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Novel *OCRL* mutations in patients with Dent-2 disease

Detlef Böckenhauer[a], Arend Bökenkamp[b], Matti Nuutinen[c], Robert Unwin[d], William van't Hoff[a], Tony Sirimanna[e], Kristina Vrljicak[f] and Michael Ludwig[g,*]

[a]*Department of Nephrology, Great Ormond Hospital for Children, London, UK*
[b]*Department of Pediatric Nephrology, VU University Medical Center, Amsterdam, The Netherlands*
[c]*Department of Pediatrics and Adolescence, Oulu University Hospital, Oulu, Finland*
[d]*UCL Centre for Nephrology, Royal Free Hospital and Campus, University College Medical School, London, UK*
[e]*Department of Audiology, Great Ormond Hospital for Children, London, UK*
[f]*Department of Nephrology, University Children's Hospital, Zagreb, Croatia*
[g]*Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Bonn, Germany*

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Abstract. Dent disease is an X-linked tubulopathy frequently caused by mutations in the *CLCN5* gene encoding the voltage-gated chloride channel and chloride/proton antiporter, ClC-5. About 15% of patients with a Dent's phenotype have mutations in the *OCRL* gene, which also causes Lowe oculocerebrorenal syndrome. To distinguish these patients from the more severe Lowe phenotype, they are diagnosed as having Dent-2 disease. We studied 14 *CLCN5*-negative patients from 12 families with a phenotype resembling Dent disease for defects in *OCRL*. In six of these kindreds three novel (c.149+1G>A, c.1126A>T, c.1547T>C) and three repeatedly observed mutations (c.166_167delTT, c.901C>T, c.1426C>T) were discovered. With the exception of a lower prevalence of nephrocalcinosis, the renal phenotype is identical with patients harboring a *CLCN5* mutation. Affected children may have some of the extra-renal symptoms of Lowe syndrome, such as peripheral cataracts, mental impairment, stunted growth or elevation of creatine kinase/lactate dehydrogenase, blurring the distinction between those two clinical entities.

Keywords: Dent-2 disease, *OCRL* gene, proximal tubulopathy, cataracts, mental retardation

1. Introduction

Dent disease, an X-linked tubulopathy, presents with low molecular weight proteinuria (LMWP; 100% of cases), hypercalciuria (in 90%) and nephrolithiasis/nephrocalcinosis (in 75%). Additional features of the renal Fanconi syndrome are often observed, but renal tubular acidosis is exceptional [1–3]. At least 30% of the patients develop chronic renal failure, mostly in young adulthood [3]. In around 60% of cases the disease is due to mutations in the *CLCN5* gene (Dent-1 disease; MIM #300009) [4,5]. This gene encodes the voltage-gated chloride channel and chloride/proton antiporter, ClC-5 [6,7], predominantly expressed in the proximal tubule and α-intercalated cells of the collecting duct. In the proximal tubule ClC-5 locates in intracellular subapical endosomes, where it facilitates chloride entry critical for the acidification of early endosomes [8,9]. Impaired ClC-5 function interferes with the recycling

*Corresponding author: Michael Ludwig, Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Sigmund-Freud-Str. 25, 53105 Bonn, Germany. Tel.: +49 228 6885 418; Fax: +49 228 6885 401; E-mail: mludwig@uni-bonn.de.

of the multi-ligand receptors megalin and cubilin, which are involved in the re-absorption of low molecular weight proteins [10]. In the distal tubule ClC-5 disruption leads to impaired clearance of calcium crystals due to mislocalization of annexin A2, a calcium-binding protein, on the surface of intercalated cells [11].

Interestingly, mutations in *OCRL*, encoding a phosphatidyl-inositol 4,5-biphosphate 5-phosphatase (PtdIns[4,5]P_2 5-phosphatase; EC 3.1.3.36), previously identified to cause the X-linked Lowe syndrome (MIM 309000) were also found to underlie some patients with a Dent-like phenotype. The name Dent-2 disease (MIM #300555) has been coined to distinguish these cases from patients with *CLCN5*-associated Dent-1 disease [4]. *OCRL* protein preferentially converts a second messenger, PtdIns[4,5]P_2, involved in cytoskeleton-plasma membrane adhesion [12], to PtdIns[4]P [13]. OCRL localizes to lysosomes and the trans-Golgi network (TGN), early endosomes, plasma membrane ruffles and clathrin-coated trafficking intermediates [14–17]. Since the TGN has the function of directing proteins to apical or basolateral membranes in epithelial cells, localization of OCRL to the TGN suggests that it may play a role in sorting and/or trafficking.

The rare oculocerebrorenal syndrome of Lowe is characterized by the presence of congenital cataracts, cognitive and behavioral impairment in the majority of cases, as well as muscular hypotonia and incomplete renal Fanconi syndrome with slowly progressive renal failure from early childhood [18,19]. Other features include postnatal growth retardation independent of kidney function, and severe non-inflammatory arthropathy, which is found in about 50% of adult Lowe patients [18,20]. The prevalence of LMWP, hypercalciuria and nephrocalcinosis is similar to the Dent-2 phenotype, but generalized aminoaciduria and renal tubular acidosis were found more frequently in Lowe than in Dent-1 and Dent-2 patients [3]. However, it is unclear, why some patients with *OCRL* mutations develop all the symptoms of classic Lowe syndrome, while others have Dent-2 disease, which is now thought to be a mild form of Lowe syndrome [3].

Defects in the *OCRL* gene have been detected in 28 Dent-2 patients and interestingly, all premature termination mutations cluster in exons 4–7, whereas missense mutations are mainly found in exons 8–15, encoding the 5-phosphatase domain [3–5,21–25]. All these mutations have been observed exclusively in Dent-2 cases except for one found in a patient with Dent-2 while his brother showed classic Lowe syndrome (3). Here, we report clinical and molecular findings from fourteen *CLCN5*-negative patients from twelve families with a phenotype resembling Dent disease.

2. Materials and methods

2.1. Patients

Clinical features suggesting the diagnosis of X-linked recessive Dent disease were observed in 14 patients from 12 families of European origin, negative for *CLCN5* mutation. At least two of the classical hallmarks had to be present: LMWP, defined by excessive urinary loss of α_1-microglobulin, β_2-microglobulin, retinol binding-protein or lysozyme; hypercalciuria defined by >4 mg/kg (0.1 mmol/kg) in a 24-hour urine collection or >0.24 g calcium per g creatinine (0.68 mmol/mmol) on a spot specimen; nephrocalcinosis/nephrolithiasis on ultrasound or computer tomography. None of the patients had the combination of bilateral congenital cataract, muscular hypotonia and cognitive and behavioral impairment, of which are classical features of Lowe syndrome.

The medical notes of patients with identified *OCRL* mutations were reviewed for renal and extrarenal features, including ophthalmological examination and mental function. One of the children (patient Va) had undergone a formal intelligence test, the other patients were considered to have normal development if they attended a regular school with at least average school achievement.

2.2. Mutation analysis

After informed consents were given, EDTA-blood samples were obtained from the patients and their relatives. Isolation of genomic DNA was carried out using the QIAmp DNA Blood Kit (QIAGEN, Hilden, Germany). All 23 exons and the alternatively spliced exon 18a of the human *OCRL* gene [26] were amplified by polymerase chain reaction (PCR; oligonucleotide sequences obtainable on request). For mutational analysis, PCR-amplified DNA products were subjected to direct automated sequencing (373 Genetic Analyzer, Applied Biosystems, Foster City, USA). Initially, both strands from the patients' amplicons were sequenced and segregation of the mutation in family members was investigated by sequencing the respective PCR product. Nucleotide numbering is according to GenBank entry NM_000276 and since it is still unknown whether

Met-1, Met-18 or Met-20 represents the initiation codon there exists controversial numbering concerning amino acid residues and nucleotides affected by mutations. According to most reports, we also chose Met-18 as amino acid number one (Swiss-Prot entry Q01968) with its coding nucleotides located at position 217-219 in entry NM_000276.

3. Results

Patients with the classical phenotype of Dent disease, but without *CLCN5* mutations were investigated for *OCRL* defects. In five cases, the screening of *OCRL* PCR products revealed a single hemizygous nucleotide transition and one patient showed a two-base pair deletion. Two novel missense mutations in exons 12 (c.1126A>T) and 15 (c1547T>C) leading to a p.Ile376Phe and p.Ile516Thr substitution, respectively, were observed (Figs 1a and 1b) and could also be detected in the respective carrier mother. Already reported missense mutations [4,5,21] were found in two further kindreds: two brothers showed the c.901C>T (p.Arg301Cys) defect and the c.1426C>T (p.Arg376Trp) mutation was discovered in another two brothers. In both these families, carrier status was confirmed in the mother. A novel splice site mutation occurred in intron 3 (c.149+1G>A), affecting the invariant G-residue at the 5'-donor site of exon 2 (Fig. 1c). Investigation of the mother also revealed carrier status in this case. The frameshift mutation c.166_167delTT (p.Leu56D*fs*X1) has also been reported once [23]. None of these mutations have ever been detected in a patient with Lowe syndrome [5].

The clinical phenotype of patients with identified *OCRL* mutations are detailed in Table 1.

4. Discussion

In the present study, we identified six different *OCRL1* mutations in eight patients from 12 families with the clinical diagnosis of Dent disease in which no *CLCN5* mutation could be found. In none of the six pedigrees reported here did the mutation occur *de novo*. In our total series of now 72 families with a Dent disease-like phenotype [28–31] the incidence of a *CLCN5* mutation of 59.7% (i.e. 43/72) was similar to the findings of Hoopes et al. [32], who detected a *CLCN5* defect in 19/32 families (59.4%). These authors reported *OCRL* defects in 5/13 *CLCN5* negative families (4) which is in line with the present series where 34.5% (i.e. 11/29) had an *OCRL* mutation. Given this, the remaining 18 patients (25% of all cases with Dent-like phenotype) may carry a *CLCN5/OCRL* mutation (e.g. in the promoter or an intron, thereby leading to a decrease in expression or a cryptic splice product) undetectable by our current assay. On the other hand, a defect in a different gene phenocopying Dent disease might be responsible. This gene(s) still await(s) identification, since several candidate genes (*CLCN4*, *CFL1*, *SLC9A6*, *TMEM27*) investigated thus far did not show any causative mutation [4,24,33,34].

As has been observed for all other truncating *OCRL* mutations in Dent-2 patients, the newly identified splice site mutation at position +1 of intron 3 affects one of the first 7 exons. Unfortunately, this mutation could not be investigated on the mRNA level but is expected to result in exon skipping or a read-through into intron 3. In both of these cases, a resultant protein will be severely truncated: the effect of exon skipping should be p.23Glu,X8 whereas a read-through will lead to a p.Glu50Gly,X21. Although this c.149+1G>A mutation represents the most 5' defect observed thus far, its phenotypic consequences are indistinguishable from other premature termination or missense mutations (Tables 1 and 2) identified in Dent-2 cases. The two novel missense mutations detected in this study affect the exonuclease-endonuclease-phosphatase domain of *OCRL* protein where all other missense mutations, except p.Glu720Asp, have been located (Table 2, Fig. 1). Although the p.Ile376Phe represents a more conservative substitution, patient IV shows more features of the classic Lowe phenotype (elevated lactate dehydrogenase, eye involvement, and cognitive/behavioral impairment) compared with the consequences of the novel non-conservative exchange found in patient VI (p.Ile516Thr) or other missense mutations.

Except one case, in which the mutation was not reported in detail [3] none of the mutations observed in Dent-2 cases has yet been found in association with classic Lowe syndrome [5]. Nonetheless, the reason(s) why different *OCRL* mutations manifest with the phenotypic spectrum observed remain to be elucidated. Although there appear to be remarkable differences in the distribution of the mutations between both phenotypes, these findings do not adequately address this question: Besides gross gene deletions, the severe Lowe phenotype has only been reported in association with mutations in exons 8–23 of the *OCRL* gene. By contrast, all frameshift mutations or splice defects leading to a premature stop codon cluster in exons 3–7 in Dent-2 patients (Table 2). Still, Dent-2 was also

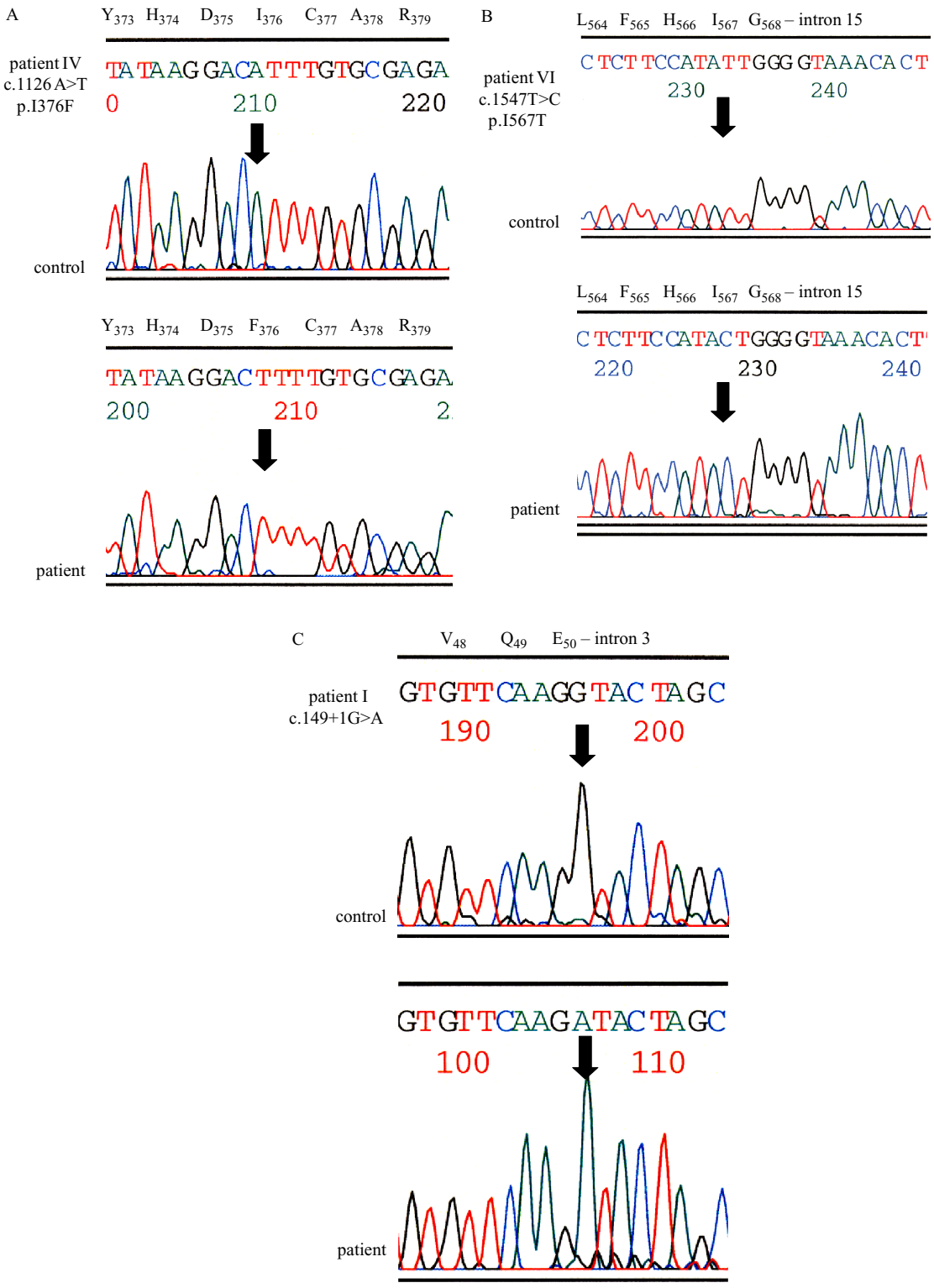

Fig. 1. Sequence analysis showing the novel *OCRL* mutations. Hemizygous substitutions are indicated by an arrow with the amino acids encoded given above.

Table 1
Clinical features of Dent-2 patients

Genetics	Patient I	Patient II	Patient IIIa *	Patient IIIb *	Patient IV	Patient Va *	Patient Vb *	Patient VI
OCRL mutation	Intron 3; c.149+1G > A	c.166_167delTT; p.Leu56AspfsX1	c.901C > T; p.Arg301Cys	c.901C > T; p.Arg301Cys	c.1126A > T; p.Ile376Phe	c. 1426C > T; p. Arg476Trp	c. 1426C > T; p. Arg476Trp	c.1547T > C; p.Ile516Thr
Family history	No (mother carrier)	No (mother carrier)	No (mother carrier)	No (mother carrier)	No (mother carrier)	No (mother carrier)	No (mother carrier)	No (mother carrier)
Extra-renal features								
Cognitive function (schooling)	Normal	Normal	Normal	Normal	Impaired **	Impaired (intelligence quotient: 77)	Normal	Normal
ocular involvement	not tested	No	No	No	Early nuclear density	No	Not tested	No
Elevated lactate dehydrogenase	Yes	No	Yes	Yes	Yes	Not tested	Not tested	Yes
Elevated creatine kinase	No	No	Yes	Yes	No	Yes	Not tested	Yes
Other	Urinary tract infections	No	Umbilical hernia	Short stature growth hormone-treatment	Depression obesity (body mass index 40)	Short stature high frequency hearing loss right ear urinary tract infections		Short stature obesity (body mass index 30) umbilical hernia
Renal features								
Chronic kidney disease stage (glomerular filtration rate) mL/min/1.73m^2 (at age in yr)	II 81e *** (17)	II 88e *** (2)	I 107e *** (12)	II 77e *** (16)	III 44 (36)	III 50 (15)	II 76e *** (5)	II 69e *** (14)
Low molecular weight proteinuria	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Hypercalciuria	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Low plasma phosphate or tubular reabsorption of phosphate/ glomerular filtration rate	No	No	No	No	No	No	No	No

(*continued*)

Table 1
Clinical features of Dent-2 patients (Continued)

	Patient I	Patient II	Patient IIIa *	Patient IIIb *	Patient IV	Patient Va *	Patient Vb *	Patient VI
Renal tubular acidosis/ plasma bicarbonate	No/26	No/22	No/24	No/23	No/26	No/28	No/25	No/25
Glycosuria	No	No	No	No	Yes	No (dipstix)	No (dipstix)	No
Aminoaciduria	No	Mild	No	No	Not tested	Mild	No	Not tested
Nephrocalcinosis/ stones	No/no	No/no	Yes/no	Yes/no	Yes/no	Yes/no	Yes/no	No/no
Medications								
Thiazide	No	Yes	No	No	No	Yes	No	No
Citrate	No	Yes	No	No	No	Yes	No	No
Phosphate	No	No	No	No	No	No	No	No

* Brothers.
** Not formally tested, but clinically impaired.
*** Estimated from plasma creatinine [µmol/l] using revised Schwartz-Haycock formula and k-factor of 0.413 (creatinine in [mg/dL]) or 36 (creatinine [µmol/l]) [27].

observed with several missense mutations in exons 9–19, exons typically implicated in Lowe syndrome.

From data established by Western blotting all frameshift/splice mutations tested were associated with the absence of OCRL protein while the missense mutations investigated (p.Arg301Cys, p.Tyr462Cys) retained some residual enzyme activity [4]. Given this, the phenotypic consequences of premature termination mutations would be expected to lead to classic Lowe syndrome, rather than to the milder form of Dent-2. Conversely, we might expect missense mutations that allow residual PtdIns(4,5)P_2 5-phosphatase activity to be responsible for Dent-2. Nonetheless, all missense mutations (except the p.Glu720Asp substitution) observed in Dent-2 patients so far affect the highly conserved exonuclease-endonuclease-phosphatase domain of the protein. As outlined in Fig. 2, this domain is conserved to a great extent in various species and conservative substitutions were found at almost all positions. This apparent evolutionary conservation as far down as *Caenorhabditis elegans* suggests a critical role for these amino acid residues for proper protein function.

The clinical phenotype of our patients is similar to that previously reported: The renal features were consistent with the selective proximal tubulopathy that we described previously in patients with *OCRL* mutations, even though these children had a clinical diagnosis of Lowe syndrome [19]. Importantly, the majority of patients presented here have evidence of extra-renal involvement in the form of elevated muscle enzymes, as described previously [35]. Interestingly, two patients (IIIa, VI) were reported to have an umbilical hernia, perhaps reflecting some muscular hypotonia, the latter being a typical feature of Lowe syndrome. Two (IV, Va) had evidence of mental impairment, one was formally tested to have an intelligence quotient of 77, the other left school early without a formal degree and at the age of 35 still lived in a supervised environment with his mother. One (case IV) had evidence of cataract (nuclear density). Two (cases IIIb and VI) had short stature, which in one patient was treated with growth hormone. All this is consistent with a phenotypic continuum within patients with Dent-2 disease and Lowe syndrome implying that Dent-2 disease actually represents a mild variant of Lowe syndrome [3]. A new finding was the sensorineural deafness, found unilaterally in one patient (Va). To our knowledge, this has not been described previously with *OCRL* mutations, and it may well be coincidental. We are not aware of data assessing hearing in patients with Lowe syndrome, but results of the neonatal hearing screen (which includes auditory brainstem

Table 2
Summarized findings of 38 Dent-2 patients with *OCRL* mutations

Exon	Mutation	Consequence	Age (years)	Elevated creatine kinase /lactate dehydrogenase	Eye involvement	Cognitive/behavioral impairment	Reference
Intron 3	c.149 +1 G > A	Splice site mutation	17	+/–	Nd	–	This study
4	c.166_167 del TT	p.Leu56Asp, *fs* X1	2.5	Nd/Nd	–	–	23
4	c.166_167 del TT	p.Leu56Asp, *fs* X1	2	–/–	–	–	This study
5	c.209 del A	p.Gln70Arg, *fs* X18	5	+/+	–	–	5
5	c.253_260 del GGAACACT	p.Glu85, *fs* X26	11	+/–	–	–	21
5	c.261_264 del TTTG	p.Cys87X	10	+/+	–	+	4
5	c.263 T > A *	p.Leu88X	13	+/Nd	–	–	24
6	c.362_363 ins A	p.Thr121Asn, *fs* X1	13 **	+/+	–	–	5
			10 **	+/+	–	–	5
6	c.362_363 ins A	p.Thr121Asn, *fs* X1	8	+/+	–	–	5
7	c.438_439 dup AA	p.Ile147Lys, *fs* X 1	8	–/–	–	–	4
7	c.447 C > A	p.Ser149X	0.5	Nd/Nd	–	–	23
7	c.456 del A	p.Gln152Arg, *fs* X15	0.1	+/+	–	+	22
7	*	p.Pro161His, *fs* X6	5	+/Nd	–	+	24
7	c.484_493 del ACCTCCACCC	p.Pro162Phe, *fs* X2	2	Nd/Nd	–	+	23
Intron 7	c.509 +1 G > A	Splice site mutation	10	Nd/Nd	–	+	23
Intron 7	c.510 –2 A > G	Splice site mutation	9	–/–	–	–	4
8	c.678 T > C	p.Phe226Ser	6.9	Nd/Nd	–	–	22
9	c.770 T > C	p.Ile257Thr	5	+/+	–	–	5
10	c.809 T > C *	p.Phe270Ser	10	–/ Nd	–	+	24
11	c.901 C > T	p.Arg301Cys	22	–/–	–	+	4
11	c.901 C > T	p.Arg301Cys	15	+/–	–	–	21
11	c.901 C > T	p.Arg301Cys	12.5 **	+/+	–	–	This study
			17 **	+/+	–	+	This study
11	c.902 G > A	p.Arg301His	12	Nd/Nd	–	–	23
11	c.911 G > A	p.Gly304Glu	6	Nd/Nd	+	+	23
12	c.1126 A > T	p.Ile376Phe	36	–/+	+	+	This study
14	c.1385 A > G	p.Tyr462Cys	9	+/+	–	+	4
15	c.1426 C > T	p.Arg476Trp	10	–/+	–	–	5
15	c.1426 C > T	p.Arg476Trp	5 **	–/–	–	–	21
			3 **	–/–	–	–	21
15	c.1426 C > T	p.Arg476Trp	15	–/Nd	Nd	–	This study
			5	Nd/Nd	Nd	–	This study
15	c.1516 G > A *	p.Asp506Asn	4	+/Nd	+	–	24
15	c.1525 C > T	p.Pro509Ser	6	Nd/Nd	Nd	Nd	25
15	c.1547T > C	p.Ile516Thr	13.8	+/+	–	–	This study
19	c.2160 G > C (or T) *	p.Glu720Asp	10 **	–/Nd	+	–	24
			11 **	–/Nd	+	–	24

* No mutation-type reported and, if possible, the expected substitution is listed.

** Brothers.

Nd = Not determined.

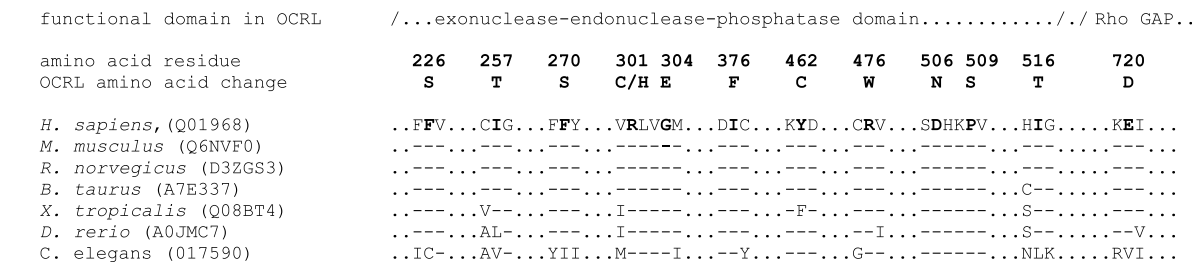

Fig. 2. Conservation of OCRL residues found mutated. Missense mutations observed in Dent-2 patients are listed with their respective amino acid position. Sequence alignment of OCRL proteins from various species is given with identical residues shown by dashes. Sequences with their respective accession number were obtained from SwissProt. Regions of functional significance in the protein (exonuclease-endonuclease-phosphatase family domain and Rho GAP-like domain) are given above.

response and otoacoustic emissions) were available for three British patients with Lowe syndrome and these were normal in all three, although otoacoustic emissions failed in one.

Summarizing all patients with documented data (Table 2), elevated creatine kinase and/or lactate dehydrogenase was present in 65% (19/29) of the Dent-2 cases, whereas it is observed in less than 10% of Dent-1 patients (31, unpublished observation). Other extrarenal manifestations, typically found in Lowe patients and never reported in Dent-1 patients, were also detected in cases with Dent-2: If tested, ocular abnormalities were noticed in ~15% (5/34) of the patients and ~30% (11/37) showed cognitive and behavioral impairment. The diagnosis Dent-2 is mainly established in very young patients and therefore these phenotypic features may manifest later in life. On the other hand, they might be detected in more cases by meticulous mental-developmental testing and ophthalmological examination.

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