OXFORD UNIVERSITY PRESS | Nephrology Dialysis Transplantation

This is the overview page

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

Journal:	*Nephrology Dialysis Transplantation*
Manuscript ID	NDT-00972-2016.R2
Manuscript Type:	Original article - Clinical Science - Chronic kidney disease
Date Submitted by the Author:	n/a
Complete List of Authors:	Zaniew, Marcin; Children's Hospital, Department of Pediatrics Böckenkamp, Arend; VU Medical Center, Department of Pediatrics Kołbuc, Marcin; Children's Hospital, Department of Pediatrics La Scola, Claudio; Azienda Ospedaliero-Universitaria "Sant'Orsola-Malpighi", Nephrology and Dialysis Unit, Department of Woman, Child and Urological Diseases Baronio, Federico; Azienda Ospedaliero-Universitaria "Sant'Orsola-Malpighi", Endocrinology Unit, Department of Woman, Child and Urological Diseases Niemirska, Anna; The Children's Memorial Health Institute, Department of Nephrology, Kidney Transplantation and Hypertension Szczepańska, Maria; Medical University of Silesia, Chair and Clinical Department of Pediatrics Bürger, Julia; University Children's Hospital, Department of General Pediatrics La Manna, Angela; Second University of Naples, Department of Paediatrics Miklaszewska, Monika; Collegium Medicum of the Jagiellonian University, Department of Pediatric Nephrology Rogowska-Kalisz, Anna; Polish Mothers Memorial Hospital Research Institute, Department of Pediatrics, Immunology and Nephrology Gellermann, Jutta; Charité University Hospital, Department of Paediatric Nephrology Zampetoglou, Argyroula; "A. and P. Kyriakou" Children's Hospital, Department of Pediatric Nephrology Wasilewska, Anna; Medical University of Białystok, Department of Pediatrics and Nephrology Roszak, Magdalena; Poznan University of Medical Sciences, Department of Computer Science and Statistics Moczko, Jerzy; Poznan University of Medical Sciences, Department of Computer Science and Statistics Krzemień, Aleksandra; Children's Hospital, Department of Nephrology Runowski, Dariusz; The Children's Memorial Health Institute, Department of Nephrology, Kidney Transplantation and Hypertension Siteń, Grzegorz; District Hospital, Dialysis Center Załuska-Leśniewska, Iga; Gdanski Uniwersytet Medyczny, Department of Pediatrics, Nephrology and Hypertension Fonduli, Patrizia; Hospital G.Brotzu, Department of Pediatric Nephrology

Zurrida, Franca; Hospital G.Brotzu, Department of Pediatric Nephrology
Paglialonga, Fabio; Pediatric Nephrology and Dialysis Unit, Fondazione IRCCS Ca\' Granda Ospedale Maggiore Policlinico
Gucev, Zoran; University Children\'s Hospital, Department of Endocrinology and Genetics
Paripović, Dušan; University Children`s Hospital, Nephrology
Rus, Rina; University Children's Hospital, Division of Nephrology
Said-Conti, Valerie; Mater Dei Hospital, Department of Pediatrics
Sartz, Lisa; Skåne University Hospital, Department of Pediatric and Adolescent Medicine
Chung, Woo Young; Inje University Busan Paik Hospital, Department of Pediatrics
Park, Se Jin; Ajou University School of Medicine, Department of Pediatrics
Lee, Jung; Ehwa University Mokdong Hospital, Department of Pediatrics
Park, Yong-Hoon; Yeungnam University College of Medicine, Department of Pediatrics
Ahn, Yo Han; Hallym University Kangnam Sacred Heart Hospital, Department of Pediatrics
Sikora, Przemysław; Medical University of Lublin, Department of Pediatric Nephrology
Stefanidis, Costantinos; "A. and P. Kyriakou" Children's Hospital, Department of Pediatric Nephrology
Tasic, Velibor; University Children\'s Hospital, Pediaric Nephrology
Konrad, Martin; University Children\'s Hospital, Department of Pediatric Nephrology
Anglani, Franca; University of Padua, Laboratory of Histomorphology and Molecular Biology of the Kidney, Department of
Addis, Maria; University of Cagliari, Department of Public Health, Clinical and Molecular Medicine
Cheong, Hae Il; Seoul National University Children\'s Hospital, Department of Pediatrics
Ludwig, Michael; University of Bonn, Department of Clinical Chemistry and Clinical Pharmacology
Bockenhauer, Detlef; University College London, Centre for Nephrology; University College London, Institute of Child Health; Great Ormond Street Hospital for Children, Department of Paediatric Nephrology

Keyword list: chronic kidney disease, Dent-2 disease, eGFR, Lowe syndrome, nephrocalcinosis, OCRL

SCHOLARONE™
Manuscripts

Original Article

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

Marcin Zaniew[1,2], Arend Bökenkamp[3], Marcin Kołbuc[1], Claudio La Scola[4], Federico Baronio[5], Anna Niemirska[6], Maria Szczepańska[7], Julia Bürger[8], Angela La Manna[9], Monika Miklaszewska[10], Anna Rogowska-Kalisz[11], Jutta Gellermann[12], Argyroula Zampetoglou[13], Anna Wasilewska[14], Magdalena Roszak[15], Jerzy Moczko[15], Aleksandra Krzemień[16], Dariusz Runowski[6], Grzegorz Siteń[17], Iga Załuska-Leśniewska[18], Patrizia Fonduli[19], Franca Zurrida[19], Fabio Paglialonga[20], Zoran Gucev[21], Dusan Paripovic[22], Rina Rus[23], Valerie Said-Conti[24], Lisa Sartz[25], Woo Yeong Chung[26], Se Jin Park[27], Jung Won Lee[28], Yong Hoon Park[29], Yo Han Ahn[30], Przemysław Sikora[2,31], Constantinos J. Stefanidis[13], Velibor Tasic[21], Martin Konrad[8], Franca Anglani[32], Maria Addis[33], Hae Il Cheong[34], Michael Ludwig[35]*, Detlef Bockenhauer[36]*

[1]Children's Hospital, Poznan, Poland

[2]Polish Registry of Inherited Tubulopathies (POLtube)

[3]Department of Pediatrics, VU Medical Center, Amsterdam, The Netherlands

[4]Nephrology and Dialysis Unit, Department of Woman, Child and Urological Diseases, Azienda Ospedaliero-Universitaria "Sant'Orsola-Malpighi", Bologna, Italy

[5]Endocrinology Unit, Department of Woman, Child and Urological Diseases, Azienda Ospedaliero-Universitaria "Sant'Orsola-Malpighi" Bologna, Italy

[6]Department of Nephrology, Kidney Transplantation and Hypertension, The Children's Memorial Health Institute, Warsaw, Poland

[7]Chair and Clinical Department of Pediatrics, SMDZ in Zabrze, SUM in Katowice, Poland

[8]Department of General Pediatrics, University Children's Hospital, Münster, Germany

[9]Department of Pediatrics, II University of Naples, Italy

[10]Department of Pediatric Nephrology, Collegium Medicum of the Jagiellonian University,

For Peer Review

Cracow, Poland

[11]Department of Pediatrics, Immunology and Nephrology, Polish Mothers Memorial Hospital Research Institute, Łódź, Poland

[12]Department of Pediatric Nephrology, Charité Universitätsmedizin Berlin, Charité Children's Hospital, Berlin, Germany

[13]Pediatric Nephrology, "A. and P. Kyriakou" Children's Hospital, Athens, Greece

[14]Department of Pediatrics and Nephrology, Medical University of Białystok, Białystok, Poland

[15]Department of Computer Science and Statistics, Poznan University of Medical Sciences, Poznan, Poland

[16]Department of Nephrology, Children's Hospital, Katowice, Poland

[17]Dialysis Center, District Hospital, Rzeszów, Poland

[18]Department of Pediatrics, Nephrology and Hypertension, Gdańsk, Poland

[19]Pediatric Nephrology, Hospital G.Brotzu, Cagliari, Italy

[20]Pediatric Nefrology and Dialysis Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy

[21]University Children's Hospital, Medical Faculty Skopje, Macedonia

[22]Nephrology Department, University Children's Hospital, Belgrade, Serbia

[23]Division of Nephrology, University Children's Hospital, Ljubljana, Slovenia

[24]Mater Dei Hospital, Msida, Malta

[25]Department of Pediatric and Adolescent Medicine, Skåne University Hospital, Lund, Sweden

[26]Department of Pediatrics, Inje University Busan Paik Hospital, Busan, Korea

[27]Department of Pediatrics, Ajou University Daewoo Hospital, Geoje, Korea

[28]Department of Pediatrics, Ehwa University Mokdong Hospital, Seoul, Korea

[29]Department of Pediatrics, Yeungnam University College of Medicine, Daegu, Korea

[30]Department of Pediatrics, Hallym University Kangnam Sacred Heart Hospital, Seoul, Korea

[31]Department of Pediatric Nephrology, Medical University of Lublin, Lublin, Poland

For Peer Review

[32]Laboratory of Histomorphology and Molecular Biology of the Kidney, Department of Medicine, University of Padova, Italy

[33]Department of Public Health, Clinical and Molecular Medicine, University of Cagliari, Italy

[34]Department of Pediatrics, Seoul National University Children's Hospital, Seoul, Korea

[35]Department of Clinical Chemistry and Clinical Pharmacology, University of Bonn, Bonn, Germany

[36]University College London, Institute of Child Health and Great Ormond Street Hospital for Children, National Health Service Trust, London, UK

*these authors contributed equally

Corresponding author:

Marcin Zaniew MD, PhD

Children's Hospital, Krysiewicza 7/8

61-825 Poznan, Poland

E-mail: zaniewmarcin@wp.pl

Phone: +48 60254826; Fax: +48 618506322

For Peer Review

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 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 to DD2 (58.8 vs. 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, eGFR, Lowe syndrome, nephrocalcinosis, *OCRL*

For Peer Review

Short summary:

The study comprises the largest, well-characterized cohort of children with *OCRL* mutations. Here, we confirmed the high prevalence of CKD in children with Lowe syndrome and Dent-2 disease and we found a difference, not previously described, in renal outcome with respect to progression of CKD between these tubulopathies. Moreover, we did not identify association between CKD and nephrocalcinosis and proteinuria, suggesting that modifying these factors will have no impact on the long-term kidney function.

For Peer Review

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 to 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.

Subjects and Methods

For Peer Review

This retrospective, multicenter study was designed to collect data obtained from pediatric 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 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 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 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), UK (n=15), Germany (n=10), Greece (n=6), Macedonia (n=3), and Kazakhstan, Malta, Serbia, Slovenia, Sweden (n=1, each).

eGFR was calculated using the original Schwartz method [10], but with a revised k-value of 26 (when serum creatinine 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-hour urine collection in some patients. Calcium excretion > 4 mg/kg/24 h was classified as hypercalciuria, as well as an elevated calcium-creatinine

For Peer Review

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]. Hypoalbuminemia 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, hypophosphatemia and hypokalemia were defined when receiving supplementation or/and with decreased blood levels, i.e. for acidosis < 22 mmol/l [15], for hypophosphatemia [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 hypophosphatemia).

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 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 3 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 dipstic 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 centers, 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 < -2.

For the genotype-phenotype analysis, we grouped mutations according to the following criteria: 1. the expected effect on the protein product (i.e. missense, unlikely to

For Peer Review

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]. 2. the localization/position of mutations, i.e. mutations were analyzed 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 analyze the data with normal distribution and homogeneous variances. The data that did not follow a Gaussian distribution were analyzed with Mann-Whitney U test or the Kruskal-Wallis test and the Dunn's post-hoc test. The relationship between variables was analyzed with the Spearman's rank correlation coefficient and by multivariate linear regression. Categorical data were analyzed with the $\chi 2$ 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 Figure and supplementary Table 1). The age of eGFR decline (break-point) was calculated by means of piecewise linear regression. CKD progression was analyzed 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

For Peer Review

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 yrs) 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%), hypophosphatemia (44%), hypokalemia (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 to a normal population. As expected, subjects with LS were shorter than those with DD2 (median height SDS: -3.98 vs. -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 compared to 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%) urinary stone obstruction. These events were almost exclusively present in patients with LS, except 2 episodes of dehydration in one patient with DD2. Seventeen individuals (16%) underwent contrast X-ray studies.

For Peer Review

Four patients (3.7%) died in the analyzed cohort (the genotypes of three patients are shown in Table 2 in suppl.). 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 apnea/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 to DD2 (58.8 vs. 87.4 ml/min/1.73 m^2, $p<0.01$). CKD stage II-V was found in the majority of patients (74%) when analyzed the entire age range 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 to DD2 (58% vs. 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) (Table 2 in suppl.).

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

For Peer Review

with uncorrected acidosis were significantly shorter than their counterparts without acidosis (height SDS: -4.08 vs. -3.03, p<0.0001).

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 Figure). 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 hypokalemic, and 13% received potassium supplementation. Overall, 34% of patients did not receive any treatment.

Eleven patients (10.4%; 9 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), their height on GH normalized. There was no apparent association with eGFR (59.7 ± 34.8 vs. 59.4 ± 25.0 ml/min/1.73m^2, before and after the therapy, respectively), although in one patient, a significant deterioration in eGFR was observed (97 vs. 35 ml/min/1.73m^2), which was in excess of the expected age-associated deterioration.

Analyses of OCRL Mutations

Table in suppl. 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 yrs) (Table 1), yet molecular result was not available for 5 patients. A total of 90 different mutations were found in the 99 mutation-positive

For Peer Review

patients. Two patients with classical features of LS and 3 patients with a clinical diagnosis of Dent disease had no detectable mutations in *OCRL* or *CLCN5*. We report 18 (20%) 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 8-24, whereas they affect exons 4-15 in DD2. We did not observe any *OCRL* mutation found in both LS and DD2.

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/1.73m^2 (44; 82.44) and 68.4 ml/min/1.73m^2 (54.5; 91), respectively, (p=0.12). The mutations were also tested for the position along the gene with the patients separated into 4 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 no significant differences in median ages between the analyzed 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 yrs), whereas other two patients at similar age (11 and 16 yrs) had CKD stage 2 and 3 respectively, and the third had normal eGFR (4.6 yrs). 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 comparable age.

Discussion

For Peer Review

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 yet unidentified factors that explain the difference between developing LS or DD2 remains to be determined. However, mutations that affect exons at the 3' end of the gene which encode the RhoGAP domain, appear to be associated with more severe CKD. As mutations of subjects with DD2 localize in exons 2-15, compared to 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 a differential activity of modyfing 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 did 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 at el. [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

For Peer Review

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 (about 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-hour proteinuria, if feasible. Similarly, calcium to osmolality ratio may be superior to Ca/Cr ratio, as shown by Richmond at 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 our cases, there is a possibility that the term nephrocalcinosis

For Peer Review

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 unfavorable 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 hypomagnesemia, 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 to get an idea on the frequency of tubular abnormalities. Quite surprisingly, glycosuria, which was absent in LS in the study of Böckenhauer et al. [5], was present in about 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 $HCO3^-$ level greater than 22 mmol/l. Importantly, we found a high proportion of patients with uncorrected acidosis, which corresponded with 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.

For Peer Review

The limitations of our study include its retrospective and multicenter nature, which is the reason for missing data and for not uniform data acquisition. The relatively small number of patients with DD2 in comparison to LS as well as restriction of the study to children, limits also 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 association between CKD and nephrocalcinosis and proteinuria, suggesting that modifying these factors will have no impact on the long-term kidney function.

Acknowledgements

We are grateful to the patients and their parents for their invaluable contributions. We thank Polish Registry of Inherited Tubulopathies (POLtube) and Inherited Kidney Disorders Working Group of European Society for Peadiatric Nephrology for the support with patient recruitment. DB is supported by The European Union, FP7 (grant agreement 2012-305608, "European Consortium for High-Throughput Research in Rare Kidney Diseases (EURenOmics)" and the National Institute for Health Research Biomedical Research Centre at Great Ormond Street Hospital for Children NHS Foundation Trust and University College London. HIC is supported by a grant (HI12C0014) from the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea.

Conflict of interest: none to declare

For Peer Review

References

1. Nussbaum RL, Orrison BM, Jänne PA, Charnas L, Chinault AC. Physical mapping and genomic structure of the Lowe syndrome gene *OCRL1*. Hum Genet 1997; 99: 145-150.
2. Hoopes RR Jr, Shrimpton AE, Knohl SJ et al. Dent disease with mutation in *OCRL1*. Am J Hum Genet 2005; 76: 260-267.
3. Mehta ZB, Pietka G, Lowe M. The cellular and physiological functions of the Lowe syndrome protein OCRL1. Traffic 2014; 15: 471-487.
4. Bökenkamp A, Ludwig M. The oculo-cerebro-renal syndrome of Lowe – an update. Pediatr Nephrol 2016 (in press).
5. Böckenhauer D, Bökenkamp A, van't Hoff W et al. Renal phenotype in Lowe syndrome: a selective proximal tubular dysfunction. Clin J Am Soc Nephrol 2008; 3: 1430-1436.
6. Bökenkamp A, Böckenhauer D, Cheong HI et al. Dent-2 disease: a mild variant of Lowe syndrome. J Pediatr 2009; 155: 94-99.
7. Shrimpton AE, Hoopes Jr. RR, Knohl SJ et al. *OCRL1* mutations in Dent-2 patients suggest a mechanism for phenotypic variability. Nephron Physiol 2009; 112: 27-36.
8. Charnas LR, Bernardini I, Rader D, Hoeg JM, Gahl WA. Clinical and laboratory findings in the oculocerebrorenal syndrome of Lowe, with special reference to growth and renal function. N Engl J Med 1991; 324: 1318-1325.
9. Hichri H, Rendu J, Monnier N et al. From Lowe syndrome to Dent disease: correlations between mutations of the *OCRL1* gene and clinical and biochemical phenotypes. Hum Mutat 2011; 32: 379-388.
10. Schwartz GJ, Brion LP, Spitzer A. The use of plasma creatinine concentration for estimating glomerular filtration rate in infants, children, and adolescents. Pediatr Clin North Am 1987; 4: 571-590.
11. K/DOQI clinical practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. Am J Kidney Dis 2002; 39: S1-266.

For Peer Review

12. Heilbron DC, Holliday MA, al-Dahwi A, Kogan BA. Expressing glomerular filtration rate in children. Pediatr Nephrol 1991; 5: 5-11.

13. Ghazali S, Barratt TM. Urinary excretion of calcium and magnesium in children. Arch Intern Med 1974; 49: 97-101.

14. Kruse K, Kracht U, Gopfert G. Renal threshold phosphate concentration (TmPO4/GFR). Arch Intern Med 1982; 57: 217-223.

15. K/DOQI Clinical Practice Guideline for Nutrition in Children with CKD: 2008 Update. Am J Kidney Dis 2009; 53, S11-104

16. Agarwal I, Kirubakaran C, Markandeyulu, Selvakumar. Quantitation of proteinuria by spot urine sampling. Indian J Clin Biochem 2004; 19(2): 45-47.

17. Recker F, Zaniew M, Böckenhauer D et al. Characterization of 28 novel patients expands the mutational and phenotypic spectrum of Lowe syndrome. Pediatr Nephrol 2015; 30: 931-943.

18. Pirruccello M, De Camilli P. Inositol 5-phosphatases: insights from the Lowe syndrome protein OCRL. Trends Biochem Sci 2012; 37: 134-143.

19. Montjean R, Aoidi R, Desbois P et al. OCRL-mutated fibroblasts from patients with Dent-2 disease ex- hibit INPP5B-independent phenotypic variability relatively to Lowe syndrome cells. Hum Mol Genet 2015; 24: 994-1006.

20. Ardissino G, Testa S, Dacco V et al. Puberty is associated with increased deterioration of renal function in patients with CKD: data from the ItalKid Project. Arch Dis Child 2012; 97(10): 885-888.

21. Cho HY, Lee BH, Choi HJ, Ha IS, Choi Y, Cheong HI. Renal manifestations of Dent disease and Lowe syndrome. Pediatr Nephrol 2008; 23: 243-249.

22. Kim HK, Kim JH, Kim YM et al. Lowe syndrome: a single center's experience in Korea. Korean J Pediatr 2014; 57: 140-148.

23. Kim HS, Cheon HW, Choe JH et al. Quantification of proteinuria in children using the urinary protein-osmolality ratio. Pediatr Nephrol 2001; 16(1): 73-76.

24. Richmond W, Colgan G, Simon S, Stuart-Hilgenfeld M, Wilson N, Alon US. Random urine calcium/osmolality in the assessment of calciuria in children with decreased muscle mass. Clin Nephrol 2005; 64(4): 264-270.

25. Sekine T, Komoda F, Miura K et al. Japanese Dent disease has a wider clinical spectrum than Dent disease in Europe/USA: genetic and clinical studies of 86 unrelated patients with low-molecular-weight proteinuria. Nephrol Dial Transplant 2014; 29(2): 376-384.

26. Konrad M, Hou J, Weber S et al. CLDN16 genotype predicts renal decline in familial hypomagnesemia with hypercalciuria and nephrocalcinosis. J Am Soc Nephrol 2008; 19: 171-181.

For Peer Review

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 of last visit (year), n=106[b]	10 (5.9; 16)	9.8 (4.8; 16)	13.8 (7; 17)	ns	1-18
Age of clinical diagnosis (year), n=102	0.95 (0.4; 5)	0.65 (0.3; 2)	5.95 (5; 10)	< 0.0001	0.1-18.5
Age of 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÷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÷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/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/1.73/m^2
CKD stage 2	27 (27.8%)	21 (26.6%)	6 (33.3%)	ns	eGFR 89-60 ml/1.73/m^2
CKD stage 3	42 (43.3%)	37 (46.8%)	5 (27.8%)	ns	eGFR 59-30 ml/1.73/m^2
CKD stage 4	6 (6.2%)	6 (7.6%)	0 (0%)	ns	eGFR 29-15 ml/1.73/m^2
CKD stage 5/ESKD	3 (3.1%)	3 (3.8%)	0 (0%)	ns	eGFR < 15 ml/1.73/m^2
Albuminemia (g/l), n=89	45 (43; 48)	45.0 (42.7; 47)	45.5 (43; 49.2)	ns	29-56
hypoalbuminemia	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
hypophosphatemia	45 (44%)	45 (52%)	0 (0%)	< 0.001	Phosphate level [15] or phosphates supplemention
actual hypophosphatemia	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

For Peer Review

					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
hypokalemia	21 (20.2%)	20 (23%)	1 (5.5%)	ns	potassium < 3.5 or potassium supplementation
actual hypokalemia	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	-
dipstic 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	-

For Peer Review

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)
[a] *p* statistical differences between patients with Lowe syndrome and Dent-2 diesease
[b] number (percentage) of patients/number of available data

Abbrevations: SDS, standard deviation score; BMI, body max index; eGFR, estimated glomerular filtration rate; CKD, chronic kidney disease; ESKD, end-stage renal 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

For Peer Review

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

Abbrevations: 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

For Peer Review

Table 3. Clinical and molecular characterization of patients with 18 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	-

For Peer Review

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

[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

Abbrevations: NC, nephrocalcinosis; NL, nephroloithiasis; HC, hypercalciuria; Ac, metabolic acidosis; P, hypophosphatemia; 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

er Review

Table 4. Type of mutations in the OCRL cohort.

	Type of mutations	Number of patients	Number of novel mutations
Lowe syndrome	missense	23	1
	nonsense	29	3
	splice site	13	1
	deletion (large deletion)	8 (3)	3 (1)
	insertion	4	3
	insertion/deletion	4	-
	total, n (%)	81 (81.8%)[a]	11 (73.3%)
Dent-2 disease	missense	10	3
	nonsense	-	-
	splice site	-	-
	deletion	4	1
	duplication	-	-
	insertion	4	-
	insertion/deletion	-	-
	total, n (%)	18 (18.2%)[a]	4 (26.7%)
Suspected Lowe syndrome – no mutation identified[b]		2	-
No genotype		5	-
Total		106 (100%)	15 (100%)

[a] percentage of all patients with known genotype (n=99)
[b] negative for *CLCN5/OCRL* mutations

For Peer Review

Legends to figures

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).

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

Figure 3. A comparison of chronic kidney disease (CKD) progression analyzed 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 carring 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).

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; 5-phosphatase; 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

For Peer Review

harbouring mutations in exons 9-16 had higher median eGFR versus those with mutations in exons 21-24 (Mann-Whitney U test, $p<0.01$).

Figure (suppl.) Raw data of eGFR in children with LS. To determine longitudinally the rate of CKD progression, height and serum creatinine were collected and when more than one measure was available, individually an averaged eGFR/year was calculated (a point in supplementary Figure). Since the data were obtained at different time points, a median of all available eGFR for all patients on a yearly basis (ages 1-19 years) was calculated (supplementary Table 1). The age of eGFR decline (break-point) was calculated by means of piecewise linear regression.

For Peer Review

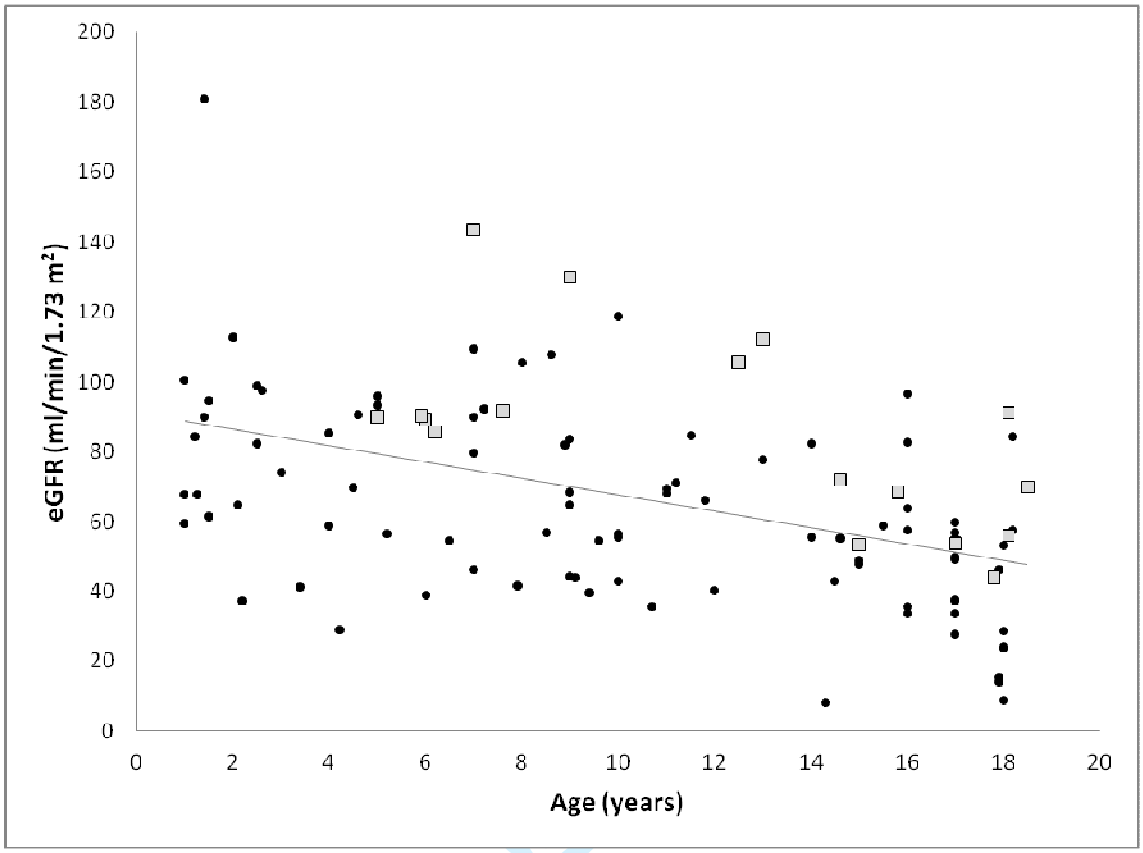


Figure 1.

For Review

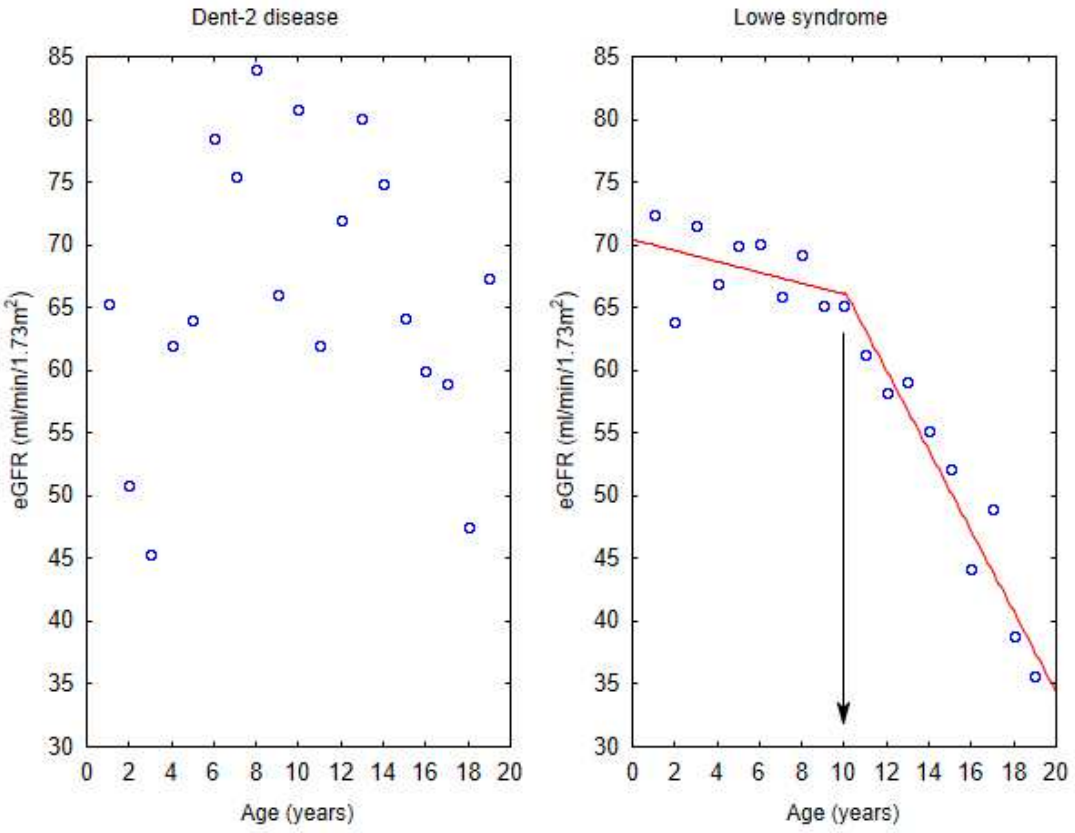
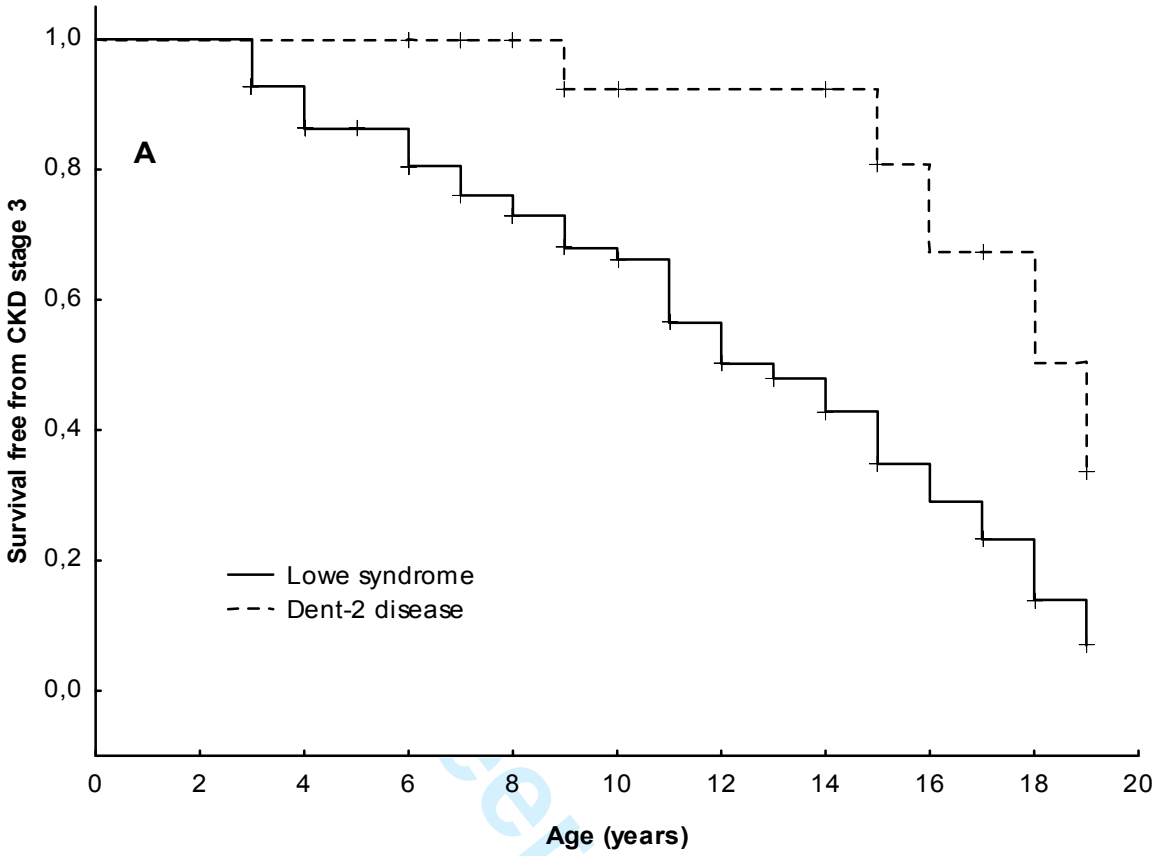


Figure 2.

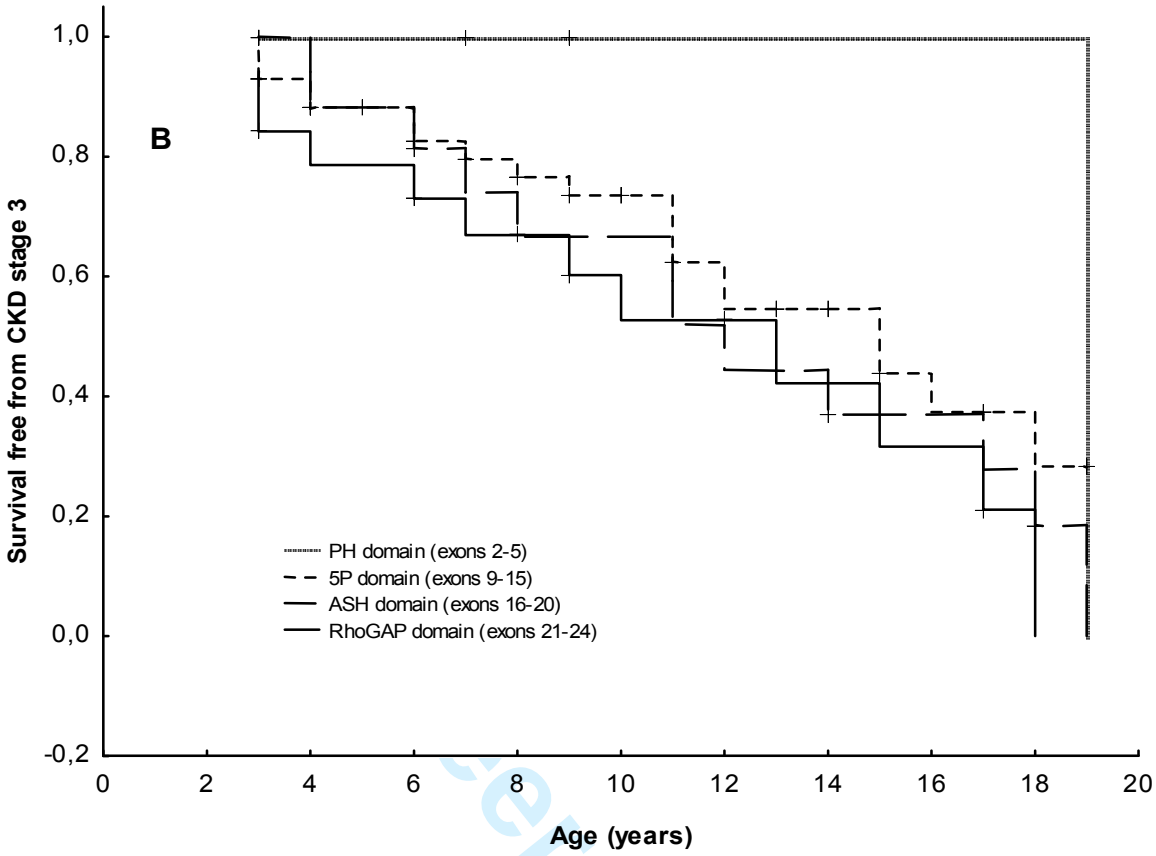
For Review



Patients at risk (n)/Age (years)	3	4.8	6.6	8.3	10.1	11.9	13.7	15.4	17.2	19
Lowe syndrome	83	62	53	44	34	27	19	12	5	2
Dent-2 disease	18	18	17	13	10	10	10	6	4	3

Figure 3A.

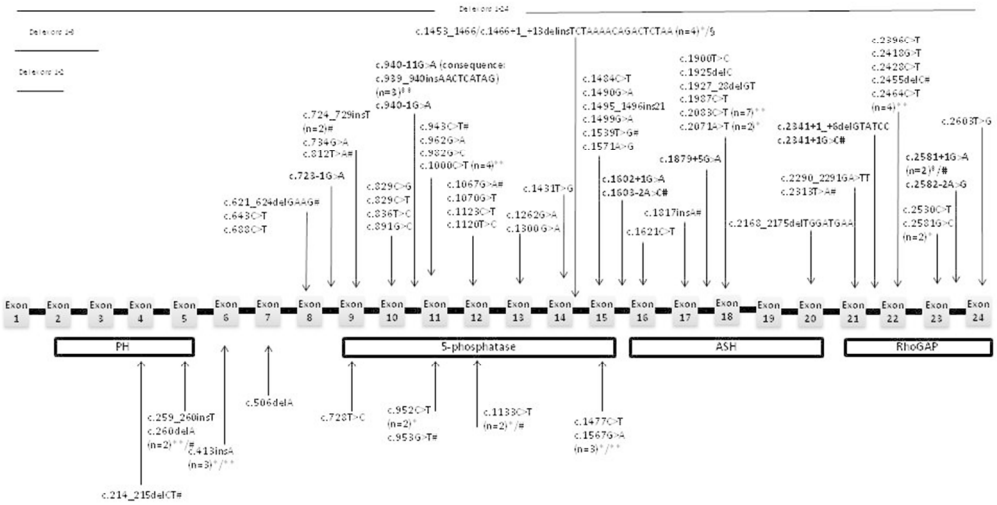
For Peer Review



Patients at risk (n)/Age (years)	3	4.8	6.6	8.3	10.1	11.9	13.7	15.4	17.2	19
PH domain	5	4	4	2	1	1	1	1	1	1
5P domain	43	33	29	24	20	16	12	7	4	3
ASH domain	18	13	11	9	9	7	6	4	3	1
RhoGAP domain	19	14	12	10	7	7	4	3	1	0

Figure 3B.

For Peer Review



254x190mm (96 x 96 DPI)

Review

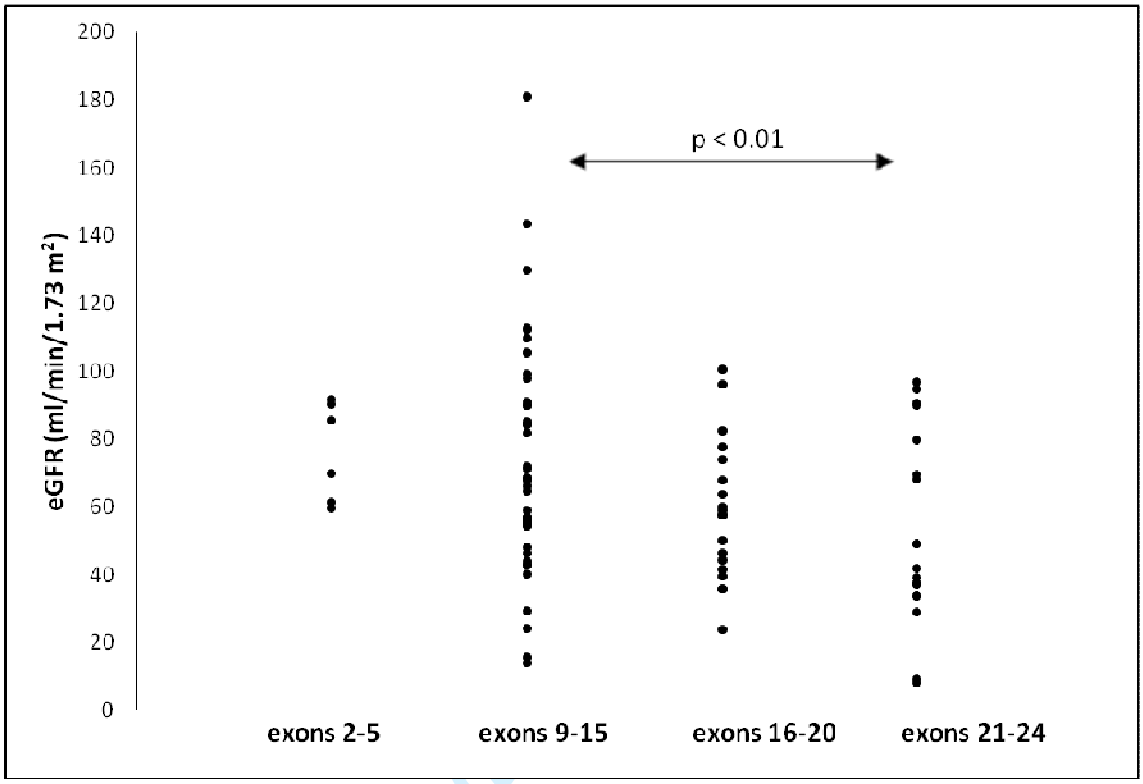


Figure 5. (corrected)

er Review

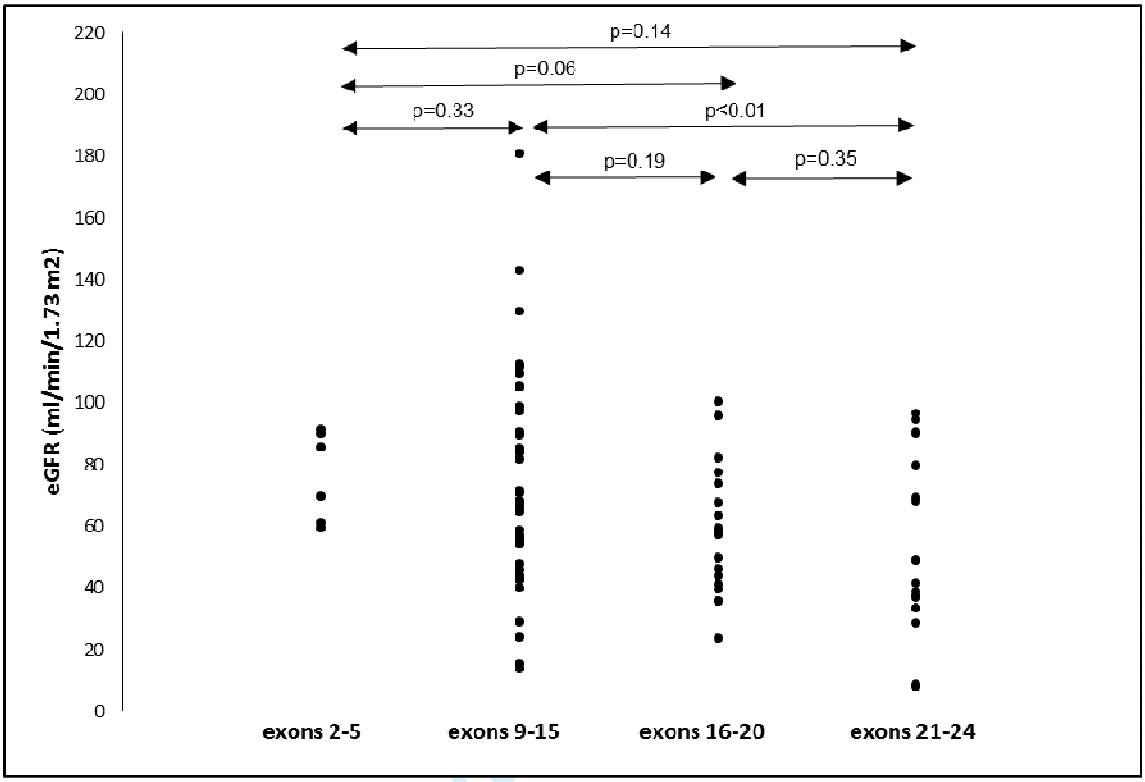


Figure 5.

er Review