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Clinical and genetic characteristics of Dent's disease type 1 in Europe

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ABSTRACT

Background. Dent's disease type 1 (DD1) is a rare X-linked nephropathy caused by *CLCN5* mutations, characterized by proximal tubule dysfunction, including low molecular weight proteinuria (LMWP), hypercalciuria, nephrolithiasis–nephrocalcinosis, progressive chronic kidney disease (CKD) and kidney failure (KF). Current management is symptomatic and does not prevent disease progression. Here we describe the contemporary DD1 picture across Europe to highlight its unmet needs.

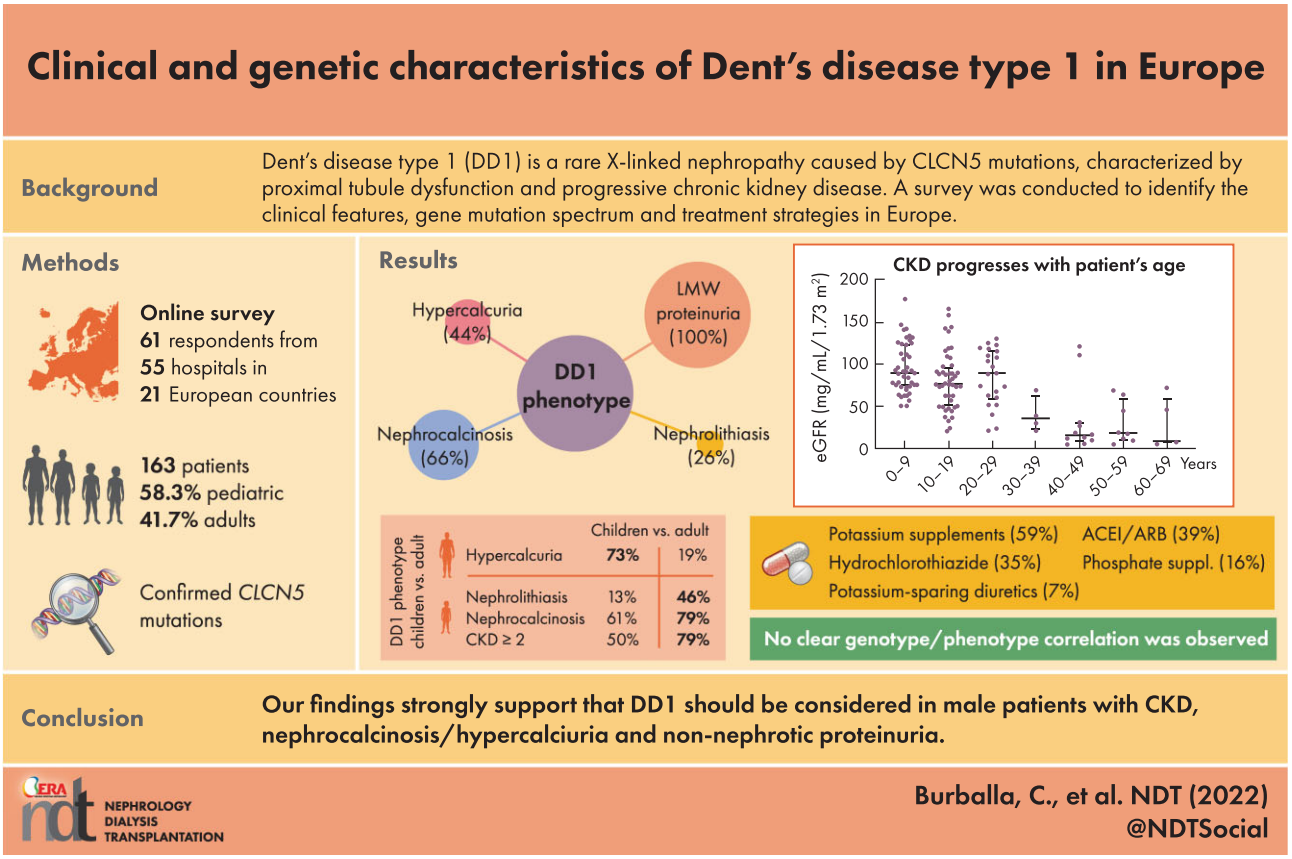
Methods. A physician-based anonymous international e-survey supported by several European nephrology networks/societies was conducted. Questions focused on DD1 clinical features, diagnostic procedure and mutation spectra.

Results. A total of 207 DD1 male patients were reported; clinical data were available for 163 with confirmed *CLCN5* mutations. Proteinuria was the most common manifestation (49.1%). During follow-up, all patients showed LMWP, 66.4% nephrocalcinosis, 44.4% hypercalciuria and 26.4% nephrolithiasis. After 5.5 years, ≈50% of patients presented with renal dysfunction, 20.7% developed CKD stage ≥3 and 11.1% developed KF. At the last visit, hypercalciuria was more frequent in paediatric patients than in adults (73.4% versus 19.0%). Conversely, nephrolithiasis, nephrocalcinosis and renal dysfunction were more prominent in adults. Furthermore, CKD progressed with age. Despite no clear phenotype/genotype correlation, decreased glomerular filtration rate was more frequent in subjects with *CLCN5* mutations affecting the pore or CBS domains compared with those with early-stop mutations.

Conclusions. Results from this large DD1 cohort confirm previous findings and provide new insights regarding age and genotype impact on CKD progression. Our data strongly support that DD1 should be considered in male patients with CKD, nephrocalcinosis/hypercalciuria and non-nephrotic proteinuria and provide additional support for new research opportunities.

Keywords: CLCN5 gene, Dent's disease 1 (DD1), low molecular weight proteinuria, nephrocalcinosis, tubulopathy

GRAPHICAL ABSTRACT



KEY LEARNING POINTS

What is already known about this subject?

- Dent's disease type 1 (DD1) is a rare X-linked renal condition caused by a defective *CLCN5* gene.
- High urinary low molecular weight proteins (LMWPs) represent the disease's hallmark, often associated with high urinary calcium/hypercalciuria, kidney stones/nephrocalcinosis and progression to chronic kidney disease (CKD).

What this study adds?

- The authors describe the current clinical practice and outcome of DD1 in Europe.
- DD1 manifestations are better characterized and new information regarding age and genetics impact on CKD progression is provided.
- Importantly, they describe how patients' symptoms change with age from children to adults.

What impact this may have on practice or policy?

- These findings provide a contemporary DD1 picture across Europe and demonstrate different phenotypes in children versus adults. In adults, DD1 should be considered in male patients with either CKD of unknown origin associated with non-nephrotic proteinuria or nephrocalcinosis/nephrolithiasis.

INTRODUCTION

Dent's disease type 1 (DD1) [Online Mendelian Inheritance in Man (OMIM) 300009] is a rare hereditary X-linked proximal tubulopathy. Low molecular weight proteinuria (LMWP) is the hallmark of the disease, and it is classically associated with hypercalciuria and nephrocalcinosis/nephrolithiasis [1–4]. Also, patients may show incomplete Fanconi syndrome (FS) with defective reabsorption of one or several other solutes (e.g. amino acids, glucose, phosphate or uric acid) [5]. Further manifestations as hypophosphatemic rickets or abnormal

electrolyte balance are occasionally present. The disease progresses to kidney failure (KF) with kidney replacement therapy (KRT) in the fourth–sixth decade of life in ≈80% of patients [4–6]. Although the disease affects almost exclusively hemizygous males, female carriers may have a mild phenotype, including LMWP or hypercalciuria [7, 8].

DD1 is caused by mutations in the *CLCN5* gene (OMIM 300008, sequence NG_007159.2), which encodes for the ClC-5 Cl^-/H^+ antiporter. More than 250 different pathogenic variants of *CLCN5* have been described as causative of DD1 [5, 6]. Several classifications have been proposed on the grounds of functional data or mutation type, but no genotype–phenotype correlation has been established [4, 9, 10]. The canonical ClC-5 is a 746-amino acid protein with 18 membrane-spanning α-helices, with both N-terminal and C-terminal facing the cytosol and two CBS (named after the presence in cystathionine-β-synthase) domains. It is mainly expressed in the proximal tubule epithelial cells (PTCs), located at the apical endosomes and the brush border, where it is thought to play a critical role in the reabsorption of solutes by regulating the acidification of the endo-lysosomal pathway [11, 12].

In the present study, as part of the European Rare Kidney Disease Reference Network (ERKNet), we conducted a survey to analyse the clinical features, management strategies, gene mutation spectrum and long-term outcome of patients with DD1 throughout Europe.

MATERIALS AND METHODS

Study design and settings

A 46-item web-based cross-sectional survey was developed using the online tool SurveyMonkey (SurveyMonkey.com). It contained multiple-choice and open-ended, non-mandatory questions, divided into five sections: sociodemographic and anthropometric data, diagnosis, updated clinical evaluation, updated blood/urinary biochemistry parameters and genetic diagnosis and management. The list of questions is provided as supplementary material (Supplementary material 1). Data were deemed adequate for analysis if >50% of the items were completed for each patient.

Patients

A total of 207 patients with confirmed DD1 diagnosis were included in the study. An additional 44 patients without a documented *CLCN5* gene pathogenic variant and/or minimum information were excluded from the analysis. Further, two female carriers were not considered in the analysis. In 33 cases, specific information about the *CLCN5* variant was not provided. Clinical and biochemical data both at diagnosis and at the last follow-up were collected.

Statistical analysis

Data are shown as frequencies (percentages) for categorical variables. Continuous variables are presented as mean ± standard deviation (SD) for normally distributed variables, according to the Kolmogorov–Smirnov/Shapiro–Wilk test and as median [interquartile range (IQR)] if non-normally distributed. Continuous variables were analysed by *t*-test or Mann–Whitney U-test; binary and categorical variables were analysed by χ^2 or Fisher's exact test, depending on the group size. *P*-values <.05 were considered statistically significant.

An expanded description of the materials and methods can be found in the Supplementary material.

RESULTS

Survey respondents

In order to analyse clinical features and gene mutation spectra of DD1 patients throughout Europe, we conducted an online survey (Supplementary material 1). A total of 62 nephrologists from 56 centres in 22 European countries participated, although the exact response rate per country could not be determined due to data protection. A total of 207 DD1 patients were reported with regular follow-up in those centres. Only male patients with at least 50% of the questionnaire data collected were considered for further analysis (Fig. 1A). Therefore we retrospectively analysed data from a final study group of 163 male patients with genetically confirmed DD1: 95 paediatric (<18 years; 58.3%) and 68 adults (>18 years; 41.7%) from 19 countries (Supplementary Fig. S1A).

Presentation at diagnosis

The median age at clinical diagnosis was 7 years (IQR 3–12). The key signs/symptoms leading to DD1 recognition were proteinuria (49.1%), nephrolithiasis (10.1%) or detection of nephrocalcinosis incidentally during ultrasound examination performed for other reasons (12.6%) (Fig. 1B). In only 11.9% of the cases was the diagnosis done after family screening, despite the presence of a positive DD1 history in 51.2% of patients by directed questioning.

Phenotypic characteristics during follow-up

At the last follow-up, all patients showed LMWP (100%), the hallmark of DD1. Nephrocalcinosis was found in 66.4% of patients and hypercalciuria in 44.4%. As a group, those patients with increased renal calcium loss had an estimated glomerular filtration rate (eGFR) >50 ml/min/1.73 m^2 (92.2 ± 30.8 ml/min/1.73 m^2). Forty-one patients (26.4%) suffered at least one kidney stone event (nephrolithiasis) at a median age of 18 years (IQR 5–29) at the first episode. A history of rickets was recorded in 16.5% of patients and 10 patients (6.5%) referred at least one fracture episode at a median age of 5 years (IQR 0–51) (Table 1).

Proximal tubular transport defects were often detected, with aminoaciduria in 45.9% of patients and glycosuria in 20.6%. Further, 28 patients (20.1%) had hypokalaemia and 40 (26.3%) had hypophosphatemia or received supplements. Interestingly, the presence of hypophosphatemia did not correlate with a history of rickets (52.6% versus 47.4%; patients with a history

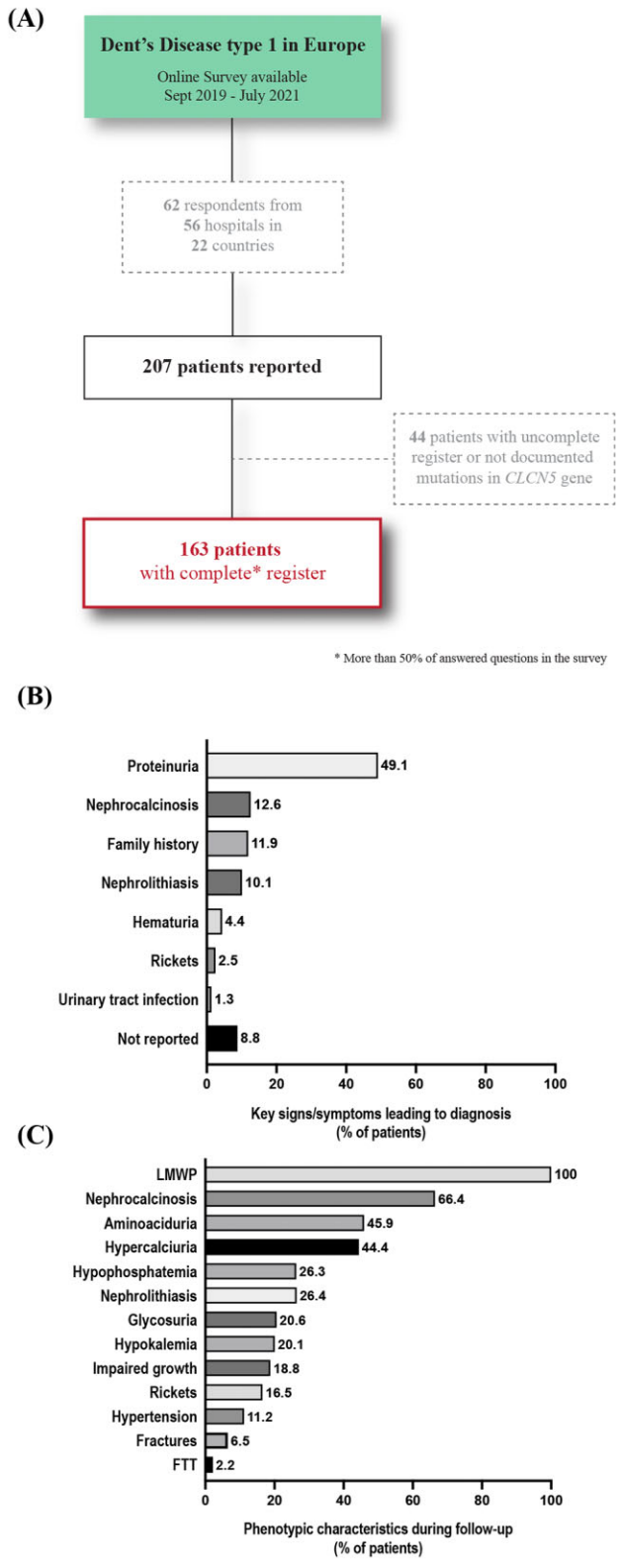


Figure 1: Description of the survey and patients' characteristics. **(A)** Scheme of the survey to study DD1 in Europe. **(B)** Key signs/symptoms leading to DD1 diagnosis. **(C)** Graphical representation of the phenotypic characteristics during follow-up. HTN: hypertension; FTT: failure to thrive.

of rickets and hypophosphatemia or normal serum phosphate, respectively; $P = .32$). Impaired growth was detected in 18.8% of patients and failure to thrive in three patients (2.2%). Few patients (11.2%) were hypertensive (Table 1, Fig. 1C).

Follow-up and kidney function

The median patient age at the last follow-up was 15 years (IQR 7.5–23.5), with a median duration since diagnosis of 5.5 years (IQR 1.7–11). Biochemical parameters at the last blood test available are shown in Table 2. At the last visit, 88 patients (61.1%) had a decreased eGFR (<90 ml/min/1.73 m^2), 46 (31.9%) developed CKD stage ≥3 and 16 (11.1%) presented KF at a median age of 51.5 years (IQR 43–63.2). Our data show that, despite individual variability, CKD progressed according to the patient's age. The median patient age among those with normal eGFR was 9.5 years, whereas the median patient age among those with CKD stage 2, 3 and 4 was 12.5, 17 and 33 years, respectively ($P < .001$) (Table 2). To further show that CKD progresses with the patient's age, we divided our cohort into different age groups and calculated the median eGFR for each. This analysis showed that the median eGFR decreased with age ($P < .001$) (Fig. 2A). Importantly, our data demonstrates the progression to KF in DD1: there were no cases of KF at <40 years of age, while the number increased drastically later, affecting 45.5% of the patients between 40 and 49 years, 75% of the patients between 50 and 59 years and reaching 100% in patients >60 years of aged.

Despite CKD progression, patients maintained with LMWP over time, and characteristically the ratio of albumin:creatinine to protein:creatinine in urine remained <1/3 in 81.1%, which highlights the value of this DD1 hallmark (Fig. 2B).

DD1 phenotype according to the patient's age

Next, we compared paediatric (<18 years) and adult patients' data at the last follow-up. We observed that DD1 clinical features changed with age (Table 3). Remarkably, only 19.0% of adult patients were hypercalciuric, compared with 73.4% of paediatric patients ($P < .001$). However, adult patients experienced more episodes of nephrolithiasis (45.6% versus 13.1%; $P < .001$) and a higher prevalence of nephrocalcinosis (78.8% versus 60.9%; $P = .04$) compared with paediatric subjects. Further, CKD became more frequent with age (paediatric versus adult patients: eGFR 88.0 ± 31.2 versus 59.2 ± 44.1 mg/ml/1.73 m^2, $P < .0001$; serum creatinine 0.69 ± 0.38 versus 1.83 ± 1.1 mg/dl, $P < .001$). also, adult patients were hypertensive more often than paediatric patients (20.0% versus 4.4%; $P = .01$) and exhibited CKD stage ≥2 at their last follow-up (78.5% versus 50.0%; $P = .003$). A higher proportion of adult patients showed hypophosphatemia (or phosphate supplementation) compared with paediatric patients (56.7% versus 21.6%; $P < .001$) and glycosuria was also more often detected in adults than children (33.3% versus 10.8%; $P = .039$). Remarkably, the protein:creatinine ratio in urine decreased from childhood to adulthood [1563 (IQR 690–2530) versus 540 (IQR 239–1712) mg/g; $P = .002$]. Nevertheless, the ratio of albumin:creatinine to protein:creatinine

Table 1: Clinical manifestation in patients with DD1.

Characteristics	Values	Patients, *n*
Age at diagnosis (years), median (IQR)	7 (3–12)	152
LMWP, *n* (%)	159 (100)	159
Hypercalciuria, *n* (%)	40 (44.4)	90
History of nephrolithiasis, *n* (%)	41 (26.4)	155
Nephrocalcinosis, *n* (%)	93 (66.4)	140
Aminoaciduria, *n* (%)	28 (45.9)	61
Glycosuria, *n* (%)	26 (20.6)	126
Hypophosphatemia or use of phosphate supplementation, *n* (%)	40 (26.3)	152
Hypokalaemia or use of potassium supplementation, *n* (%)	28 (20.1)	140
History of rickets, *n* (%)	25 (16.5)	151
History of fractures, *n* (%)	10 (6.5)	152
History of failure to thrive, *n* (%)	3 (2.2)	135
History of short stature length/height-for-age, *n* (%)	22 (18.8)	117
Hypertension, *n* (%)	17 (11.2)	152

Table 2: Kidney function and electrolyte balance parameters at the last follow-up.

Parameters	Values	Patients, *n*
Age at last follow-up (years), median (IQR)	15 (7.5–23.5)	153
Serum creatinine (mg/dl), median (IQR)	0.87 (0.54–1.37)	117
eGFR (ml/min/1.73 m^2), mean ± SD	82.3 ± 43.4	142
Serum potassium (mmol/L), mean ± SD)	3.94 ± 0.5	138
Total serum calcium (mg/dl), mean ± SD)	9.13 ± 2.2	134
Serum phosphate (mg/dl), median (IQR)	3.7 (2.8–4.3)	124
Protein:creatinine ratio (mg/g), median (IQR)	1415 (500–2353)	103
Albumin:creatinine ratio (mg/g), median (IQR)	223 (80–400)	59
Urine calcium:creatinine ratio (mg/mg), median (IQR)	0.26 (0.17–0.46)	93
CKD stage		
CKD 2, *n*/*N* (%)	42/144 (29.2)	144
Age at CKD 2 (years), median (IQR)	12.5 (7–18)	144
CKD 3, *n*/*N* (%)	19/144 (13.2)	144
Age at CKD 3 (years), median (IQR)	17 (13–21)	144
CKD 4, *n*/*N* (%)	11/144 (7.6)	144
Age at CKD 4 (years), median (IQR)	33 (26–45)	144
CKD 5 or KRT, *n*/*N* (%)	16 /144 (11.1)	144
Age at CKD 5 (years), median (IQR)	51.5 (43–63.2)	144

remained <30% for most of the paediatric and adult patients (Fig. 2C). In summary, DD1 paediatric patients exhibited a clinical picture of LMWP associated with hypercalciuria and less frequently hypophosphatemia, with normal eGFR or very mild CKD, whereas adults mainly manifested with CKD and nephrocalcinosis/nephrolithiasis.

CLCN5 variants spectrum

Genetic data for 130 patients was provided. Seventy-four variants in *CLCN5* were reported, of which 28 were novel (Supplementary Table S1) [2, 3, 10, 13]. The most common variants detected were nonsense and missense variants (40.3% and 33.3%, respectively), and less frequently, splice-site variants, frameshift variants, large deletions and in-frame variants (Fig. 3A). Interestingly, plotting these variants on the ClC-5 protein sequence (746 amino acids), revealed clustering around the pore-forming domains (47.4% of the variants, especially missense variants) and the CBS domains (involved in intracellular trafficking and protein–protein interactions; 26.3%, mainly nonsense variants; Fig. 3B) [12].

Phenotype and mutation severity

We used the same algorithm as in a previous report [4] to classify *CLCN5* variants as severe variants (nonsense, frameshift, large deletion or splice-site variants; 63.6% of patients) or moderate variants (missense and in-frame variants; 36.4% of patients). No differences regarding the age of diagnosis or DD1 manifestations between both subgroups were observed. The only exception was that hypophosphatemia was reported more commonly in patients with moderate variants than in those with severe variants (60.8% versus 24.2%; $P = .004$); however, that information could only be collected from a small number of patients and should be interpreted with caution. Finally, grouped renal function based on eGFR at the last follow-up did not differ according to mutation severity (Table 4]. Further analysis considering only truncating *CLCN5* variants as severe (38.1% of the patients) showed that a history of nephrolithiasis was reported more frequently in patients carrying non-truncating variants (30.0% versus 10.6%; $P = .018$), with no statistical differences for the rest of the analysed clinical manifestations (Supplementary Table S2).

Next, considering the distribution of the variants on the ClC-5 protein sequence and to better assess any

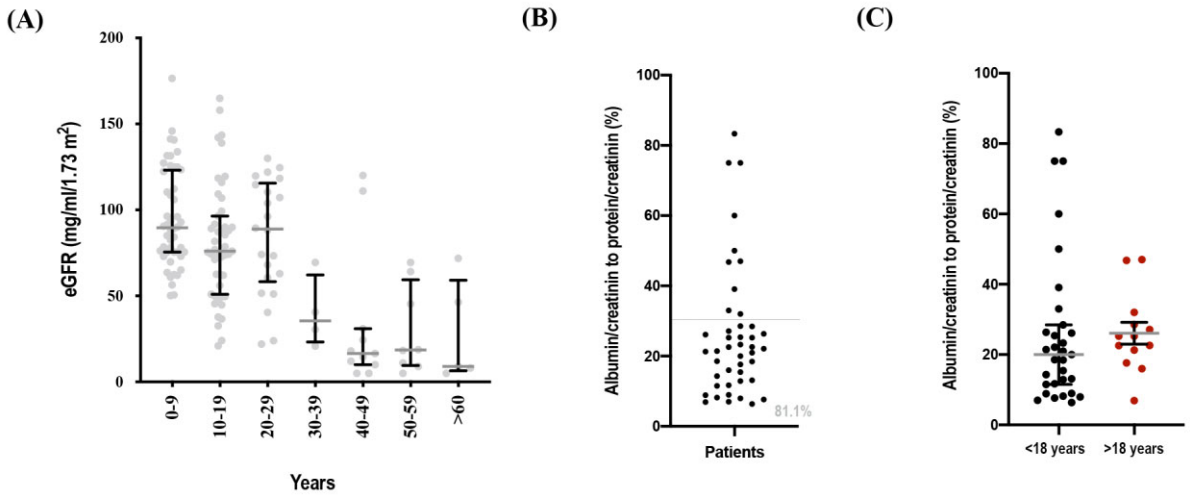


Figure 2: Phenotypic characteristics at the last follow-up. **(A)** Dot plot of eGFR (*y*-axis) according to age (*x*-axis) at the last follow-up. Patients were divided into groups according to their age. Each point represents a single patient. **(B)** Albumin:creatinine to protein:creatinine levels (%). Each point represents a single patient. **(C)** Albumin:creatinine to protein:creatinine levels (%) in paediatric (<18 years; black dots) and adult (>18 years; red dots) patients. Each point represents a single patient. *$P < .01$.

Table 3: Phenotype and kidney function at the last follow-up according to age.

Characteristics	<18 years old ($n = 94$)	>18 years old ($n = 56$)	*P*-value
Age at diagnosis (years), median (IQR)	4 (1–7)	12 (6.7–18.5)	**<.001**
History of nephrolithiasis, *n*/*N* (%)	12/91 (13.1)	26/57 (45.6)	**<.001**
Nephrocalcinosis, *n*/*N* (%)	50/82 (60.9)	41/52 (78.8)	**.04**
History of rickets, *n*/*N* (%)	15/85 (17.6)	9/59 (15.2)	.06
History of fractures, *n*/*N* (%)	5/89 (5.6)	4/56 (7.1)	.29
Hypertension, *n*/*N* (%)	4/90 (4.4)	11/55 (20)	**.01**
Age at last follow-up (years), median (IQR)	8 (5–12)	29 (20–33.5)	**<.001**
Serum creatinine (mg/dl), mean ± SD	0.69 ± 0.38	1.83 ± 1.11	**<.001**
Hypophosphatemia or use of phosphate supplementation, *n*/*N* (%)	18/83 (21.6)	21/37 (56.7)	**<.001**
Hypokalaemia or use of potassium supplementation, *n*/*N* (%)	21/89 (23.5)	7/48 (14.5)	.31
Protein:creatinine ratio (mg/g), median (IQR)	1563 (690–2530)	540 (239–1712)	**.002**
Albumin:creatinine ratio (mg/g), median (IQR)	270 (82–502)	170 (72.8–353)	.53
Hypercalciuria, *n*/*N* (%)	36/69 (73.4)	4/21 (19.0)	**<.001**
Aminoaciduria, *n*/*N* (%)	21/40 (52.1)	6/16 (37.5)	.22
Glycosuria, *n*/*N* (%)	8/74 (10.8)	15/45 (33.3)	**.039**
CKD stages 2–5, *n*/*N* (%)	44/88 (50)	44/56 (78.5)	**.003**

Statistically significant values in bold.

genotype–phenotype correlation, we decided to classify *CLCN5* variants according to their effect on the protein as early stop variants, variants affecting the pore, variants affecting the CBS domains and other. Remarkably, this functional division showed a possible correlation between the type of mutation and renal function deterioration (Fig. 3C) that should be studied thoroughly in the future. In more detail, our results showed that 55.5% of the patients with early stop variants had CKD stage 1, while this percentage was greatly reduced in patients with variants affecting the pore (27.0%), the CBS (35.4%) or other types of variants (31.5%) ($P = .039$; 'other' was excluded from the analysis due to the small number of cases) (Fig. 3C). Accordingly, 44.5% of patients with early stop variants presented with CKD stage ≥2, a condition that was present in 73.0% of those with pore variants, 64.6% of those with CBS variants and 68.5% with other variants. There were no differences in the ages between groups and CKD stages (Supplementary Table S3).

Management of the disease

At the time of the study, 43 patients (34.9%) received hydrochlorothiazide and 49 (38.9%) received angiotensin-converting enzyme inhibitors (ACEis) or angiotensin receptor blockers (ARBs). As for other treatments, 11 patients (7.1%) received potassium-sparing diuretics, 16 patients (16.0%) received phosphate supplements and 58.8% of patients with available data were receiving potassium citrate or other potassium supplements. Further analysis confirmed that there were no major differences in treatment between paediatric and adult patients (Fig. 3D).

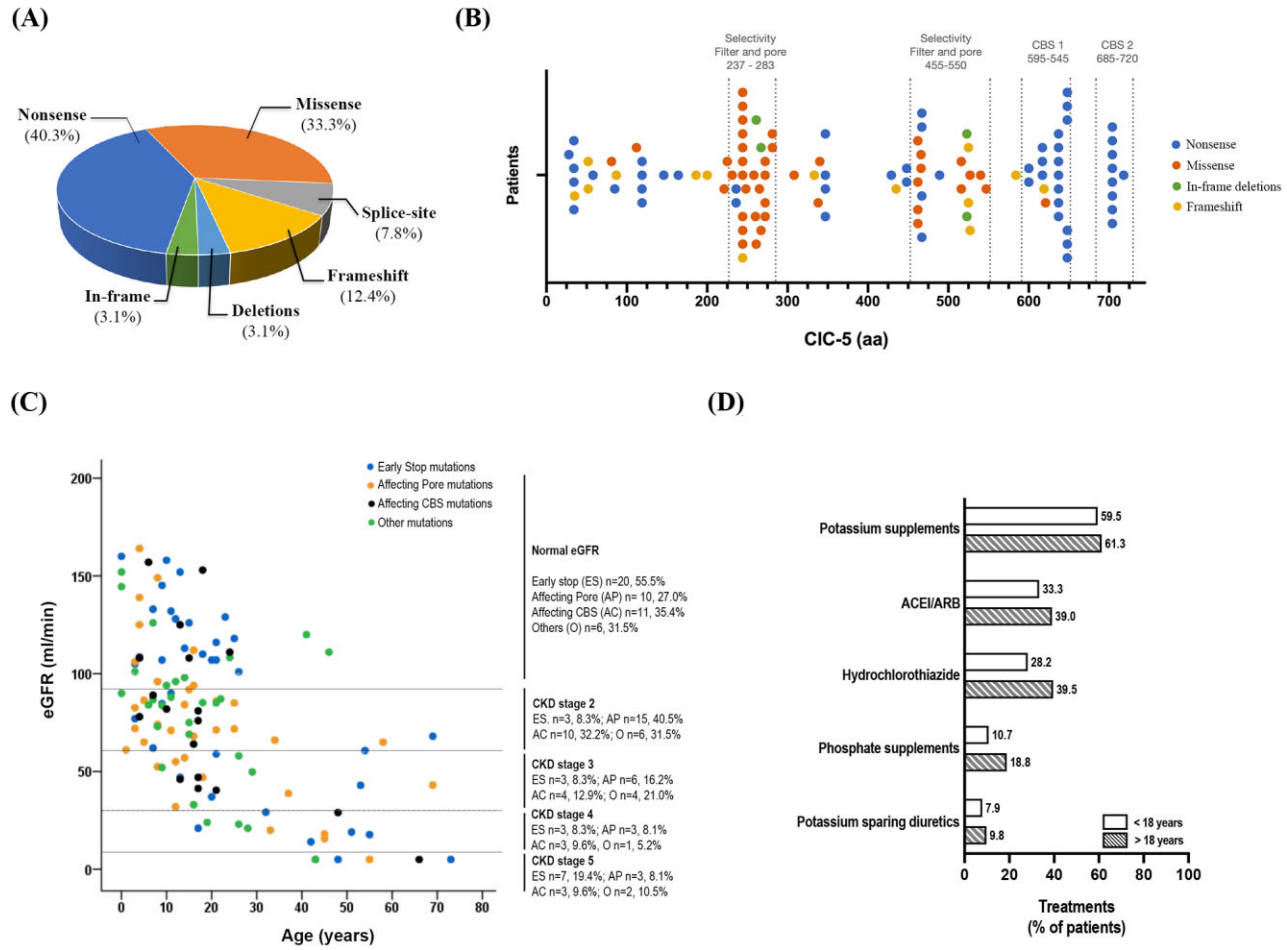


Figure 3: Variants in *CLCN5* gene and genotype–phenotype correlation. **(A)** Classification of variants on *CLCN5*. **(B)** Distribution of variants on ClC-5 amino acid sequence (transcript 3 was used). **(C)** eGFR according to age at the last follow-up and variant distribution. Each point represents a single patient. Blue dots represent early stop variants, orange dots represent pore-affecting variants, black dots represent variants affecting the CBS domains and green dots represent other types of variants. Dashed lines represent the different CKD stages. **(D)** Treatments used by clinicians in our cohort. HTN: hypertension, mut: mutation, ES: early stop; AP: affecting pore; AC: affecting CBS domains; O: others.

DISCUSSION

We have provided a snapshot of the contemporary situation of DD1 in Europe by describing the clinical characteristics and management in a large cohort of 163 genetically confirmed DD1 male patients at diagnosis and their long-term outcomes. Inclusion of a significant proportion of adult subjects (41.7%) facilitated the description of disease phenotype variation over time, differences between affected children and adults and progression of CKD according to age and genetic features. LMWP remained the hallmark of DD1 in the long-term, even in advanced CKD stages. Our data raise the need to increase DD1 awareness and promote early diagnosis, adequate management and research effort to discover new treatments to halt disease burden.

DD1 phenotype and diagnosis

A wide range of key signs led to DD1 diagnosis in our cohort, with isolated proteinuria being the most common (48.8%), which is in accordance with previous descriptions of DD1 [14–21]. However, we only observed the classic triad of LMWP, hypercalciuria and nephrocalcinosis/nephrolithiasis [17] in 26.1% of patients. These findings are aligned with previous European series [4, 5, 14], although hypercalciuria prevalence was lower in our cohort compared with others [5]. Environmental and other genetic factors may explain this difference, but it may also be related to a higher proportion of CKD stage ≥2 observed in our study, as hypercalciuria decreased in parallel with CKD progression [4]. Similar to previous reports [8, 20, 22], incomplete FS was also observed in our series, with aminoaciduria found in 45.9% of patients and glycosuria in 20.6% [23], and to a lesser extent, a history of rickets and impaired growth. Although disruption of calcium–phosphorus balance has been considered a potential cause, hypophosphatemia was not associated with bone abnormalities in our cohort, in agreement with others [24]. A lack of vitamin D reabsorption due to downregulation of megalin/cubilin could be involved as well [25, 26], but further research is needed to confirm this.

Comparison of adult versus paediatric patients revealed that the calcium:creatinine ratio in urine and proteinuria decreased according to the eGFR and age. Likewise, adults presented

Table 4: Phenotype and kidney function at the last follow-up according to *CLCN5* variant severity.

Characteristics	Moderate variants ($n = 42$)	Severe variants ($n = 70$)	*P*-value
Age at diagnosis (years), median (IQR)	6.5 (1–11)	7 (3–12)	.29
History of nephrolithiasis, *n*/*N* (%)	11/40 (27.5)	12/68 (17.6)	.42
Nephrocalcinosis, *n*/*N* (%)	22/33 (66.6)	41/60 (68.3)	.61
History of rickets, *n*/*N* (%)	5/40 (11.1)	12/68 (20.8)	.67
History of fractures, *n*/*N* (%)	2/40 (5)	5/67 (7.4)	.87
Hypertension, *n*/*N* (%)	4/38 (10.5)	9/67 (13.2)	.49
Age at last follow-up (years), median (IQR)	14.5 (7.7–21)	15 (8–21.7)	.64
Serum creatinine (mg/dl), mean ± SD	1.2 ± 0.8	1.01 ± 0.75	.29
Hypophosphatemia, *n*/*N* (%)	14/23 (60.8)	12/49 (24.2)	**.004**
Hypokalaemia or use of potassium supplements, *n*/*N* (%)	9/36 (25)	14/57 (24.5)	.81
Protein:creatinine ratio (mg/g), median (IQR)	703.1 (445–1712)	1465 (500–2572)	.15
Albumin:creatinine ratio (mg/g), median (IQR)	246 (77.7–404)	108.3 (43.5–302.5)	.09
Hypercalciuria, *n*/*N* (%)	7/21 (33.3)	20/40 (50)	.35
Aminoaciduria, *n*/*N* (%)	6/8 (75)	14/31 (45.1)	.13
Glycosuria, *n*/*N* (%)	5/32 (15.6)	12/56 (21.4)	.62
CKD stage			
CKD 2, *n*/*N* (%)	14/42 (33.3)	19/70 (27.1)	
Age at CKD 2 (years), median (IQR)	18.5 (6.7–25)	9 (6.2–15.7)	.12
CKD 3, *n*/*N* (%)	8/42 (19.1)	8/70 (11.4)	
Age at CKD 3 (years), median (IQR)	17.5 (12.5–33)	20 (14.5–27.5)	.81
CKD 4, *n*/*N* (%)	3/42 (7.1)	5/70 (7.1)	
Age at CKD 4 (years), median (IQR)	33 (30–39)	26 (19–32)	.39
CKD 5, *n*/*N* (%)	3/42 (7.1)	9/70 (12.9)	
Age at CKD 5 (years), median (IQR)	55 (49–62)	53 (45.5–67.5)	.91

Statistically significant values in bold.

more frequently with nephrocalcinosis and nephrolithiasis than younger patients. Indeed, we did not find the rate of hypokalaemia previously described in adults [4], which may be explained by different management, but also by a higher rate of advanced CKD in our series and subsequent loss of the tubular phenotype. We also observed that hypophosphatemia (or the use of phosphate supplementation) was more common in adult patients. However, both children and adults remained with proteinuria, and a low albumin:creatinine ratio was observed despite CKD progression. Therefore, although the manifestation of DD1 was characterized by proximal tubular dysfunction in children and by CKD in affected adults, detection of LMWP or low albuminuria in male patients with proteinuria remained the disease hallmark, pointing to its specific diagnostic value [17, 18]. Further, one major contribution of our study is that even with large interindividual variability, grouped analysis showed that CKD progression in DD1 was age dependent, and older patients had worse kidney function despite a similar therapeutic approach.

Interestingly, the median age at diagnosis was 4 years earlier than in other European cohorts [4, 14], possibly related to expertise and genetic availability in reference centres. Few patients were identified after family screening despite a positive history, which confirms the need for increasing DD1 awareness. Similar to other authors, we suggest that males with non-nephrotic proteinuria should be checked for LMWP and potentially for *CLCN5* variants [16] to avoid delayed diagnosis and poor management [18, 19].

CLCN5 variants

CLCN5 nonsense variants were most common in our patients (43%), followed by missense variants (33%). Although we did not find a clear phenotype–genotype association, we observed a tendency in patients with severe *CLCN5* gene variants to reach late CKD stages at a younger age than those with moderate variants. Also, patients with severe variants presented less hypophosphatemia than patients with moderate variants. Similarly, patients with truncating variants also reported fewer episodes of nephrolithiasis than those with non-truncating *CLCN5* variants. This could be due to the CKD phenotype prevailing over the tubular phenotype in patients with severe variants. An interesting finding of our study is that missense variants accumulated in the pore-forming regions, while nonsense variants were more frequent in the CBS domains and the N-terminus domain. This could be the result of selective pressure against deleterious variants. In addition, it was satisfying to find, for the first time in DD1, a correlation between the domain affected by the mutation (early stop, pore or CBS domains) and kidney function. Patients with early stop variants present more frequently with CKD stage 1 than other patients. A possible explanation for this, which could be the basis for further research on genotype–phenotype correlation in DD1, is that a malfunctioning ClC-5 (e.g. affected pore) is more dangerous for proximal tubule cells than a partial or non-functioning protein, the cells of which may compensate by expressing other members of the ClC family. This may also explain the finding that patients carrying non-truncating variants of *CLCN5* present more commonly with a history of nephrolithiasis at the last follow-up than patients with truncating *CLCN5* variants.

DD1 management in Europe

Currently there is no specific therapy for DD1 patients, so pharmacological intervention generally aims to reduce

proteinuria and hypercalciuria or rickets and prevent nephrolithiasis/nephrocalcinosis [4, 13]. As our results reveal, clinical approaches in Europe are very variable and individually tailored. Thiazide diuretics are commonly used since they have proved effective against hypercalciuria in DD1 patients [27]. Potassium citrate was also used, since a high-citrate diet was shown to slow progression of CKD in *Clcn5* knockout mice [28]. Some patients were treated with ACEIs/ARBs. Although they are not effective for tubular proteinuria, they have proved effective in a few DD1 cases, hypothetically because glomerular damage was present in these patients [4, 19, 29]. We did not find any major difference in the treatment between adult and paediatric patients, which supports that phenotypic variability between children and adults is better explained by disease progression than by treatment.

Strengths

The most remarkable strength of our study is the large number of well-characterized and genetically confirmed contemporary DD1 patients, including children and adults, with similar therapeutic management throughout Europe. We describe that the classic clinical triad of LMWP, hypercalciuria and nephrocalcinosis/nephrolithiasis was only observed in 26.1% of patients, highlighting the DD1 phenotype variability. Further, we provided new data regarding patient phenotypes, *CLCN5* gene variants and progression to CKD.

Limitations

The main limitation of this study is its retrospective and cross-sectional nature, which prevented an estimation of the rate of eGFR decline in DD1. Further, due to data variability and differences in the number of cases per country, it was not possible to calculate DD1 European prevalence. On the other hand, we cannot ignore that selection bias may have occurred due to participation of expert nephrology centres and a larger proportion of paediatric patients included, which may not represent the average practice. Another limitation of this study is the young age of the individuals included in the adult group, which could blur the differences between paediatric and adult DD1 patients. Further, hypercalciuria was estimated by calcium:creatinine ratios since measurement of daily calcium excretion was not available and thus accurate correlation with eGFR could not be determined. Moreover, the interindividual variability of the treatment does not allow proper analysis of the effects of the therapies applied. Finally, for data protection, specific information on *CLCN5* variants was not described, to avoid tracking of individual DD1 patients.

In conclusion, DD1 has a heterogeneous presentation and as patients grow older there is a blurring of the main phenotypic traits, yet it progresses to CKD, which makes diagnosis challenging unless LMWP or low albuminuria in male patients with proteinuria is detected. CKD progression and severity is related to patient age, which highlights the unmet need of specific treatments. We have found no clear genotype–phenotype correlation in our European cohort, so we postulate that differences in progression may be because of other factors such as functional disparities among variants, which deserve further research.

SUPPLEMENTARY DATA

Supplementary data is available at *ndt* online.

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AUTHORS' CONTRIBUTIONS

C.B. and G.A. conceived and designed the research studies. C.B., L.P., F.L., K.S., P.V.A., M.B., D.B., L.M., A.B.T., F.T., M.P., P.C., D.D., D.C., L.K.K., M.F., A.P., I.C., G.C., M.G., B.M., T.W., F.S. and G.A. provided data from patients. C.B., G.C.R. and G.A. analysed the data. C.B., G.C.R. and G.A. drafted the manuscript. All authors read, reviewed and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and in its online supplementary material.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

APPENDIX

The following persons and institutions participated in the DENT Study Group: Hopital Femme Mère Enfant (Lyon, France): Justine Bacchetta; Pediatric Nephrology Dialysis and Transplant Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy): Fabio Paglialonga; Nefrologia Pediatrica, Dialisi e Trapianto, Università di Padova (Padova, Italy): Luisa Murer; Children's Clinical University Hospital (Riga, Latvia): Ilze Andersone; Renal Services Centre, Newcastle upon Tyne Hospitals NHS Foundation Trust (Newcastle, UK): John A. Sayer; Necker Hospital

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REFERENCES

1. Dent CE, Friedman M. Hypercalcuric rickets associated with renal tubular damage. *Arch Dis Child* 1964;**39**:240–9.
2. Thakker RV. Pathogenesis of Dent's disease and related syndromes of X-linked nephrolithiasis. *Kidney Int* 2000;**57**:787–93.
3. Ludwig M, Utsch B, Monnens LAH. Recent advances in understanding the clinical and genetic heterogeneity of Dent's disease. *Nephrol Dial Transplant* 2006;**21**:2708–17.
4. Blanchard A, Curis E, Guyon-Roger T *et al.* Observations of a large Dent disease cohort. *Kidney Int* 2016;**90**:430–9.
5. Gianesello L, Del Prete D, Anglani F *et al.* Genetics and phenotypic heterogeneity of Dent disease: the dark side of the moon. *Hum Genet* 2021;**140**:401–21.
6. Mansour-Hendili L, Blanchard A, Le Pottier N *et al.* Mutation update of the *CLCN5* gene responsible for Dent disease 1. *Hum Mutat* 2015;**36**:743–52.
7. Reinhart SC, Norden AG, Lapsley M *et al.* Characterization of carrier females and affected males with X-linked recessive nephrolithiasis. *J Am Soc Nephrol* 1995;**5**:1451–61.
8. Wrong OM, Norden AG, Feest TG. Dent's disease; a familial proximal renal tubular syndrome with low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, metabolic bone disease, progressive renal failure and a marked male predominance. *QJM* 1994;**87**:473–93.
9. Smith AJ, Reed AAC, Loh NY *et al.* Characterization of Dent's disease mutations of CLC-5 reveals a correlation between functional and cell biological consequences and protein structure. *Am J Physiol Renal Physiol* 2009;**296**:F390–7.
10. Lourdel S, Grand T, Burgos J *et al.* ClC-5 mutations associated with Dent's disease: a major role of the dimer interface. *Pflugers Arch* 2012;**463**:247–56.
11. Devuyst O, Christie PT, Courtoy PJ *et al.* Intra-renal and subcellular distribution of the human chloride channel, CLC-5, reveals a pathophysiological basis for Dent's disease. *Hum Mol Genet* 1999;**8**:247–57.
12. Carr G, Simmons N, Sayer J. A role for CBS domain 2 in trafficking of chloride channel CLC-5. *Biochem Biophys Res Commun* 2003;**310**:600–5.
13. Devuyst O, Luciani A. Chloride transporters and receptor-mediated endocytosis in the renal proximal tubule. *J Physiol* 2015;**593**:4151–64.
14. Tosetto E, Ghiggeri GM, Emma F *et al.* Phenotypic and genetic heterogeneity in Dent's disease—the results of an Italian collaborative study. *Nephrol Dial Transplant* 2006;**21**:2452–63.
15. Ye Q, Shen Q, Rao J *et al.* Multicenter study of the clinical features and mutation gene spectrum of Chinese children with Dent disease. *Clin Genet* 2020;**97**:407–17.
16. Beara-Lasic L, Cogal A, Mara K *et al.* Prevalence of low molecular weight proteinuria and Dent disease 1 CLCN5 mutations in proteinuric cohorts. *Pediatr Nephrol* 2020;**35**:633–40.
17. Devuyst O, Thakker RV. Dent's disease. *Orphanet J Rare Dis* 2010;**5**:28.
18. van Berkel Y, Ludwig M, van Wijk JAE *et al.* Proteinuria in Dent disease: a review of the literature. *Pediatr Nephrol* 2017;**32**:1851–9.
19. Wang X, Anglani F, Beara-Lasic L *et al.* Glomerular pathology in dent disease and its association with kidney function. *Clin J Am Soc Nephrol* 2016;**11**:2168–76.
20. Hodgin JB, Corey HE, Kaplan BS *et al.* Dent disease presenting as partial Fanconi syndrome and hypercalciuria. *Kidney Int* 2008;**73**:1320–3.
21. De Matteis MA, Staiano L, Emma F *et al.* The 5-phosphatase OCRL in Lowe syndrome and Dent disease 2. *Nat Rev Nephrol* 2017;**13**:455–70.
22. Ramos-Trujillo E, Claverie-Martin F, Garcia-Nieto V *et al.* Dent's disease: identification of seven new pathogenic mutations in the CLCN5 gene. *J Pediatr Genet* 2013;**2**:133–40.
23. Pereira-Moreira R, Muscelli E. Effect of insulin on proximal tubules handling of glucose: a systematic review. *J Diabetes Res* 2020;**2020**:8492467.
24. Hoopes RR, Raja KM, Koich A *et al.* Evidence for genetic heterogeneity in Dent's disease. *Kidney Int* 2004;**65**:1615–20.
25. Nielsen R, Christensen EI, Birn H. Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. *Kidney Int* 2016;**89**:58–67.
26. Anglani F, D'Angelo A, Bertizzolo LM *et al.* Nephrolithiasis, kidney failure and bone disorders in Dent disease patients with and without CLCN5 mutations. *Springerplus* 2015;**4**:492.
27. Raja KA, Schurman S, D'Mello RG *et al.* Responsiveness of hypercalciuria to thiazide in Dent's disease. *J Am Soc Nephrol* 2002;**13**:2938–44.
28. Cebotaru V, Kaul S, Devuyst O *et al.* High citrate diet delays progression of renal insufficiency in the ClC-5 knockout mouse model of Dent's disease. *Kidney Int* 2005;**68**:642–52.
29. Zaniew M, Mizerska-Wasiak M, Załuska-Leśniewska I *et al.* Dent disease in Poland: what we have learned so far? *Int Urol Nephrol* 2017;**49**:2005–17.
30. Mejia N, Santos F, Claverie-Martin F *et al.* RenalTube: a network tool for clinical and genetic diagnosis of primary tubulopathies. *Eur J Pediatr* 2013;**172**:775–80. https://doi.org/10.1007/s00431-013-1934-6
31. World Health Organization. Child growth standards: length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development. https://www.who.int/publications-detail-redirect/924154693X (9 May 2022, date last accessed)
32. Matos V, vanMelle G, Boulat O *et al.* Urinary phosphate/creatinine, calcium/creatinine, and magnesium/creatinine ratios in a healthy pediatric population. *J Pediatr* 1997;**131**:252–7 https://doi.org/10.1016/S0022-3476(97)70162-8
33. Habbig S, Beck BB, Hoppe B. Nephrocalcinosis and urolithiasis in children. *Kidney Int* 2011;**80**:1278–91. https://doi.org/10.1038/ki.2011.336

34. Pottel H, Hoste L, Martens F. A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol* 2012;**27**:973–9. https://doi.org/10.1007/s00467-011-2081-9
35. Levey AS, Stevens LA, Schmid CH *et al.* A new equation to estimate glomerular filtration rate. *Ann Intern Med* 2009;**150**:604–12. https://doi.org/10.7326/0003-4819-150-9-200905050-00006
36. Gianesello L, Ceol M, Bertoldi L *et al.* Genetic analyses in Dent disease and characterization of CLCN5 mutations in kidney biopsies. *Int J Mol Sci* 2020;**21**:516. https://doi.org/10.3390/ijms21020516
37. Hogg RJ, Portman RJ, Milliner D *et al.* Evaluation and management of proteinuria and nephrotic syndrome in children: recommendations from a pediatric nephrology panel established at the National Kidney Foundation conference on proteinuria, albuminuria, risk, assessment, detection, and elimination (PARADE). *Pediatrics* 2000;**105**:1242–9.

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