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From Lowe syndrome to Dent disease: correlations between mutations of the OCRL1 gene and clinical and biochemical phenotypes

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3 **From Lowe syndrome to Dent disease: correlations between mutations of the OCRL1**
4 **gene and clinical and biochemical phenotypes**
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ABSTRACT

Mutations of OCRL1 are associated with both the oculocerebrorenal syndrome Lowe, a multi-systemic and Dent-2 disease, a renal tubulopathy. We have identified a mutation in 130 Lowe syndrome families and 6 affected by Dent-2 disease with 51 of these mutations being novel. No founding effect was evidenced for recurrent mutations. Two mutations initially reported as causing Dent-2 disease were identified in patients, including two brothers, presenting with Lowe syndrome thus extending the clinical variability of OCRL1 mutations.

mRNA levels, protein content and PiP₂-ase activities were analyzed in patient's fibroblasts. Although mRNA levels were normal in cells harbouring a missense mutation, the OCRL1 content was markedly lowered suggesting that enzymatic deficiency resulted mainly from protein degradation rather than a catalytic inactivation as usually reported. Analysis of a splicing mutation that led to the elimination of the initiation codon evidenced the presence of shortened forms of OCRL1 that might result from the use of alternative initiation codons. The specific mapping of the frameshift and nonsense mutations, exclusively identified in exons 1-7 and exons 8-23 respectively for Dent disease and Lowe syndrome together with the possible use of alternative initiation codons might be related to their clinical expression i.e. Lowe syndrome or Dent-2 disease.

KEYWORDS :

OCRL1 – Lowe syndrome – Dent 2 disease – Phosphatidylinositol 4,5 biphosphate

INTRODUCTION

Lowe syndrome

The oculocerebrorenal syndrome of Lowe (OCRL [MIM#309000]) is a rare X-linked multisystem disorder presenting with major abnormalities in the eyes, the kidneys and the central nervous system [Lowe et al., 1952; Loi, 2006]. OCRL is a rare disease with a prevalence estimated between 1 and 2 boys per million people. Ocular abnormalities include a constant prenatal development of cataracts and frequent associated signs such as glaucoma, microphthalmos, decreased visual acuity and corneal keloid formation. Neonatal hypotonia, intellectual impairment and areflexia are also cardinal features. The majority of patients have a cognitive delay and behavioural troubles including temper tantrums and aggressiveness are frequently noted. Brain magnetic resonance imaging (MRI) may show periventricular cystic lesions [Schneider et al., 2001; Loi, 2006]. Fanconi syndrome, a generalized impairment of the proximal tubular cells functions, is a major feature.

Onset of the tubular dysfunction can vary between patients and the severity tends to worsen with age. Low molecular weight proteinuria (LWMP) is invariably present and aminoaciduria, hypercalciuria and bicarbonaturia are frequently included. Progressive glomerular dysfunction leads usually to renal failure. Skeletal muscle abnormalities may develop as secondary consequences of hypotonia or renal dysfunction. Nontender joint swelling and subcutaneous nodules are also frequently described in affected patients and may reflect a primary abnormality of connective tissue growth. Lowe syndrome results from mutations of the *OCRL1* gene (MIM #300535) that encodes a phosphatidyl inositol 4,5 biphosphate (PI(4,5)P₂) phosphatase.

Dent disease

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3 Dent's disease (Dent-1, MIM#300009), is a X-linked proximal renal tubulopathy,
4 characterized by LWMP, hypercalciuria and progressive renal insufficiency. In addition, other
5 features of proximal tubular dysfunction such as glycosuria, aminoaciduria, phosphaturia,
6 uricosuria or complete Fanconi syndrome may also be present [Dent and Friedman, 1964;
7 Thakker, 2000]. Except for rickets noted in some patients; no extra-renal manifestations of the
8 disease have been reported.

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10 Mutations in the *CLCN5* gene (MIM #300008) encoding the renal voltage-
11 gated chloride channel CLC-5 have been reported in 60% of patients with Dent's disease
12 [Lloyd et al., 1996, Hoopes et al., 2004]. CLC-5 is mainly localized in subapical endosomes of
13 epithelial kidney cells and contributes to the acidification of intra-endosomal compartments
14 and participates in membrane recycling in the proximal tubule [Devuyst et al., 1999; Piwon et
15 al., 2000]. A recent study showed that mutations in the *OCRL1* gene can lead to a Dent-like
16 phenotype (Dent-2 disease, DD-2, MIM #300555) that can present as a clinical intermediate
17 between Lowe syndrome and Dent disease [Hoopes et al., 2005; Bökenkamp et al., 2009].
18 Nevertheless, DD-2 patients harbouring an OCRL1 mutation displayed comparable decrease
19 in PI(4,5)P2ase activity and OCRL1 content to those reported for patients affected by Lowe
20 syndrome [Hoopes et al., 2005; this study].

21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 **OCRL1**

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50 OCRL1 is a type II inositol polyphosphate 5-phosphatase that participates into the
51 Lowe, 2005]. OCRL1 has been originally localized to the Trans-Golgi Network and to
52 lysosomes [Olivos-Glander et al., 1995; Dressman et al., 2000; Ungewickell et al., 2004] and
53 more recently to endosomes [Choudury et al., 2005; Hyvola et al., 2006; Erdmann et al.,
54 2007]. Abnormalities in the actin cytoskeleton have been demonstrated in Lowe fibroblasts

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3 [Suchy and Nussbaum, 2002] and OCRL1 was recently proposed to play a role in regulating
4 membrane trafficking and actin dynamics [Lowe, 2005; Faucherre et al, 2005].
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8 The OCRL1 gene spans 52278 bp on chromosome Xq24.26 [ENSG00000122126] and
9 contains 24 exons with exon 18a as an alternatively spliced exon mostly expressed in brain
10 [Attree et al., 1992; Nussbaum et al., 1997]. The ATG start codon was initially assigned to
11 exon 2 [Attree et al, 1992], however recent reports mapped the initiation codon in exon 1
12 [Hyvola et al., 2006; Erdmann et al, 2007; Mao et al., 2009]. Accordingly the 5156 bp cDNA
13 (NM_000276) encodes a protein monomer of 901 amino-acids (MW = 103 226 kDa).
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25 This paper reports 51 novel mutations of the *OCRL1* gene associated either with Lowe
26 syndrome or Dent-2. Correlations between the mutations and their expression at the mRNA,
27 functional and protein levels will be discussed in regard to their clinical expression. Based on
28 these data, putative mechanisms to explain how mutations of the OCRL1 protein express as
29 heterogeneous clinical entities will be discussed.
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39 MATERIAL AND METHODS

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46 A panel of 175 non-related families addressed to the laboratory for suspicion of Lowe
47 syndrome has been investigated. Among the male patients addressed for suspicion of Lowe
48 syndrome, 124 index cases presented with full criteria of Lowe syndrome. 15 mothers of
49 deceased children likely to be affected by Lowe syndrome were also investigated for
50 mutations in the *OCRL1* gene. French clinical centres addressed 58% of the families with the
51 remaining families originating from Europe (18%), Middle-East (5%), North-America (5%),
52 Asia (6%) and Australia (3%).
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3 41 patients affected by Dent's disease were additionally included in the study. All
4 patients originated from France, they did not show cataract or noticed neurological troubles
5 and did not harbour mutations in the *CLCN5* gene.
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10 Blood samples and, whenever possible, fibroblasts, were obtained from the affected
11 patients and/or from their relatives. An informed consent allowing genetic studies was
12 obtained from all patients included in the study.
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21 **Mutation detection**

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23 Genomic DNA was isolated using standard procedures. The 24 exons of the *OCRL1*
24 gene were amplified from genomic DNA as described previously [Monnier et al., 2000]. For
25 mutational analysis, PCR-amplified DNA products were subjected to direct automated
26 sequencing on an ABI 3130 DNA Analyser (Life Technologies, Carlsbad, CA, USA).
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33 Alternatively RNA was extracted from 2-5 10^6 fibroblast cells using the Trizol®
34 reagent (Life Technologies, Carlsbad, CA, USA). RNA was dissolved in 50 μ l of RNase-
35 free water. Reverse transcription was performed by using 0.2 mg of total RNA at 48°C for 60
36 minutes with 50 U of Expand Reverse Transcriptase (Roche, Basel, Switzerland) and in the
37 presence of oligo-dT and the following specific primer (5'-
38 AACTTTGGCTTGGCAATATAAGTC). The resulting cDNA was then amplified and
39 sequenced as previously described.²⁴ Mutations responsible for aberrant sized transcripts were
40 characterized by direct sequencing of the corresponding exon and intron-exon junctions.
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52 Mutation assignment was based on the cDNA sequence (Genbank # NM_000276)
53 using the first coding ATG of exon 1 as initiation codon according to international guidelines
54 for description of sequences variants (<http://www.hgvs.org/mutnomen>). This resulted in an in
55 frame addition of 51 nucleotides at the 5' end of the cDNA and of 17 amino acids at the N
56 terminal end of the protein in comparison with the initial nomenclature [Attree et al., 1992].
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3 None of the novel variants identified in this study were found after screening of 200
4 chromosomes from the general population. Genomic rearrangements were evidenced using
5 data obtained from PCR screening or MLPA analysis in probands. In families harbouring a
6 genomic rearrangement, the carrier status of mothers was determined using a homemade
7 MLPA® assay (MRC Holland, Amsterdam, The Netherlands) [Coutton et al, 2010].
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18 **Quantitative transcript analysis**

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20 Quantitative PCR was performed using 100 ng of cDNA in a final volume of 25 µl
21 and specific primers for the *OCRL1* gene (F-5'-CGAGCTGTATCAGCGATGTC; R-5'-
22 GGAGGCCTCAGGAGAAGACT) and the *GAPDH* gene used as control (F-5'-
23 CATCAAGAAGGTGGTGAAGC; R-5'- GAGCTTGACAAAGTGGTCGT). The *OCRL1*
24 primers allowed the amplification of a 197 bp fragment spanning exon 21 to 23. Duplicated
25 samples were PCR were run on a iQ Cyclor apparatus in presence of the iQ SYBR Green
26 Supermix containing 500 nM primers (Biorad, Hercules, CA, USA).
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39 **Enzymatic assay for PI(4,5)P₂ phosphatase activity**

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41 Activity was assayed as described previously on cell extracts prepared by freeze-
42 thawing of fibroblasts obtained from affected and non affected patients except that each assay
43 included 50 µg of fibroblast protein and that quantification was performed by direct analysis
44 of the TLC plates using a Beta Imager 2000 (Biospace, Paris, France) [Suchy et al., 1995;
45 Satre et al., 1999].
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56 **Western blot analysis**

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58 For western blot analysis, aliquots of cell extracts (25 µg of protein) were resolved by
59 an 8% SDS-polyacrylamide gel electrophoresis and electro-blotted onto Immobilon P transfer
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3 membrane (Millipore, Billerica, MA, USA). OCRL1 was revealed using polyclonal
4 antibodies raised in rabbit against the N-terminal region (amino-acids 18-217) and a
5
6 peroxidase-labelled mouse anti-rabbit detection system. As control we used the signals
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8 obtained using polyclonal antibodies raised against the β -subunit of the mitochondrial
9
10 ATPase. The amount of OCRL1 present in each sample was determined by quantitative
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12 western blot analysis using a ChemiDoc XRS apparatus and the Quantity One[®] software
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14 (BioRad, Hercules, CA, USA), after correction of protein loading by Coomassie blue staining.
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24 Results

25 Mutation analysis

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27 We have identified a mutation in the *OCRL1* gene in 130 out of the 175 families
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29 included for Lowe's syndrome and in 6 out of the 41 Dent disease families. 51 of these
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31 mutations were novel (Table 1) with four of them identified in patients affected by Dent-2
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33 disease. Extensive genomic and cDNA sequencing analysis led to the correction of
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35 boundaries of exons 8, 9, 11, 12, 13, 14 in reference to those reported in the Lowe Syndrome
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37 Mutation Database²⁸ and to the use of a cDNA numbering based on the use of initiation codon
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39 in exon 1 (Supplementary Table S1).
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47 Near to 200 mutations have been identified so far in the *OCRL1* gene in association
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49 with Lowe syndrome or Dent-2 disease (Supplementary Table S2). Frameshift, splicing or
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51 non-sense mutations leading to a premature termination of the protein represented 63% of the
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53 mutations whereas missense mutations and genomic deletion accounted respectively for 33%
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55 and 4% of the molecular variations. No noticeable differences in this distribution were noticed
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57 when comparing Lowe syndrome or Dent-2 Disease. 33 recurrent mutations were identified
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59 and accounted for the disease in 41% of the genotyped families. 17 mutations have been
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3 reported in more than 3 families (n=3: p.Ile274Thr, p.Arg318Cys, p.Arg334Stop,
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5 p.Gln388Stop, p.Arg822Stop; n=4: c.940-11G>A, p.Gly421Glu, p.Arg500Stop,
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7 p.Arg541Stop, c.1694_1695insCCTT, c.2360_2361delTG, p.Arg844Stop; n=5:
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9 p.Arg663Stop, p.Arg695Stop, p.Arg810Stop; n=8: p.Arg500Gln; n=10: c.2581G>A). As it
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11 could be inferred from data presented in supplementary Table S2, none of these mutations
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13 appeared to be population specific.
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18 Genetic investigation of 98 mothers of patients affected by Lowe syndrome showed
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20 the occurrence of a *de novo* mutation in 36 cases (37.2 %) whereas 3 cases of mosaicism were
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22 characterized in our panel of genotyped families indicating that, although not frequent,
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24 mosaicism must be taken in account for genetic counselling.
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28 As shown in Figure 1, 53 out of the 66 missense mutations reported to date mapped to
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30 the PI(4,5)P₂ phosphatase domain spanning exons 9-15. They involved amino-acids defined
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32 as major determinants for specificity of the inositol polyphosphate 5-phosphatase activity
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34 [Whisstock et al., 2000; Tsujishita et al., 2001]. The remaining missense mutations were
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36 identified in the C-terminal ASH-RhoGAP-like domain spanning exons 16 to 23 [Peck et al.,
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38 2002; Faucherre et al., 2003, Choudhury et al., 2005; Hyvola et al., 2006; Erdmann et al.,
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40 2007] and involved amino acids located in well conserved domains (supplementary Figure
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42 S1). Noticeably, no missense mutations have been identified so far in exons 1 to 8.
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48 Frameshift mutations or nonsense mutations leading to premature termination of the
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50 protein have been characterized in all exons but exons 2 and 3 (Fig 1). While nine
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52 substitutions affected directly the consensus acceptor or donor sites at the exon-intron
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54 junctions, seven additional intronic mutations induced abnormal splicing: c.40-14A>G,
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56 c.238+4701G>A, c.940-11G>A, c.1467-3C>G, c.1879+5G>A, c.1880-5del16,
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58 c.2469+2_6delinsA, c.2581+4A>G. Transcript analysis showed that 3 substitutions affecting
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60 the last nucleotide of exons 9, 14 and 22: c.824G>C, c.1466G>A, c.2581G>A affected the

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3 splicing. Thus, the p.Gly275Ala, p.Ser489Asn, p.Ala861Thr mutations, initially reported as
4 missense mutations [Kawano et al., 1998; Monnier et al., 2000] led thus primarily to a
5 premature termination of the protein.
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10 Noticeably, while frameshift and nonsense mutations associated with Lowe syndrome
11 concentrated in exons exons 8 to 23, all frameshift and nonsense mutations causative of Dent
12 2 disease have been characterized so far only in the first seven exons.
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21 **Functional expression of mutations**

22 *mRNA expression*

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28 As shown in Figure 2 no significant differences in the level of mRNA expression of
29 the *OCRL1* gene could be evidenced in patients harbouring a missense mutation when
30 compared to controls. At difference, patients harbouring stop, frameshift or splicing mutations
31 showed a very low mRNA content that indicated either a decreased transcription or more
32 likely a mRNA instability resulting from a non sense mediated decay mechanism. No
33 difference was observed between patients affected by Lowe syndrome or Dent-2 disease.
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35 Noticeably, one patient harbouring a c.[40-14A>G] splicing mutation showed a normal
36 amount of mRNA (Figure 2, arrow). As shown in panel A of the figure 3, the c.40-14A>G
37 mutation resulted in the production of two abnormal transcripts. Sequencing analysis showed
38 that whereas both transcripts could be amplified and quantified, they both lacked the 3' end of
39 exon 1 that contains the ATG initiation codon (supplementary Fig S2).
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54 *PIP2ase activity*

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3 PI (4,5)P₂ phosphatase activity was measured in 12 patients harbouring a missense
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6 mutation and in 11 patients harbouring a nonsense or a frameshift mutation that led to a
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8 premature termination of the protein. As presented in table 2 the enzymatic activities
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10 measured in patients with either a missense mutation or a mutation leading to a premature
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12 termination of the protein showed a mean inhibition of 85-90% when compared to PI (4,5)P₂
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14 phosphatase activity determined in fibroblasts originating either from healthy individuals or
15
16 from patients addressed for a suspicion of Lowe syndrome and for whom no mutation had
17
18 been identified in the *OCRL1* gene. No significant differences between the residual activities
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20 measured in fibroblasts harbouring a missense mutation and those harbouring a nonsense or
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22 frameshift mutation were evidenced. Likewise no differences of PI (4,5)P₂ phosphatase
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24 activities could be evidenced between Lowe syndrome or Dent-2 patients (not shown).
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27 Residual activities measured in mutant fibroblasts were likely to correspond to non specific
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29 hydrolysis of PI (4,5)P₂ by other inositol polyphosphate phosphatases present in fibroblasts
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31 since they were also determined in cells fully devoid of OCRL1 as a consequence of a
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33 genomic deletion of the *OCRL1* gene [Lin et al., 1997; Monnier et al., 2000; this paper].
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42 *OCRL1* protein analysis

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45 The amount of OCRL1 was estimated in total cell extracts using either the total
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47 protein amount or the β -ATPase signals for normalisation. As shown in Figure 4, patients
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49 harbouring a missense mutation in the *OCRL1* gene (lanes 2-6) had a markedly reduced
50
51 content of OCRL1 when compared to controls (lanes 1, 7, 8, 12). Two bands were revealed
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53 by antibodies, the upper band at \approx 105 kDa corresponds to the native OCRL1 while the lower
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55 band at \approx 75 kDa corresponds to a proteolytic product [Lichter-Konecki et al., 2006]. As
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57 expected, cells harbouring stop and frameshift mutations and characterized by a low mRNA
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59 content showed an almost complete absence of OCRL1 (lanes 9,10,11). For quantification
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3 studies of OCRL1 presented in table 3, both bands were taken in account. Unexpectedly, most
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5 missense mutations resulted in a drastic decrease of the OCRL1 content although their mRNA
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7 content was unaffected (Fig 2). The decrease in OCRL1 was observed whether missense
8
9 mutation mapped to the central phosphatase domain or to the C-terminal Rho-Gap domain
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11 (Fig 4). This indicated that missense mutations not only could affect the catalytic properties
12
13 of OCRL1 but also could affect the stability of the protein. Noticeably, expression of the c.40-
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15 14A>G resulted in the presence of a protein doublet at ≈ 80 kDa with no detectable bands at
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17 100 or 75kDa (Fig 3, panel B, lane 2).
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26 **Clinical expression of mutations**

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28 All but two of the 124 male patients that presented with classical features of the
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30 disease i.e. congenital bilateral cataract, central nervous system symptoms and kidney
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32 troubles harboured a mutation in the *OCRL1* gene. However as no cDNA analysis could be
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34 performed for these two patients, we cannot exclude the presence of a mutation not detected
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36 by genomic sequencing. At contrast, no mutation in the *OCRL1* gene was identified in the 36
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38 additional male patients presenting with an incomplete panel of oculocerebrorenal symptoms
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40 although highly suggestive of Lowe syndrome. When tested PI(4,5)P₂ phosphatase activity
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42 also excluded the diagnosis of Lowe syndrome in these patients.
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48 6 out of the 41 patients included on the basis of diagnosis of a Dent disease not
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50 associated to the *CLC5* gene carried a mutation of the *OCRL1* gene. None of these 6 patients
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52 had ocular or CNS symptoms.
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55 A few mutations that mapped to the same amino acid or to amino acids located at
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57 close vicinity were identified in patients addressed either for diagnosis of Lowe syndrome or
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59 Dent disease (Table 4). The p.Ile274Thr initially described in a patient presenting with the
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3 phenotype of Dent disease [Utsch et al., 2006] was identified in two families, one presenting
4 with a classical Dent disease and one diagnosed as a Lowe syndrome family in which the two
5 affected brothers had mental retardation and developmental with one of them having a
6 congenital cataract. Likewise, the p.Arg318Cys mutation previously reported in 2 families
7 with Dent disease [Hoopes et al., 2005; Sekine et al., 2007] was identified in a patient
8 presenting with Dent disease and in another patient initially diagnosed as Lowe syndrome
9 patient on the basis of renal and CNS symptoms although ocular symptoms at 24 y of age
10 were restricted to a severe myopia. Three mutations mapped to amino acids closely located in
11 the Rho-GAP domain i.e. p.Ala797Pro, p.Pro799Leu and p.Pro801Leu. While all the three
12 mutations were associated with proximal tubulopathy, only two of them (p.Ala797Pro and
13 p.Pro801Leu) were associated with congenital cataract and SNC symptoms, typical features
14 of the Lowe syndrome.
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35 CONCLUSIONS AND DISCUSSION

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38 A major consequence of mutations affecting the *OCRL1* gene was the loss of
39 PI(4,5)P₂ase activity regardless of the Lowe or Dent-2 clinical phenotypes associated with
40 mutations in patients and regardless of the pathogenic mechanisms.
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45 Nonsense mutations, genomic deletions, frameshift or splicing mutations leading to
46 the occurrence of a premature termination codon represent 2/3 of the *OCRL1* mutations
47 identified to date. Expression studies of these mutations indicated that all but one of the
48 mutations tested resulted in an almost complete absence of mRNA and protein (Fig. 2 and
49 table 3).
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58 Interestingly, the splicing c.40-14G>A mutation allowed an apparently normal
59 quantitative production of mRNA. However and as shown in Fig. 3 (panel A) the mutation led
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3 to the synthesis of two abnormal transcripts lacking the normal initiation codon in exon 1 (see
4
5 supplementary Fig. S2). As shown in panel B of Fig. 3 two peptides with a molecular weight
6
7 close to 80 kDa were evidenced in fibroblasts harbouring the c.40-14 A>G mutation. Based
8
9 on bioinformatics analyses, it has been recently proposed that a differential splicing of the
10
11 *OCRL1* gene associated with the possible use of an alternative initiation of translation in exon
12
13 8 that would allow the synthesis of a shortened OCRL1 might occur in different tissues and at
14
15 different developmental stages [Schrimpton et al., 2009]. Remarkably, frameshift and
16
17 nonsense mutations associated so far with Dent-2 disease were exclusively identified in the
18
19 first 7 exons of the *OCRL1* gene while all frameshift and nonsense mutations causing Lowe
20
21 syndrome mapped to exons 8-23 (Fig 1). There are only three methionyl residues in the
22
23 sequence spanning exon 3 to 10 at position 158, 187 and 206, respectively in exons 7 and 8,
24
25 that will keep the *OCRL1* reading frame. As suggested by Schrimpton [Schrimpton et al.,
26
27 2009], use of these methionyl residues as alternative initiation codons will allow the
28
29 synthesis of 85.425 kDa, 82.159 kDa and 79.976 kDa peptides respectively, molecular
30
31 weights compatibles with the observed bands at ≈ 80 kDa in Fig 3. Such proteins will lack the
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33 PH domain and a major clathrin binding site [Mao et al., 2009] and are likely to display
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35 modified interactions with membranes and other proteins.
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44 While most missense mutations mapped to the phosphatase domain of *OCRL1*
45
46 spanning exons 9-15, 14 missense mutations or in-frame deletion have been identified in the
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48 C-terminal ASH (ASPM, SPD-2, Hydin)-RhoGAP-like domain spanning exons 16 to 23 (Fig
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50 1). Eight mutations mapped to the ASH/Rab binding domain spanning exons 17-19 (amino
51
52 acids 539-752) close to residues that have been shown to modulate Rab binding and targeting
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54 of *OCRL1* to Golgi and endosomes [Hyvola et al., 2006]. The six missense mutations in
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56 exons 20-23 affected amino acids that mapped to a region in close interaction with the ASH
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58 domain [Erdman et al., 2007]. Mutations in exon 21 clustered to a domain showing significant
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3 homologies with GAP related proteins (Supplementary Fig S1). Although interaction with
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5 Rac 1 a member of the Rho family has been reported [Erdman et al., 2007] and low Rac GAP
6
7 activity measured *in vitro* [Faucherre et al., 2003], whether OCRL1 functions as a GAP *in*
8
9 *vivo* is still debated [Lowe, 2005]. Expression studies of the p.I768N and p.A797P mutations
10
11 in COS7 cells suggested that the Rho-GAP like domain is important for the enzymatic
12
13 activity of OCRL1 while being catalytically inactive by itself and showed that this domain
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15 could also interact with small G-proteins of the Arf family [Lichter-Konecki et al., 2006].
16
17 Therefore and as suggested by their effect on activity and OCRL1 content, these last two
18
19 residues might play a critical role for the folding of the protein. The ASH-RhoGAP-like
20
21 domain has been recently shown to interact with the Rab5 effector APPL1 [Erdmann et al.,
22
23 2007; McCrea et al., 2008] and with Ses1 and Ses2, two closely related endocytic proteins
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25 [Swan et al., 2010]. Isothermal titration calorimetry studies performed using peptides
26
27 corresponding to the interacting domains of Ses1 and APPL1 with OCRL1 suggested that
28
29 binding of Ses and APPL1 are mutually exclusive and abolished by the same mutations at
30
31 positions 591, 634, 799, 801 in the ASH-RhoGAP-like domain that also disrupt APPL1
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33 binding. This suggested that Lowe syndrome and Dent disease might result from
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35 perturbations at multiple sites within the endocytic pathway [Swan et al., 2010]. Noticeably,
36
37 most variants affected amino acids conserved among OCRL1 species and INPP5B but much
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39 less conserved among other polyphosphate phosphatases. This may suggest that interaction
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41 with Rab proteins is a specific feature of OCRL1 and INPP5B.
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51 The decrease of the PI(4,5)P₂ase activity in mutant cells harbouring a missense
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53 mutation was usually associated with a deleterious effect of the missense mutations on
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55 phosphatase activity, whether they mapped to the catalytic PI(4,5)P₂ase domain or to the Rho-
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57 GAP domain [Lin et al., 1997., Hyvola et al., 2006., Schrimpton et al., 2009]. However and as
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59 illustrated in Fig. 4 and Table 3, the consequence of missense mutations is mostly a decrease
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3 of the amount of OCRL1 present in the cell. This is likely to result from an abnormal
4
5 processing of the protein since OCRL1 mRNA levels were normal in the cells harbouring
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7 these missense mutations (Fig. 2). The protein degradation might be the consequence of the
8
9 activation of endoplasmic reticulum-associated degradation (ERAD) or of unfolded protein
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11 response (UPR) as an answer to the accumulation of unfolded or misfolded mutant OCRL1 in
12
13 the endoplasmic reticulum [Schröder and Kaufman., 2005]. Conclusions of functional studies
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15 based on expression mutant OCRL1 in cellular models must thus take in account protein
16
17 degradation processes when analysing the physiological relevance of the different mutations.
18
19 Nevertheless, a small amount (20-30%) of OCRL1 was detected in a few mutant cells
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21 although PI(4,5)P₂ase activity was almost totally absent. In these situations, the overall
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23 decrease of PI(4,5)P₂ase activity is likely to result from two processes: a decrease of the
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25 amount of OCRL1 through protein degradation and a catalytic inactivation. A few missense
26
27 mutations e.g. p.Phe242Ser, p.Ala797Pro resulted in the presence of both a small amount of
28
29 protein and a residual PiP₂ase activity. Noticeably, although the Lowe patients harbouring
30
31 these mutations presented with classical eye and kidney features, their neurological symptoms
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33 were less severe and their degree of mental retardation was moderate. This may reflect a
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35 differential dependence of tissues or of developmental stages toward PI(4,5)P₂ homeostasis.
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37 Differences in clinical expression that were observed between patients harbouring different
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39 mutations might thus reflect variability in protein synthesis and/or catalytic properties in the
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41 different tissues depending on the nature of the mutation beside variability of the individual
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43 genetic background.
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53 Mutations of the *OCRL1* gene have been associated with two distinct clinical
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55 phenotypes i.e. Lowe syndrome and Dent disease. Although apparently undistinguishable
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57 from the Dent phenotype associated to the *CLCN5* gene, Dent phenotype associated with
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59 OCRL1 mutations has been referred as Dent-2 disease to specify the genetic cause of the
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3 disease and may represent a mild variant of Lowe syndrome [Bökenkamp et al., 2009].
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5 Accordingly, it has been proposed that a phenotypic continuum exists between Dent-2 disease
6
7 and Lowe syndrome. This continuum was not only observed between patients harbouring
8
9 different OCRL1 mutations but also occurred between patients harbouring the same mutation
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11 as presented in table 4. Clinical phenotypes range from patients affected by severe Lowe
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13 syndrome with typical ocular, neurological and renal features to Dent-2 patients presenting
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15 only with renal impairment and comprise atypical forms of Lowe syndrome presenting with
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17 incomplete eye symptoms or moderate neurological troubles. Presence of a residual
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19 PI(4,5)P₂ase activity has been documented during this study in patients presenting usually
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21 with a moderate clinical expression. However, it must be noted that ocular and neurological
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23 symptoms were documented even in Lowe patients showing a residual PI(4,5)P₂ase activity
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25 up to 20% of the normal values in their fibroblasts. On the other hand, it must be kept in mind
26
27 that mild and severe presentations of Lowe syndrome together with pure real forms of Dent
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29 disease were associated with a complete loss of the OCRL1 enzymatic activity. A possible
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31 explanation of this clinical variability might be the presence of modifying factors
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33 (compensatory phosphatases, interacting proteins...) whose expression will depend on the
34
35 genetic background of the different patients. The main clinical difference observed between
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37 two brothers presenting with a Lowe syndrome and harbouring the same p.Ile274Thr
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39 mutation e.g. a documented unilateral cataract for the youngest and no signs of cataract for
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41 the eldest one would thus likely result from such modifying factors. Along this line we have
42
43 investigated a possible role of INPP5B, a phosphatase. No significant variations in the
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45 INPP5B content could be evidenced when comparing fibroblast extracts originating from
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47 healthy controls or patients affected either by Lowe syndrome or Dent-2 disease. This was in
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49 agreement with our data showing that no significant differences of the PiP₂-ase activity could
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51 be evidenced between Lowe syndrome and Dent-2 disease patients (table 2).
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3 The report of truncated forms of OCRL1 that might result from the use of alternative
4 initiation codons open also new perspectives to explain clinical variability associated with
5 OCRL1 mutations. However these investigations were performed on skin fibroblasts and
6 using antibodies that may not recognize all forms of truncated or alternatively spliced
7 OCRL1. It will be very important to extend these studies to other cell types that are more
8 affected by the disease e.g. renal and ocular cells and to use a larger panel of antibodies
9 directed against different domains of the protein in order to gain new insights regarding the
10 differential pathological mechanisms leading to the different clinical phenotypes.
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LEGENDS OF FIGURES

Figure 1. Mapping of mutations associated with Lowe syndrome and Dent's disease in the *OCRL1* gene.

Exons, including the alternatively spliced exon 18a, are numbered 1 to 23 and are drawn to scale at the exception of the non-coding sequence of exon 23. The catalytic phosphatase domain is indicated in black, the PH domain in hatched, the ASH-Rab binding domain in light grey, the Rho-GAP like domain as a punctuated pattern and the 5' and 3' untranslated regions are shown in dark grey. LIDIA and LIDLE refer to motives involved in clathrin binding [Mao et al., 2009] and FEDNF to amino-acids involved in AP-2 clathrin adaptator binding [Ungewickell et al., 2004]. Frameshift and nonsense mutations are indicated using their cDNA numbering above the schematic representation of the cDNA while missense mutations and in frame deletions are presented below the cDNA and designated by their protein numbering. Mutations indicated in bold correspond to nonsense mutations. Stars refer to intronic mutations leading to splicing defects. Horizontal bars indicated gross genomic deletions.

Figure 2. Quantification of mRNA *OCRL1* expression in patients harbouring a mutation of the *OCRL1* gene.

OCRL1 and *GAPDH* mRNAs were isolated from fibroblasts and quantified as described in Material and Methods. Horizontal black bar represent the mean and upper and lower boxes the 2 SD limits. Arrow points to the ratio value measured in a patient affected by Dent Disease and harbouring a c.40-14G>A mutation.

Figure 3. mRNA and protein expression of the c.40-14 A>G mutation.

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3 Panel A. cDNA obtained from fibroblasts harbouring the c.40-14A>G mutation was amplified
4 using primers allowing amplification of a sequence encompassing exons 1, 2 and 3.
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7 Amplification products were analyzed using a 2% agarose gel. Amplification of the mutant
8 cDNA (lane 1) generated two bands respectively long of 288 and 391 bp (arrows) while
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10 amplification of the normal sequence (lane 2) generated a 488 bp fragment as expected.
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14 Panel B. Western blotting of fibroblast extracts was performed as described in Material and
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16 Methods. Lanes 1, 4: control fibroblasts; lane 2: c.40-14A>G mutant fibroblasts; lane 3:
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18 p.Ile274Thr mutant fibroblasts.
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24 Figure 4. Western blot analysis of OCRL1 in control and patient fibroblasts.
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27 Fibroblasts extract (25 µg) prepared as described in Material and Methods was loaded in each
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29 lane. Upper panel: western blot analysis using polyclonal antibodies directed against OCRL1.
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31 Lane M: molecular weight markers, lanes 1, 7, 8, 12 : control patients, lane 2: p.Glu468Gly,
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33 lane 3: p.Arg318Cys, lane 4: p.Asn373Tyr, lane 5: p.Pro801Cys, lane 6: p.Pro799Leu, lane
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35 9: p. Arg810Stop, lane 10: c.2469+2T>G, lane 11: c.825-2A>G. Lower panel: protein loading
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37 in the different lanes was demonstrated by a western blot analysis of the same blot with the
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39 use of antibody against the β subunit of the mitochondrial ATPase .
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47 Figure S1. Conservation of residues located in the phosphatidylinositol phosphatase catalytic
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49 domain and in the Rab and Rho-GAP domains of OCRL1 and related proteins.

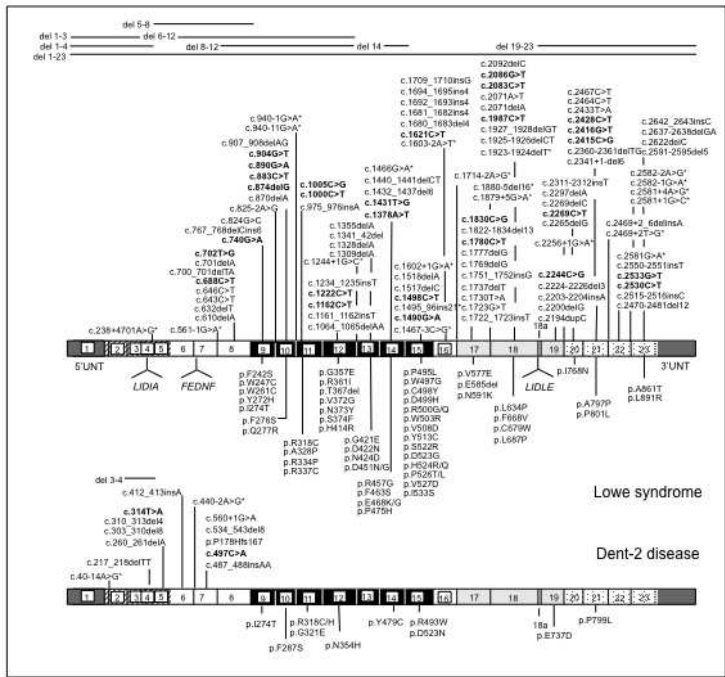
50
51 Amino acids alignment is given with identical residues shown by dashes. Mutated residues
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53 are shown in bold. Numbering of the amino-acids refer to the human OCRL1 sequence
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55 (ENSP00000360154). Upper amino acids represent the missense mutations identified in
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57 patients affected by Lowe syndrome and Dent disease (underlined character). Sequences were
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59 obtained from SwissProt and Genbank with the following accession number. OCRL1: H.
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3 sapiens Q01968 , M. musculus Q6NVF0, C. elegans NM 060183, D. melanogaster NP
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5 569962; INPP5B: NP005531; INPPL1: NP001558; SHIP: NP001017915; Synaptojanin:
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7 NP003886; ARHGAP10: AAI09030; RHOGAP4: NP001657; Oligophrenin: O60890;
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9 RACGAP1: AAH32754 .
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15 Figure S2. Schematic representation of the splicing consequences of the c.40-14G>A
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17 mutation.
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20 Element involved in the splicing (donor and acceptor sites) of normal and mutant exons 1, 2
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22 and 3 are indicated in the sequences (bold character). The c.40-14A>G mutation (underlined
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24 character) revealed a cryptic donor site in exon 1 located in position -82 that was used for
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26 both mutated transcripts. In mutant transcript #1 splicing used a cryptic acceptor site of intron
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28 1 at position -24 while exon 2 was completely skipped in mutant transcript #3. This
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30 representation was based on sequencing data obtained after cloning of the different transcripts
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32 that have been evidenced.
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275x190mm (72 x 72 DPI)

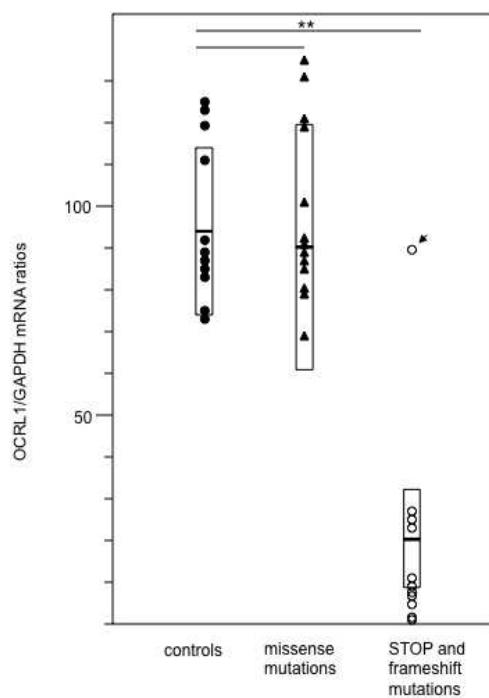


Figure 2
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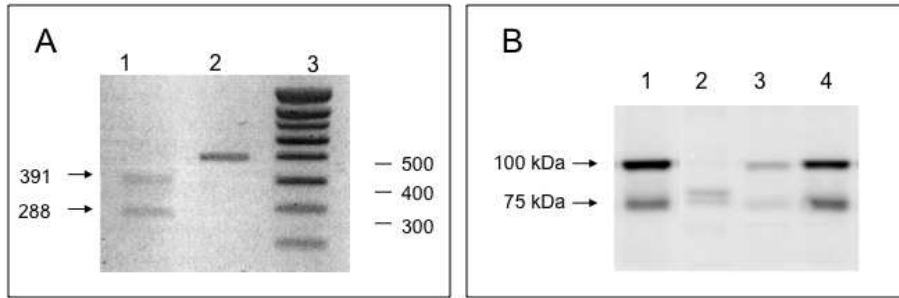


Figure 3
254x190mm (72 x 72 DPI)

review

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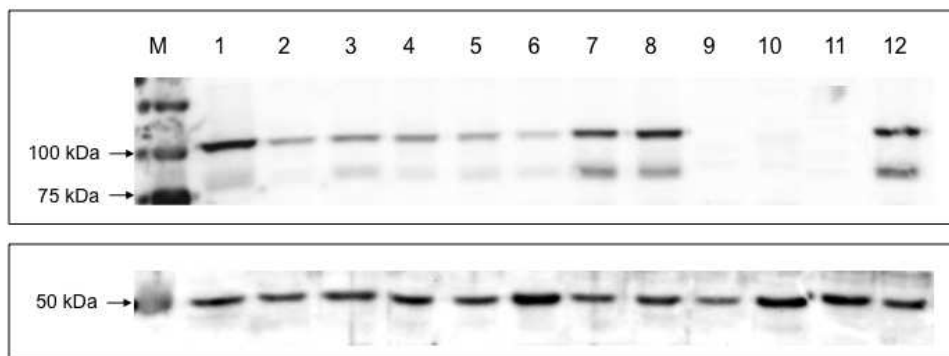


Figure 4
254x190mm (72 x 72 DPI)

review

Human Mutation

Patient ID	Exon	Nucleotide change ^a	Protein change ^b	Enzyme activity ^c	Disease status	Age ^d	Ocular symptoms ^e		Neurological symptoms ^e			Renal symptoms ^e						
							CC	G	NH	DD	MR	FS	PT	RF	AA	LMWP	P	Ca
05den10	1	c.40-14A>G	Splicing defect	1.6	Dent	7	-		-						+		+	
09ls009	i1	c.238+4701A>G	Splicing defect		Lowe	7	+			+			+			+		
04ls15	8	c.632delT	p.Val211AspfsX38		Lowe	6	+	+	+	+	+/-				+		+	
06ls37	8	c.688C>T	p.Arg230X		Lowe	2	+		+	+			+		+			
05ls26	8	c.702T>G	p.Tyr234X		Lowe	1												
02ls42	9	c.725T>C	p.Phe242Ser	2.6	Lowe	30	+	+	+	+	+		+			+		+
02ls25 ^f	9	c.821T>C	p.Ile274Thr		Lowe	43	-			+	+/-			+		+		
02ls26 ^f				Lowe	34	+		+	+/-		+		+					
08ls008	i9	c.825-2A>G	Splicing defect		Lowe	17	+			+	+		+			+		
06ls59	10	c.830A>G	p.Gln277Arg		Lowe	7	+			+	+		+		+	+	+	
06ls54	11	c.952C>T	p.Arg318Cys		Lowe	23	-	-	+	+	+	+	+	+	+		+	+
07den01				Dent	7	-		-									+	+
10ls011	11	c.1005C>G	p.Tyr335X		Lowe	5	+	-	+	+	+		+			+	+	
06ls32 ^g	11	c.1009C>T c.1082G>T	p.Arg337Cys p.Arg361Ile	1.4	Lowe	1	+		+							+	+	
04den01	12	c.1060A>C	p.Asn354His		Dent	27	-		-									+
98ls44	12	c.1115T>G	p.Val372Gly		Lowe	22	+	+		+		+	+					
06ls15	12	c.1117A>T	p.Asn373Tyr	1.4	Lowe	13	+		+	+	+	+	+		+	+		
98ls48	12	c.1121C>T	p.Ser374Phe		Lowe	3	+		+	+		+	+		+	+	+	
10ls016 ^f	12	c.1241A>G	p.His414Arg		Lowe	2	+	+	+	+	+	+				+	+	+
10ls017 ^f				Lowe	5	+	+	+	+	+	+	+					+	+
09ls014	13	c.1341_1342delCT	p.Leu448Glu fsX17		Lowe	1	+		+			+				+	+	
03ls38	13	c.1351G>A	p.Asp451Asn		Lowe	30	+	+		+		+	+					
00ls37	13	c.1355delA	p.Gln452Arg fsX1		Lowe	12	+			+								
00ls03	14	c.1369C>G	p.Arg457Gly	0,6	Lowe	20	+			+	+	+						
06ls17	14	c.1378A>T	p.Lys460X		Lowe	1	+	+	+		+	+	+		+	+	+	
03ls35	14	c.1402G>A	p.Glu468Lys		Lowe	5	+		+		+	+	+			+	+	
07ls20	14	c.1403A>G	p.Glu468Gly		Lowe	5	+		+	+		+				+	+	
04ls11	14	c.1440-1441delCT	p.Asp463Asp fsX1		Lowe	0.1	+	+	+			+				+	+	
06ls19	i14	c.1467-3C>G	Splicing defect		Lowe	7	+								+		+	
02ls50	15	c.1484C>A	p.Pro495Leu	1.8	Lowe	3	+		+				+			+		
04ls05	15	c.1495G>C	p.Asp499His		Lowe	5	+		+	+		+						
06ls18	15	c.1507T>C	p.Trp503Arg		Lowe	0.4	+	+	+	+					+			
05ls21	16	c.1681_1682insGACT	p.Phe561X		Lowe	2	+	+	+	+	+	+	+		+		+	
00ls29	16	c.1692_1693insCCTT	p.Leu565Pro fsX11		Lowe	12	+	+		+	+	+						
07ls20	17	c.1773C>A	p.Asn591Lys		Lowe	13	+	+	+	+	+				+	+		
03ls23	17	c.1780C>T	p.Gln594X		Lowe	0.9	+		+	+	+	+					+	
04ls21	i17	c.1879+5G>A	Splicing defect	0.3	Lowe	32	+				+/-							

00ls23	18	c.1927_1928delGT	p.Val643AsnfsX8	1.8	Lowe	0.3	+		+	+			+		+	+			
02ls35	18	c.2086G>T	p.Glu696X		Lowe	20	+			+	+		+			+		+	
07ls10	18	c.2092delC	p.Pro681LeufsX66		Lowe	dead	+		+										
08ls011	19	c.2194dupC	p.Leu732PfsX38		Lowe	1	+	+	+	+	+	+	+			+			
02ls11	19	c.2200delG	p.Val734PhefsX8		Lowe	6	+		+			+							
08ls15	19	c.2224_2226delGTA	p.Val742del		Lowe	10	+	+	+	+	+	+	+	+	+	+	+	+	
98ls54	20	c.2269C>T	p.Gln757X		Lowe	23	+		+	+	+	+	+/-	+	+	+	+	+	
05ls29	20	c.2311-2312insT	p.Cys771LeufsX8		Lowe	7	+		+	+	+	+	+				+	+	
09ls001					Lowe	2	+		+	+				+			+		
04ls17	21	c.2464C>T	p.Arg822X		Lowe	2	+	+	+	+				+			+		
05ls17	i21	c.2469+2T>G	Splicing defect	0.3	Lowe	1	+		+	+	+/-		+	+			+		
01ls19	i22	c.2581+1G>C	Splicing defect		Lowe	1.5	+	+											
03ls14	i22	c.2582-1G>A	Splicing defect		Lowe	23	+					+		+				+	
06ls65	i22	c.2582-2A>G	Splicing defect		Lowe	6	+		+	+	+	+	+	+			+	+	
08ls03	23	c.2591_2595del6	p.Gln879_Thr880>HisfsX4		Lowe	28	+	+	+	+	+		+			+	+		
02ls08	23	c.2637_2638del	p.Gln862HisfsX3		Lowe	12	+				+								
09ls013	23	c.2672T>G	p.Leu891Arg		Lowe	10	+		+	+			+		+				
06den06	3-4	del exon 3-4	Frameshift deletion		Dent	9	-		-	-	-			+			+		+
00ls05	19-23	del exons 19-23	Frameshift deletion	1.8	Lowe	30	+		+	+	+	+	+						

^a Numbering was based on the cDNA sequence with +1 corresponding to the A of the ATG initiation codon of translation in the reference sequence.

^b Expected consequences of the mutations with codon 1 corresponding to the ATG initiation codon of translation.

^c Enzyme activity measured in controls was N = 9.6 +/- 2.3 nanomoles PI(4,5)P₂ hydrolyzed/min/mg

^d Age at the time of the molecular investigation

^e CC: congenital cataract, G: glaucoma, NH: neonatal hypotonia, DD: developmental delay, MR: mental retardation, FS: Fanconi syndrome, PT : proximal tubulopathy, RF: renal failure, AA: amino-aciduria, LMWP: low molecular weight proteinuria; P: hypophosphatemia/hyperphosphaturia, Ca : hypercalciuria.

(+) sign present, (-) sign absent, () sign not documented at the time of the last examination

^f Patients 02ls25 and 02ls26, and 10ls06 and 10ls017 are brothers

^g Variants c.1009C>T and c.1082G>T are on the same allele

Table 1 : Novel mutations in the OCRL1 gene

	enzyme activity ^a (nanomol PI(4,5)P ₂ hydrolyzed mg ⁻¹ min ⁻¹) (+/- SEM)
Patients with missense mutations ^b	1.39 +/- 0.99 (n = 12)
Patients with nonsense or frameshift mutations ^b	1.54 +/- 0.63 (n = 11)
Patients with no OCRL1 mutation identified ^c	9.43 +/- 1.47 (n = 12)
Control individuals	9.62 +/- 2.31 (n=13)

^a n value represent the number of independent patients tested, all measurements

^b 19 patients were affected by Lowe syndrome, 4 presented with Dent-2 disease

^c these patients were addressed to the laboratory for molecular and biochemical investigations. on the basis of suspicion of Lowe syndrome

Table 2. PI(4,5)P₂ phosphatase activity in patients with OCRL1 mutations

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	OCRL1 / β ATPase ^a relative units (+/- SEM)
Patients with nonsense or frameshift mutations	0.05 +/- 0.02 (n = 11)
Patients with missense mutations	0.35 +/- 0.14 (n = 12)
Control individuals	1.70 +/- 0.36 (n = 13)

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^a n value represent the number of independent patients tested

Table 3. OCRL1 content in fibroblasts originating from patients with OCRL1 mutations

Patient ID	Clinical diagnosis ^a	Mutation	Age ^b	PiP ₂ ase activity ^c	Ocular symptoms	SNC symptomss	Renal symptoms
98ls18	Lowe	del exons 1-4	3		Congenital bilateral cataract	Neonatal hypotonia, convulsions	Fanconi syndrome
06den06	Dent	del exon 3-4	14		None	None	LMW proteinuria, hypercalciuria
02ls25 ^d	Lowe	p.Ile274Thr	43		None	Moderate mental retardation allowing an autonomous life	Nephrotic syndrome, proteinuria, renal failure
02ls26 ^d	Lowe	p.Ile274Thr	34		Congenital unilateral cataract	Moderate mental retardation allowing an autonomous life	Renal failure, proteinuria
05den11	Dent	p.Ile274Thr	2		None	Developmental delay	Proximal tubulopathy, proteinuria, hypercalciuria
06ls59	Lowe	p.Arg318Cys	24		Severe myopia	Neonatal hypotonia, severe mental retardation	Fanconi syndrome, proteinuria, hyperphosphaturia, renal failure
07den01	Dent	p.Arg318Cys	4		None	None	Tubular proteinuria, hypercalciuria
98ls80	Lowe	p.Ala797Pro	57	2.9	Congenital bilateral cataract	Moderate mental retardation and mild psychomotor troubles	Fanconi syndrome
04den05	Dent	p.Pro799Leu	21	0.8	None	None	LMW proteinuria, hypercalciuria, nephrocalcinosis, hyperphosphaturia, aminoaciduria
06ls27	Lowe	p.Pro801Leu	1	1.6	Congenital bilateral cataract	Neonatal hypotonia, moderate developmental delay, no psychomotor troubles	Glomerular and tubular proteinuria

^a initial clinical diagnosis

^b age at the time of the molecular investigation

^c enzyme activity measured in controls was N = 9.6 +/- 2.3 nanomoles PI(4,5)P₂ hydrolyzed/min/mg

^d patients 02ls25 and 02ls26 are brothers

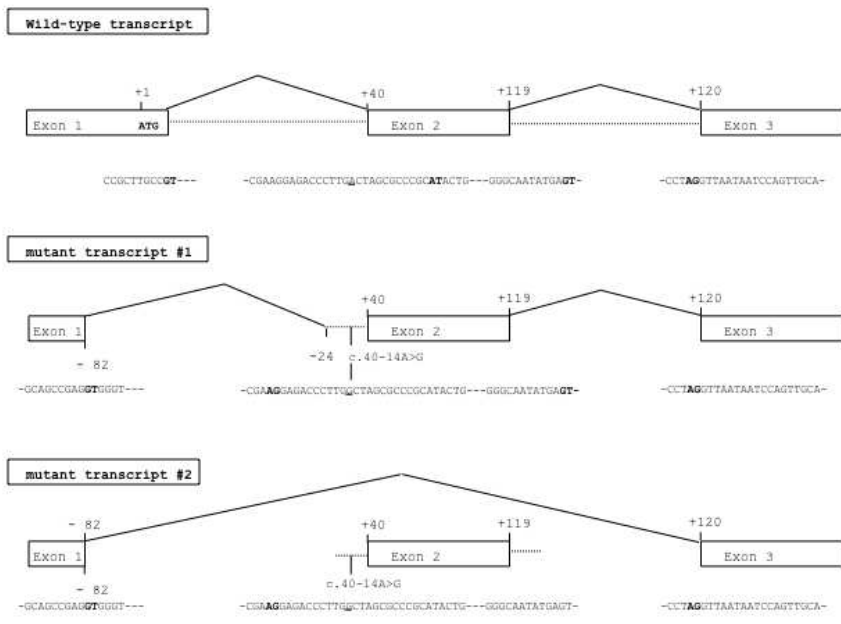
Table 4. Clinical expression of OCRL1 mutations in patients affected by Lowe syndrome or Dent disease

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	EXON 17			EXON 18				
		E	del	K	V	W	P	
OCRL1 H. sapiens	562	LPSLELSRREFVFEN	VKFRQLQK	KFPQISNNGQ	662	DRGKDYFLTIS	682	SCFGTSLLEALCR
OCRL1 M. musculus		-----F-----				-----G		
OCRL1 C. elegans		--QVQ--LN--VD--GI--NYLEPKTRSVIVQ--V--K				---R---IP--T		----ST--K--L5
INPP5B H. sapiens		I--VS--K--C--Q--YM--KV--S--T--H--				-----SVS		----SPIHT--Y
INPPL1 H. sapiens		IEAIVKTASRTH-F.IE-YSTCL-EYKH-FEND				GTRKRELYEW--		AGAKSKAPSVS-
SHIP H. sapiens		CYATLKTKSQTK-V.LE-HSSCL-S-VK-QE-E				KTRKRELYDFVK		SSGPKTKRS-TS
Synaptojanin H. sapiens		-V-IKS-LP-NN-FDDALIDELQQ-ASFGEVI				QPS555G-GT5		PCQSPITISEGFV
		EXON 20			EXON 21			
		N			P L L			
OCRL1 H. sapiens	765	LQQI IDC	787	VAEALLIFLEAL	EPVICYELY			
OCRL1 M. musculus		-----						
OCRL1 C. elegans		FNR-RSA			LYS--IRL-DS-D--I-LEGMF			
INPP5B H. sapiens		FEM-R--			-----L--S-----ST-			
ARRGAP10 H. sapiens		-MDVNT-			ITS--KQY-RS---LMT---H			
RHOGAP4 H. sapiens		FERGEPD			--GV-KLYFRS-EP-LFPPD-F			
Oligophrenin H. sapiens		FFDPKCP			ITSS-KFY-RN-S---MT-R-H			
RACGAP1 H. sapiens		FLRVNTV			ICSL-KD--RN-K--LLTFR-N			

Supplementary Figure 1
254x190mm (72 x 72 DPI)

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Supplementary Figure 2
254x190mm (72 x 72 DPI)

Review

exon	Previous numbering ^a	Corrected numbering ^b
1	to -13	-169 to 39
2	-12 to 68	40 to 119
3	69 to 148	120 to 199
4	149 to 187	200 to 238
5	188 to 298	239 to 349
6	299 to 388	350 to 439
7	389 to 509	440 to 560
8	510 to 670	561 to 722
9	671 to 773	723 to 824
10	774 to 888	825 to 939
11	889 to 1004	940 to 1056
12	1005 to 1193	1057 to 1244
13	1194 to 1304	1245 to 1356
14	1305 to 1415	1357 to 1466
15	1416 to 1551	1467 to 1602
16	1552 to 1662	1603 to 1713
17	1663 to 1828	1714 to 1879
18	1829 to 2064	1880 to 2115
18a	2065 to 2088	2116 to 2140
19	2089 to 2205	2141 to 2256
20	2206 to 2290	2257 to 2341
21	2291 to 2418	2342 to 2469
22	2419 to 2530	2470 to 2581
23	2531 to 4935	2582 to 4986

^a Previous numbering [Attree et al.,1992; LSMD, 2010]

^b Conversion of previous numbering using the ATG start codon of exon 1 (+51 bp), bold numbers correspond to exons for which boundaries have been corrected

Supplementary table 1. Numbering of exon boundaries

Exon Intron	Nucleotide change ^a	Protein change ^b	Enzyme activity N = 1 ^c	Protein ^d	Number of patients ^e	Geographical origin ^f	Disease status	References
1	c.40-14A>G	Splicing defect	0.17		1	France	Dent-2	This paper
4	c.217_218delTT	p.Leu73AspfsX1			1	North-America	Dent-2	Schimpton et al., 2009
i4	c.238+4701A>G	Splicing defect			1	UK	Lowe	This paper
5	c.260_261delA	p.Gln87ArgfsX88			2	Turkey	Dent-2	Utsch et al., 2006
5	c.303_310del8	p.Glu101GludefX27			1	Japan	Dent-2	Sekine et al., 2007
5	c.310_313delTGTT	p.Cys104X	0.10	-	1	Germany	Dent-2	Hoopes et al., 2005
5	c.314T>A	p.Leu105X			1	Italy	Dent-2	Tosetto et al, 2009
6	c.412_413insA	T138 AsnfsX122			2	Germany	Dent-2	Utsch et al., 2006
i6	c.440-2A>G	Splicing defect	0.11	-	1	Germany	Dent-2	Hoopes et al., 2005
7	c.487_488insAA	p.K163KfsX2	0.09	-	1	Germany	Dent-2	Hoopes et al., 2005
7	c.497C>G	p.Ser166X			1	North-America	Dent-2	Schimpton et al., 2009
7		p.Pro178HisfsX167			1	Italy	Dent-2	Tosetto et al, 2009
7	c.534_543del	p.Pro178ProfX3			1	North-America	Dent-2	Schimpton et al., 2009
7	c.560+1G>A	p.Met187IlefsX1			1	North-America	Dent-2	Schimpton et al., 2009
i7	c.561-1G>A	Splicing defect	0.10		1	North-America	Lowe	LSMD, 2010
8	c.610delA	p.Gln204ThrfsX45 ^d			1	Italy	Lowe	Addis et al., 2004
8	c.632delT	p.Val211AspfsX38			1	Spain	Lowe	This paper
8	c.643C>T	p.Gln215X			1	North-America	Lowe	LSMD, 2010
8	c.646delT	p.Ser216LeufsX39			1	ni	Lowe	LSMD, 2010
8	c.688C>T	p.Arg230X			1	Australia	Lowe	This paper
8	c.700_701delTA	p.Tyr234CysfsX21			1	ni	Lowe	LSMD, 2010
8	c.701delA	p.Tyr234LeufsX15			1	ni	Lowe	LSMD, 2010
8	c.702T>G	p.Tyr234X			1	Spain	Lowe	This paper
9	c.725T>C	p.Phe242Ser	0.13	++	1	France	Lowe	This paper
9	c.740G>A	p.Trp247X			1	ni	Lowe	LSMD, 2010
9	c.741G>T	p.Trp247Cys			1	Italy	Lowe	Addis et al., 2004
9	c.767_768delCinsAGACTT	p.Ser256_Gly257delinsfsX10			1	North-America	Lowe	LSMD, 2010
9	c.783G>C	p.Trp261Cys			1	ni	Lowe	LSMD, 2010
9	c.814T>C	p.Tyr272His			1	North-America	Lowe	LSMD, 2010
9	c.821T>C	p.Ile274Thr			1	France	Lowe	This paper
9	c.821T>C	p.Ile274Thr			2	Germany, France	Dent-2	Utsch et al., 2006 ; this paper
9	c.824G>C	Splicing defect / p.Gly275Ala			1	France	Lowe	Monnier et al., 2000
i9	c.825-2A>G	Splicing defect			1	French	Lowe	This paper
10	c.827T>C	p.Phe276Ser	0.05	+++	1	Morocco	Lowe	Chabaa et al., 2006
10	c.830A>G	p.Gln277Arg			1	India	Lowe	Sehti et al., 2008
10	c.860T>C	p.Phe287Ser			1	Italy	Dent-2	Tosetto et al, 2009
10	c.870delA	p.Glu290AspfsX2				ni	Lowe	LSMD, 2010

10	c.874delG	p.Val292X	0,05	-	1	North-America	Low	Lin et al., 1997
10	c.883C>T	p.Gln295X			1	North-America	Low	Lin et al., 1997
10	c.890G>A	p.Trp297X			1	Germany	Low	LSMD, 2010
10	c.904G>T	p.Glu302X			1	India	Low	Sehti et al., 2008
10	c.907_908delAG	p.Arg303ArgfsX9			1	France	Low	Monnier et al., 2000
i10	c.940-11G>A	Splicing defect	0,13-0.17	-	5	China, France Norway, Japan	Low	Chou et al., 2005 ; this paper
i10	c.940-1G>A	Splicing defect			1	ni	Low	Lin et al., 1998
11	c.952C>T	p.Arg318Cys	0.16-0.18	+++	3	Germany, France Japan	Dent-2	Hoopes et al., 2005 ; Sekine et al., 2007 ; this paper
11	c.952C>T	p.Arg318Cys			1	France	Low	This paper
11	c.953G>A	p.Arg318His			1	North-America	Dent-2	Schimpton et al., 2009
11	c.962G>A	p.Gly321Glu			1	North-America	Dent-2	Schimpton et al., 2009
11	c.975-976insA	p.Leu325LeufsX14			1	France	Low	Satre et al., 1999
11	c.982G>C	p.Ala328Pro			1	ni	Low	Lin et al., 1998
11	c.1000C>T	p.Arg334X			3	Spain, France	Low	Satre et al., 1999 ; Monnier et al., 2000
11	c.1001G>C	p.Arg334Pro			1	ni	Low	LSMD, 2010
11	c.1005C>G	p.Tyr335X			1	France	Low	This paper
11	c.1009C>T	p.Arg337Cys	0.15		1	France	Low	This paper
12	c.1060A>C	p.Asn354His			1	France	Dent-2	This paper
12	c.1064_1014delAA	p.Lys355ArgdelfsX29			1	ni	Low	LSMD, 2010
12	c.1070G>A	p.Gly357Glu			2	France	Low	Monnier et al., 2000
12	c.1082G>T	p.Arg361Ile			1	France	Low	This paper
12	c.1099-1101delACC	p.Thr367del	0.01	+	2	North-America Spain	Low	Lin et al., 1997 ; Satre et al., 1999
12	c.1115T>G	p.Val372Gly			1	France	Low	This paper
12	c.1117A>T	p.Asn373Tyr	0.15	++	1	France	Low	This paper
12	c.1121C>T	p.Ser374Phe			1	France	Low	This paper
12	c.1123C>T	p.His375Tyr	0.0	+	1	North-America	Low	Kubota et al., 1998
12	c.1161_1162insT	p.Gln388SerfsX4			1	ni	Low	LSMD, 2010
12	c.1162C>T	p.Gln388X	0.12	-	3	North-America Japan, Germany	Low	Lin et al., 1997 ; Kubota et al., 1998 ; Röschinger et al., 2000
12	c.1222C>T	p.Gln408X			2	Japan	Low	Kubota et al., 1998
12	c.1234_1235insT	p.Met412IlefsX4			1	India	Low	Sehti et al., 2008
12	c.1241A>G	p.His414Arg			2	Kosovo	Low	This paper
i12	c.1244+1G>C	Splicing defect			1	Italy	Low	Addis et al., 2004
13	c.1262G>A	p.Gly421Glu	0.05-0.19	+	4	Canada, Switzerland	Low	Monnier et al., 2000 ; this paper
13	c.1264G>A	p.Asp422Asn			1	ni	Low	LSMD, 2010
13	c.1270A>G	p.Asn424Asp			1	France	Low	Monnier et al., 2000
13	c.1309delA	p.Ser437ValfsX12			1		Low	LSMD, 2010

1	13	c.1341_1342delCT	p.Leu448Glu	X17			1	France	Low	This paper
2	13	c.1328delA	p.Asp443Ala	X6			1	France	Low	Monnier et al., 2000
3	13	c.1351G>A	p.Asp451Asn				1	France	Low	This paper
4	13	c.1352A>G	p.Asp451Gly		0.02	+	1	North-America	Low	Lin et al., 1997
5	13	c.1355delA	p.Gln452Arg	X2			1	Australia	Low	This paper
6	14	c.1369C>G	p.Arg457Gly		0,06	+/-	1	Canada	Low	This paper
7	14	c.1378A>T	p.Lys460X				1	Spain	Low	This paper
8	14	c.1387T>C	p.Phe463Ser				1	North-America	Low	Lin et al., 1997
9	14	c.1402G>A	p.Glu468Lys				1	France	Low	This paper
10	14	c.1403A>G	p.Glu468Gly			++	1	France	Low	This paper
11	14	c.1424G>A	p.Pro475His				1	China	Low	Chou et al., 2005
12	14	c.1431T>G	P.Tyr477X				2	Italy	Low	Addis et al., 2004
13	14	c.1432_1437del6	p.Lys479_Tyr480del				1	Germany	Low	Röschinger et al., 2000
14	14	c.1436A>G	p.Tyr479Cys		0.13	++	1	Germany	Dent-2	Hoopes et al., 2005
15	14	c.1440-1441delCT	p.Asp480Asp	X1			1	Australia	Low	This paper
16	14	c.1466G>A	Splicing defect / p.Ser489Asn				1	France	Low	Monnier et al., 2000
17	i14	c.1467-3C>G	Splicing mutation				1	UK	Low	This paper
18	15	c.1477C>T	p.Arg493Trp				2	Germany, Japan	Dent-2	Utsch et al., 2006 ; Sekine et al., 2007
19	15	c.1484C>A	p.Pro495Leu		0,12	+	1	Belgium	Low	This paper
20	15	c.1489T>G	p.Trp497Gly				1	ni	Low	Schneider et al., 2001
21	15	c.1490G>A	p.Trp497X				1	ni	Low	LSMD, 2010
22	15	c.1493G>A	p.Cys498Tyr				2	France	Low	Monnier et al., 2000
23	15	c.1495G>C	p.Asp499His				1	Norway	Low	This paper
24	15	c.1495-1496ins21	p.Asp499delinsGRVPATCY				1	ni	Low	Böckenhauer et al., 2008
25	15	c.1498C>G	p.Arg500Gly				2	France, ni	Low	Satre et al., 1999
26	15	c.1498C>T	p.Arg500X				5	Serbia, France, India	Low	Monnier et al., 2000 ; this paper
27	15	c.1499G>A	p.Arg500Gln		0.1	+	9	North-America Japan, Germany, France	Low	Lin et al., 1997 ; Kawano et al., 1998 ; Röschinger et al., 2000 ; LSMD, 2010 ; this paper
28	15	c.1507T>C	p.Trp503Arg				1	France	Low	This paper
29	15	c.1517delC	p.Thr506Lys	X13			1	Basque country	Low	Monnier et al., 2000
30	15	c.1518delA	p.Thr506Thr	X13	0.08	-	1	North-America	Low	Lin et al., 1997
31	15	c.1523T>A	p.Val508Asp				1	North-America	Low	Lin et al., 1998
32	15	c.1538A>G	p.Tyr513Cys		0,10	++++	1	North-America	Low	Lin et al., 1998
33	15	c.1566C>G	p.Ser522Arg				1	Japan	Low	Kubota et al., 1998
34	15	c.1567G>A	p.Asp523Asn				1	Italy	Dent-2	Tosetto et al, 2009
35	15	c.1568A>G	p.Asp523Gly				1	ni	Low	LSMD, 2010
36	15	c.1571A>G	p.His524Arg		0.03	++	1	North-America	Low	Lin et al., 1997
37	15	c.1572C>G	p.His524Gln		0.1	-	1	Japan	Low	Kawano et al., 1998
38	15	c.1576C>A	p.Pro526Thr				1	ni	Low	LSMD, 2010

15	c.1577C>T	p.Pro526Leu			1	Germany	Lowe	Röschinger et al., 2000
15	c.1580T>A	p.Val527Asp			1	Italy	Lowe	Addis et al., 2004
15	c.1598T>G	p.Ile533Ser	deficient		1	ni	Lowe	LSMD, 2010
i15	c.1602+1G>A	Splicing defect	0.17	+	2	Israel, France	Lowe	Monnier et al., 2000 ; this paper
i15	c.1603-2A>G	Splicing defect			1	ni	Lowe	Böckenhauer et al., 2008
16	c.1621C>T	p.Arg541X			5	France, India, ni	Lowe	LSMD, 2010 ; this paper
16	c.1680_1683delCTTC	p.Asp560AspfsX4			1	France	Lowe	Satre et al., 1999
16	c.1681_1682insGACT	p.Phe561X			1	Spain	Lowe	This paper
16	c.1692_1693insCCTT	p.Leu564ProfsX11			1	Morocco	Lowe	This paper
16	c.16494_1695insCCTT	p.Leu565PhefsX11			4	ni	Lowe	Addis et al., 2004
16	c.1709_1710insG	p.Arg570ArgfsX6			1	France	Lowe	Monnier et al., 2000
i16	1714-2A>G	Splicing defect			2	Belgium, ni	Lowe	LSMD, 2010 ; this paper
17	c.1722-1723insT	p.Glu575X			1	Germany	Lowe	Röschinger et al., 2000
17	c.1723G>T	p.Glu575X			1	Lebanon	Lowe	Satre et al., 1999
17	c.1730T>A	Splicing defect / p.Val577Glu			1	Italy	Lowe	Addis et al., 2004
17	c.1737delT	p.Phe579PhefsX64			1	ni	Lowe	LSMD, 2010
17	c.1751_1752insG	p.Glu585LysfsX21			1	France	Lowe	Monnier et al., 2000
17	c.1753_1755delGAG	p.Glu585del			1	France	Lowe	Monnier et al., 2000
17	c.1769delG	P.Ser590ThrfsX53			1	ni	Lowe	LSMD, 2010
17	c.1773C>A	p.Asn591Lys			1	Turkey	Lowe	This paper
17	c.1777delG	p.Gly593AspfsX51			1	Spain	Lowe	Satre et al., 1999
17	c.1780C>T	p.Gln594X			1	Arabia	Lowe	This paper
17	1822_1834del13nt	p.Ser608_Lys312>SerfsX32			1	France	Lowe	Monnier et al., 2000
17	1830C>G	p.Tyr613X			1	ni	Lowe	LSMD, 2010
i17	c.1879+5G>A	Splicing defect	0.03	-	1	France	Lowe	This paper
i17	c.1880-5_1880-21del16	p.Asn627fsX16			1	Italy	Lowe	Addis et al., 2004
18	c.1901T>C	p.Leu634Pro			1	The Netherlands	Lowe	Böckenhauer et al., 2008
18	c.1923-1924delT	p.Ser642CysfsX4			1	ni	Lowe	Böckenhauer et al., 2008
18	c.1925_1926delCT	p.Ser642CysfsX9			1	North-America	Lowe	Lin et al., 1997
18	c.1927_1928delGT	p.Val643AsnfsX9	0.21	+/-	1	France	Lowe	This paper
18	c.1987C>T	p.Arg663X	0.04	-	5	North-America Finland, France	Lowe	Lin et al., 1997 ; Monnier et al., 2000 ; LSMD, 2010
18	c.2002T>G	p.Phe668Val			1	France	Lowe	Swan et al., 2010
18	c.2037T>G	p.Cys679Trp			1	Italy	Lowe	McCrea et al., 2008
18	c.2060T>C	p.Leu687Pro			1	ni	Lowe	LSMD, 2010
18	c.2071A>T	p.Lys691X			2	ni	Lowe	Böckenhauer et al., 2008
18	c.2071delA	p.Lys691LysfsX13			1	France	Lowe	Monnier et al., 2000
18	c.2083C>T	p.Arg695X			5	North-America Kuwait, France	Lowe	Lin et al., 1998 ; Monnier et al., 2000 ; Addis et al., 2004
18	c.2086G>T	p.Glu696X			1	Belgium	Lowe	This paper
18	c.2092delC	p.Pro698LeufsX66				Israel	Lowe	This paper

19	c.2194dupC	p.Leu732ProfsX38			1	UK	Low	This paper
19	c.2200delG	p.Val734PhefsX8			1	Belgium	Low	This paper
19	c.2203_2204insA	p.Pro735HisfsX35			1	North-America	Low	Lin et al., 1998
19	c.2212G>C	p.Glu737Asp			1	Italy	Dent-2	Tosetto et al, 2009
19	c.2224_2226delGTA	p.Val742del			1	Cuba	Low	This paper
19	c.2244C>G	p.Tyr748X			1	ni	Low	LSMD, 2010
i19	c.2256+1G>A	Splicing defect			1	Germany	Low	Keilhauer et al., 2007
20	c.2265delG	p.Leu755LeufsX70			1	Belgium	Low	Monnier et al., 2000
20	c.2269C>T	p.Gln757X			1	France	Low	This paper
20	c.2269delC	p.Gln757ArgfsX68			1	ni	Low	LSMD, 2010
20	c.2297delA	p.Gln766fsX58			1	Italy	Low	Addis et al., 2004
20	c.2303T>A	p.Ile768Asn			1	France	Low	Monnier et al., 2000
20	c.2311-2312insT	p.Cys771LeufsX8			2	France, Mali	Low	This paper
21	c.2341+1_del6	Splicing defect			1	ni	Low	Böckenhauer et al., 2008
21	c.2360_2361delTG	p.Val787GlyfsX2	0.10-0.16	-	4	North-America, India	Low	Lin et al., 1997 ; Lin et al., 1998 ; this paper
21	c.2389G>C	p.Ala797Pro	0.39	++	1	France	Low	Monnier et al., 2000
21	c.2396C>T	p.Pro799Leu	0.09	++	1	France	Dent-2	Swan et al., 2010
21	c.2402C>T	p.Pro801Leu	0.22		1	France	Low	Swan et al., 2010
21	c.2415C>G	p.Tyr805X			2	France	Low	Monnier et al., 2000 ; this paper
21	c.2416G>T	p.Glu806X			1	ni	Low	LSMD, 2010
21	c.2428C>T	p.Arg810X	0.27		5	UK, India	Low	Monnier et al., 2000 ; this paper
21	c.2433T>A	p.Cys811X			1	ni	Low	LSMD, 2010
21	c.2464C>T	p.Arg822X			3	Germany Lebanon, ni	Low	This paper
21	c.2467C>T	p.Gln823X	0,05	-	1	North-America	Low	Lin et al., 1998
i21	c.2469+2T>G	Splicing defect	0.03		1	French	Low	This paper
i21	c.2469+2_6delinsA	Splicing defect			1	ni	Low	LSMD, 2010
22	c.2470_2481del12	p.Val824_Gln27del			1	North-America	Low	Leahey et al., 1993
22	c.2515_2516insC	p.Leu839SerfsX12			1	ni	Low	LSMD, 2010
22	c.2530C>T	p.Arg844X			4	North-America	Low	Leahey et al., 1993
22	c.2533G>T	p.Glu845X			1	France	Low	Monnier et al., 2000
22	c.2550_2551insT	p.Glu851X	0.07	+	1	North-America	Low	Gropman et al., 2000
22	c.2553_7 del	p.Glu851GlufsX1			1	ni	Low	Böckenhauer et al., 2008
22	c.2581G>A	Splicing defect, p.Ala861Thr	0.14	-	10	Japan, Brasil, Italy Belgium, France, India	Low	Kawano et al., 1998 ; Monnier et al., 2000 ; Addis et al., 2004 ; Böckenhauer et al., 2008 ; LSMD, 2010 ; this paper
i22	c.2581+1G>C	Splicing defect			1	Spain	Low	This paper
i22	c.2581+4A>G	Splicing defect			1	ni	Low	LSMD, 2010
i22	c.2582-1G>A	Splicing defect			1	Spain	Low	This paper
i22	c.2582-2A>G	Splicing defect			1	India	Low	Sehti et al., 2008

23	c.2591_2595delTCACT	p.Phe864X			1	France	Lowe	This paper
23	c.2622delC	p.Asn874AsnfsX34	0,29		2	France, ni	Lowe	Satre et al., 1999 ; LSMD, 2010
23	c.2637_2638delGA	p.Gln879_Thr880>HisfsX4			1	Grece	Lowe	This paper
23	c.2642_2643insC	p.Pro881ProfsX2			1	ni	Lowe	LSMD, 2010
23	c.2672T>G	p.Leu891Arg			1	Maroco	Lowe	This paper
1-3	del exons 1-3	Genomic deletion, frameshift			1	France	Lowe	Monnier et al., 2000
1-4	del exons 1-4	Genomic deletion, frameshift			1	France	Lowe	Monnier et al., 2000
3-4	del exon 3-4	Genomic deletion, frameshift			1	France	Dent-2	This paper
5-8	del exons 5-8	Genomic deletion, frameshift			1	France	Lowe	Monnier et al., 2000
6-12	del exons 6-12	Genomic deletion, frameshift			2	North-America	Lowe	LSMD, 2010
8-12	del exons 8-12	Genomic deletion			1	Belgium	Lowe	Monnier et al., 2000
14	del exon 14	Genomic deletion, frameshift	0,10	-	1	North-America	Lowe	Lin et al., 1997
19-23	del exons 19-23	Genomic deletion, frameshift	0,19		1	Canada	Lowe	This paper
1-23	del OCRL	Genomic deletion,			2	Australia, Italy	Lowe	Peverall et al., 2000 ; Addis et al., 2007

Supplementary Table S3 : Reported mutations in the OCRL1 gene

^a Numbering was based on the cDNA sequence (#NM_000276) with +1 corresponding to the A of the ATG initiation codon of translation in the reference sequence.

^b Expected consequences of the mutations with codon 1 corresponding to the ATG initiation codon of translation.

^c The enzyme activities were normalized versus the normal activity values indicated by the different authors with 1 being the normal average value measured in controls. When 2 or more patients have been tested for enzyme activity, extreme values are indicated.

^d The amount of OCRL1 present in patients' fibroblasts was estimated by western blot with +++++ indicating a normal amount and - a complete absence of the protein.

^e Total number of independent patients reported with the specific mutation.

^f ni: geographical origin of the patient not indicated