurrence risk will be difficult but can be bypassed if the mutation in a sporadic case is identified and screened regardless of the mother's status.

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Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Généthon, http://www.genethon.fr
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