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Original Article

Long-term renal outcome in children with *OCRL* mutations: retrospective analysis of a large international cohort

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ABSTRACT

Background. Lowe syndrome (LS) and Dent-2 disease (DD2) are disorders associated with mutations in the *OCRL* gene and characterized by progressive chronic kidney disease (CKD). Here, we aimed to investigate the long-term renal outcome and identify potential determinants of CKD and its progression in children with these tubulopathies.

Methods. Retrospective analyses were conducted of clinical and genetic data in a cohort of 106 boys (LS: 88 and DD2: 18). For genotype–phenotype analysis, we grouped mutations according to their type and localization. To investigate progression of CKD we used survival analysis by Kaplan–Meier method using stage 3 CKD as the end-point.

Results. Median estimated glomerular filtration rate (eGFR) was lower in the LS group compared with DD2 (58.8 versus 87.4 mL/min/1.73 m^2, $P < 0.01$). CKD stage II–V was found in 82% of patients, of these 58% and 28% had moderate-to-severe CKD in LS and DD2, respectively. Three patients (3%), all with LS, developed stage 5 of CKD. Survival analysis showed that LS was also associated with a faster CKD progression than DD2 ($P < 0.01$). On multivariate analysis, eGFR was dependent only on age ($b = -0.46$, $P < 0.001$). Localization, but not type of mutations, tended to correlate with eGFR. There was also no significant association between presence of nephrocalcinosis, hypercalciuria, proteinuria and number of adverse clinical events and CKD.

Conclusions. CKD is commonly found in children with *OCRL* mutations. CKD progression was strongly related to the underlying diagnosis but did not associate with clinical parameters, such as nephrocalcinosis or proteinuria.

Keywords: chronic kidney disease, Dent-2 disease, Lowe syndrome, nephrocalcinosis, *OCRL*

ORIGINAL ARTICLE

INTRODUCTION

The oculocerebrorenal syndrome of Lowe (LS) (OMIM #309000) and its milder variant, Dent-2 disease (DD2) (OMIM #300555) are ultrarare X-linked disorders associated with mutations in *OCRL*, which encodes the enzyme inositol polyphosphate 5-phosphatase, OCRL1 [1, 2]. OCRL1 is involved in proximal tubular endocytosis and is also reported to play a role in the maturation of polarized epithelial cells and in cytokinesis and ciliogenesis [3, 4]. Despite being caused by mutations in the same gene, LS is characterized by multi-organ involvement with the triad of congenital cataracts, neurological abnormalities and a selective tubular dysfunction of variable extent, whilst the DD2 phenotype is restricted mainly to a proximal tubulopathy [5–7]. The life span of an affected individual with LS rarely exceeds 40 years and is mainly limited by progressive kidney failure [8]. Severity of chronic kidney disease (CKD) is variable and the factors determining this variability remain to be elucidated.

A genotype–phenotype effect has been observed in some disorders, but this has not been shown in LS and DD2 so far [9]. However, there is a distinct distribution of mutations along the *OCRL* gene. In DD2, most mutations have been detected 5′ of exon 8, whereas in classic LS, mutations concentrate in exons 8–24 [9]. Besides the extra-renal manifestations, there also appears to be a difference with regards to renal involvement in these two phenotypes in that the prevalence of renal failure is lower in patients with DD2 compared with LS (32 and 74%, respectively) [6].

In the present study, we conducted retrospective analyses of genetic and clinical data in a large, well-characterized cohort of children with *OCRL* mutations. We aimed to compare the long-term renal outcome between the LS and DD2 groups and to investigate possible determinants of CKD progression, ranging from clinical factors, such as nephrocalcinosis, to a potential genotype effect.

MATERIALS AND METHODS

This retrospective, multicentre study was designed to collect data obtained from paediatric patients (<19 years of age) with OCRL-related disorders. A spreadsheet with requested information was sent to clinicians. We requested the most recent data, including anthropometrical and biochemical parameters as well as clinical data obtained throughout the observation period [presence of hypertension, episodes of dehydration, stone obstruction, urinary tract infections, acute kidney injuries (AKI), numbers of contrast X-ray studies, presumed cause of death]. Additionally, the physicians were asked to provide details of treatments, including height and serum creatinine before and on growth hormone (GH) therapy. To determine longitudinally the rate of CKD progression, height and serum creatinine were obtained and individually an averaged estimated glomerular filtration rate (eGFR)/year was calculated.

Between September 2014 and December 2015, data on 107 patients (boys) with LS and DD2 were collected. Finally, due to lack of the height parameter in one patient, 106 children were included into the analysis. Eighty-eight cases (83%) presented with full oculo cerebro renal criteria, whereas the remaining patients, defined by clinicians as DD2, showed a milder phenotype of LS ($n = 6$; 5.7%) or exhibited renal tubulopathy only ($n = 12$; 11.3%). The patients originated from: Poland ($n = 27$), Korea ($n = 24$), Italy ($n = 16$), the UK ($n = 15$), Germany ($n = 10$), Greece ($n = 6$), Macedonia ($n = 3$), and Kazakhstan, Malta, Serbia, Slovenia and Sweden ($n = 1$, each).

eGFR was calculated using the original Schwartz method [10], but with a revised k-value of 26 (when serum creatinine is in μmol/L) for patients with LS, as suggested previously [5].

The subject's severity of CKD was classified by strata defined by the National Kidney Foundation Kidney Disease Outcomes Quality Initiative (K/DOQI) CKD staging system [11], taking into account physiologically lower GFR in children <2 years [12].

Urine analysis was performed on spot samples and calciuria, phosphaturia, glycosuria, aminoaciduria, untimed proteinuria, creatininuria and low-molecular weight proteinuria (LMWP) was determined by dipstick and/or formal local laboratory methods. Calciuria and proteinuria was also assessed by 24-h urine collection in some patients. Calcium excretion >4 mg/kg/24 h was classified as hypercalciuria, as well as an elevated calcium-to-creatinine ratio (Ca/Cr; mg/mg) in a spot urine sample with age-appropriate references [13]. Hyperphosphaturia was designated as the presence of a decreased tubular reabsorption of phosphate (<80%) or tubular maximum for phosphate reabsorption normalized to the age-appropriate lower limit of normal [14]. Hypoalbuminaemia was defined as serum albumin <35 g/L and hyperparathyroidism as serum intact parathyroid hormone >66 pg/mL. Plasma bicarbonates, phosphate and potassium values were assessed and acidosis, hypophosphataemia and hypokalaemia were defined when receiving supplementation or/and with decreased blood levels, i.e. for acidosis <22 mmol/L [15], for hypophosphataemia [15] and for potassium <3.5 mmol/L. Fanconi syndrome was recognized when a constellation of proximal renal tubular abnormalities was present (i.e. LMWP, hypercalciuria, acidosis, glycosuria, aminoaciduria and hypophosphataemia).

Urinary protein-to-creatinine ratio (P/Cr; mg/mg) and daily protein excretion assessed in 24-h urine collection (mg/m^2/h) were used to define the level of proteinuria. Patients were identified as having normal ratios (P/Cr < 0.2), significant proteinuria (P/Cr 0.2–2) or high grade proteinuria (P/Cr > 2). Daily proteinuria was graded into three groups: <4 mg/m^2/h, 4–40 mg/m^2/h and >40 mg/m^2/h. In a few cases (n = 13), only dipstick proteinuria was available and was graded as 1+ (closest to 30 mg/dL), 2+ (closest to 100 mg/dL), 3+ (closest to 300 mg/dL) and 4+ (>2000 mg/dL). To integrate the different categories of proteinuria submitted by the various centres, we adopted a grading system (grade 0 – P/Cr ratio <0.2 or <4 mg/m^2/h or <1+; grade 1 – P/Cr ratio 0.2–2 or 4–40 mg/m^2/h or 2+; grade 2 – P/Cr ratio >2 or >40 mg/m^2/h or 3+/4+) [16].

Nephrocalcinosis and nephrolithiasis were assessed from ultrasound and/or CT/X-ray reports. Height and body mass index (BMI) data in the European children were transformed into standard deviation scores (SDS) with reference to the World Health Organization growth charts (http://www.who.int/growthref/en). For children of Asian ethnicity, SDS data were reported directly by the treating physicians. Short stature was defined as height SDS less than −2.

For the genotype–phenotype analysis, we grouped mutations according to the following criteria: (i) the expected effect on the protein product (i.e. missense, unlikely to cause complete lack of protein production and truncated mutations, which comprise nonsense, frameshift, splice-site mutations and exonic deletions, assumed to produce no or a truncated protein product) [9]. (ii) The localization/position of mutations, i.e. mutations were analysed with respect to recognized functional OCRL1 domains [i.e. exons 2–5 for pleckstrin homology domain (PH); exons 9–15 for a central 5-phosphatase domain; exons 16–20 for ASPM-SPD2-Hydin (ASH); and exons 21–24 for Rho GTPase activating (RhoGAP) domain] [9].

Statistical analysis

Data were presented as a mean (standard deviation) or as median (upper/lower quartiles) as appropriate for continuous variables and as absolute numbers and/or percentages for categorical variables. One-way ANOVA and unpaired t-test were used to analyse the data with normal distribution and homogeneous variances. The data that did not follow a Gaussian distribution were analysed with Mann–Whitney U-test or the Kruskal–Wallis test and the Dunn's *post hoc* test. The relationship between variables was analysed with the Spearman's rank correlation coefficient and by multivariate linear regression. Categorical data were analysed with the χ^2 test or the Fisher-Freeman-Halton test. For the longitudinal analysis, a median of all available eGFR for all patients at each age (1–19 years) was calculated and was shown as trend lines over time (details are provided in Supplementary data, Figure S1 and Table S1). The age of eGFR decline (break-point) was calculated by means of piecewise linear regression. CKD progression was analysed by the Kaplan–Meier method in a univariate analysis. The end-point was the first measurement of eGFR <60 mL/min/1.73 m^2. Data were censored at the last available eGFR measurement, and differences between subgroups were assessed by the log-rank test. All results were considered significant at $P < 0.05$. Statistical calculations were performed using STATISTICA 10.0 PL (StatSoft Polska, Kraków, Poland).

RESULTS

The characteristics of 106 children (all boys) from 97 families are displayed in Table 1. The median age at last follow-up was 10 years (5.9; 16) and was similar between the LS and DD2 groups. The frequencies of abnormalities of tubular function reflected previous studies [5, 6, 17] and were as follows: LMWP (100%), hypercalciuria (80%), nephrocalcinosis (52%), nephrolithiasis (24%), metabolic acidosis (68%), hypophosphataemia (44%), hypokalaemia (20%), aminoaciduria (65%) and glycosuria (16%). An apparent complete renal Fanconi syndrome was identified in only six patients with LS (5.7%). Short stature was noted in 84% of patients when compared with a normal population. As expected, subjects with LS were shorter than those with DD2 (median height SDS: −3.98 versus −2.11, $P < 0.001$). Moreover, phosphate wasting and acidosis were observed more frequently in LS than in DD2 (see Table 1).

All patients presented with proteinuria. As LMWP was not reported/determined in all patients this parameter was not studied in detail. Twenty percent of children had significant proteinuria (grade 1 in 16/82 patients), and the rest of the patients had severe proteinuria (grade 2 in 66/82 patients). Urinary P/Cr ratio was commonly used for the assessment of proteinuria in our cohort (n = 63). The median P/Cr ratio was 3.9 mg/mg (2.7; 7.1), and was greater in patients with LS

Table 1. Characterization of the entire cohort ($n = 106$) and the subgroups, i.e. Lowe syndrome ($n = 88$) and Dent-2 disease ($n = 18$)

Parameter	OCRL (all)	Lowe syndrome	Dent-2 disease	P[a]	Range/normal
Ethnicity, European	81 (76%)	68 (77%)	13 (72%)	ns	–
Age at last visit (years), $n = 106$	10 (5.9; 16)	9.8 (4.8; 16)	13.8 (7; 17)	ns	1–18.5
Age at clinical diagnosis (year), $n = 102$	0.95 (0.4; 5)	0.65 (0.3; 2)	5.95 (5; 10)	<0.0001	0.1–18
Age at molecular diagnosis (year), $n = 93$	2.6 (0.8; 9.5)	1.8 (0.8; 7)	9.4 (6.2; 12)	<0.0001	0.1–19
Height SDS, $n = 106$	−3.74 (−5.72; −2.75)	−3.98 (−6.04; −3.29)	−2.11 (−3.23; −1.1)	<0.0001	−10.15 to 0.47
Growth retardation	89 (84%)	80 (91%)	9 (50%)	<0.001	<−2 SDS
BMI SDS, $n = 99$	0.55 (−0.8; 1.68)	0.55 (−1.21; 1.52)	0.85 (0.02; 2.03)	ns	−4.26 to 2.96
Nephrocalcinosis, $n = 104$	54 (52%)	45 (52%)	9 (50%)	ns	–
Nephrolithiasis, $n = 104$	25 (24%)	19 (22%)	6 (33%)	ns	–
Serum creatinine (μmol/L), $n = 106$	51.6 (31.8; 77.8)	50.6 (30.9; 76)	59.2 (42.4; 107.8)	ns	11.5–415.5
eGFR (mL/min/1.73 m^2), $n = 106$	62.5 (46.2; 85.6)	58.8 (43; 83)	87.4 (56; 91.6)	<0.01	8.2–181
CKD (>2 years)	97	79	18	–	–
CKD stage 1	19 (19.6%)	12 (15.2%)	7 (38.9%)	<0.05	eGFR > 90 mL/min/1.73 m^2
CKD stage 2	27 (27.8%)	21 (26.6%)	6 (33.3%)	ns	eGFR 89–60 mL/min/1.73 m^2
CKD stage 3	42 (43.3%)	37 (46.8%)	5 (27.8%)	ns	eGFR 59–30 mL/min/1.73 m^2
CKD stage 4	6 (6.2%)	6 (7.6%)	0 (0%)	ns	eGFR 29–15 mL/min/1.73 m^2
CKD stage 5/ESKD	3 (3.1%)	3 (3.8%)	0 (0%)	ns	eGFR < 15 mL/min/1.73 m^2
Albuminaemia (g/L), $n = 89$	45 (43; 48)	45.0 (42.7; 47)	45.5 (43; 49.2)	ns	29–56
Hypoalbuminaemia	4 (4.5%)	4 (5%)	0 (0%)	ns	<35 g/L
Serum phosphate (mmol/L), $n = 103$	1.27 (1.07; 1.49)	1.23 (1.03; 1.42)	1.42 (1.29; 1.58)	<0.05	0.31–2.02
Hypophosphataemia	45 (44%)	45 (52%)	0 (0%)	<0.001	Phosphate level [15] or phosphate supplementation
Actual hypophosphataemia	34 (33%)	34 (39.5%)	0 (0%)	<0.05	Phosphate level [15]
Hyperphosphaturia, $n = 68$	34 (50%)	33 (57%)	1 (10%)	<0.05	TRP >80% or TmP/GFR [14]
Bicarbonate concentration (mmol/L), $n = 101$	22 (19; 24)	21 (19; 23.9)	24.4 (23; 25)	<0.0001	13.7–33
Acidosis	69 (68%)	66 (79.5%)	3 (17%)	<0.0001	$HCO3^-$ <22 mmol/ or alkali supplementation
Actual acidosis	50 (49.5%)	49 (59%)	1 (5.5%)	<0.001	$HCO3^-$ <22 mmol/L [15]
Serum potassium (mmol/L), $n = 104$	4.0 (3.6; 4.2)	4.0 (3.6; 4.2)	4.1 (3.9; 4.2)	ns	2.7–5.8
Hypokalaemia	21 (20.2%)	20 (23%)	1 (5.5%)	ns	Potassium <3.5 or potassium supplementation
Actual hypokalaemia	15 (14.4%)	15 (17%)	0 (0%)	ns	>3.5 mmol/L
iPTH (pg/mL), $n = 76$	31.7 (17.8; 52.5)	36.9 (18; 53.5)	21.3 (10.9; 38.4)	ns	2.8–174
Hyperparathyroidism	11 (14.5%)	10 (16%)	1 (8%)	ns	>66 pg/mL
Proteinuria				–	–
LMWP, $n = 77$	77 (100%)	60 (100%)	17 (100%)	ns	–
Dipstick proteinuria (mg/dL), $n = 13$	154 (111; 170)	154 (111; 170)	–	–	50–513
P/Cr ratio (mg/mg), $n = 63$	3.9 (2.7; 7.10)	4.8 (3.3; 7.6)	2.2 (1.6; 3.6)	<0.0001	0.3–16.7
P/Cr ratio 0.2–2.0	9 (14%)	3 (6.4%)	6 (37.5%)	<0.01	–
P/Cr ratio >2.0	54 (86%)	44 (93.6%)	10 (62.5%)		–
Daily excretion of protein (mg/m^2/h), $n = 18$	45 (28.7; 68)	42 (12.5; 84)	48 (40; 58)	ns	8–100; <4 mg/m^2/h
Proteinuria grading	82	65	17	ns	–
Grade 1	16 (20%)	13 (20%)	3 (18%)		–
Grade 2	66 (80%)	52 (80%)	14 (82%)		–
Hypercalciuria, $n = 95$	76 (80%)	64 (83%)	12 (67%)	ns	–
Daily excretion (mg/kg/24 h), $n = 8$	6.0 (4.6; 7.05)	6.2 (4.6; 7.5)	4.5 (4.5; 4.5)	ns	2.4–8.9; <4 mg/kg/24 h
Ca/Cr ratio (mg/mg), $n = 87$	0.39 (0.29; 0.66)	0.46 (0.3; 0.74)	0.38 (0.15; 0.44)	<0.05	0.09–1.68 [13]
Glycosuria, $n = 104$	17 (16%)	13 (15%)	4 (22%)	ns	–
Aminoaciduria, $n = 77$	50 (65%)	46 (78%)	4 (22%)	ns	–
Fanconi syndrome, $n = 106$	6 (5.7%)	6 (6.8%)	0 (0%)	ns	–
Hypertension, $n = 106$	0 (0%)	0 (0%)	0 (0%)	ns	–
Death, $n = 106$	4 (3.8%)	4 (4.5%)	0 (0%)	ns	–
Treatments					–
Alkali therapy	60 (57%)	58 (66%)	2 (11%)	<0.001	–
Bicarbonates supplementation	45 (42%)	45 (51%)	0 (0%)	<0.001	–
Citrates supplementation	40 (38%)	38 (43%)	2 (11%)	<0.05	–
Phosphate supplementation	24 (23%)	24 (27%)	0 (0%)	<0.05	–
Potassium supplementation	13 (13%)	12 (14%)	1 (5.5%)	ns	–
Thiazides	7 (7%)	6 (6.8%)	1 (5.5%)	ns	–
ACE/ARB	17 (16%)	15 (17%)	2 (11%)	ns	–
Vitamin D	30 (28%)	30 (34%)	0 (0%)	<0.05	–
GH	11 (10.4%)	9 (10%)	2 (11%)	ns	–
Not treated	36 (34%)	22 (25%)	14 (78%)	<0.001	–

Data are given as median and lower/upper quartiles/range (continuous variables) or as absolute numbers and percentages (categorical variables).
SDS, standard deviation score; BMI, body max index; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; ESKD, end-stage kidney disease; TRP, tubular reabsorption of phosphate; TmP/GFR, tubular maximum for phosphate reabsorption; iPTH, intact parathyroid hormone; LMWP, low-molecular weight proteinuria; P/Cr ratio, protein-to-creatinine; Ca/Cr ratio, calcium-to-creatinine; ACE, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blocker; GH, growth hormone; ns, not significant.
[a]P statistical differences between patients with Lowe syndrome and Dent-2 disease.

compared with DD2 ($P < 0.0001$). Notably, P/Cr ratio did not correspond either to the severity of CKD or eGFR. Importantly, this parameter highly correlated with BMI SDS ($r = -0.58$, $P < 0.0001$).

Clinical course and causes of death

Ten patients (9.4%) experienced at least one episode of urinary tract infection (9 patients had recurrent infections, i.e. >2 episodes), 18 patients (17%) had one episode of dehydration requiring hospitalization (7 patients had ≥2 episodes), 11 patients (10.4%) had AKI and 3 patients (2.8%) had urinary stone obstruction. These events were almost exclusively present in patients with LS, except two episodes of dehydration in one patient with DD2. Seventeen individuals (16%) underwent contrast X-ray studies.

Four patients (3.7%) died in the analysed cohort (the genotypes of three patients are shown in Supplementary data, Table S2). In one previously reported patient (ls13) [17], death occurred at the age of 18 months and was related to pneumonia, while the other three patients died at later ages: one (ls25.2; 11.5 years) due to apnoea/deep acidosis, and the other two, including the patient ls25.4 (17–18 years) due to sudden death of unknown causes. Notably, the two patients (ls25.2 and ls25.4) were from the same family (cousins) and had the same complex genotype described in detail elsewhere [17].

Chronic kidney disease

Median eGFR was lower in the LS group compared with DD2 (58.8 versus 87.4 mL/min/1.73 m^2, $P < 0.01$). CKD stage 2–5 was found in the majority of patients (74%) when the entire age range was analysed, and was evident in 82% of patients when the analysis was restricted to those >2 years ($n = 97$). Overall, nearly half of the patients (49%) had moderate (eGFR 30–59 mL/min/1.73 m^2) and 9.3% severe CKD (eGFR <30 mL/min/1.73 m^2). Moderate-to-severe CKD was more common in LS compared with DD2 (58 versus 28%). End-stage kidney disease (ESKD) was found only in LS individuals. Two patients (ls66 and ls76), who developed ESKD at ages of 14 and 16 years, respectively, harboured mutations in exon 22 (p.Arg822* and p.Glu806Asp, respectively), while the third patient (ls25.4), with ESKD at the age of 17 years, had a complex mutation in exon 14/intron 14 (p.Arg486Serfs). Importantly, his cousin (ls25.3) with the same genotype had severe CKD (eGFR of 15.5 mL/min/1.73 m^2) (Supplementary data, Table S2).

In the univariate analysis (Table 2), there was a significant relationship between eGFR and Ca/Cr ratio ($r = 0.32$, $P < 0.01$) as well as between eGFR and age in the whole group ($r = -0.5$, $P < 0.0001$; Figure 1). After the multivariate analysis, only age remained significantly correlated ($b = -0.46$, $P < 0.001$). Both age and Ca/Cr corresponded also with CKD stages. The presence of nephrocalcinosis, hypercalciuria, proteinuria, the number of adverse clinical events (mentioned above) and contrast X-ray studies had no evident effect on renal function. Height SDS was highly correlated with bicarbonate levels ($r = 0.35$, $P < 0.0001$) and the patients with uncorrected acidosis were significantly shorter than their counterparts without acidosis (height SDS: −4.08 versus −3.03, $P < 0.0001$).

Table 2. Univariate correlations between eGFR and other variables in the entire cohort

Variable	Correlation coefficient	P
Age	−0.5	<0.0001
Age of clinical diagnosis	−0.06	0.57
Age of molecular diagnosis	−0.20	0.10
Height SDS	0.05	0.57
BMI SDS	−0.2	0.05
Bicarbonate concentration	−0.12	0.24
Albuminemia	−0.13	0.19
Serum phosphate	0.02	0.82
Serum potassium	0.09	0.38
iPTH	0.00	0.99
P/Cr	−0.06	0.64
Ca/Cr	0.32	<0.01

eGFR, estimated glomerular filtration rate; SDS, standard deviation score; BMI, body max index; iPTH, intact parathyroid hormone; P/Cr ratio, protein-to-creatinine; Ca/Cr ratio, calcium-to-creatinine.

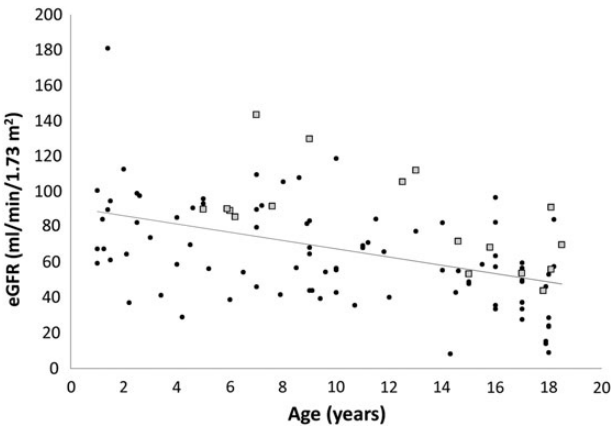

FIGURE 1: A scatter plot of eGFR versus age in children with Dent-2 disease (squares) and Lowe syndrome (black dots) ($r = -0.5$, $P < 0.0001$, for the entire group).

In the longitudinal analysis, in children with LS eGFR linearly falls with age and a clear break-point of eGFR decline occurs at the age of 10 years, whereas in subjects with DD2 eGFR remained stable during childhood (Figure 2 and Supplementary data, Figure S1). Renal survival censored by Kaplan–Meier analysis showed that LS was also associated with a faster progression to stage 3 of CKD ($P < 0.01$; Figure 3A).

Treatment

Table 1 summarizes participants' treatment characteristics. The analysis of therapy in our group showed variable treatments. Overall, acidosis was treated in 57% of patients with alkali therapy, yet 49.5% remained acidotic. A third (33%) of patients were hypophosphataemic, yet only 23% received phosphate supplementation. Similarly, 14.4% were hypokalaemic, and 13% received potassium supplementation. Overall, 34% of patients did not receive any treatment.

Eleven patients (10.4%; nine patients with LS) had been treated with GH for a median time of 64 months (range 30–89), and all but one had experienced an increase in height. Mean height SDS increased from −4.08 ± 1.30 to −3.20 ± 1.36 ($P < 0.001$). Yet, eight patients were still short-statured. In DD2 patients

who had a slight growth deficiency (height SDS −2.2 and −2.8), height on GH normalized. There was no apparent association with eGFR (59.7 ± 34.8 versus 59.4 ± 25.0 mL/min/1.73 m^2, before and after the therapy, respectively), although in one patient, a significant deterioration in eGFR was observed (97 versus 35 mL/min/1.73 m^2), which was in excess of the expected age-associated deterioration.

Analyses of *OCRL* mutations

Supplementary data, Table S2 shows a summary of the mutations and their predicted effects on OCRL1 protein. In our study cohort, 104 patients (98%) had a molecular diagnosis, which was made at a median time of 2.6 years (0.8; 9.5) (Table 1), yet a molecular result was not available for five patients. A total of 90 different mutations were found in the 99 mutation-positive patients. Two patients with classical features of LS and three patients with a clinical diagnosis of Dent disease had no detectable mutations in *OCRL* or *CLCN5*. We report 17 (19%) previously unreported mutations in 21 patients associated either with LS or DD2. The phenotypic features of patients harbouring novel mutations are presented in Table 3. As evident in Table 4, the mutations are diverse. More than two-thirds of patients (66.6%) carry truncating mutations. As demonstrated in the genogram (Figure 4), mutations are scattered throughout the gene, and mutations in LS locate between exons 8 and 24, whereas they affect exons 4–15 in DD2. We did not observe any *OCRL* mutation found in both LS and DD2.

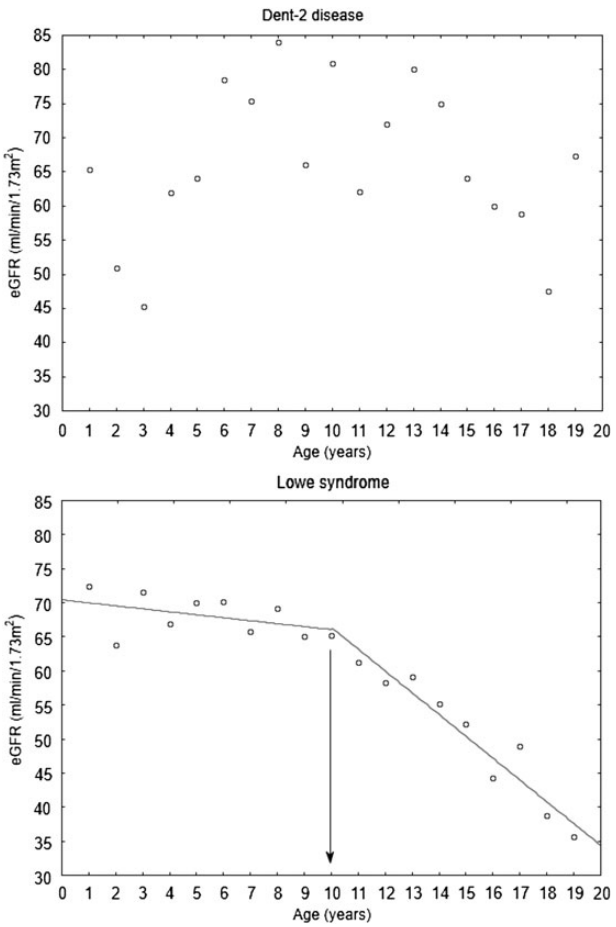

FIGURE 2: Longitudinal analysis of renal function in children with Dent-2 disease (upper panel) and Lowe syndrome (LS; lower panel). An arrow indicates a break-point of eGFR decline in LS (10 years of age).

Genotype–phenotype correlation

The median eGFR for carriers of a truncating mutation (n = 66) and for a missense mutation (n = 33) was 58.82 mL/min/1.73 m^2 (44; 82.44) and 68.4 mL/min/1.73 m^2 (54.5; 91), respectively (P = 0.12). The mutations were also tested for the position along the gene with the patients separated into four groups, which refer to the respective OCRL1 domains (Figure 4). The cross-sectional analysis showed a tendency for a decrease in eGFR along the gene (P < 0.05; Figure 5). There were

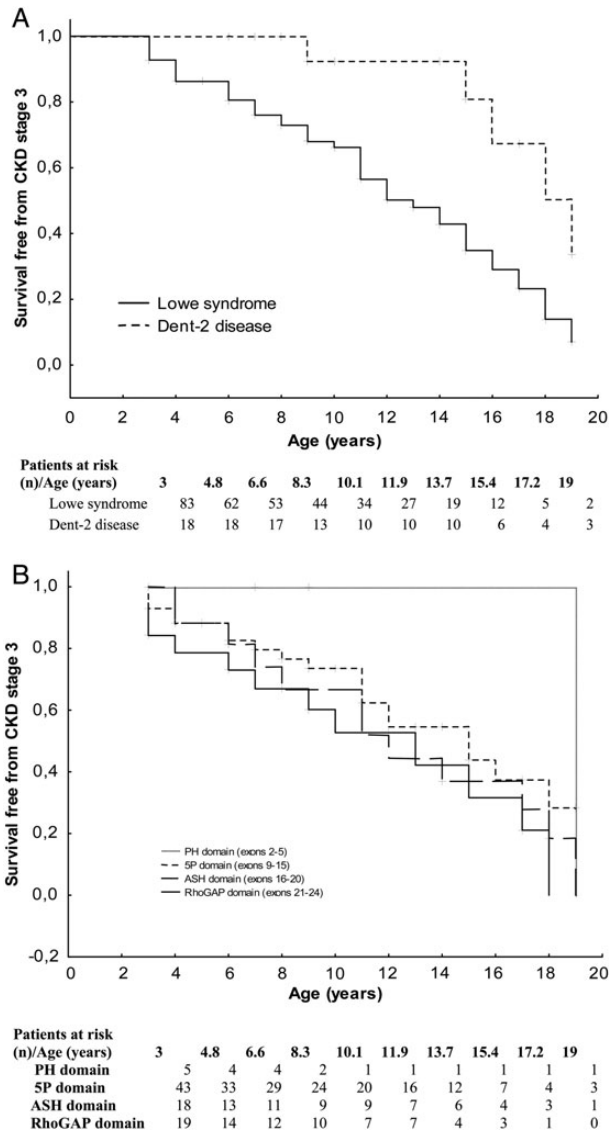

FIGURE 3: A comparison of chronic kidney disease (CKD) progression analysed by Kaplan–Meier survival with the end-point at stage 3 of CKD between children with Lowe syndrome and Dent-2 disease (P < 0.01) (**A**), and between patients carrying mutations with respect to the functional OCRL1 domains (**B**; P = 0.36) (PH, pleckstrin homology; 5P, 5-phosphatase; ASH, ASPM-SPD2-Hydin; RhoGAP, Rho GTPase activating).

no significant differences in median ages between the analysed mutational groups. Similar analyses were done by Kaplan–Meier method. Renal survival was not different between mutational types (P = 0.24) and the mutations' localization (P = 0.36; Figure 3B).

Furthermore, we observed strong inter-familial variability of eGFR. For example, among four patients with the same genotype (c.2464C>T; p.Arg822*), only one (ls66) had ESKD (14.3 years), whereas two other patients of similar age (11 and 16 years) had CKD stage 2 and 3 respectively, and the third had normal eGFR (4.6 years). We also found evidence of significant intra-familial variation of eGFR as illustrated in one of the families (ls25) with four affected boys, of whom two had severe CKD, while the other two had stage 2 of CKD at a comparable age.

DISCUSSION

This study presents a comprehensive analysis of molecular defects, clinical phenotypes and treatments with respect to CKD. The most important finding from this analysis was that among OCRL patients, those diagnosed with LS have worse renal function than those with DD2 and a faster rate of CKD progression in LS. Whether this is related to genotype or to other as yet unidentified factors that explain the difference between developing

Table 4. Type of mutations in the OCRL cohort

	Type of mutations	Number of patients	Number of novel mutations
Lowe syndrome	Missense	23	2
	Nonsense	29	3
	Splice site	13	3
	Deletion (large deletion)	8 (3)	3 (1)
	Insertion	4	2
	Insertion/deletion	4	–
	Total, *n* (%)	81 (81.8%)[a]	13 (76.5%)
Dent-2 disease	Missense	10	2
	Nonsense	–	–
	Splice site	–	–
	Deletion	4	2
	Insertion	4	–
	Insertion/deletion	–	–
	Total, *n* (%)	18 (18.2%)[a]	4 (23.5%)
Suspected Lowe syndrome—no mutation identified[b]		2	–
No genotype		5	–
Total		106 (100%)	17 (100%)

[a]Percentage of all patients with known genotype ($n = 99$).
[b]Negative for *CLCN5/OCRL* mutations.

Table 3. Clinical and molecular characterization of patients with 17 novel mutations

Patient	Age[a]	Location	Mutation[b]	Consequence[b]	Cataract/glaucoma	Neurological symptoms	Renal symptoms[c]								Other features
							NC/NL	HC	Ac	P	Gly	AA	FS	eGFR	
d13	5.9	Exon 4	c.214_215delCT	p.Leu72Phe*fs**2	n/n	n	n/n	y	n	n	n	N/D	n	90	–
d7	18	Exon 5	c.260delA	p.Gln87Arg*fs**19	n/n	N/A	n/n	n	n	n	n	N/D	n	70	–
d6	6.2	Exon 5	c.260delA	p.Gln87Arg*fs**19	n/n	N/A	n/n	y	n	n	n	N/D	n	85	–
ls38	9	Exon 8	c.621_624delGAAG	p.Lys208Ser*fs**31	y/y	N/A	n/n	N/A	y	y	n	n	n	44	–
ls29	14	Exon 9	c.724_729insT	p.Val/244Cys*fs**12	y/y	N/A	n/n	y	y	y	n	n	n	55	–
ls8	10	Exon 9	c.724_729insT	p.Val244Cys*fs**12	y/n	HP, DD	y/n	y	y	y	n	y	n	56	–
ls4	2	Exon 9	c.812T>A	p.Ile271Asn	y/y	HP, DD	n/n	y	y	y	y	y	y	113	Rickets, facial dysmorphia
ls12	15.5	Exon 11	c.943C>T	p.Gln315*	y/y	DD	y/n	n	y	y	n	y	n	59	Rickets, facial dysmorphia
d12	5	Exon 11	c.953G>T	p.Arg318Leu	n/n	n	y/y	y	y	n	n	n	n	90	–
ls75	2.6	Exon 12	c.1067G>A	p.Gly356Asp	y/n	HP, DD	n/y	y	n	y	n	n	n	98	Cholestasis
d8.1[d]	9	Exon 12	c.1133C>T	p.Ala378Val	n/n	n	y/y	y	n	n	n	N/D	n	130	–
d8.2[d]	7	Exon 12	c.1133C>T	p.Ala378Val	n/n	n	n/n	n	n	n	n	N/D	n	143	–
ls41	1	Exon 15	c.1539T>G	p.Tyr513*	y/y	N/A	N/A	N/A	y	n	n	N/D	n	68	–
ls44	17	Intron 15	c.1603-2A>C	Splice defect	y/n	N/A	y/n	y	n	y	n	N/D	n	50	–
ls72	1.2	Exon 17	c.1817insA	p.Asn606Lys*fs**1	y/n	HP, DD	n/n	n	y	y	n	y	n	68	Rickets, facial dysmorphia
ls7	7	Exon 21	c.2313T>A	p.Cys771*	y/y	HP, DD	y/n	y	y	n	n	y	n	80	–
ls71	7	Intron 21	c.2341+1G>C	Splice defect	y/n	DD	n/n	y	n	n	n	y	n	90	Facial dysmorphia
ls10	15	Exon 22	c.2455del C	p.Arg819Gly*fs**5	y/n	HP, DD	y/n	n	y	y	n	y	n	49	–
ls6.1[e]	17	Intron 23	c.2581+1G>A	Splice defect	y/n	N/A	y/n	y	y	n	n	y	n	37	–
ls6.2[e]	17	Intron 23	c.2581+1G>A	Splice defect	y/n	N/A	y/n	y	y	y	n	n	n	34	–
ls73	1.5	Exons 1–2	deletion	–	y/n	HP, DD	y/y	n	y	y	n	y	n	61	Rickets

NC, nephrocalcinosis; NL, nephrolithiasis; HC, hypercalciuria; Ac, metabolic acidosis; P, hypophosphataemia; Gly, glycosuria; AA, aminoaciduria; FS, Fanconi syndrome; eGFR, estimated glomerular filtration rate; HP, muscular hypotonia; DD, developmental delay; N/A, not applicable; N/D, not done.
[a]Age at the last observation.
[b]Numbering according to the cDNA sequence (GenBank NM_000276.3) with the A of the first coding methionine as no. 1.
[c]All patients have low-molecular weight proteinuria.
[d]Patients d8.1 and d8.2 are brothers.
[e]Patients d6.1 and d6.2 are cousins.

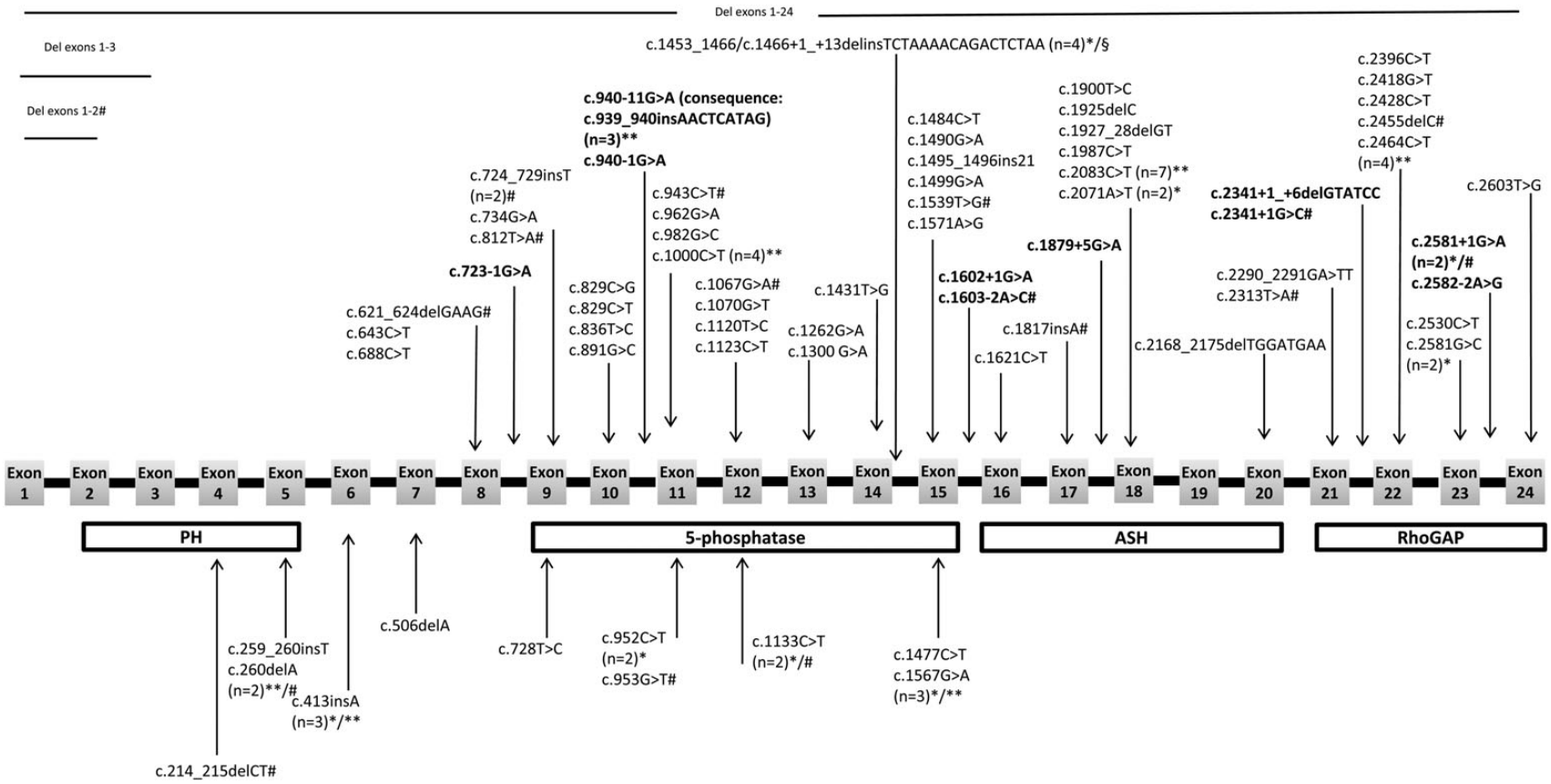

FIGURE 4: Distribution of mutations (at DNA level) in the *OCRL* gene in our patients with Lowe syndrome ($n = 81$) and Dent-2 disease ($n = 18$) (above and below the genogram, respectively). The boxes represent the exons (not to scale). Intronic mutations are indicated in bold, while horizontal line show gross genomic deletions (del). The domains are shown below respective, coding exons (PH, pleckstrin homology; ASH, ASPM-SPD2-Hydin; RhoGAP, Rho GTPase activating). *Familial mutations; **recurrent mutations; [#]novel mutations; [§]a complex mutation; described elsewhere [17].

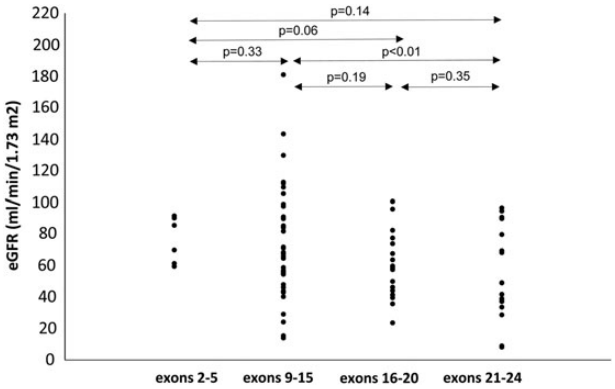

FIGURE 5: The differences in eGFR across the *OCRL* gene by affected domains [exons 2–5 (PH domain, $n = 6$); exons 9–15 (5-phosphatase domain, $n = 46$); exons 16–20 (ASH domain, $n = 19$); exons 21–24 (RhoGAP domain, $n = 19$)] (Kruskal–Wallis test, $P < 0.05$). The patients harbouring mutations in exons 9–16 had higher median eGFR versus those with mutations in exons 21–24 (Mann–Whitney *U* test, $P < 0.01$). PH, pleckstrin homology; ASH, ASPM-SPD2-Hydin; RhoGAP, Rho GTPase activating; eGFR, estimated glomerular filtration rate.

LS or DD2 remains to be determined. However, mutations that affect exons at the 3′ end of the gene that encode the RhoGAP domain appear to be associated with more severe CKD. As mutations of subjects with DD2 localize in exons 2–15, compared with exons 8–24 in LS patients [9], mutation localization does appear to correlate with disease severity. However, we also observed a strong inter- and intra-familial variability of eGFR in patients with the same mutation. Suboptimal medical treatment, which in fact was demonstrated in our study, might be a significant determinant of the variability. This clinical variation in renal function might also be explainable by the individual ability to compensate for the loss of OCRL1 function. It has been suggested that this occurs through INPP5B, an inositol 5-phosphatase, which shares nearly all functional domains with OCRL1 [18]. It is questionable, however, whether this mechanism does explain the phenotypic differences, as Montjean *et al.* [19] observed identical expression not only of OCRL, but also of INPP5B at the RNA and protein levels in fibroblasts from both DD2 and LS patients. On the other hand, they demonstrated an intermediate phenotype of DD2 fibroblasts in terms of the F-actin network, alpha-actinin and primary cilia. Hence, it has been proposed that a differential activity of modifying factors might contribute to the clinical variability between patients.

Slowly progressive renal failure is a hallmark of LS and DD2. However, no report documented the longitudinal course of renal function in patients with LS and DD2. In this study, we carried out for the first time longitudinal analyses of eGFR and found that in subjects with LS, renal function starts to decrease at around 10 years of age, predicting ESKD in the fourth decade (not shown) as was originally predicted by Charnas *et al.* [8]. Unexpectedly, we demonstrated that in DD2, eGFR does not change significantly with time in childhood. Indeed, we are not aware of a patient with DD2 and ESKD, though the data are limited in adults [4]. One might speculate that the observed decline in eGFR in LS might result simply from the natural deterioration of damaged nephrons, but not due to puberty, which was demonstrated in patients with CKD with renal hypodysplasia [20]. In this regard, the limitation of our study is lack of puberty status that might account for the rate of renal function decline.

In this study, we showed a high prevalence of CKD. Importantly, a high percentage (~50%) of our patients had moderate-to-severe CKD. As suggested by Böckenhauer *et al.* [5], we used for LS a k-value of 26, derived from the formal GFR measurements, because creatinine values can be misleading in patients with abnormal muscle mass. When using original k-values, the calculation of eGFR tends to overestimate this value, thereby resulting in an underappreciation of CKD, as reported in previous case series [21, 22]. This imprecision in measurement of eGFR might cause a high proportion of CKD patients misdiagnosed, so that some abnormalities and interventions related to CKD may be missed and/or delayed. In view of the abnormal muscle mass of these patients, cystatin C should preferably be used to monitor GFR in LS patients while making use of the most recent IFCC calibrated GFR estimating equations [4].

This cohort study also provides an interesting observation regarding proteinuria. Here, we found that the level of P/Cr ratio did not correspond with renal function. This finding calls into question the usefulness of this parameter in the population we studied. Importantly, this parameter and also urinary Ca/Cr ratio negatively correlated with BMI SDS (data not shown), which suggests that ratios, based on urine creatinine excretion, might be largely dependent on muscle mass, which is very low especially in children with LS. These parameters may be misleading, so that they cannot be reliably used in this group. In this regard, urinary protein to osmolality ratio may be preferable [23] or ideally 24-h proteinuria, if feasible. Similarly, calcium to osmolality ratio may be superior to Ca/Cr ratio, as shown by Richmond *et al.* [24].

Nephrocalcinosis is another factor that is suspected to contribute to CKD progression, but again, our data could not show any association. Importantly, since nephrocalcinosis was assessed by ultrasound in most of our cases, there is a possibility that the term nephrocalcinosis may have referred to just hyperechogenic kidneys. So, we cannot exclude the overrepresentation of patients with renal calcifications. On the other hand, the frequency of this abnormality (52%) was comparable to that reported by others [6, 17]. Interestingly, our data showed that LS patients are more susceptible to severe dehydration, AKI and urinary tract infections. These observations are important and should inform the management of these patients. It clearly cautions against the use of thiazides and angiotensin-converting enzyme inhibitors or angiotensin receptor blockers. These agents are typically prescribed to modify calci- and proteinuria under the assumption that these accelerate CKD progression. Yet, our data argue against this and the treatment could instead only predispose them to unfavourable events, such as dehydration and AKI. Our observations may be relevant beyond LS/DD2, as we are not aware of any data in tubulopathies with nephrocalcinosis like Dent-1 disease [25], familial hypomagnesaemia, hypercalciuria and nephrocalcinosis [26]

that could evidently demonstrate the impact of nephrocalcinosis on the presence or progression of CKD.

Although the complete data set of proximal abnormalities was not available for all patients, the large cohort allowed us to get an idea of the frequency of tubular abnormalities. Quite surprisingly, glycosuria, which was absent in LS in the study of Böckenhauer *et al.* [5], was present in ∼16% of our patients. Since this parameter was assessed by dipstick and not tested repeatedly, its frequency shown in our study may be misleading. One should be aware of the usefulness of this abnormality as this is the key finding in predicting Fanconi syndrome, which was in turn evident in 5.7%. Glycosuria is easy to detect in a spot urine by dipstick; however, in case of polyuria, a formal urine glucose determination is recommended.

An intriguing observation is the apparently suboptimal treatment in a substantial portion of patients. Only 51% of our patients met K/DOQI guidelines [15] and had an $HCO3^-$ level >22 mmol/L. Importantly, we found a high proportion of patients with uncorrected acidosis, which corresponded with a high frequency of growth impairment and CKD. Potentially, part of the growth deficiency may result from acidosis. Indeed, in our study in the LS group height strongly correlated with actual acidosis. Yet, no relationship could be disclosed between height and eGFR.

The limitations of our study include its retrospective and multicentre nature, which is the reason for partially incomplete and non-uniform data acquisition. The relatively small number of patients with DD2 in comparison with LS, as well as restriction of the study to children, also limits the power of our study to demonstrate renal survival.

In summary, we confirmed the high prevalence of CKD in children with *OCRL* mutations and found a difference in clinical outcome with respect to progression of CKD associated with different OCRL phenotypes. We did not identify an association between CKD and nephrocalcinosis and proteinuria, suggesting that modifying these factors will have no impact on the long-term kidney function.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

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CONFLICT OF INTEREST STATEMENT

None declared.

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