# Molekulargenetische Untersuchungen proximaler renaler Tubulopathien anhand des Lowe-Syndroms

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**Für meine Familie**

## Inhaltsverzeichnis

# 1. Abkürzungsverzeichnis

| | |
|---|---|
| **DNA** | desoxyribonucleic acid (Desoxyribonukleinsäure) |
| **eGFR** | estimated (geschätzte) glomeruläre Filtrationsrate |
| **OCRL** | okulo-cerebro-renales Lowe-Syndrom |
| **OMIM** | Online Mendelian Inheritance in Man – Datenbank |
| **PCR** | polymerase chain reaction (Polymerase-Kettenreaktion) |
| **POLtube** | Polish Registry of Inherited Tubulopathies |

# 2. Zusammenfassung

## 2.1 Einleitung

Die klassische Form des okulo-cerebro-renalen Lowe-Syndroms (OCRL) (OMIM #309000), welches 1952 erstmals beschrieben wurde (Lowe et al., 1952), ist eine X-chromosomale Multisystemerkrankung mit einer geschätzten Prävalenz von 1: 500.000 (Lowe et al., 1952; Loi et al., 2006; Bökenkamp et al., 2014). Sie setzt sich aus einer Trias aus kongenitalem Katarakt, mentaler und verhaltenssozialer Retardierung und Dysfunktion des renalen Tubulus, die langsam bis zum Nierenversagen führt, zusammen. Meist treten okuläre Manifestationen und eine muskuläre Hypotonie mit Geburt auf, wohingegen die renale Beteiligung meist in den ersten Monaten im Rahmen einer Leichtgewichtkettenproteinurie postnatal bemerkbar wird. Weitere Merkmale des Lowe-Syndroms können eine Wachstumsretardierung, eine Areflexie und eine Gelenkschwellung mit hämorrhagischer Beteiligung im Sinne einer Thrombozytenfunktionsstörung sein. Magnetresonanztomographisch zeigen sich periventrikuläre zystische Läsionen (Recker et al., 2013). Die Therapie des Lowe-Syndroms beschränkt sich heutzutage hauptsächlich auf eine symptomatische Therapie, wobei die betroffenen Patienten nur in den wenigsten Fällen älter als 40 Jahre werden.

Die molekulare Ursache des Lowe-Syndroms liegt in einer Mutation des OCRL-Gens, welches für das Enzym Inositolpolyphosphat-5-phosphatase (IPP-5P) codiert (Attree et al., 1992). Dieses Enzym konvertiert Phosphatidylinositol-4,5-biphosphonat (PIP2) zu Phophatidylinositol-4-phosphat (Raucher et al., 2000; Zhang et al., 1995). Es ist an der Adhäsion von Zytoskelettplasmamembranen und an verschiedensten Kompartementen im endozytischen Netzwerk beteiligt und spielt somit eine Rolle bei der Zytokinese und Ziliogenese. Eine Dysregulation der verschiedenen OCRL-abhängigen Prozesse erklärt das variable klinische Erscheinungsbild, das für das Lowe-Syndrom kennzeichnend ist.

Im Formenkreis der renalen Tubulopathien gibt es ebenfalls die sogenannte Dent-Erkrankung, die mit renaler Tubulopathie, Nephrolithiasis und progredienter Nieren-insuffizienz assoziiert wird. Hierfür zeigen sich Mutationen im CLCN5-Gen ver-antwortlich. Interessanterweise wurden OCRL-Mutationen in Patienten mit einer Dent-Erkrankung entdeckt, die nun als Dent-2 bezeichnet werden (Szczepanska et al., 2014). Die Entdeckung von Patienten mit klinisch mildem Lowe-Syndrom und ohne okuläre

Beteiligung lässt darauf schließen, dass Dent-2-Erkrankungen eine milde Form des Lowe-Syndroms darstellen (Hoopes et al., 2005; Utsch et al., 2006). Die beobachteten Phänotypunterschiede bei Patienten mit Lowe-Syndrom und Dent-2-Erkrankung legen die Vermutung nahe, dass es individuelle Unterschiede in der Kompensationsmöglichkeit des Enzymverlusts gibt.

Die dargestellten Studien (Publikation A und B) hatten die Identifizierung neuer OCRL-Mutationen in einem Patientenkollektiv von insgesamt 29 Patienten, die an einem Lowe-Syndrom erkrankt waren, zum Ziel. Im beobachteten Kollektiv konnten ebenfalls zum ersten Mal die exakten Bruchpunkte einer kompletten Deletion des OCRL-Genes bei einem auffälligen Patienten bestimmt werden. Zudem konnten in dem genannten Patientenkollektiv erstmalig phänotypische Merkmale beschrieben werden, die bisher nicht mit dem Lowe-Syndrom assoziiert waren. Weiterhin wurde untersucht, ob OCRL-Mutationen ein phänotypisches Kontinuum aufzeigen, welches selektiv bzw. zeitabhängig auftreten kann und ob eine Genotyp–Phänotyp-Korrelation besteht.

## 2.2 Material und Methoden

In Studie A wurde eine Patientenkohorte von 28, nicht verwandten Familien untersucht, welche alle einen Indexpatienten aufwiesen, der mit hoher Wahrscheinlichkeit aufgrund des klinischen Erscheinungsbildes an einem Lowe-Syndrom erkrankt war. Alle Indexpatienten zeigten neben den typischen okulären Symptomen (Katarakt/Glaukom), eine muskuläre Hypotonie und kognitive sowie verhaltenssoziale Auffälligkeiten, die mit einer proximalen Tubulopathie gepaart waren. Die Mehrheit des Kollektives, das einen Altersmedian von 7,75 Jahren aufwies, wurde aus Polen (n=19) rekrutiert. Die übrigen Patienten stammten aus Großbritannien (n=1), Malta (n=1), Mazedonien (n=1), Schweiz (n=1), Türkei (n=1) und Indien (n=1). Die Daten hierfür wurden im Rahmen internationaler Register und Fachgesellschaften erhoben.

Die phänotypischen Merkmale wurden anhand klinischer Mess-, Labor- und Standardwerte bestimmt. Eine Leichtgewichtkettenproteinurie wurde anhand eines Verlustes von $\alpha$1-Mikroglobulin, $\beta$2-Mikroglobulin oder retinolbindendem Protein diagnostiziert. Eine renale tubuläre Azidose wurde mit Hilfe der Werte des

Plasmabicarbonates bestimmt und Aminoazidurie anhand eines Ionenaustauschchromatopgraphen ermittelt. Eine Calciumausscheidung von über 4mg/kg/24h (0,1 mmol/kg/24 h) wurde als Hypercalcämie klassifiziert. Ein chronisches Nierenversagen im Kollektiv wurde als eGFR < 90 ml/min per 1,73 $m^2$ definiert. Gerinnungsphysiologische Auffälligkeiten der Thrombozyten wurden anhand des PFA-100 Systems ermittelt (Siemens Healthcare, Erlangen, Deutschland).

Die genomische DNA wurde mit einem QIAmp Extraktionsset (Qiagen, Hilden, Deutschland) extrahiert. Alle 24 OCRL Exons wurden im Kollektiv per Polymerase-Kettenreaktion (PCR) amplifiziert. Die Analyse wurde mittels Sanger-Sequenzierung durchgeführt (3130XL Genetic Analyzer; Applied Biosystems, Foster City, Kalifornien, USA). Die Nukleotidbenennung erfolgte anhand der GenBank-No. NM_000276.3.

Zur genauen Deletionsanalyse bei Indexpatient 28 wurde ein SurePrint G3 ISCA CGH+SNP 180k Array mit der Agilent Feature Extraction Software Version 11.5 genutzt (Agilent CytoGenomics 2.5; Agilent, Santa Clara, Kalifornien, USA). Anhand eines sog. Primerwalkings konnte final ein PCR-Produkt zur Bestimmung des Bruchpunktes amplifiziert und sequenziert werden.

In Publikation B wurde die genomische DNA eines Patienten aus Sri Lanka ebenfalls mit dem QIAmp Extraktionssets (Qiagen, Hilden, Deutschland) extrahiert. Alle 24 Exons des OCRL-Gens wurden per Polymerase-Kettenreaktion (PCR) amplifiziert. Die Analyse wurde ebenfalls mittels Sanger-Sequenzierung durchgeführt (3130XL Genetic Analyzer; Applied Biosystems, Foster City, Kalifornien, USA). Die Nukleotidbenennung wurde, wie beschrieben, auch mit der GenBank-No. NM_000276.3 durchgeführt.

## 2.3 Ergebnisse

Die genetischen Analysen in Publikation A identifizierten in dem Kollektiv bei allen Patienten einen Defekt im OCRL-Gen.

Es konnten zehn bisher nicht beschriebene Mutationen in den Exons 8–24 nachgewiesen werden. Das Mutationsspektrum im gescreenten Kollektiv setzt sich aus zehn missense-Mutationen (35,7 %), acht nonsense-Mutationen (32,1 %) und fünf intronischen Splice-Mutationen (17,9 %) zusammen. Bei drei Patienten (10,7 %) konnten kleine Insertionen bzw. Deletionen gefunden werden. Weiterhin zeigte sich bei einem Patienten ein

p.Asp523 Asn-Austausch, der ebenfalls bei Dent-2-erkrankten Patienten vorliegen kann. Bei diesem X-chromosomalen Erbgang wurden ebenfalls 23 Mütter der Patienten des Lowe-Patientenkollektivs untersucht. Hier zeigte sich, dass 16 Patientenmütter auch Träger des entsprechenden Gendefektes waren. Nur bei 7 Müttern ließ sich eine de-novo-Mutation (30,4 %) darstellen.

Ein Patient zeigte eine größere Deletion, wobei die letzten 14 Nukleotide von Exon 14 und die ersten 13 Nukleotide von Intron 14 deletiert waren. Darüber hinaus zeigte sich bei diesem Patienten eine 17-bp große Insertion. Bei einem weiteren Patienten konnte kein PCR-Produkt des OCRL-Gens amplifiziert werden, so daß die Vermutung einer vollständigen Gendeletion nahe lag. Die zunächst durchgeführte Arrayanalyse zeigte hier den Verlust von ca. 103kb, wobei die Deletion sowohl das OCRL-Gen mit flankierenden 3'– und 5'-Sequenzen als auch Teile des SMARCA1-Gen beinhaltete. Mittels Primerwalking konnte bei diesem Patienten schließlich ein, den Bruckpunkt beinhaltendes PCR-Produkt amplifiziert werden. Eine Sanger-Sequenzierung definierte dann eine Deletion von genau 67865 Basenpaare (bp), die ausschließlich das komplette OCRL-Gen erfaßt.

Die meisten der untersuchten Patienten und deren klinische Daten wurden durch die *Polish Registry of Inherited Tubulopathies* (POLtube) rekrutiert. Bei bisher 20 beschriebenen Lowe-Patienten in Polen wurde mittels Hochrechnung auf die Allgemeinbevölkerung eine Prävalenz von circa 1: 2 000 000 in Polen ermittelt.

Alle Patienten wiesen perinatal bzw. im weiteren Lebensverlauf einen kongenitalen Katarakt auf. Ebenfalls zeigte sich bei dem gesamten Kollektiv eine Proteinurie, wovon nur 11 von 28 (39,3%) eine Leichtgewichtkettenproteinurie aufwiesen. Alle Patienten wiesen die typisch charakteristischen Symptome eines Lowe-Syndromes wie eine Nephrokalzinose bzw. Nephrolithiasis, eine Aminoazidurie, eine Glykosurie und eine renale tubuläre Azidose auf. In 64,3 % ließ sich ebenfalls eine chronische Nierenerkrankung Stufe 2 oder größer nachweisen.

Weiterhin ließen sich in dem Patientenkollektiv auch hämatologische Auffälligkeiten nachweisen, die zuvor von Lasne et al. erstmals beschrieben wurden (Lasne et al., 2010). Dabei handelt es sich um Thrombozytopenien, eine hypochrome mikrozytäre Anämie und Plättchenfunktionsstörungen.

Darüber hinaus konnte bei zwei Patienten zum ersten Mal das klinische Erscheinungsbild der Hyperosmie und Hyperakusis in Zusammenhang mit einem Lowe-Syndrom beschrieben werden. Weitere Untersuchungen konnten leider aufgrund des fehlenden Erscheinens der Patienten nicht durchgeführt werden.
In Publikation B konnte bei dem Patienten aus Sri Lanka eine, bisher nicht in der Literatur beschriebene, hemizygote Mutation c. 1427C>T (pThr476Ile) gefunden werden, die ebenfalls bei der Mutter des Patienten als Trägerin dieser Mutation nachgewiesen werden konnte.

## 2.4 Diskussion

Die Identifizierung des Mutationsspektrums der untersuchten OCRL-Mutationen und die Anzahl der de-novo-Mutationen in Publikation A bestätigt die in der Literatur beschriebenen Befunde für die Prävalenz verschiedener OCRL-Mutationen (Hichri et al., 2011). Es konnten circa ein Drittel de-novo-Mutationen gefunden werden, welche hauptsächlich die OCRL-Exons 8 – 24 betreffen. Bisher sind in der Literatur nur zehn große Deletionen (Hichri et al., 2011) bei Lowe-Patienten beschrieben, wobei nur zwei das gesamte OCRL-Gen betreffen (Hichri et al., 2011; Peverall et al., 2000; Addis et al., 2007). Erstmal konnte in Publikation A eine Deletion mit ihren exakten Bruchpunkten bei einem Lowe-Patienten bestimmt und analysiert werden. Hier zeigte sich der Verdacht eines Replikations-slippage, welches nur einen einzelnen Strang betrifft. In diesem Fall zeigte sich im involvierten Bereich vierfache eine Alu-Sequenz, welche bereits bei dem Gen LPAR6 in der Literatur den gleichen Effekt zeigte und als Markersequenz für die betreffenden Deletionen dienen könnte (Mahmoudi et al., 2012).
Im Hinblick auf die Prävalenz des Lowe-Syndroms sind in der Literatur keine genauen Daten beschrieben. Die amerikanische und italienische Lowe-Gesellschaft schätzen die Prävalenz auf 1: 500 000 (Loi, 2006). In der Studie zeigte sich im Hinblick auf Polen eine Prävalenz von 1: 2 000 000. Dies kann einerseits bedeuten, dass die Erkrankung seltener als bisher angenommen vorkommt oder aufgrund des variablen klinischen Erscheinungsbildes nicht richtig diagnostiziert wird (Keilhauer et al., 2007; Pasternack et al., 2013).
Besonders im Hinblick auf die renalen Phänotypen zeigte sich eine selektive tubuläre Dysfunktion, welche im beschriebenen Kollektiv von Publikation A nachvollzogen werden kann (Böckenhauer et al., 2008). Die Variabilität der renalen Manifestation steigt mit dem

Alter, wobei kein Lowe-Patient in diesem Kollektiv die komplette Manifestation eines renalen Fanconi-Syndroms zeigt (Böckenhauer et al., 2008; Kleta et al., 2008). In der Literatur wird die renale tubuläre Azidose bei nur etwa einem Drittel der Lowe-Patienten beschrieben (Bökenkamp et al., 2009). Die Beobachtung, dass in der vorliegenden Arbeit zwei Drittel des Patientenkollektives eine renale tubuläre Azidose aufwiesen, verdeutlicht die Variabilität des Erscheinungsbildes, aber auch die Bedeutung von einheitlichen und genau definierten Kriterien zur Bestimmung einer renalen tubulären Azidose.

Eine chronische Nierenerkrankung wird mit einer hohen Prävalenz bei Lowe-Patienten gefunden. Zu ihrer Diagnose benötigt man die akkurate glomeruläre Filtrationsrate (GFR), wobei ein korrigierter k-Wert von 26 zur Kalkulation der geschätzten glomerulären Filtrationsrate (eGFR) in der Schwartz-Haycock-Formel genutzt wird. Die Ursache hierfür liegt in der niedrigen Muskelmasse bei Lowe-Patienten (Böckenhauer et al., 2008). Ein Ignorieren dieses Sachverhaltes kann zur Fehldiagnose und zum Nicht-Erkennen der Erkrankung führen. Eine Korrelation zwischen der eGFR und der Körpergröße konnte bei Lowe-Patienten bislang nicht nachgewiesen werden (Bökenkamp et al., 2009).
Es zeigte sich weiterhin, dass bei der Identifizierung von Lowe-Patienten auch die hämatologischen Parameter entscheidend sein können. Matzaris et al. beschrieben das Vorkommen des OCRL-1-Proteins in humanen Thrombozyten, sodass eine Korrelation mit Blutungsstörungen bei Lowe-Patienten nachzuweisen war (Matzaris et al., 1994). Im besonderen bei der Carbamazepin-Behandlung von Krampfanfällen, welche bei Lowe-Patienten gehäuft vorkommen können, kann sich eine Verstärkung des hämatologischen Störungsbildes zeigen, sodass bei der Therapie von Lowe-Patienten dies bedacht werden muss (Verrotti et al., 2014).
Als erstmals beschriebenes klinisches Erscheinungsbild zeigte sich in Publikation A eine Hyperakusis. In der Literatur wird über eine Interaktion des Proteins SNX9 mit der Aktinpolymerisation berichtet, welche eine entscheidende Rolle für die Stereozilien in der cutikulären Platte im Ohr spielt (Cao et al., 2013). In diesem Kontext wurde die Bindung des OCRL1-Proteins direkt an SNX9 in Fibroblasten beschrieben (Nandez et al., 2014). Das OCRL1-Protein spielt somit eine wichtige Rolle bei der Aktinzusammensetzung des Innenohres. In dieser Publikation konnte nachgewiesen werden, dass das klinische Bild des Lowe-Syndroms auch akustische Auffälligkeiten beinhalten kann

Eine genaue Phänotyp-Genotyp-Bestimmung lässt das Lowe-Syndrom aufgrund der großen klinischen Variabilität nicht zu. Allerdings zeigte sich ein Kontinuum des Phänotyps, welches bisher in der Literatur nur bei Dent-2-Patienten spezifisch beschrieben wurde. In Studie A zeigte sich nun erstmals dieses Kontinuum auch bei Lowe-Patienten. Weiterhin kann sich in der magnetresonanztomographischen Diagnostik bei Lowe-Patienten eine Oligogyrierung und eine vereinfacht gyrale Musterung darstellen, welche sowohl als Hinweis auf Krampfanfälle als auch kognitive und mentale Einschränkungen verstanden werden kann (Hanefeld et al., 1999; Pang et al., 2008).
Eine weitere Besonderheit, die das in der Publikation A untersuchte Kollektiv aufzeigt und ebenfalls in der Literatur beschrieben wurde, ist eine Konzentration der Mutationen in den ersten sieben Exons bei Dent-2 und den Exons 8 – 24 bei Lowe-Patienten (Hichri et al., 2011; Böckenhauer et al., 2012). Dies legt den Verdacht von sogenannten Hotspots für Mutationen im OCRL-Gen nahe und muss in Zukunft genauer untersucht werden. Hotspots sind einzelne Stellen oder Bereiche eines Gens, an dem gehäuft und meist die gleichen Mutationen auftreten können. Dieser Mechanismus kann aufgrund eines Gründereffekts erklärbar sein. Ein Zusammenhang dieses Effektes mit den dargestellten Untersuchungen des Lowe-Syndroms als X-chromosomale Erkrankung, konnte jedoch nicht nachweisen werden und gilt, aufgrund der geringen Reproduktionsrate der Patienten, als eher unwahrscheinlich (Ten Kate et al., 1984).
In Publikation B wurde eine neue und in der Literatur nicht beschriebene Mutation bei einem Patienten aus Sri Lanka nachgewiesen. Diese erweitert das bisher beschriebene Mutationsspektrum, welches sowohl aus missense, nonsense, splice site Muationen, als auch aus Insertionen und Deletionen besteht.

## 2.5 Ausblick

Die vorliegenden Studien zeigen, dass genetische Faktoren bei der Entstehung des Lowe-Syndroms eine essenzielle Rolle spielen und viele Mutationen bisher noch nicht bekannt sind.
Durch die am Institut für Klinische Chemie und Klinische Pharmakologie durchgeführten genetischen Analysen konnten neue Mutationen im OCRL-Gen identifiziert werden, die bislang nicht in der Literatur beschrieben waren und die die Möglichkeit neuer Screeningmethoden für mögliche Mutations-Hotspots eröffnen.

Anhand der Größe des Kollektivs und der Anzahl an erkrankten Patienten einer Nationalität, konnte erstmals aufgrund der Kollektivgröße eine Prävalenz der Erkrankung ermittelt werden. Die bisher angenommene Prävalenz ergab sich lediglich aus Schätzungen der entsprechenden Lowe-Selbsthilfegruppen.

Ebenfalls ließen sich im Rahmen der Studie Hotspots der Genloci in den Exons 8–24 nachvollziehen. Es zeigte sich, dass hier ein besonderes Augenmerk beim zukünftigen Screening des Lowe-Syndroms liegen sollte. Ein Gründereffekt, der sich häufig bei repetitiven Mutationen zeigt, konnte anhand des Kollektives nicht nachgewiesen werden.

Weiterhin konnten erstmals die phänotypischen Charakteristika von Lowe-Patienten genauer untersucht werden. Anhand der dargestellten Resultate muß angenommen werden, dass sowohl einzelne modifizierende Effekte bestimmter Genloci als auch Umwelteinflüsse einen Einfluss auf die Entwicklung und Ausprägung bei Lowe- und Dent-2 Patienten haben können. Es werden weitere Studien benötigt um diese Einflüsse in Zukunft zu untersuchen und eventuelle Therapiemöglichkeiten zu entwickeln.

Ebenfalls wurde ersichtlich, dass die Diagnostik und Erkennung von renalen Tubulopathien und deren phänotypischen Merkmalen aktuell unzureichend ist. In Zukunft sollten bessere Screeningmethoden entwickelt und Schulungen hinsichtlich des Formenkreises dieser Erkrankungen angeboten werden.

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# 3. Publikation A

Pediatr Nephrol
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ORIGINAL ARTICLE

# Characterization of 28 novel patients expands the mutational and phenotypic spectrum of Lowe syndrome

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**Abstract**
*Background* The oculocerebrorenal syndrome of Lowe (OCRL) is a rare X-linked multi-systemic disorder, almost always characterized by the triad of congenital cataract, cognitive and behavioral impairment and a proximal tubulopathy.

*Methods* Twenty-eight novel patients with suspected Lowe syndrome were studied.
*Results* All patients carried *OCRL* gene defects with mutational hot spots at CpG dinucleotides. Mutations previously unknown in Lowe syndrome were observed in ten of the 28 patients, and carriership was identified in 30.4 % of the

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mothers investigated. Mapping the exact breakpoints of a complete *OCRL* gene deletion revealed involvement of several flanking repeat elements. We noted a similar pattern of documented clinically relevant symptoms, and even though the patient cohort comprised relatively young patients, 32 % of these patients already showed advanced chronic kidney disease. Thrombocytopenia was seen in several patients, and hyperosmia and/or hyperacusis were reported recurrently. A p.Asp523Asn mutation in a Polish patient, associated with the typical cerebrorenal spectrum but with late cataract (10 year), was also evident in two milder affected Italian brothers with ocular involvement of similar progression.

*Conclusions* We have identified clinical features in 28 patients with suspected Lowe syndrome that had not been recognized in Lowe syndrome prior to our study. We also provide further evidence that *OCRL* mutations cause a phenotypic continuum with selective and/or time-dependent organ involvement. At least some of these mutants might exhibit a genotype–phenotype correlation.

**Keywords** Oculocerebrorenal syndrome of Lowe · *OCRL* · Cataract · CpG dinucleotides · Hyperosmia · Hyperacusis · Thrombocytopenia

## Introduction

The oculocerebrorenal syndrome of Lowe (OMIM #309000), first reported by Lowe and colleagues in 1952 [1], is a rare X-linked multi-systemic disorder with an estimated prevalence of 1 in 500,000 in the general population [2]. Lowe syndrome is characterized by the triad of congenital cataracts, cognitive and behavioral impairment and a renal proximal tubulopathy in almost all patients. The ocular manifestations and severe hypotonia mostly present at birth, whereas renal involvement appears within the first months of life [2–4], with low-molecular-weight proteinuria (LMWP) having been demonstrated already in the neonatal period [5]. Additional features observed in Lowe syndrome include postnatal growth retardation, independent of kidney function, areflexia, nontender joint swelling and subcutaneous nodules. A debilitating arthropathy aggravates the syndrome in about 50 % of adult patients [3]. Occasionally, brain magnetic resonance imaging (MRI) identifies white matter abnormalities, mainly representing periventricular cystic lesions [6, 7]. A recent retrospective clinical survey noted an increased risk for hemorrhage in patients with Lowe syndrome, attributable to defects in platelet function [8]. Treatment of Lowe syndrome is mainly symptomatic, and the life span of an affected individual rarely exceeds 40 years, mainly limited by progressive kidney failure. However, deaths from other complications may occur at all ages due to infection, dehydration and pneumonia [9].

The causative gene of oculocerebrorenal syndrome of Lowe (*OCRL*) encodes the enzyme inositol polyphosphate 5-phosphatase, OCRL-1 [10], and more than 170 different mutations have been observed in patients with classic Lowe syndrome [11]. However, *OCRL* mutations are not only found in classic Lowe syndrome, but also in milder affected patients [11–14] who are classified as having Dent-2 disease (OMIM #300555) [15]. OCRL-1 localizes to multiple compartments in the endocyctic network (early endosomes, clathrin-coated pits, Golgi apparatus and the basal body) and also plays a role in the maturation of polarized epithelial cells and in cytokinesis and ciliogenesis (for a detailed review, see [16, 17]). Hence, a dysregulation of all these OCRL-1-dependent processes may explain the pleiotropic clinical presentation seen in Lowe syndrome. Moreover, the observed phenotypic continuum within patients with Dent-2 disease and Lowe syndrome suggests individual differences in the ability to compensate for loss of enzyme function [15]. However, the reason(s) why some *OCRL* mutations manifest with the respective phenotypic spectrum remain(s) to be elucidated.

In the study reported here, we conducted a clinical and genetic analysis of 28 newly identified and unrelated patients with suspected Lowe syndrome. These investigations revealed novel phenotypic features that had not been associated with the disease in previous studies and identified an as-yet unknown *OCRL* mutation in more than one-third of the cases. We have mapped, for the first time, the exact breakpoints of a complete *OCRL* gene deletion involving several flanking repeat elements. CpG dinucleotides represent mutational hot spots in the gene, and for one mutant we were able to establish a striking genotype–phenotype correlation.

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## Material and methods

### Subjects

Twenty-eight unrelated families with an index patient with clinical suspicion of Lowe syndrome were investigated. For all patients, the diagnosis of Lowe syndrome was based on clinical criteria; that is, all affected boys showed characteristic eye symptoms (cataract/glaucoma), muscular hypotonia and/or cognitive and behavioral impairment and a proximal tubulopathy. Twenty-three mothers of these children were also investigated for carriership. The majority of the patients (median age 7.75 years, range 0.7–20 years) were enrolled from Poland ($n$=19); the remaining patients were from the UK ($n$=3), Gibraltar ($n$=1), Malta ($n$=1), Macedonia ($n$=1), Switzerland ($n$=1), Turkey ($n$=1) and India ($n$=1). The study was approved by the respective local ethics committees, and after informed consent was given, blood samples were obtained from each patient and, if available, his mother.

### Assessment of phenotypic features

Urine analysis was performed on spot samples, and glycosuria was determined by dipstick and/or formal laboratory methods. Aminoaciduria was assessed by ion exchange chromatography. LMWP was defined by excessive urinary loss of α1-microglobulin, β2-microglobulin or retinol-binding protein. Calcium excretion exceeding 4 mg/kg/24 h (0.1 mmol/kg/24 h) was classified as hypercalciuria, as was an elevated calcium–creatinine ratio in a spot urine sample using age-appropriate reference values [18]. Renal tubular acidosis (RTA) was assessed by plasma bicarbonate values and the need for bicarbonate supplementation. Potassium wasting was defined as repeated serum potassium levels below the normal limits used by the local laboratory and/or the need for potassium supplementation. Phosphate wasting was defined as the need for phosphate supplementation or as a hypophosphatemia according to local laboratory values in the presence of a decreased tubular reabsorption rate of phosphate (<80 %) or tubular maximum for phosphate reabsorption [TmP/glomerular filtration rate (GFR)] normalized to the age-appropriate lower limit of normal [19].

Estimated GFR (eGFR) was calculated using the original Schwartz method [20]. A lower $k$-value of 26 was used with serum creatinine (in μmol/l) as suggested previously [3]. Chronic kidney disease (CKD) was defined as an eGFR of <90 ml/min per 1.73 $m^2$. Nephrocalcinosis was assessed by ultrasonography. MRI had been performed in selected patients based on clinical indication. The standard deviation score for height and body mass index (BMI) were calculated from the World Health Organization growth charts (http://www.who.int/growthref/en). Short stature was defined as a height SDS of less than −2. Prolonged closure times due to platelet dysfunction were assessed with the PFA-100 system (Siemens Healthcare, Erlangen, Germany).

### Mutation detection

Genomic DNA was extracted using standard procedures of the QIAamp extraction kit (Qiagen, Hilden, Germany). All 23 exons and the alternatively spliced exon 18a of the human *OCRL* gene [10] were amplified by PCR (oligonucleotide sequences are obtainable upon request). For the mutational analysis, the PCR-amplified DNA products were subjected to direct automated sequencing (3130XL Genetic Analyzer; Applied Biosystems, Foster City, CA). Initially, both strands from the patients' amplicons were sequenced, and carriership in the mothers was investigated by sequencing the respective PCR product. Nucleotide numbering is according to GenBank entry NM_000276.3.

### Deletion analysis

Array analysis in patient no. 28 was carried out using the SurePrint G3 ISCA CGH+SNP 180k Array and Agilent Feature Extraction Software version 11.5 (Agilent CytoGenomics 2.5; Agilent, Santa Clara, CA). This array includes markers with a median genomic distance of 4.3 kb in defined regions of clinical significance [International Standards for Cytogenomic Arrays (ISCA); http://iscaconsortium.org]; other regions are covered with a median genomic distance of markers of 30.5 kb. Primers used for amplification and sequence analysis of the junction fragment and to map the exact breakpoint were F-5′-GATT GGGTCCTTGTCCTGGG-3′ and R-5′-GTAAAAAGCACA AAACAGGGGC-3′.

## Results

### Mutation and carrier analysis

*OCRL* gene analysis in the 28 Lowe syndrome patients revealed the presence of a single defect in all cases. As outlined in Table 1, we detected ten previously unreported mutations, all of which are located in exons 8–23 and comprise ten missense (35.7 %), eight nonsense (32.1 %) and five intronic mutations (17.9 %) affecting the respective consensus motif for splice site recognition. An additional effect on correct splicing can also be assumed for two of the missense mutations (p.Ala861Pro, p.Ala861Thr) observed. In three patients

**Table 1** Type and occurrence of *OCRL* mutations detected

| Patient | Mutation[a] | Location | Consequence[a] | De novo | Reported yet[b] |
|---|---|---|---|---|---|
| 1, JC | c.668C>T[c] | Exon 8 | p.Arg230* | Yes | Yes |
| 2, JL | c.723-1G>A | Intron 8 | splice defect | No | No |
| 3, ZC | c.836T>C | Exon 10 | p.Leu279Pro | ? | No |
| 4, SN | c.940-11G>A[c] | Intron 10 | splice defect | No | Yes |
| 5, DJ | c.940-11G>A[c] | Intron 10 | splice defect | No | Yes |
| 6, NY | c.1000C>T | Exon 11 | p.Arg334* | ? | Yes |
| 7, EZ | c.1070G>T | Exon 12 | p.Gly357Val | Yes | No |
| 8, KS | c.1123C>T | Exon 12 | p.His375Tyr | No | No |
| 9, JS | c.1453_66/c.1466+1_+13del insTCTAAAACAGACTC TAA | Exon 14, intron 14 | p.Arg486Ser*fs* ?[d] | No | No |
| 10, AS | c.1490G>A | Exon 15 | p.Trp497* | No | Yes |
| 11, CF | c.1499G>A[c] | Exon 15 | p.Arg500Gln | Yes | Yes |
| 12, MS | c.1567G>A[c] | Exon 15 | p.Asp523Asn[c] | No | Yes |
| 13, RD | c.1567G>T | Exon 15 | p.Asp523Tyr | No | No |
| 14, JK | c.1621C>T[c] | Exon 16 | p.Arg541* | No | Yes |
| 15, KSo | c.1879+5G>A | Intron 17 | splice defect | No | Yes |
| 16, CE | c.1925_26delTC | Exon 18 | p.Ser642Cys*fs*X9 | ? | Yes |
| 17, PM | c.1927_28delGT | Exon 18 | p.Val643Asn*fs*X8 | No | Yes |
| 18, JP | c.2083C>T[c] | Exon 18 | p.Arg695* | Yes | Yes |
| 19, MZ | c.2083C>T[c] | Exon 18 | p.Arg695* | Yes | Yes |
| 20, SP | c.2313T>A | Exon 20 | p.Cys771* | Yes | No |
| 21, BG | c.2416G>T | Exon 21 | p.Glu806* | ? | Yes |
| 22, BK | c.2418G>T | Exon 21 | p.Glu806Asp | Yes | No |
| 23, PM | c.2464C>T[c] | Exon 21 | p.Arg822* | No | Yes |
| 24, KC | c.2581G>C | Exon 22 | p.Ala861Pro, splice defect | ? | No |
| 25, RC | c.2582-2A>G | Intron 22 | splice defect | No | Yes |
| 26, DS | c.2581G>A[c] | Exon 23 | p.Ala861Thr, splice defect | No | Yes |
| 27, MH | c.2581G>A[c] | Exon 23 | p.Ala861Thr, splice defect | No | Yes |
| 28, MJ | complete gene deletion | – | – | No | No |

*OCRL*, Causative gene of oculocerebrorenal syndrome of Lowe

[a] Numbering according to the cDNA sequence (GenBank NM_000276.3) with the A of the first coding methionine as no. 1

[b] According to Hichri et al. [11]

[c] Mutation at CpG dinucleotide

[d] Additional effect of this mutation could not be predicted

[e] This mutation has also been observed in a patient with Dent-2 disease [21] and his brother (this study)

(10.7 %), small insertions and/or deletions could be detected. One patient showed a complex mutation in which the last 14 nucleotides of exon 14 and the first 13 nucleotides of intron 14 were deleted, together with a 17-bp insertion (Table 1). This mutation predicts a p.Arg486Ser substitution and a frameshift. Since the mutation also eliminates the normal 3′-splice site, exon skipping, cryptic splice site usage or a read-through may occur, which can only be verified on the mRNA level. However, a RNA sample was not available. One patient carried a complete gene deletion, as outlined below. In one of our patients (no. 12) a p.Asp523Asn exchange was detected; this same mutation was recently observed in a patient with Dent-2 disease [21].

Investigation of 23 mothers of the Lowe syndrome patients revealed carriership in 16 females and the de novo occurrence of the respective mutation in seven cases (30.4 %). The c.2083C>T (p.Arg695*; Table 1) mutation was observed twice in our patient cohort (nos. 18, 19) and occurred de novo in both these patients. The c.2581G>A mutation was observed in patients of Polish (no. 26) and Indian origin (no. 27), also making an independent appearance most likely. One mutation (c.940-11G>A) was detected in two apparently unrelated families of Polish origin (nos. 4, 5); here, an independent origin could not be proven and, hence, a common founder responsible for this recurrence could not be excluded.

## Breakpoint analysis

Failure to amplify *OCRL* gene products by our standard procedure suggested a complete gene deletion in patient 28. Array analysis confirmed this assumption and revealed a

maximum loss of a 103-kb fragment that comprised the *OCRL* gene together with 3′- and 5′-flanking sequences (arr[hg19]Xq25-q26.1-128.641.304x1,128.667.163-128.725.786x0, 128.743.853x1), as well as part of the *SMARCA1* gene. Primer walking identified a junction fragment of 1.752 bp in this patient and his carrier mother, and sequence analysis defined a deletion of 67.865 bp, excluding an involvement of *SMARCA1*. The first deleted nucleotide resides at position 128.664.721, in the center of an Alu-Jb repeat, whereas the 3′-breakpoint (last deleted nucleotide at bp 128.732.585) locates between two members (L2B and L1MB2) of the family of long interspersed nuclear elements (LINEs) (Fig. 1). Microhomology of two basepairs (ApC) between the proximal and distal breakpoints could be observed, with one of these ApC-dinucleotides being maintained.

## Prevalence of the disorder

Of the patients investigated in this study, 19 were of Polish origin, and the largest part of the data was collected by the Polish Registry of Inherited Tubulopathies (POLtube). There has also been one previously reported case [22], bringing the total number of reported patients with Lowe syndrome in Poland to 20. This limited study cohort allows a very rough estimate of the prevalence of Lowe syndrome in Poland (general population of 38,544,513): approximately 1 in 2 million in the general population. However, this estimate is

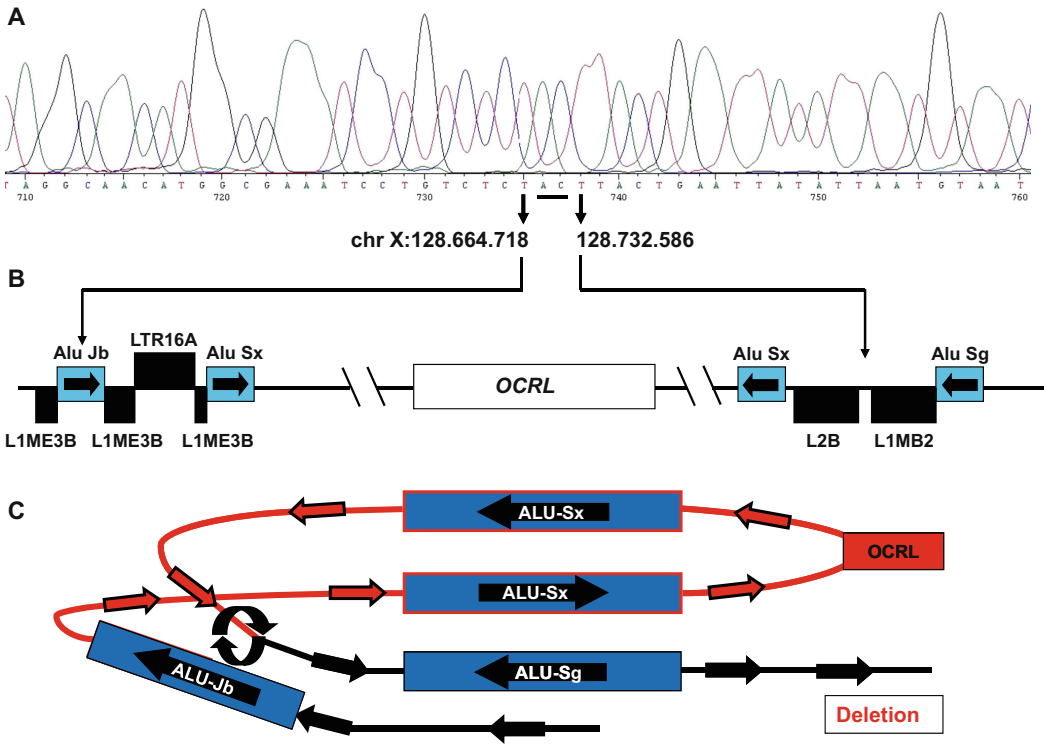


**Fig. 1** Breakpoint analysis of a complete *OCRL* gene (causative gene of oculocerebrorenal syndrome of Lowe) deletion in patient no. 28. **a** Sequence analysis of the junction fragment showing the X-location of the residues retained (*arrows*). Microhomology of two basepairs located between the proximal and distal breakpoints can be observed, with one of the ApC-dinucleotides (*underlining*) being maintained. **b** Distribution of repetitive elements in the breakpoint flanking regions comprising (partial) repeats: L1ME3B, L2B and L1MB2 belong to the family of long interspersed nuclear elements (*LINEs*), and LTR16A is a member of long-terminal-repeat elements of the endogenous retroviral ERVL family; four short interspersed nuclear elements of the Alu family are shown with their orientation indicated by *arrows* (http://www.repeatmasker.org). **c** Owing to the orientation of the Alu repeats, a 'triple-barrel' structure involving one strand may have formed during replication. Thus, the complete *OCRL* gene with its flanking sequences (*red line*) would have been deleted following illegitimate recombination secondary to a slip–replication mechanism [25]

based on the assumption that all cases of Lowe syndrome have been correctly diagnosed and captured in POLtube.

### Phenotypic findings

Clinical features reported in our patient cohort are listed in Tables 2 and 3. Congenital cataract was noted in all patients except one (no.12), who subsequently developed this feature at the age of 10 years. All patients presented with proteinuria, although LMWP was not determined in 11/28 (39.3 %) cases. According to the data available, hypercalciuria was observed in 67.9 % (19/28) of patients, nephrocalcinosis and/or nephrolithiasis in 42.9 % (12/28), aminoaciduria in 72.0 % (18/25), glycosuria in 3.6 % (1/28) and RTA in 78.6 % (22/28). Phosphate wasting was noted in 57.7 % (15/26), and 11/25 (44.0 %) patients showed excess potassium loss resulting in hypokalemia. Median eGFR was just below the normal range at 78 ml/min/1.73 $m^2$ (range: 9–120 ml/min/1.73 $m^2$, $n$=23) and CKD at stage 2 or higher (eGFR <90 ml/min) was detected in 18 patients (64.3 %). Of the 28 patients, 32.1 % (9/28) had moderate CKD (eGFR 30–59 ml/min/1.73 $m^2$) and 10.7 % (3/28) had severe CKD (eGFR <30 ml/min/1.73 $m^2$).

Data on height and BMI were available for 27 and 20 patients, respectively. Short stature was noted in 81.5 % (22/27) of patients when compared with a normal population. Median height SDS was −3.94 (range −0.32 to −6.84), whereas median BMI SDS corrected for height age was 0.17 (range 3.92–1.9).

Age was inversely related to eGFR ($r$=−0.61, $p$<0.002), but not with height SDS ($r$=−0.49, $p$<0.1). There was a direct relationship between age and the number of features of renal manifestations ($r$=0.49, $p$<0.007), thereby demonstrating progression of tubular dysfunction with age. There was no correlation between eGFR and nephrocalcinosis ($r$=−0.06; $p$=0.7). The severity of nephrocalcinosis could not be correlated as we obtained no data relating to its degree.

Hematological abnormalities were observed in nine (32.1 %) cases: thrombocytopenia was noted in three Polish patients (nos. 3, 5, 23) and a low-normal platelet count was noted in three other Polish patients (nos. 10, 11, 12). A hypochromic, microcytic anemia [hemoglobin (Hb) 8.8 g/dl] with a normal iron profile (serum Fe, ferritin, transferrin saturation) and an unremarkable thalassemia screen was recorded for the patient from Malta (no. 11). One patient (no. 1) showed microcytosis with a mean corpuscular volume of 69 fl (age-appropriate normal value 74 fl) with a normal Hb level (11.8 g/dl) and iron profile. A platelet-activation anomaly, as described by Lasne et al. [8], was detected in two patients (nos. 1, 6) from England and Gibraltar.

In two patients (nos. 4 and 17), hyperosmia and/or hyperacusis were noted several times by the parents. To our knowledge, this is the first report of these two conditions in patients with Lowe syndrome The parents reported a sensitivity to certain frequency and volume ranges of domestic and environmental noise and a heightened sense of some types of smells. Unfortunately, these conditions could not be verified by any formal assay, since the patients did not appear following invitation.

One of our patients (no. 11) died aged 18 months with respiratory failure following a severe right upper lobe pneumonia, probably due to aspiration.

## Discussion

### OCRL gene analysis

The mutational spectrum observed in our patient cohort is in line with the data reviewed by Hichri et al. [11] who reported similar values for the respective type of *OCRL* mutations. Similar to these authors we also found a de novo mutation in roughly one-third of cases. With the exception of the p.Asp523Asn substitution (see below), none of the mutations observed in this study have been detected in patients with Dent-2 disease as yet and, as expected, all point mutations and small deletions/insertions were detected in *OCRL* exons 8–23.

A total of ten large deletions [11], with two comprising the complete *OCRL* gene have been described in previous studies [23, 24]. Here, we report a patient with a complete gene deletion that we investigated in detail for the first time by precisely mapping its exact breakpoints (Fig. 1a). This deletion involved half of an Alu-Jb repeat in the 5′-region of the gene, and its 3′-break centred between two members of the family of LINEs (L2B and L1MB2; Fig. 1b), suggesting replication slippage involving one strand only. Four Alu sequences are orientated in opposite directions, and owing to this arrangement, the formation of a ‘triple-barrel’ structure involving one strand may have occurred (Fig. 1c). This would have allowed strand slipping, deleting the *OCRL* gene with some flanking sequences, a mechanism recently proposed for a complete deletion of the *LPAR6* gene [25]. Since the gene flanking regions are enriched for repeat elements, a similar mechanism may have been involved in the other two gross *OCRL* deletions.

### Prevalence of Lowe syndrome

No data reporting the incidence of Lowe syndrome have been published to date. Based on the observations of the American Lowe’s syndrome Association and the Italian Association of Lowe’s Syndrome, a prevalence of 1 in 500,000 in the general population has been estimated [2]. In our study, we obtained a value for Poland of 1 in 2 million in the general population, suggesting that either the disease is even rarer than previously estimated, or the disease is still underdiagnosed, probably due

**Table 2** Clinical findings detected in Lowe patients

| Patient | Age[a] (years) | Low-molecular weight proteinuria | Hypercalciuria | Nephro-calcinosis/ lithiasis | Aminoaciduria | Renal tubular acidosis | Phosphate wasting | Potassium wasting | Glycosuria | CKD eGFR | Short stature (height SDS) | Cataract | Cognitive/ behavioral impairment | Other features |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1, JC | 2.5 | + | + | − | nd | Mild | − | − | − | + (57) | + (−3.73) | + | Mild | Microcytosis, platelet dysfunction |
| 2, JL | 7.5 | nd (P) | + | + | + | + | − | + | − | + (51) | + (−4.45) | + | + | Facial dysmorphism |
| 3, ZC | 17.5 | + | − | − | + | + | − | + | − | + (22) | + (−5.85) | + | +, Seizures | Thrombocytopenia |
| 4, SN | 7 | + | + | − | − | Mild | + | − | − | + (86) | + (−3.78) | + | Tantrums, auto-agression, no speech | Cryptorchidism (l), hyperacusis, hyperosmia, joint hypermobility |
| 5, DJ | 2 | + | − | − | + | − | − | − | − | + (83) | − (−1.32) | + | + | Thrombocytopenia |
| 6, NY | 11 | + | + | − | + | + | − | − | − | + (38) | + (3.56) | + | Severe | Platelet dysfunction, renal stones |
| 7, EZ | 4 | nd (P) | − | − | − | + | + | + | − | + (62) | + (−3.89) | + | Severe | Encephalo-myelocele |
| 8, KS | 8.5 | + | − | − | + | + | − | + | − | − (92) | + (−5.67) | + | +, Seizures | Rickets |
| 9, JS | 16.5 | nd (P) | − | + | nd | Severe | + | + | − | + (80) | + (−6.23) | + | + | Rickets, recurrent fractures |
| 10, AS | 0.7 | nd (P) | − | − | − | + | − | − | + | − (101) | − (−0.64) | + | +, Seizures | Low normal platelet count, rickets, facial dysmorphism, dysmorphic shape of thorax |
| 11, CF | 1 | + | + | − | + | + | + | + | − | − | + (−6.84) | + | + | VUR, hydro-uretero nephrosis, low-normal platelet count, hypochromic microcytic anemia |
| 12, MS | 12 | nd (P) | + | + | Mild | − | − | − | − | + (77) | − (−0.38) | +, at 10 years of age | + | See Table 4 |
| 13, RD | 4 | + | + | + | + | + | + | + | − | + (88) | + (−5.13) | + | + | Recurrent fractures |
| 14, JK | 20 | nd (P) | + | + | + | + | + | − | − | + (43) | + (−4.8) | + | +, Seizures | Facial dysmorphism, rickets |
| 15,KSo | 1 | + | − | − | + | + | + | − | − | − (101) | + (−4.52) | + | + | Cryptorchidism, joint hypermobiliy, ventriculomegaly, periventricular cysts |
| 16, CE | 17.7 | + | + | − | Moderate | + | + | − | − | + (55) | + (−3.52) | + | Developmental delay | − |
| 17, PM | 3 | + | + | + | − | Mild | − | − | − | + (74) | + (−2.18) | + | Developmental delay, tantrums, aggressiveness | Cryptorchidism, hyperacusis, rickets, joint hypermobility |
| 18, JP | 17.5 | + | − | − | + | + | nd | + | − | + (27) | + (−3.86) | + | + | Rickets |
| 19, MZ | 4 | + | − | − | + | − | − | − | − | − (91) | − (−0.32) | + | Grand mal epilepsy | − |
| 20, SP | 3.7 | + | + | + | − | − | − | − | − | nd | nd | + | + | − |
| 21, BG | 11 | + | + | + | + | + | + | − | − | − (120) | + (−4.21) | + | Severe | − |
| 22, BK | 18 | + | + | + | − | − | + | + | − | + (9) | + (−5.80) | + | +, Seizures | Rickets, facial dysmorphism |

**Table 2** (continued)

| Patient | Age[a] (years) | Low-molecular weight proteinuria | Hypercalciuria | Nephrocalcinosis/lithiasis | Aminoaciduria | Renal tubular acidosis | Phosphate wasting | Potassium wasting | Glycosuria | CKD eGFR | Short stature (height SDS) | Cataract | Cognitive/behavioral impairment | Other features |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 23, PM | 15.5 | nd (P) | + | – | + | + | nd | nd | – | – | + (−4.18) | + | Severe, tantrums, aggressivenes | Ventriculomegaly |
| 24, KC | 18 | nd (P) | + | + | nd | – | + | nd | – | + (50) | + (−3.94) | + | +, Seizures | Thrombocytopenia, osteoporosis, facial dysmorphism |
| 25, RC | 15 | nd (P) | + | + | + | + | + | + | – | + (78) | + (−5.18) | + | +, Seizures | Rickets |
| 26, DS | 3 | nd (P) | + | – | – | + | + | nd | – | + (85) | − (−1.91) | + | +, Severe | ASD, VSD, cryptorchidism |
| 27, MH | 2.5 | + | + | – | + | + | + | – | – | nd | + (> −3) | + | +, Severe wasting/stunting | Cryptorchidism |
| 28, MJ | 8 | nd (P) | + | + | + | + | + | + | – | – (97) | + (−3.72) | + | + | Rickets |

SDS, standard deviation score; CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate (in ml/min per 1.73 $m^2$); nd, Not determined; P, proteinuria present, low molecular-weight proteinuria (LMWP) not determined; VUR, vesicoureteral reflux; ASD, atrial septal defect; VSD, ventricular septal defect

[a] Current age and all laboratory values were measured at this age

to the absence of cardinal features such as congenital cataract [26, 27]. As our study involves only pediatric nephrologists, underreporting of patients treated by pediatric neurologists cannot be excluded.

## Clinical findings in patients with Lowe syndrome

In this study, we evaluated renal phenotypes in detail, and our results confirmed a selective tubular dysfunction, as described by Böckenhauer et al. [3]. Of note, we observed an increase in the number of different renal manifestations with increasing age. However, as reported, no patient had the complete renal Fanconi syndrome [3, 4]. This observation is of clinical relevance, as minimal tubular manifestation in early childhood should not preclude a diagnosis of Lowe syndrome. Indeed, in our group of patients, four children aged <4 years (nos. 5, 10, 19, 20), had three or fewer abnormal tubular features. In terms of specific abnormalities, we found the most prominent differences in the presence of RTA, which was observed in two-thirds of our patients compared to one-third as reported by Bökenkamp et al. [15], as well as different frequencies for phosphate and potassium wasting (Table 3). These differences may be explained by differences in patient age, different criteria used to define the above abnormalities as well as the severity of CKD. In fact, it is well known that tubular dysfunction may be aggravated with renal failure [3].

We also observed a high prevalence of CKD (64.3 %), which is in line with results from previous studies [3, 15]. Importantly, a high percentage (32.1 %) of our patients had moderate to severe CKD. As suggested by Böckenhauer et al. [3], we used a *k*-value of 26, which allows for more accurate estimation of eGFR in patients with Lowe syndrome due to their abnormally low muscle mass. When original *k*-values are used, the calculated eGFR overestimates the true GFR, thereby resulting in underrecognition of CKD, as reported earlier [8, 28, 29]. Consequently, a high proportion of patients with Lowe syndrome may remain underdiagnosed in terms of CKD, and some abnormalities related to CKD may be missed. For example, severe growth deficiency in this tubulopathy may result, at least to some extent, from CKD-related metabolic abnormalities, although no relationship between short stature and eGFR has ever been demonstrated—in either a previous study [15] or in our present study.

Despite normal routine coagulation tests, Lasne et al. [8] detected prolonged closure times with a platelet-function analyzer in all six patients with Lowe syndrome in their study. Matzaris et al. [30] demonstrated OCRL-1 protein in human platelets, providing the link between bleeding disorders and Lowe syndrome. As patients with Lowe syndrome may have an increased risk of hemorrhage, coagulation should be tested, particularly in view of surgery. The platelet-activation anomaly was also detected in two of our patients (nos. 1, 6). Moreover, we found three patients (nos. 3, 5, 24) with

**Table 3** Phenotypic findings in our series of 28 patients with Lowe syndrome compared with those reported in the literature

| Phenotypic feature | Observed in our cohort | Presence in patients with Lowe syndrome [15] |
|---|---|---|
| Cataract | 100 % | almost 100 % |
| Intellectual impairment | 100 % | 90 % |
| Growth retardation (mean height SDS) | −3.7 | −3.08 |
| Low-molecular-weight proteinuria | 100 % | 100 % |
| Hypercalciuria | 67.9 % | 83 % |
| Nephrocalcinosis/nephrolithiasis | 42.9 % | 44 % |
| Aminoaciduria | 72.0 % | 82 % |
| Renal tubular acidosis | 78.6 % | 33 % |
| Phosphate wasting | 57.7 % | 43 % |
| Potassium wasting | 44.0 % | 21 % |
| Glycosuria | 3.6 % | 7 % |
| Chronic kidney disease (eGFR <90 ml/min/1.73 $m^2$) | 64.3 % | 74 % |

thrombocytopenia; however, this condition may have been induced by carbamazepine in patient 3 [31] who had received this anticonvulsant for treatment of partial seizures. In their very recently published study, Verrotti et al. [32] report that carbamazepine is associated with hematological disorders that range from mild thrombocytopenia or neutropenia to anemia and red cell aplasia, ultimately to bone marrow suppression. Of note, patients 5 and 24 in our study did not receive any anticonvulsant drug, and three further patients (nos. 10, 11, 12), also without anticonvulsant therapy, showed a tendency for thrombocytopenia or low-normal platelet counts. These findings indicate that depletion of OCRl-1 protein may also

**Table 4** Phenotypic findings in three patients carrying the *OCRL* p.Asp523Asn mutation

| Phenotypic feature | Patient no. 4, Italian[a,b] | Brother of patient no. 4, Italian[b] | Patient no. 12, Polish |
|---|---|---|---|
| Age at diagnosis | 8 years | 2 years | 2 years |
| Eye involvement | + (cataract at 8 years) | + (megalocornea and optic disc nuance at 5 years) | + (cataract at 10 years) |
| Intellectual impairment | − | − | + |
| Muscular hypotonia | − | − | + |
| Growth retardation | + (Growth hormone deficienty at 12 years) | − | − |
| Elevated lactate dehydrogenase/creatinine kinase | +/− | +/+ | +/+ |
| Low molecular weight proteinuria | + | + | + |
| Hypercalciuria | + | + | + |
| Rickets | − | − | − |
| Nephrocalcinosis/nephrolithiasis | −/− | −/− | −/+ |
| Renal tubular acidosis | − | − | − |
| Aminoaciduria | + | + | trace |
| Phosphate wasting | − | − | − |
| Potassium wasting | − | − | − |
| Glycosuria | − | − | − |
| Renal failure | − | − | - (But chronic kidney disease) |
| Other | Renal cyst (diagnosis at 7 years) | Brain magnetic resonance imaging: oligogyria | Facial dysmorphy, low-normal platelet count, elevated serum transaminases, bilirubin, and cholesterol |

[a] Initially reported in Tosetto et al. [21]

[b] Patients not listed in Tables 1 and 2

have an effect on platelet maturation and/or platelet half-life. Hence, as outlined by Verrotti et al. [32], careful concomitant hematological monitoring is needed, especially when carbamazepine, phenytoin and valproic acid are used.

Hyperacusis, characterized by over-sensitivity to certain frequency and volume ranges of sound, was noted in two patients (nos. 4 and 17) by the respective parents. In the inner ear, stereocilia are supported on the cuticular plate, a rigid platform formed by a meshwork of actin filaments in the apical cytoplasm of the hair cell at the level of the junction between the hair and its supporting cells. Actin filaments descend from the stereocilium into the cuticular plate as a rootlet, which is cross-linked into the actin meshwork [33]. F-actin dynamics in stereocilia and the cuticular plate are highly controlled by a network of proteins involving two mammalian orthologs (FCHSD1 and FCHSD2) of *Drosophila* Nervous Wreck [34]. FCHSD1 interacts with Sorting Nexin 9 (SNX9), an adaptor protein that couples late-stage endocytic coated pits to actin polymerization [34]. Since OCRL-1 has been shown to directly bind to SNX9 in Lowe fibroblasts [35], OCRL-1 depletion may also interfere with the correct assembly of actin in the inner ear.

A similar mechanism might also impair cilia formation on olfactory receptor cells and hence lead to hyperosmia, an increased olfactory acuity, which was observed in patient no. 4. According to recent findings, patients with Lowe syndrome display characteristics of a ciliopathy, with their cells showing defects in the assembly of primary cilia and in the regulation of cilia length [36, 37]. Of interest, anosmia, although opposite to hyperosmia, is known to be one of the clinical features of ciliopathies [38]. Our observations could not be verified by formal tests, but further studies should systematically address these findings in order to establish if there is a link between Lowe syndrome and olfactory and auditive dysfunction.

## Genotype–phenotype correlation

The type of mutation in Lowe syndrome and its milder form, Dent-2 disease, cannot be correlated with the clinical severity of the conditions. Accordingly, a phenotypic continuum has been proposed [15], illustrated by two mutations (p.Ile274Thr, p.Arg318Cys), which initially were associated with only the mild Dent-2 phenotype [12–14, 39, 40]. However, Hichri et al. [11] showed that these mutations can also cause the severe

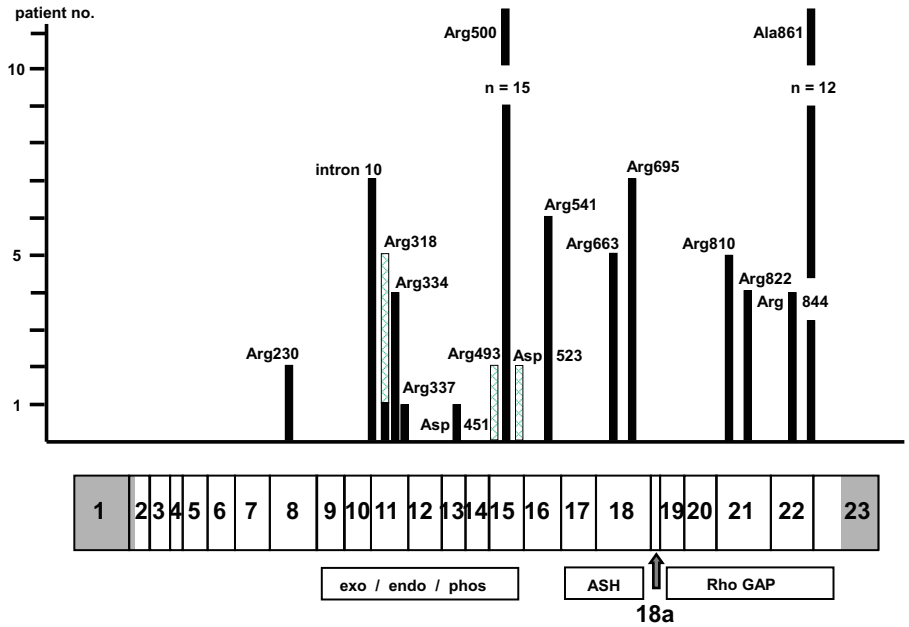


**Fig. 2** Location and frequency of *OCRL* mutations affecting CpG dinucleotides. *Black columns* Number of patients with classic Lowe syndrome, *hatched columns* occurrence of *OCRL* mutations in milder affected Dent-2 patients. A schematic diagram of *OCRL1* cDNA is shown *below.* Exons are numbered 1–23, and alternative splicing of exon 18a leads to the in-frame inclusion of eight additional amino acids. Except for exon 23, the cDNA is drawn to scale and untranslated regions are depicted in *gray* [10]. Regions of functional significance in the protein [exonuclease–endonuclease–phosphatase family domain (*exo/endo/phos*), ASPM, SPD-2, Hydin domain (*ASH*), and *Rho GAP*-like domain] are depicted *below the cDNA*

classic phenotype, even within the same family. In this section, we report several cases of incomplete or evolving Lowe phenotype, underlining the clinical variability of the disease.

Although cataract is typically one of the first findings in Lowe syndrome and may even be detected prenatally, patient 12 only displayed the cerebral and renal manifestations of Lowe syndrome (Tables 2, 4), while cataract was first noted at the age of 10 years. Interestingly, the p.Asp523Asn mutation observed in this patient has seen reported previously in an Italian patient [21], and his brother also showed the milder Dent-2 phenotype (Table 4). Both of these patients showed no cognitive and intellectual impairment and no muscular hypotonia at the time of the study, but ocular involvement followed a similar course as that in our patient, with the older brother developing cataract at the age of 8 years while megalocornea and optic disc nuance were noted in his younger brother at the age of 5 years. Of note, patients with megalocornea often develop presenile cataract [41, 42]. Moreover, brain MRI revealed oligogyria, a simplified gyral pattern, in this patient. This feature deserves further attention since it may lead to cognitive and mental impairment, as well as seizures [43, 44].

Selective and/or growth-dependent organ involvement among the *OCRL* mutations is also indicated by two cases, where a premature termination mutation (p.Gln199X) [27] and a splice variant (IVS19+1G>A) [26] were associated with a delayed diagnosis of Lowe syndrome at the age of 13 and 29 years, respectively. Both these patients had remained undiagnosed due to the absence of congenital cataract [26] or even any ocular involvement at all [27]. Also, only minimal signs of tubular dysfunction and a mild behavioral and cognitive phenotype were found in association with an *OCRL* p.Glu851X nonsense mutation [45], and a mild renal phenotype was also described due to an c.1244+5G>A defect [46].

Summarizing all these observations, one might expect effects of as yet to be identified modifier loci and/or environmental factors to influence the development and/or progression of clinical features in Lowe syndrome and its milder variant, Dent-2 disease.

## Mutational hot spots in the *OCRL* gene

A still unexplained, but well-known finding is the location of premature *OCRL* termination mutations. These affect only the first seven exons in Dent-2 disease and concentrate in exons 8–23 in classic Lowe syndrome [11, 14], as in our study. Other mutations are scattered throughout the *OCRL* gene, and none of these mutations seem to predominate in a given ethnic background. However, a closer look reveals the well-known enhancement of C>T/G>A transitions at CpG dinucleotides mainly affecting arginine codons due to their nucleotide composition [47, 48]. In our study, mutations in CpG dinucleotides account for 42.9 % ($n=12$) of the mutations observed, and a de novo origin could be proven in four of these cases (Table 1). This value is much higher than the data by Hichri et al. [11] who reported transitions at CpG dinucleotides in 70/281 (24.9 %) patients, possibly reflecting differences in sample size. Nevertheless, if data were combined, these occurrences represent 82/308 (26.6 %) of all reported *OCRL* mutations (Fig. 2).

Mutations repeatedly observed may either reflect a founder effect or repeat de novo events, a question that could not be solved in case of our intron 10 (c.940-11G>A) mutation, which was observed twice in Polish patients. A rough estimate of the lifetime of a mutation in X-linked recessive diseases was performed by Ten Kate [49], who calculated that the half-life period of a mutation in Duchenne muscular dystrophy [fitness ($f$) of affected carriers = 0] is less than two generations and that after four generations >90 % of the mutations have disappeared. Since a similar value of $f$ can be assumed for patients with Lowe syndrome, the occurrence of an identical mutation in two unrelated families is probably due to coincidence, and a founder effect is less likely.

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# 4. Publikation B

Lowe syndrome: Case report of a patient with a ... *Sri Lanka Journal of Child Health*, 2017; **46**(3): 281-283

## Lowe syndrome: Case report of a patient with a novel mutation in the *OCRL* gene

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(Key words: Lowe syndrome, OCRL gene, oculocerebrorenal syndrome, congenital cataract)

**Introduction**
The oculocerebrorenal (OCRL) syndrome of Lowe is an X linked, multisystem disorder characterised by a triad of abnormalities in the eyes, the nervous system and the renal tubules[1]. It is caused by a mutation in the *OCRL* gene which encodes an inositol polyphosphate 5-phosphatase. This enzyme has been detected on vesicular structures of the endosomal system and the Golgi complex, and plays a main role in cellular metabolism. The deficiency of this enzyme impairs the maturation of polarizing epithelium in neurons and glia, renal proximal tubule and lens[2]. Prevalence of this syndrome has been estimated as 1 in 500,000 [3].

**Case report**
This baby boy was born of a non-consanguineous marriage in January 2013 at the Base Hospital, Kahawatta and bilateral cataracts were detected at birth. On the 4th day of life, baby was referred to the Eye Surgeon, Lady Ridgeway Hospital (LRH), Colombo. He underwent bilateral trabeculectomy at the age of 2 months. At the age of 1 week, baby was referred to the Department of Chemical Pathology for necessary biochemical investigations of cataract by the eye unit. There is a history of delayed milestones. His family tree (Figure 1) revealed his maternal cousin to share the clinical features.

On physical examination, his weight was 6.2 kg less than - 3SD), height was 64 cm (less than -3 SD) and head circumference was 45 cm (10th centile). He had frontal bossing, flat nasal bridge, upward nystagmus, microcephaly, hypermobility of joints, hypotonia, severe global developmental delay and failure to thrive (Figure 2).

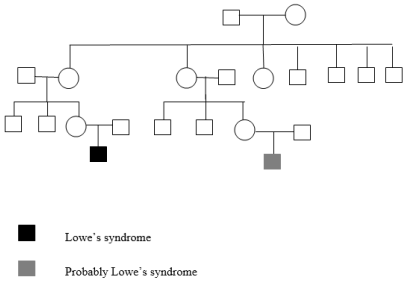


**Figure 1: Family tree**

**Figure 2:** Showing microcephaly, frontal bossing, flat nasal bridge and cataract
**Permission given by parents to publish photograph*

The clinical laboratory investigations showed hyperchloraemic metabolic acidosis with normal serum creatinine. Serum alkaline phosphatase, fasting phosphate, tubular reabsorption of phosphate and corrected calcium were 717 U/L (80 - 480), 1.06 mmol/L (1.45 - 2.16), 84% (> 85%) and 2.61 mmol/L (2.2 - 2.7) respectively. Serum creatine kinase, lactate dehydrogenase and aspartate aminotransferase were elevated. 25-hydroxy vitamin D level was 46.2 nmol/L (deficient ≤50) and parathormone (PTH) level was

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66.5 pg/ml (10–65). His urine investigations are summarised in table 1. X-ray wrist showed delayed bone age. Ultra sound scan of the brain was normal.

**Table 1: Urine biochemical investigations**

| Type of test | Result (ref. range) |
|---|---|
| ***Spot test*** | |
| pH | 7.3 (6 - 6.5) |
| Uric acid: creatinine ratio (mmol/mmol) | 1.64 (0.5 - 1.4) |
| Alanine (µmol/L) | 7076 (767 - 6090) |
| Citrulline (µmol/L) | 803 (22 - 181) |
| Lysine (µmol/L) | 2760 (189 - 850) |
| Cystine | Positive |
| Tyrosine | Positive |
| Clinistix test for glucose | Negative |
| ***24 hour excretion*** | |
| Protein (mg/m$^2$/hour) | 92 (<4) |
| Calcium (mmol/kg/day) | 0.12 (<0.1) |
| Phosphate (mmol/kg/day) | 0.7 (0.48 - 0.64) |

He was treated with Joule's solution and Polycitra. The clinical diagnosis of Lowe syndrome was confirmed by DNA analysis. This revealed a hemizygous mutation c. 1427C>T (p.Thr476Ile) in exon 14 and carriership was confirmed in the mother. Slit lamp examination of eyes of his mother did not reveal any abnormalities and her renal tubular functions were normal.

**Discussion**

Patients with OCRL syndrome, typically present in infancy with congenital bilateral cataract, growth failure and mental retardation. Cataract manifests early in embryogenesis. Congenital or childhood glaucoma has been observed in 50% of Lowe syndrome patients[4]. Neurologic manifestations include seizures, repetitive behaviour, intellectual disability and variable degree of mental retardation. Renal tubular dysfunctions include low molecular weight proteinuria, albuminuria, aminoaciduria, hypercalciuria and phosphaturia which may lead to chronic renal failure[5].

Our patient was clinically diagnosed on the basis of typical oculocerebrorenal manifestations. Impaired proximal tubular reabsorption led to generalised aminoaciduria, proteinuria, phosphaturia, hyperuricosuria and metabolic acidosis. He did not have glycosuria which is consistent with other reported cases of Lowe and indicates selective proximal dysfunction in Lowe syndrome patients[2]. Our patient also had vitamin D deficiency. Usually Lowe patients develop vitamin D dependent rickets due to impaired activation of 1-alpha hydroxylase with normal 25-hydroxy vitamin D level[6]. In our case vitamin D deficiency led to high PTH and ALP levels. X-rays did not show evidence of rickets.

Hyperphosphaturia and metabolic acidosis due to bicarbonate loss are part of the proximal tubulopathy of Lowe syndrome or result from PTH action on renal tubules. Hypercalciuria may be a manifestation of either tubulopathy or vitamin D deficiency. Though our patient had hypercalciuria, there was no radiological evidence of urolithiasis or nephrocalcinosis often reported in OCRL patients[7]. Severe hypotonia is reported in the literature[3] and our patient also had hypotonia with elevated CK, LDH and AST despite of normal liver function. Elevation of these enzymes could be due to muscle involvement. According to the literature carrier mothers with a mild phenotype developed aminoaciduria following ornithine loading[8] and often show eye lesions by slit lamp examination[9]. We could not perform ornithine loading test but eye examination did not detect any abnormalities. As she is a carrier, she has 25% of possibility of having an affected boy and 25% of possibility of having a carrier girl in future pregnancies. His 5-year-old cousin was clinically diagnosed as Lowe syndrome and is on treatment however we could not perform genetic analysis on him.

More than 200 mutations in the *OCRL* gene have been described in Lowe syndrome patients including nonsense, splice-site, missense mutations and insertions and deletions[10]. The missense mutation (c. 1427C>T) in exon 14 of the *OCRL* gene observed in our patient had not been previously reported. Probably it may be a novel mutation in Sri Lanka.

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Review Article

# Lowe syndrome/Dent-2 disease: A comprehensive review of known and novel aspects

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**Abstract.** The oculocerebrorenal syndrome of Lowe is a rare X-linked multisystemic disorder characterized by the triad of congenital cataracts, cognitive and behavioral impairment and a renal proximal tubulopathy in almost all of the patients. Whereas the ocular manifestations and severe hypotonia are present at birth, the renal involvement appears within the first months of life. Patients show progressive growth retardation and may develop a debilitating arthropathy. Treatment is symptomatic and life span rarely exceeds 40 yr. The causative *OCRL* gene, encodes an inositol polyphosphate 5-phosphatase. *OCRL* mutations were not only found in classic Lowe syndrome, but also in milder affected patients, classified as having Dent-2 disease. There is a phenotypic continuum within patients with Dent-2 disease and Lowe syndrome, suggesting that there are individual differences in the ability to compensate for loss of enzyme function. Researchers have conducted a large amount of work to understand the etiology responsible for the disease. However, the mechanisms leading to the clinical manifestations are still poorly understood and we are far from an effective therapy. In this review, we have included well-established findings and the most recent progress in understanding Lowe syndrome and Dent-2 disease.

Keywords: Congenital cataracts, cognitive and behavioral impairment, *OCRL* gene, oculocerebrorenal syndrome, inositol polyphosphate 5-phosphatase, proximal tubulopathy

## 1. Introduction

The classic form of the oculocerebrorenal syndrome of Lowe, first described by Lowe et al. in 1952 [1], presents with the triad of congenital cataracts, mental retardation, and renal tubular dysfunction with slowly progressive renal failure [2,3]. Other features include postnatal growth retardation independent of kidney function, areflexia, nontender joint swelling, subcutaneous nodules, and arthropathy, which can be observed in about 50% of adult Lowe syndrome patients [3]. Periventricular cystic lesions may be identified in some cases on brain magnetic resonance imaging and a recent retrospective clinical survey identified defects in platelet function with increased risk for hemorrhage [4–6]. The molecular basis was later identified as X-linked mutations in the gene *OCRL*, encoding an inositol

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polyphosphate 5-phosphatase (IPP-5P) [7]. Interestingly, mutations in *OCRL* were also found to underlie some patients with a Dent-like disease, now called Dent-2 disease, raising the question of how mutations in the same gene could cause two seemingly distinct diseases [8,9]. The discovery of a Lowe syndrome case with a more selective organ involvement without any ocular involvement and patients with Dent-2 disease presenting with mild extra-renal Lowe syndrome-like symptoms (peripheral cortical lens opacities, stunted growth, mild intellectual impairment, elevation of serum creatine kinase (CK)/lactate dehydrogenase (LDH), implied that Dent-2 disease actually represents a mild form of Lowe syndrome [8–12].

In this review, we will describe the knowledge of the molecular background, phenotypic features and therapeutic approaches currently used to ameliorate disease manifestations.

## 2. Prevalence of the disorder

Lowe syndrome is a very rare, pan-ethnic disease and no data reporting its incidence have been published yet. Dependent on the observations of the American Lowe's syndrome Association and the Italian Association of Lowe's syndrome, the prevalence has been estimated to be 1 in 500,000 in the general population [3].

## 3. Genetic background

The *OCRL* locus, affected by mutations in Lowe syndrome and Dent-2 disease patients, was initially mapped by linkage analysis and the identification of two balanced de novo X;autosome translocations with a breakpoint at Xq25–26 in two unrelated Lowe carriers [13–18]. Subsequently, the *OCRL* gene was identified by positional cloning and its genomic structure, comprising 24 exons occupying 52 Kb, has been elucidated [7,19]. The coding region includes exons 1–23 and alternative splicing of exon 18a enlarges the resultant 893 amino acids-long protein by eight (in frame) additional amino acids [19]. This alternative splicing seems to be realized in a tissue-specific pattern [20,21]. The reader should keep in mind that *OCRL* nucleotide and amino acid numbering has been updated following the data of Hichri et al. [22].

The majority of patients (63%) with Lowe syndrome display frameshift, nonsense or splice defects leading to mRNA decay or premature termination of the resultant OCRL-1 protein. Missense mutations and gross deletions account for 33% and 4% of the cases, respectively [22]. In the milder affected (Dent-2 disease cases), 43% carry frameshift and nonsense mutations that cluster in exons 1–7, whereas the several missense mutations detected yet, are spread throughout exons 9–21 [12,22]. No mutation, affecting the alternative exon 18a has been reported so far. *OCRL* gene analysis usually comprises all exons with their corresponding splice sites. With the application of this strategy, the causative defect remains undetected in 10–20% of the patients with suspected Lowe syndrome [22; own unpublished observation]. Hence, promoter, polyA-site or deep intronic mutations might be detected in the remaining cases.

The reason(s) why different *OCRL* mutations manifest with the phenotypic spectrum observed remain(s) to be elucidated. Although there appear to be remarkable differences in the distribution of mutations between Dent-2 disease and classic Lowe syndrome phenotype, this finding not adequately addresses this question.

Interestingly, a 615 Kb duplication comprising seven genes, including *OCRL*, was detected in a boy with autism and short stature [23]. Although both these features can also be part of the Lowe syndrome spectrum caused by deficiency of OCRL-1, they do not necessarily indicate OCRL-1 dysfunction. Hence, this boy cannot be diagnosed as Lowe syndrome case.

## 4. OCRL-1 function

OCRL-1 protein, an IPP-5P [7] preferentially converts phosphatidylinositol 4,5-bisphosphate (PIP2), involved in cytoskeleton-plasma membrane adhesion, to phosphatidylinositol 4-phosphate [24,25]. Amongst others, inositol 1,4,5-trisphosphate and inositol 1,3,4,5-tetrakisphosphate are further accepted subtrates and are converted into inositol 1,4-bisphosphate and inositol 1,3,4-trisphosphate, respectively. OCRL-1 contains several domains involved in various protein-protein interactions. These include an N-terminal pleckstrin homology (PH) domain, a central 5-phosphatase domain, an ASPM-SPD2-hydin (ASH) domain, and a C-terminal RhoGAP-like domain [26]. These interactions and the several functions of OCRL-1 observed in cellular processes are outlined below.

### *4.1. Endocytosis*

OCRL-1 was initially reported to localize to the Golgi complex [27]. Zhang et al. [28] reported its lysosomal

location in a kidney tubule cell line, however this finding was not observed in subsequent studies [29,30]. Instead, OCRL-1 has been detected on vesicular structures of the endosomal system and the Golgi complex and also on plasma membrane ruffles and clathrin coated pits [29–33]. These findings support a role for OCRL-1 in endocytic trafficking including a possible function in endosome to trans-Golgi network (TGN) traffic. The TGN directs proteins to apical or basolateral membranes in epithelial cells, hence, localization of OCRL-1 to the TGN implicates a role in trafficking from this compartment. This role may be in delivery of cargo to the endosomes, but could also be to the apical or basolateral membrane [29–34].

OCRL-1 has two clathrin binding sites, the RhoGAP domain and the N-terminal PH domain [33,35]. Both play a role in linking OCRL-1 to clathrin, whereby the RhoGAP domain is probably the more important domain. OCRL-1 has also been shown to interact with inositol phosphatase interacting protein of 27 kDa (IPIP27) A and B (also referred to as Ses1 and 2), regulators of cargo recycling in the endocytic pathway [36,37]. These two proteins, together with the endocytic adaptor protein with phosphotyrosine binding, PH domains and leucine zipper motif (APPL1) harbor a common OCRL-1-binding phenylalanine and histidine motif in their C-terminal regions and bind to the C-terminal ASH and RhoGAP-like domain of OCRL-1 [33,36–38]. IPIP27A and B have been identified as key regulators of endosomal trafficking linking OCRL-1 to the recycling of receptors at sorting and recycling endosomes [38]. In this context, van Rhaden et al. [39] provided evidence that OCRL-1 regulates a Rac1-cofilin signaling cascade mediating retrograde transport of the mannose 6-phosphate receptor from endosomes to the TGN. Cofilin, which dependent on PIP2 dephosphorylation, regulates F-actin dynamics, seems to be controlled by OCRL-1 and the cofilin mediated actin turnover, essential for endosome trafficking, is likely Rac1 dependent [39].

OCRL-1 also directly interacts with Rac1 GTPase in the TGN and binds to several Rab GTPases with the strongest interaction with Golgi-associated Rab1 and Rab6 and endosomal Rab5 [31,40]. Rab binding through the OCRL-1 ASH domain is required for targeting OCRL-1 to Golgi and endosomes and directly stimulates its 5-phosphatase activity [40,41]. A Rab5- and APPL1-dependent recruitment of OCRL-1 to phagosomes has also been reported by Bohdanowicz et al. [42].

The functional consequences of all these interactions with regard to the Lowe syndrome phenotype in almost all cases remain to be elucidated. The ASH-RhoGAP domain regulates the most of these protein-protein interactions, including the multiple clathrin-dependent trafficking processes [30,32,35]. In that context, McCrea et al. [43] tested several *OCRL* missense mutations affecting this domain. Whereas binding to clathrin and Rac was not affected by these amino acid substitutions, all mutations caused disturbance of APPL1 binding to OCRL-1. This prevented a correct colocalization of OCRL-1/APPL1 on endosomes, pointing to a substantial role of this interaction in the etiology of Lowe syndrome [33,43].

### *4.2. Actin cytoskeleton*

Suchy and Nussbaum [44] found that actin remodeling is disrupted in fibroblasts from Lowe syndrome patients. These authors further reported an abnormal distribution of gelsolin and alpha-actinin, proteins also involved in actin dynamics. Actin remodeling is strongly activated by calcium and a specific increase in bradykinin-induced calcium mobilization was found in fibroblasts from Lowe syndrome patients [45]. Hence, and as mentioned above, changes in actin dynamics and/or altered membrane traffic may be involved in the pathology of Lowe syndrome.

### *4.3. Cell migration and cell-cell contacts*

OCRL-1 has also been detected in membrane ruffles and lamellipodia of COS-7 cells and in fibroblasts from Lowe syndrome patients [46]. The translocation of OCRL-1 to membrane ruffles was found to be mediated by Rac GTPase activation upon growth factor stimulation [46]. As outlined by Faucherre et al. [46], these findings suggest an interference with cellular properties such as cell migration and formation of cell-cell contacts, which depend on ruffling and lamellipodia within OCRL-1 depleted cells.

### *4.4. Polarity*

Another role of OCRL-1 has been revealed in epithelial cells at the early stages of polarization [47,48]. Here, OCRL-1 localized to junctions and formed complexes with key junctional components. In Madin-Darby canine kidney and human intestinal epithelial Caco-2 cells, the authors detected requirement of OCRL-1 for correct

organization of apical and basolateral surfaces. A further observation supported a polarity defect as these epithelial cells failed to form cysts in 3-dimensional cultures. Inhibition of this maturation might depend on mistargeting apical cargo from recycling apical endosomes to the lateral membrane. Cell types mainly affected in Lowe syndrome, i.e. neurons and glia, and renal proximal tubule and lens epithelium, are highly polarized and the impairment of maturation of the polarizing epithelium might contribute to the phenotypic features observed.

### *4.5. Cytokinesis*

Most recent studies with the Drosophila ortholog dOCRL revealed another role for OCRL-1 in the cytokinesis machinery and its implication in cell division, supported by findings in HeLa cells and Lowe syndrome renal cells where Rab35 controls cytokinesis abscission through OCRL-1 function [49–51]. As shown by Dambournet et al. [51], a p.Gly421Glu mutation, found in a Lowe syndrome patient, caused an increase in PIP2 levels and F-actin at the intracellular bridge, important for the last step of cell division. These findings are consistent with the observation of abnormal F-actin dynamics in fibroblasts from patient cells [44]. As the addition of F-actin depolymerizing agents at a dose not perturbing wild type cells, was able to restore normal amounts of F-actin in Lowe syndrome patient cells, a therapeutic approach was suggested - that some phenotypic features due to OCRL-1 deficiency might be correctable by reducing increased F-actin levels [51].

### *4.6. Ciliogenesis*

OCRL-1 deficiency affects the same major tissues (brain, eye, and kidney) as ciliopathy syndromes and recent studies revealed an involvement of OCRL-1 in the regulation of ciliogenesis and trafficking processes to the cilia [48,52,53]. Coon et al. [52] showed that OCRL-1 is involved in protein trafficking to the primary cilia in a Rab8- and IPIP27/Ses-dependent manner and that primary cilia assembly is impaired in patients' fibroblast cells and zebrafish Ocrl morphants. This finding was corroborated in that Ocrl knockdown in zebrafish was associated with developmental defects consistent with disruption of ciliary function, including body axis curvature, pericardial edema, hydrocephaly and impaired renal clearance [52,53]. However, whereas two studies reported reduced cilia length and number in Lowe syndrome affected fibroblasts and OCRL-1 depleted retinal pigment epithelial cells and kidney tubular cells, Rbaibi et al. [48] detected increased cilia length in Madin-Darby canine kidney epithelial cells [52,53]. Although these disparate findings might be explained by a tissue-specific effect, it is still unclear how OCRL-1 depletion in ciliary biogenesis and function may play a role in the pathogenesis of Lowe syndrome. The current knowledge has been extensively discussed by Conduit et al. [54] who assumed that Lowe syndrome may rather represent a ciliopathy-associated syndrome.

In conclusion, OCRL-1 localizes not only to multiple compartments in the endocyctic network (early endosomes, clathrin-coated pits, Golgi apparatus and the basal body) but plays also a role in the maturation of polarized epithelial cells and in cytokinesis and ciliogenesis. A deregulation of all these PIP2-dependent processes may explain the pleiotropic phenotypic features seen in Lowe syndrome.

## 5. Genotype-phenotype correlation

The type of mutation often predicts the severity of the respective disease. In case of Lowe syndrome the grade of severity cannot be correlated to the defect observed. Hichri et al. [22] reported two mutations (p.Ile274Thr, p.Arg318Cys) that have been associated with mild Lowe syndrome (Dent-2 disease) [8,9,12,55,56] but can also cause the severe phenotype even within the same family. A premature termination mutation (p.Gln199X) and a splice variant (IVS19+1G>A) were found to be associated with Lowe syndrome at patient's ages of 13 yr and 29 yr, respectively [10,57]. Both these cases had remained undiagnosed due to absence of congenital cataract or even any ocular involvement [10,57]. On the other hand, only minimal signs of tubular dysfunction and a mild behavioral and cognitive phenotype were found in association with an *OCRL* p.Glu851X nonsense mutation and a mild renal phenotype was also described due to an c.1244+5G>A defect [58,59]. These findings are somewhat surprising, since expression studies performed thus far, showed that all except two (p.E851X [58], c.40-14A>G [8]) of the frameshift and nonsense mutations tested, resulted in an almost complete absence of *OCRL* mRNA and IPP-5P activity [8,22,60,61].

The main restriction of the Lowe syndrome phenotype to certain epithelial cells of the lens, kidney, and

the brain remains to be elucidated. OCRL-1 protein may act in a specific manner in these polarized cells and/or its deficiency might be compensated by another IPP-5P, like the inositol polyphosphate 5-phosphatase (INPP5B), in unaffected tissues [62]. With this assumption, individual variability in such compensation would explain the differing extent of symptoms. However, knockout mice for either *Ocrl* or *Inpp5b* provided no clue in that they were viable with no obvious defects in lens, kidney, or brain [63]. Instead, murine *Ocrl* and *Inpp5b* double-knockouts led to an early embryonic lethal phenotype, implying a functional overlap of these enzymes in mice. The finding of species-specific differences in *Inpp5b* expression and splice-site choice might be an explanation why *Inpp5b* and *INPP5B* differ in their ability to compensate for OCRL-1 deficiency [64]. Indeed, $Ocrl^{-/-}$ mice that expressed human *INPP5B*, but not the murine ortholog, showed reduced postnatal growth and cardinal features of renal tubulopathy [65]. In conclusion, one might expect effects of yet to be identified modifier loci influencing the phenotype of Lowe syndrome and Dent-2 disease.

## 6. Lowe phenotype

### 6.1. Eye

Congenital bilateral cataract is present at birth in most of the patients and surgical removal with prompt optical correction is recommended [66–68]. This defect manifests early in embryogenesis due to defective formation and subsequent degeneration of the primary posterior lens fibers [67]. Congenital or childhood glaucoma, observed in around 50% of Lowe syndrome patients but not reported in Dent-2 disease, should be detected in the first year of life in most cases [68,69]. Some are due to cataract surgery. Corneal opacity (keloid) and a progressive course of corneal keloids have been observed [70,71]. Other findings were nystagmus, enophthalmos, anterior polar cataract, subcapsular fibrous plaque, capsular excrescences, bladder cells and posterior lenticonus [67].

More mildly affected (Dent-2 disease) patients may show no pathologic findings but discrete peripheral opacity between nucleus and cortex, as well as mild bilateral nuclear sclerosis have been observed [11]. As mentioned above, Lowe syndrome may remain undiagnosed due to absence of congenital cataract or even any ocular involvement [10,57].

### 6.2. Brain and nervous system

#### 6.2.1. Neuroradiological features

Studies of several Lowe syndrome patients have shown a wide range of heterogeneity in the white matter changes ranging from diffuse high-intensity signal to no demonstrable changes at all. In all cases reported, hypointense areas were detected in T1-weighted images whereas they were hyperintense in T2- and proton density-weighted images [72,73]. These images are due to gliosis, as has been revealed by proton magnetic resonance imaging (MRI) spectroscopy where prominent myoinositol peaks, a glial marker suggesting the presence of gliosis, were present [74]. In addition, even in fluid-attenuated inversion recovery images, deep white matter in the periatrial regions was observed [75].

In the central nervous system, the accumulation of lysosomal products leads to a dilatation of perivascular spaces, whereas extracellular release of lysosomal enzymes can lead to toxic gliotic reactions [76]. In comparison to this, the spectra from the lesion sites the peaks of the major metabolites including N-acetyl-aspartate, choline and creatinine appeared normal [75]. However, the dual pattern seen in MRI, with a mixture of cystic lesions and resembling those of mucopolysaccharidoses, is due to the accumulation of PIP2. Alteration of deep white matter as a result of reactive gliosis or demyelination might be due to the toxicity of the extracellulary released lysosomal enzymes [76].

MRI also revealed the appearance of cysts in the periventricular white matter that correspond to perivascular lacunes [5,74]. These diffuse supratentorial white matter abnormalities consist of two types: The first one is a punctate lesion with signal characteristics similar to those of cerebrospinal fluid. The second pattern consists of patchy white matter abnormalities that are hypointense in T1-weighted and hyperintense in T2-weighted and proton-density images [73]. Even apparent diffusion coefficient maps demonstrate hyperintense periatrial lesions although $b = 1000 s/mm^2$ images are negative. Thus, these lesions are responsible for such high apparent diffusion coefficient values ($1.76$ and $1.66 \times 10^{-3} mm^2/s$). In the case reported by Sener [75]. it was summed up that the high values are a combination of both hyperintensities and punctate cysts, being characteristic for either gliosis or demyelination. This main feature allows a reinforcement of the clinical diagnosis.

Onur et al. [73] reported a tigroid pattern in the MRI in one patient with preserved white matter areas with hypointense radially oriented stripes within the hyperintense cerebral white matter on T2-weighted images.

This tigroid skin pattern of demyelination has already been described in Pelizaeus-Merbacher disease, globoid cell leukodystrophy and metachromatic leukodystrophy, but never before in Lowe syndrome. Histopathologically, the white matter foci in this pattern are thought to be residual islets of preserved myelin, especially around the blood vessels.

The neuropathological findings reported in the literature are quite variable and consist of pachygyria, polymicrogyria, neuronal migration disorder, diffuse fibrillary gliosis of the centrum semiovale and patchy demyelination and cerebellar white matter [77]. De Carvalho-Neto et al. [74] reported brain weight below normal, atrophy, ventricular enlargement and the thinning of the corpus callosum. These meanings suggest fibrotic tissue without any inflammatory changes. Other MRI findings consist of dilated lateral ventricles and multiple, bilateral well-defined bright spots on T2- and proton density-weighted images [78].

*6.2.2. Seizures*

A wide variability in seizure types has been reported by Charnas [79], who found 15 out of 40 Lowe syndrome patients with a history of seizures. These included febrile convulsions, myoclonic seizures and infantile spasms, staring spells, and mixed staring and generalized tonic-clonic seizures as the most common type. Recently, atonic seizures accompanied by focally initiated secondary generalized epileptic discharges were added to this list [80].

Seizure control was obtained in 13 of the 15 patients reported by Charnas [79], applying phenobarbital and/or phenytoin, or valproate. In two cases, phenytoin alone was reported to be effective for controlling brief tonic seizures and generalized tonic convulsions [78]. Atonic seizures were reported to be resistant to valproate/phenytoin/clonazepam therapy, but responded when lamotrigine was added instead of phenytoin [80]. Use of the newly-developed vagus nerve stimulation is under investigation for individuals with Lowe syndrome [81].

*6.2.3. Repetitive behavior in Lowe syndrome*

Patients with Lowe syndrome often show a very characteristic pattern of behaviors that can interfere with everyday functioning. Arron et al. [82] reported self-injurious behavior and patients harbor a greater chance for physical aggression. Individuals display higher scores on measures of autistic-like repetitive behavior, over activity and impulsivity. A very special feature existing in Lowe syndrome patients was inserting objects or body parts as well as repeated painful biting and even eye-poking [10,82]. The last feature might be attributable to visual impairment or may reflect intraocular pain, for instance, from poorly controlled glaucoma. There is also a great inability to concentrate or focus and unusual preoccupations or obsessions [81]. Further, more than 80% of patients show several maladaptive behaviors including stubbornness, temper tantrums and complex repetitive movements that interfere with adaptive functioning and are significantly worse than observed in other visually impaired or comparably mentally retarded individuals [83]. Lowe syndrome patients scored significantly higher than controls on all measures of maladaptive behavior including aggression, irritability and stereotypy, reflected in repetitive, purposeless movements. Typically, this stereotypy is shown by hand flapping [83]. Some evidence suggests that the most difficult period for behavior problems is between the ages of 8-13 yr. In single cases, these problems can even continue in adulthood. Here, medication therapy may be effective including antidepressant and/or antipsychotic medications [81].

*6.2.4. Intellectual disability*

IQ was tested in 47 Lowe syndrome patients. Here, mean IQ was in the range of 40-54 and 25% of the affected males showed an IQ of ≥70 [83].

*6.2.5. Muscle hypotonia*

Muscle involvement in Lowe syndrome includes severe hypotonia, present at birth and absence of deep tendon reflexes [3,4]. Muscle biopsy from two Lowe syndrome patients revealed selective type 1 fiber atrophy and additionally mild type 1 fiber predominance [84], a feature also reported by Gobernado et al. [85]. It remains to be elucidated, if these findings contribute to the elevation of serum CK, LDH and aspartate aminotransferase despite normal liver function [86].

The etiology of all these pathological central nervous system features is still unclear. OCRL-1 is highly expressed in brain and here only the longer splice variant represents the expressed transcript [20,21]. This isoform was shown to bind clathrin with higher affinity than the shorter isoform b, and is significantly more enriched in clathrin-coated trafficking intermediates [21]. Most recently, a zebrafish model was shown to recapitulate several of the neurological findings in Lowe syndrome, e.g. susceptibility to seizures and cystic brain lesions [87]. The authors showed that loss of IPP-5P activity impaired cell survival and reduced cell proliferation within the developing neurological tissue. Using this

model, a novel role for OCRL-1 in maintaining Akt signaling and cell survival during embryogenesis was also identified [87].

### 6.3. Orthopedic manifestations

Skeletal manifestations, frequently reported in Lowe syndrome, are osteomalacia and rickets and despite the presence of normal serum concentrations of vitamin D metabolites, calcium and phosphorus bone demineralization and recurrent fractures may occur [88–91]. Rickets should be treated with vitamin D and oral phosphate supplements. It should be noted, that typically treatment requires 1-alpha hydroxylated vitamin D, as uptake of the vitamin D/vitamin D-binding protein complex is necessary for activation by 25-hydroxyvitamin D-1 alpha hydroxylase, mitochondrial [92]. This may explain the presence of rickets despite normal vitamin D levels.

Also often noted were tenosynovitis, joint hypermobility, joint effusions and contractures [88–90]. Studying six Lowe syndrome patients, Holtgrewe and Kalen [93] added scoliosis, kyphosis, platyspondyly, dislocated and/or subluxation of hips, and cervical spine anomalies to the list of orthopedic deformities. Lowe syndrome patients may develop a debilitating arthropathy [89,94], not seen in Dent-2 disease patients yet. These features require correct treatment of rickets and the therapy of scoliosis, present in about half of the patients [81].

### 6.4. Growth failure

Lowe syndrome patients were of short stature when compared with a control population. Impaired growth may vary from slight stunting in mildly affected patients to the well-recognized growth retardation seen in the severe manifestation. A bone age between height age and chronologic age and a direct relationship between age and growth failure in patients with Lowe syndrome was reported by Charnas et al. [86]. These findings were confirmed by Bökenkamp et al. [11], who also observed a similar growth pattern in patients with Dent-2 disease and Lowe syndrome. The underlying pathophysiology of growth failure is yet unclear, although it does not seem to reflect chronic renal insufficiency as height standard deviation scores were not related to glomerular filtration rate (GFR) when adjusted for diagnosis and age [11]. One may speculate that untreated metabolic acidosis or electrolyte disturbances might account for growth failure in Lowe syndrome patients.

Growth failure has been treated with human growth hormone (GH) in some cases but whether this really changes final height is questionable [12,81]. Whereas GH application in a single patient showed no effect on growth at all (D. Böckenhauer, personal communication), therapy with recombinant human GH, together with intravenous bisphosphonate (pamidronate disodium), was reported to significantly improve bone turnover and bone mineral density in another case [95]. However, whether the change in bone density is permanent or temporary is still unknown. Given these uncertainties, the invasive (daily injections) and expensive GH treatment should be carefully considered.

### 6.5. Oral and dental manifestations

Anomalies concerning oral and dental features in Lowe syndrome have rarely been reported and primarily described in isolated cases. Rodrigues Santos et al. [96], Brooks and Ahmad [97], and Ruellas et al. [98], have reviewed the many different oral manifestations seen in the patients. These include frequently observed eruption cysts or rather specified dental cysts [96,99–102] and a plenitude of jaw anomalies, dental abnormalities, periodontal findings and circumoral features yet observed [97].

However, all these articles mainly describe phenotypic findings without addressing their clinical regime. This is somewhat surprising since, as outlined in a recent report, orthodontic treatment can substantially improve dental esthetics, occlusal function and facial profile, hence, the quality of live [98]. Prolonged bleeding following tooth extractions has been reported [96,103]. This observation reflects a recent retrospective clinical survey of Lowe syndrome cases, which identified defects in platelet function with increased risk for hemorrhage as outlined below [17].

### 6.6. Kidney

Renal disease in Lowe syndrome is primarily attributable to specific tubular dysfunctions of variable extent with only low molecular-weight proteinuria and albuminuria present in all patients [2]. There is an obvious negative relationship between age and GFR [2,11,86]. This may lead to chronic renal failure and end-stage renal disease requiring dialysis or even transplantation.

Renal failure was observed in 32% of the milder affected (Dent-2) patients and in 74% with classic Lowe syndrome [11]. As suggested by Copelovitch et al. [104], focal segmental glomerulosclerosis (FSGS) may occur secondary to tubular dysfunction or damage. Indeed, FSGS has been reported in Lowe syndrome and Dent-2 patients [105,106]. Symptoms related to tubular dysfunction are consistent with an impairment of intracellular trafficking as a key mechanism and include:

*6.6.1. Low molecular-weight proteinuria (LMWP)*

Proteinuria was noted in the original report of Lowe syndrome [1] however, it was not assessed according to size. Today, the presence of low molecular-weight proteinuria is known as a uniform abnormality in Lowe syndrome/Dent-2 disease patients and can be identified just after birth [107]. Especially retinol binding protein (RBP), with a mean elevation of approximately 1000-fold above the upper normal limit, has been established as highly sensitive marker for impairment of tubular protein absorption [2,108]. All patients show moderately elevated urinary albumin. Reabsorption of both these proteins occurs in the proximal tubule via the megalin receptor pathway [109–111]. Whether the different levels of elevation may depend on slight differences in their reabsorption pathways (albumin is bound also by cubilin) or rather reflect the difficulties in the assessment of urinary albumin, which is obviously strongly affected by glomerular function, is yet unknown [110]. On the other hand, one can argue that retinol binding protein is freely filtered and exclusively reabsorbed in the proximal tubule, whereas only a fraction of albumin is filtered. This alone may explain the discrepancy in the magnitude of urinary levels. Cui et al. [34] found no defect in the trafficking and function of megalin upon OCRL-1 siRNA knockdown and assumed that the renal manifestations in Lowe syndrome are downstream from endocytosis or postendocytic membrane trafficking. However, the paper by Vicinanza et al. [111] reported the opposite, i.e. a megalin trafficking defect.

*6.6.2. Aminoaciduria*

Organic aciduria (which included amino acids) has been part of the initial description [1] and has been detected in most patients with Lowe syndrome [2,86, 112]. In one report, it was noted to spare branched-chain amino acids, but results typically showed generalized aminoaciduria, although with considerable variability between patients [86]. Whereas aminoaciduria is observed in around 82% of classic Lowe syndrome patients, it manifests in only 52% affected with Dent-2 disease [2,11].

*6.6.3. Lysosomal enzymuria/hyperenzymemia*

Urinary lysosomal enzymuria by determination of N-acetyl-beta-d-glucosaminidase has only rarely been assessed in with patients Lowe syndrome but in those cases uniformly showed elevated levels [2,107,113]. This feature was linked to altered intracellular trafficking of the cation-independent-mannose-6-phosphate receptor, required for the directed recognition and vesicular transport of lysosomal proteins [114]. Norden et al. [113] proposed a mis-trafficking via a default pathway delivering lysosomal enzymes from the Golgi to the apical membrane of proximal tubule cells and excluded cell necrosis as a possible cause. On the other hand, Nielsen et al. [115] reported evidence against a primary defect in intracellular sorting. Hence, the underlying mechanism still remains to be elucidated.

Ungewickell and Majerus [116] measured plasma levels of seven lysosomal enzymes and found a 1.6- to 2.0-fold increase of all enzymes in all patients tested. These authors also postulated a defect in lysosomal enzyme trafficking, leading to the observed increase and suggested that this may cause a tissue damage in Lowe patients. The increased enzyme levels however, could not be attributed to renal insufficiency [116].

*6.6.4. Hypercalciuria/nephrocalcinosis*

Urinary calcium excretion had not been assessed in the original three patients described by Lowe et al. [1], and was not noted in a later series of 23 patients [86]. However, other studies described it as uniformly present in Lowe syndrome and Dent-2 disease patients [2,11,117–119]. Therapy with potassium citrate might be useful to reduce renal calcium excretion and to prevent nephrocalcinosis. Since citrate is metabolized to bicarbonate, citrate treatment may also correct co-existing tubular acidosis. When applying thiazide, which should be considered very carefully, hypokalemia and hyponatremia should be monitored. These patients have already impaired proximal tubular reabsorption with consequent polyuria and salt wasting. Hence, to pharmacologically paralyze another tubular segment makes them extremely vulnerable to hypovolemia. To our knowledge, only one Dent-2 disease patient has been reported by Vrljičak et al. [120] who has been treated with hydrochlorothiazide in combination with amiloride and K-Na-citrate. Here, the authors reported a good response in reducing calciuria.

Nephrocalcinosis and/or calculi occurred in 67% of patients (nine of 15 assessed), reported by Böckenhauer et al. [2] and in 39% of Dent-2 disease cases [11]. Interestingly, this finding is not explainable by the degree of hypercalciuria, nor was it obviously related to age [2]. Hence, the transport pathway for calcium in the proximal tubule, although yet to be defined, is clearly affected by OCRL-1 dysfunction. However, a most recent observation provided evidence for a direct effect of *OCRL* mutations on intestinal calcium transport. Wu et al. [121] investigated, whether the intestinal calcium channel transient receptor potential, vanilloid subfamily, subtype 6 (TRPV6) is regulated by OCRL-1. TRPV6 mediates active calcium absorption and its expression at the apical surface of intestinal epithelial cells increases in response to 1,25-dihydroxyvitamin D3. The authors showed that the Rab binding domain of OCRL-1 was involved in regulating the trafficking of TRPV6 and examined the effect of several Dent-2 disease *OCRL* missense mutations. In a *Xenopus laevis* oocyte expression system, OCRL-1 suppressed TRPV6 activity through modulation of PIP2 levels. All mutants reduced this suppression and increased TRPV6-mediated intestinal calcium absorption, thereby, together with the altered calcium reabsorption in the proximal tubule, providing another clue for hypercalciuria in Dent-2 disease/Lowe syndrome [121].

*6.6.5. Acidosis*

Metabolic acidosis was present in all three children described by Lowe et al. [1]. In a subsequent review of 70 cases, 65 had values for blood carbon dioxide content available, which was normal in 12 (18%) [112]. Similarly, in later reviews, 35% (8/23) and 56% (9/16) of the children did not require alkali substitution to maintain acid-base balance [2,86]. Renal tubular acidosis was detected in only 1 out of 27 patients with mild Lowe syndrome (Dent-2 disease) but in one-third (11/33) of classic Lowe syndrome cases [11]. However, as outlined by Böckenhauer et al. [2] plasma total carbon dioxide concentration was typically found at the lower end of normal. These authors suggested that a more detailed investigation of renal ammonia production and urine acidification, as performed originally, might well reveal subclinical abnormalities [1]. Interestingly, Lowe et al. [1] argued that the decreased production of ammonia actually set his patients apart from those with renal Fanconi syndrome, in whom a strongly increased amount of ammonia were detected [122].

*6.6.6. Phosphaturia*

Phosphaturia was not noted in the first reported patients who had low to normal serum phosphate levels [1]. In an extensive review, 61% (14/23) did not require phosphate supplementation [86]. Four of seven Korean patients had hypophosphatemia and in another series, phosphate wasting was detected in 24% (6/25) of Dent-2 disease patients and in 43% (16/37) with classic Lowe syndrome [11,119]. Obviously, assessment of phosphaturia may be complicated by the often-present elevated parathyroid hormone levels (PTH). TmP/GFR (tubular maximum for phosphate reabsorption) values in the patients reported by Böckenhauer et al. [2] all were obtained while PTH levels were normal, and seven of 16 patients investigated required 1-OH cholecalciferol substitution to keep PTH in the normal range. Thus, the low serum phosphate values in the original series may be partly due to secondary phosphaturia mediated by PTH, rather than primary phosphaturia from tubular dysfunction.

*6.6.7. Glycosuria*

In reviews of Lowe syndrome patients, glucose was only occasionally detected in the urine and then in marginal amounts [2,11,86,112,119]. This is obviously in contrast to other cases of renal Fanconi syndrome, in whom glycosuria is a defining feature and argues strongly for a selective proximal dysfunction in Lowe syndrome [94].

*6.6.8. Poor renal accumulation of technetium-99 m-dimercaptosuccinate (99mTc-DMSA) in Lowe syndrome proximal tubular dysfunction*

An abnormal handling of 99mTc-DMSA has been reported in children with idiopathic tubular proteinuria (Japanese Dent's disease) [123]. Poor accumulation of 99mTc-DMSA in the kidneys with high bladder content of the radionuclide was also found in a total of seven patients with Lowe syndrome and three Dent-2 disease cases [124,125]. A similar abnormal radionucleotide distribution could be detected in other tubular disorders, like Fanconi syndrome, distal renal tubular acidosis and nephronophtisis, even in case of a normal GFR [126–128]. There is still uncertainty how 99mTc-DMSA reaches the bladder since its handling is very complex in the kidneys: it is filtered via the glomerulus and taken up from both the brush border and basolateral membrane of renal tubular cells, in a cotransporter mode together with sodium [129,130]. Hence,

the presence of 99mTc-DMSA in the bladder might be due to an impaired tubular reabsorption as it is known for sodium, phosphate, uric acid, glucose, low molecular weight proteins, and amino acids. Since 99mTc-DMSA visualization of the kidneys occurs by uptake into tubular cells, poor visualization of the kidneys might be explained by failure to extract from the blood or excretion into the tubular lumen. Irrespective of these open questions, 99mTc-DMSA renal scans might be used as diagnostic tool to evaluate functional tubular mass, which depends on the renal blood flow and proximal tubular cell membrane transport function in Lowe patients. It would also be interesting, if an abnormal pattern might also be detectable in female carriers of *OCRL* mutations.

## 7. Further laboratory findings

### *7.1. Elevated serum CK/LDH*

Levels of serum CK, LDH or both were elevated in most patients with Dent-2 disease and all cases with classic Lowe syndrome for whom these measures were available [11,12,124]. The origin of the CK and LDH level elevation is yet unclear and may reflect muscle involvement or nonspecific damage in cell membranes in the kidney because both, CK and LDH, were reported to be involved in the metabolism of proximal tubular cells [36,82].

### *7.2. Hypocarnitinemia*

In about one-third of Lowe patients a decrease in plasma carnitine concentration has been observed, that can be treated by oral carnitine therapy [131].

## 8. Hemostasis

Aside from normal coagulation tests, Lasne et al. [6] detected prolonged closure times with a platelet-function analyzer in all six Lowe patients tested. Matzaris et al. [62] initially detected OCRL-1 in human platelets and these novel findings provide evidence that the platelet-activation anomaly observed might be due to an impaired Rho-dependent signaling caused by decreased OCRL-1 protein. Since data implicate an increased hemorrhagic risk, Lowe syndrome patients should be tested for hemostasis defects, particularly in view of surgery. It has been shown that tranexamic acid improves platelet function in patients with chronic renal failure and it also ameliorates platelet dysfunction in Lowe syndrome patients (D. Böckenhauer, personal communication) [132]. This should be considered pre-surgery or in case of an actively bleeding.

## 9. Reproductive anomalies

About one-third of the patients show cryptorchidism, but in most instances testes descend into the scrotum without hormone therapy or surgical intervention. Delayed onset of puberty has been reported [81].

## 10. Dermatological findings

There are just a few reports regarding Lowe syndrome and dermatological features. Erdoğan et al. [80] found a benign cyst skin tumor, unlike a vellus hair cyst. The interior material originated from mature hair follicles. Epidermal examination issued a nodular lesion and diagnosis revealed trichoepitelioma. Based on the biochemical deficiencies, an *OCRL* mutation was assumed. Nandekar et al. [133] reported that benign skin findings may be more common in Lowe syndrome. In one case, an eruptive vellus hair cyst was presented and abnormal high levels of extracellular lysosomal enzymes, leaving these enzymes free to cause tissue destruction, were found. The authors suggested, that the cyst formation is a result of localized reaction intended to wall off the destructive enzymes, which means the loss of cellular control due to accumulation of PIP2 and the extracellular release of lysosomal enzymes. Otherwise, cyst formation may be due to polarity defects as in ciliopathies, since OCRL-1 has been shown to be involved in ciliogenesis [48,52,53].

A further case presented with multiple eruption cysts in the oral cavity and hematoma formation [98]. Won et al. [134] found various skin lesions reported to be skin colored, deep seated, with soft cystic masses on the temporal and occipital scalp. The histological examination showed each cyst to be lined by true epidermis composed of several layers of stratified squamous epithelium and a granular layer containing keratinous materials arranged in laminated layers. Most recently, a Lowe syndrome patient

presenting with multiple skin folds and an increase in subcutaneous fat has been reported [135].

## 11. Unusual findings in association with Lowe syndrome

In a patient with tubular proteinuria, dysmorphic features, rickets, growth delay, mild cognitive and behavioral impairment, and peripapillary optic nerve atrophy with grey papillae, the co-inheritance of a *CLCN5*- and *OCRL*-mutation was found by Addis et al. [136]. The *OCRL* splice defect observed (c.388+3A>G) locates in the region typically affected in Dent-2 disease cases and the authors suggested, that here the phenotype resulted from synergic interaction between the two mutations. In two Lowe patients who died at the age of 45 and 4 d, respectively, pronounced glomerular changes attributable to congenital nephrotic syndrome, were reported [137].

Elevated high-density lipoprotein cholesterol (HDL-C) levels are present in around 65% of Lowe syndrome patients [82]. In three patients with elevated HDL-C, Asami et al. [138] detected a heterozygous p.Asp442Gly mutation in the gene encoding cholesteryl ester transfer protein (CETP) in one of these patients and assumed this as a possible cause of increased serum HDL-C. The finding of homozygous cystinuria and Lowe syndrome in one family was described by Bailey et al. [139]. In one patient, where diagnosis was only based on decreased IPP-5P activity in cultured skin fibroblasts and not on *OCRL* gene analysis, anal atresia has been reported in association with Lowe syndrome [102]. Further findings were sensorineural deafness, found unilaterally in one patient [12]. Congenital diaphragmatic hernia has been described once in association with classic Lowe syndrome whereas umbilical hernia was found twice in patients with Dent-2 disease [12,140]. However, all these occurrences might rather represent a coincidence than features attributable to an OCRL-1 defect.

## 12. Female carriers

The investigation of 98 mothers of Lowe syndrome patients revealed a de novo mutation in 37.2% of the cases and hence one might assume maternal carriers in around two-third of the occurrences [22]. As in other X-linked diseases, carriers may show a mild phenotype. Following ornithine loading, aminoaciduria was reported in one carrier and other features, i.e. [141]. LMW proteinuria might at least in part, be attributable to unfavorable lionization. In most of the carriers, slit lamp examination reveals punctuate white to grey opacities, distributed in a radial fashion in all layers of the lenticular cortex [142–144].

Heterozygous females may manifest a more complete phenotype due to several mechanisms and a total of ten cases have been reported in the literature. Cytogenetic abnormalities like a reciprocal translocation involving the X-chromosome have been found in two Lowe syndrome carriers [17,18]. Otherwise, the cause might be attributable to a 45,X karyotype, uniparental disomy or an extremely skewed X-inactivation, like the pattern with a ratio of 100:0 detected in a carrier with the full Lowe syndrome phenotype [145]. The causative defect was not ascertained in a further seven cases [146–152]. On the other hand, completely skewed X-inactivation of the *OCRL* mutation carrying X-chromosome was observed in a healthy carrier [136].

## 13. Genetic counseling

It should be kept in mind, that there is a large phenotypic continuum within *OCRL* positive patients and that there may be selective organ involvement with i.e. absence of any ocular involvement [10]. As outlined above, Lowe syndrome can be attributed to a de novo mutation in around one-third of the cases [22]. Although not frequent, the presence of germline mosaicism has to be taken into account. Mosaicism for a single point mutation has been observed in five Lowe families yet and two families showed mosaicism with two de novo events [10,22,153–155]. In one of these cases, triple mosaicism, affecting a single nucleotide residue was observed in the carrier mother [155].

## 14. Prenatal diagnosis

In families, where the *OCRL* mutation is known, genetic diagnosis can be performed following chorionic villi or amniotic fluid sampling [156]. In other cases, early detection of Lowe syndrome might be achieved by measuring the IPP-5P activity in cultured amniocytes, elevated maternal serum and amniotic fluid alpha-fetal protein, or by the diagnosis of fetal cataract [157–161]. A recent report described increased fetal nuchal translucency in two cases with Lowe syndrome [162].

## 15. Perspectives

In conclusions, without the invaluable contributions of the several Lowe syndrome self-help groups many of the current findings of this very rare disorder would not have been obtained. Although a lot of experimental results, phenotypic descriptions and therapeutic approaches have been published, we still only pieced a few parts of the puzzle in the sixty years since the first description of Lowe syndrome [1]. Utilization of zebrafish and novel mouse models may reveal hitherto unrecognized mechanisms by which OCRL-1 deficiency leads to the phenotypic spectrum of the syndrome. This will also lead to the recognition of further proteins involved in the networks affected. The better understanding of disease etiology may provide the basis for developing therapies and preventive strategies i.e. for renal function. Further studies will help to clarify, if the phenotypic variability is either caused by different effects of mutations/variants in other genes and/or might be attributable to environmental effects.

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# 6. Publikation D

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CLINICAL UTILITY GENE CARD

# Clinical utility gene card for: Lowe syndrome

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## DISEASE CHARACTERISTICS

**1.1 Name of the disease (synonyms)**
Lowe syndrome.
Oculocerebrorenal syndrome.
Oculo-cerebro-renal syndrome.
OCR syndrome.

**1.2 OMIM# of the disease**
Lowe syndrome (MIM #309000).

**1.3 Name of the analyzed genes or DNA/chromosome segments**
*OCRL*, Xq25–q26.1.

**1.4 OMIM# of the gene(s)**
*OCRL*, 300535.

**1.5. Mutational spectrum**
The *OCRL* gene was identified by positional cloning[1] and its genomic structure, comprising 24 exons occupying 52 kb, has been elucidated.[2] Since then, more than 200 Lowe syndrome patients with *OCRL* defects have been identified, which were extensively reviewed by Hichri *et al.*[3] Disease-causing variants are scattered throughout the gene and the majority of patients (63%) display frameshift, nonsense or splice defects[3] leading to mRNA decay or premature termination of the resultant OCRL-1 protein. Missense variants and gross deletions account for 33 and 4% of the cases, respectively.[3] No variant affecting the alternative exon 19 has been reported so far.

Human wild-type *OCRL* gene and OCRL-1 protein with their corresponding exon and amino acid numbering are deposited in GenBank, acc. nos. NM_000276.3 and AAB03839. *OCRL* variants are included in the Human Gene Mutation Database (http://www.hgmd.org/) or can be obtained via the Leiden Open Variation Database (http://www.ncbi.nlm.nih.gov/lovd/home.php?select_db=OCRL).

**1.6 Analytical methods**
Bi-directional Sanger sequencing of PCR-amplified products comprising the total coding region and the exon–intron boundaries of the *OCRL* gene. For detection of genomic *OCRL* rearrangements and/or precise gene quantification, the multiplex ligation-dependent probe amplification might be used.[4]

**1.7 Analytical validation**
Confirmation of the detected variant at least from a second amplicon, preferentially from an independent biological sample of the index case. Pathogenicity of novel missense variants has to be verified by (i) testing a set of at least 100 chromosomes from normal ethnically matched controls, (ii) considering its deposition in SNP databases and (iii) using *in-silico* prediction methods. Gene transcripts should be analyzed in case of splice variants. The gold standard is analysis of functional consequences of the respective *OCRL* variant in cell models, performed in a few laboratories in the world.[3,5–7] In case of suspected splice site variants, *OCRL* mRNA should be analyzed.

**1.8 Estimated frequency of the disease (incidence at birth ('birth prevalence') or population prevalence)**
1:500,000.[8]

**If known to be variable between ethnic groups, please report)**
Not applicable.

**1.9 Diagnostic setting**

| | Yes | No |
|---|---|---|
| A. (Differential) diagnostics | ☒ | ☐ |
| B. Predictive testing | ☒ | ☐ |
| C. Risk assessment in relatives | ☒ | ☐ |
| D. Prenatal | ☒ | ☐ |

Comment: In affected boys, bilateral cataracts, one of the cardinal symptoms of Lowe syndrome, develop *in utero* and are almost invariably present at birth.[3,8,9] Other ocular findings include microphthalmia, enophtalmos and glaucoma, the latter developing in the first three decades in 50–60% of the patients. About 25% of Lowe syndrome patients have corneal scarring and keloids.

CNS pathology manifests as neonatal and infantile hypotonia with areflexia and delay in motor development.[8] Seizures are observed in about half of the patients. Typically, patients have mildly elevated creatine kinase and/or lactate dehydrogenase levels.[10] Intellectual disability is a cardinal finding with only 10% of patients having normal intelligence. Behavioral abnormalities (stereotypic behavior, self-injury, tantrums, aggression/irritability, repetitive non-purposeful movements) are common, too.

The renal phenotype is characterized by proximal tubular dysfunction. Impaired reabsorption of low-molecular-weight proteins is

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present in all patients, while disturbances in other tubular functions are variable:[10,11] Generalized aminoaciduria in 80%, phosphate and potassium wasting (in 40 and 20%, respectively), proximal renal tubular acidosis in 35% and slowly progressive renal failure leading to end-stage renal failure in the second and third decade. Unlike other tubular functions, glucose reabsorption is less affected. Like patients with Dent disease (see below), the majority of patients have hypercalciuria (80%), while nephrocalcinosis is observed less often (40–50%). The pattern of tubular dysfunction in the two forms of Dent disease and Lowe syndrome was compared by Bokenkamp *et al.*[10]

Additional features of Lowe syndrome are postnatal growth retardation and a debilitating non-inflammatory teno-arthropathy present in 50% of adult patients.

In a subgroup of patients with *OCRL* variants, the clinical phenotype is dominated by the renal manifestations of the disease and the ocular and cerebral findings are very subtle. These patients are classified as having Dent-2 disease (MIM #300555).[10,12] Reported extra-renal abnormalities were mild growth retardation, clinically unapparent cataract in 2/28, subtle mental retardation in 9/23 and elevated creatine kinase or lactate dehydrogenase in all.[10] In selected cases the classification as Lowe syndrome or Dent-2 disease can be somewhat arbitrary. The characteristics of the two forms of Dent disease and Lowe syndrome are summarized in Table 1.

## 2. TEST CHARACTERISTICS

### 2.1 Analytical sensitivity
**(proportion of positive tests if the genotype is present)**

Close to 100%. The sensitivity of sequence analysis of PCR-amplified products approaches 100%. Many variants have been tested functionally, and the pathogenicity of most variants has been predicted by publically available algorithms. Nonetheless, errors may occur due to allele dropout and variants outside the coding region in the promoter, polyA site, enhancers or intronic variants may be missed.

### 2.2 Analytical specificity
**(proportion of negative tests if the genotype is not present)**

Nearly 100%. In rare cases, variants may erroneously be interpreted as pathogenic.

**Table 1 Clinical and laboratory characteristics of Lowe syndrome compared with Dent-1 (*CLCN5* mutation) and Dent-2 (*ORCL* mutation) disease[8–10]**

| | *Dent-1 (CLCN5+)* | *Dent-2 (OCRL+)* | *Lowe (OCRL+)* |
|---|---|---|---|
| Cataract | No | 10% (asymptomatic) | Almost 100% |
| Intellectual impairment | No | 30% (mild) | 90% |
| Growth retardation | No | Postnatal, mild (−1 to −2 SD) | Postnatal, severe (−2 to −6 SD) |
| Elevated LDH or CK | 36% | 100% | 100% |
| LMW-PU | 100% | 100% | 100% |
| Hypercalciuria | 90% | 86% | 83% |
| Nephrocalcinosis | 75% | 39% | 44% |
| Aminoaciduria | 41% | 52% | 82% |
| RTA | 3% | 4% | 33% |
| Phosphate wasting | 22% | 24% | 43% |
| Potassium wasting | 15% | 6% | 21% |
| Glycosuria | 17% | 11% | 7% |
| Renal failure | 30% | 32% | 74% |

Abbreviations: CK, creatine kinase; LDH, lactate dehydrogenase; LMW-PU, low-molecular-weight proteinuria; RTA, renal tubular acidosis; SD, standard deviation.

### 2.3. Clinical sensitivity
**(Proportion of positive tests if the disease is present)**

Variants in *OCRL* account for 80–90% of cases with a phenotype of Lowe syndrome.[3]

### 2.4 Clinical specificity
**(proportion of negative tests if the disease is not present)**

100%.

### 2.5 Positive clinical predictive value
**(lifetime risk to develop the disease if test is positive)**

Almost 100%. Still, a small number of patients with *OCRL* variants have a predominantly renal phenotype and are classified as having Dent-2 disease on clinical grounds. In a large series, 6 out of 136 families with an *OCRL* variant were classified as Dent-2.[3] There have been incidental reports of Lowe syndrome and Dent-2 in patients harboring the same variant, even within one family.[3]

### 2.6 Negative clinical predictive value
**(Probability of not developing the disease if the test is negative)**

Almost 100%, still in some patients with clinical features of Lowe syndrome no *OCRL* variants were detected.[3]

## 3. CLINICAL UTILITY

### 3.1 (Differential) diagnostics: the person is clinically affected

***3.1.1 Can a diagnosis be made other than through a genetic test?***

| | | |
|---|---|---|
| No | ☐ (continue with 3.1.4) | |
| Yes | ☒ | |
| | Clinically | ☒ |
| | Imaging | ☐ |
| | Endoscopy | ☐ |
| | Biochemistry | ☒ |
| | Electrophysiology | ☐ |
| | Other (please describe): | Ophthalmological examination |

Comment: The combination of all three diagnostic domains is diagnostic. Q1

Slit-lamp examination can be used for identification of female carriers.[8]

***3.1.2. Describe the burden of alternative diagnostic methods to the patient***

The alternative methods to diagnose Lowe syndrome are generally non-invasive. The diagnosis of Lowe syndrome is suggested by congenital cataract, which is almost uniformly present. Recognition of the cerebral manifestations is based on a thorough neurological examination. The biochemical diagnosis of Lowe syndrome is not invasive (spot urine for low-molecular-weight proteinuria, hypercalciuria and variable presence of other proximal tubular dysfunctions).

***3.1.3 How is the cost effectiveness of alternative diagnostic methods to be judged?***

The cost effectiveness of the physical examination in combination with an ophthalmological examination and simple biochemical tests is high.

***3.1.4 Will disease management be influenced by the result of a genetic test?***

Not for Lowe syndrome. In patients with the mild Dent-2 phenotype, who might be missed clinically, nephrological follow-up is warranted.[12]

**3.2 Predictive setting: the tested person is clinically unaffected but carries an increased risk based on family history**

***3.2.1 Will the result of a genetic test influence lifestyle and prevention?***

Not for Lowe syndrome. In case of the milder Dent-2 phenotype, nephrological follow-up should be initiated.[12]

***3.2.2 Which options in view of lifestyle and prevention does a person at risk have if no genetic test has been done?***

Urine could be tested for the presence of low-molecular-weight proteinuria, which is an obligate finding in Dent-2 disease. In case of Dent-2 disease, nephrological follow-up should be initiated.[12]

**3.3 Genetic risk assessment in family members of a diseased person**

***3.3.1 Does the result of a genetic test resolve the genetic situation in that family?***

Yes.

***3.3.2 Can a genetic test in the index patient save genetic or other tests in family members?***

Yes. If the index case has known mutations, siblings, parents and other family members can be screened for disease by ophthalmological examination and urine analysis for low-molecular-weight proteinuria.

***3.3.3 Does a positive genetic test result in the index patient enable a predictive test in a family member?***

Yes. Still, variable clinical expression has to be considered (cf. 2.5).

**3.4 Prenatal diagnosis**

***3.4.1 Does a positive genetic test result in the index patient enable a prenatal diagnosis?***

Yes. Still, variable clinical expression has to be considered (cf. 2.5).

## 4. IF APPLICABLE, FURTHER CONSEQUENCES OF TESTING

Please assume that the result of a genetic test has no immediate medical consequences. Is there any evidence that a genetic test is nevertheless useful for a patient or his/her relatives:

Establishing an unequivocal molecular diagnosis may be helpful for the family.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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