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

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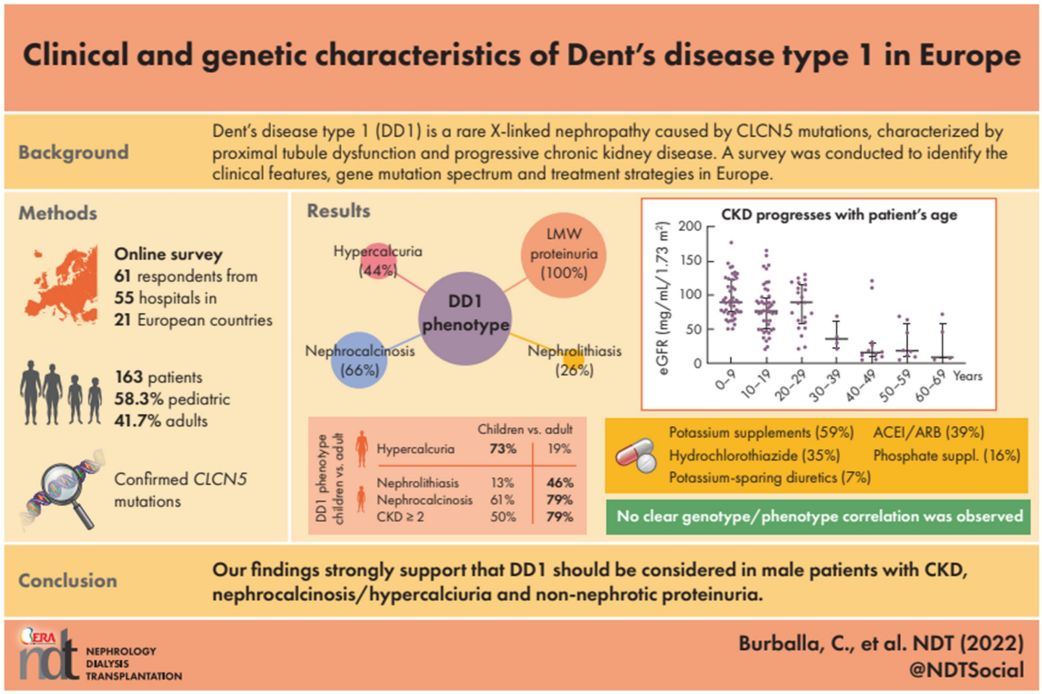
Running title: Dent's Disease 1 in Europe

GRAPHICAL ABSTRACT



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 spectrum.

Results.

Two-hundred seven DD1 male patients were reported, being clinical data available for 163 with confirmed *CLCN5* mutations. Proteinuria was the most common leading 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 renal dysfunction, 20.7% developed CKD ≥3, and 11.1% KF. At last visit, hypercalciuria was more frequent in pediatric patients than in adults (73.4% vs. 19.0%). Conversely, nephrolithiasis, nephrocalcinosis and renal dysfunction were more prominent in adults. Furthermore, CKD progressed with age. Despite no clear phenotype/genotype correlation was observed, decreased glomerular filtration rate was more frequent in subjects with *CLCN5* mutations affecting the pore or CBS domains compared to 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.

KEY LEARNING POINTS

What is already known about this subject?

- Dent's disease 1 (DD1) is a rare X-linked renal condition caused by defective CLCN5 gene.
- High urine low-molecular-weight proteins (LMWP) represents 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?

- In this anonymous international e-survey of 159 DD1 patients, the authors describe the current clinical practice and outcome of DD1 in Europe.
- They have better characterized DD1 manifestations and provided new information regarding age and genetics impact on CKD progression.
- Importantly, they describe how patients' symptoms change from pediatric to adults.

What impact this may have on practice or policy?

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

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

INTRODUCTION

Dent's disease type 1 (DD1) (OMIM #300009) is a hereditary rare 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). Besides, patients may show an incomplete Fanconi syndrome (FS) with defective reabsorption of one or several other solutes (e.g. aminoacids, 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 to sixth decade of life in approximately 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 *CLCN5* gene (MIM#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 yet (4,9,10). The canonic 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 (PTC), 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 analyze the clinical features, management strategies, gene mutation spectrum and long-term outcome of patients with Dent's disease type 1 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: socio-demographic and anthropometric data; diagnosis; updated clinical evaluation; updated blood/urinary biochemistry parameters, genetic diagnosis and management. The list of questions is provided as supplementary material (**Supplementary material 1**). Data were deemed adequate for analysis if more than 50% of the items were completed for each patient.

Patients

Two hundred seven patients with confirmed DD1 diagnosis were included in the study. Additional 44 reported 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 deviations (SD) for normally distributed variables, according to Kolmogorov-Smirnov/ Shapiro-Wilk test and as median (interquartile range (IQR)) if non-normally distributed. Continuous variables were analyzed by t-test or U-Mann-Whitney test; binary and categorical variables by chi-square or Fisher exact test, depending on group size. A p value <0.05 was considered statistically significant.

Expanded description of the materials and methods can be found in the supplemental material.

RESULTS

Survey respondents

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

Presentation at diagnosis

Median age of clinical diagnosis was 7 [3-12] years. Main 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%) (**Figure 1B**). Only in 11.9% of the cases, the diagnosis was done after family screening, despite presence of positive DD1 history in 51.2% of patients addressed by directed questioning.

Phenotypic characteristics during follow-up

At 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 eGFR higher than 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 [5-29] years at the first episode. History of rickets was recorded in 16.5% of patients and ten patients (6.5%) referred at least one fracture episode at a median age of 5 [0-51] years (**Table 1**).

Proximal tubular transport defects were often detected: aminoaciduria in 45.9% of patients and glycosuria in 20.6%. Further, 28 patients (20.1%) had hypokalemia and 40 (26.3%) hypophosphatemia or received supplements. Interestingly, presence of hypophosphatemia did not correlate with history of rickets (52.6%; vs. 47.4%; patients with history of rickets and hypophosphatemia or normal serum phosphate respectively; p=0.32). Impaired growth was detected in 18.8% of patients and failure to thrive in three patients more (2.2%). Few patients (11.2%) were hypertensive (**Table 1**, **Figure 1C**).

Follow-up and kidney function

Median patient age at the last follow-up was 15 [7.5-23.5] years, with a median duration since diagnosis of 5.5 [1.7-11] years. Biochemical parameters at the last blood test available are shown in Table 2. At the last visit, 88 patients (61.1%) had 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 [43-63.2] years. Our data show that, despite individual variability, CKD progressed according to patient's age. Median patient's age among those with normal eGFR was 9.5 years, whereas median patient age among those with CKD2, CKD3 and CKD4 was 12.5, 17 and 33 years respectively (p<0.001) (**Table 2**). To further show that CKD progresses with patient's age, we divided our cohort in different age groups and calculated the median eGFR for each one. This analysis showed that median eGFR decreased with age (p<0.001) (**Figure 2A**). Importantly, our data demonstrates the progression to KF in DD1: there are no cases of KF below 40 years of age, while the numbers increase drastically later, affecting 45.5% of the patients between 40-49 years, 75% of the patients between 50-59 years, and finally reaching 100% in patients >60 years old.

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

DD1 phenotype according to patients' age

Next, we compared pediatric (<18 years) and adult patients' data at last follow-up. We observed that DD1 clinical features changed with age (**Table 3**). Remarkably, only 19.0% of adult patients were hypercalciuric in comparison with 73.4% of pediatric patients (p<0.001). However, adult patients experienced more episodes of nephrolithiasis (45.6% vs. 13.1%; p<0.001) and a higher prevalence of nephrocalcinosis (78.8% vs. 60.9%; p=0.04) compared to pediatric subjects. Further, CKD became more frequent with age (Pediatric vs. adult patients: eGFR 88.0±31.2 mg/ml/1.73 m^2 vs. 59.2±44.1 mg/ml/1.73 m^2, p<0.0001; serum creatinine 0.69±0.38 mg/dL vs. 1.83±1.1 mg/dL, p<0.001). Besides, adult patients were hypertensive more often than pediatric patients (20.0% vs. 4.4%; p=0.01) and exhibited CKD≥2 at their last follow-up (78.5% vs. 50.0%; p=0.003). A higher proportion of adult patients showed hypophosphatemia (or phosphate supplementation) compared to pediatric patients (56.7% vs. 21.6%; p<0.001), and glycosuria was also more often detected in adults than children (33.3% vs. 10.8%; p=0.039). Remarkably, protein/creatinine ratio in urine decreased from childhood to adulthood (1563 [690-2530] mg/g vs. 540 [239-1712] mg/g p=0.002). Nevertheless, the ratio albumin/creatinine to protein/creatinine remained below 30% for the most of pediatric and adult patients (**Figure 2C**). In

summary, DD1 pediatric 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, from which 28 were novel (2,3,10,13) (**Supplementary table 1**). The most common type of 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 (**Figure 3A**). Interestingly, plotting these variants on the ClC-5 protein sequence (746 aa), 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 (12)) (26.3%, mainly nonsense variants) (**Figure 3B**).

Phenotype and mutation severity

We used the same algorithm as in a previous report (4) to classify *CLCN5* variants as: (A) Severe variants (nonsense, frameshift, large deletion, or splice-site variants; 63.6% of patients); and (B) Moderate variants (missense and in-frame variants; 36.4% of patients). No differences regarding 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% vs. 24.2%; p=0.004); 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 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 history of nephrolithiasis was reported more frequently in patients carrying non-truncating variants (30.0% vs. 10.6%, p=0.018), with no statistical differences for the rest of the analyzed clinical manifestations (**Supplementary table 2**).

Next, taking into account the distribution of the variants on ClC-5 protein sequence and to better assess any genotype-phenotype correlation, we decided to classify *CLCN5* variants according to their effect on the protein as (A) Early stop variants, (B) Variants affecting the pore, (C) Variants affecting the CBS domains, and (D) other. Remarkably, this functional division showed a possible correlation between the type of mutation and renal function deterioration (**Figure 3C**) that should be studied deeply in the future. In more detail, our results showed that 55.5% of the patients with early stop variants had CKD1, while this percentage was strongly reduced in patients with variants affecting the pore (27.0%), the CBS (35.4%) or other type of variants (31.5%) (p=0.039, "others" was excluded from the analysis due to the low number of cases) (**Figure 3C**). Accordingly, 44.5% of patients with early stop variants presented CKD≥2, a condition that was present in 73.0% of those with variants in the pore, 64.6% of those with variants affecting the CBS or 68.5% with other variants. There were no differences in the age between groups and CKD stages (**Supplementary table 3**).

Management of the disease

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

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 long-term outcome. 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 on the long-term, even in advanced CKD stages. Our data raises the need to increase DD1 awareness, promote early diagnosis, adequate management, and research effort for discovering new treatments to halt disease burden.

DD1 phenotype and diagnosis

A wide range of key signs led to DD1 diagnosis in our cohort, being isolated proteinuria the most common one (48.8%), which is in accordance with previous descriptions of DD1 (26–33). However, we only observed the classic triad of LMWP, hypercalciuria and nephrocalcinosis/nephrolithiasis (29) in 26.1% of the patients. These findings are aligned to previous European series (4,5,26), although hypercalciuria prevalence was lower in our cohort compared to others (5). Environmental and other genetic factors may explain this difference, but it can be also related to higher CKD≥2 proportion observed in our study as hypercalciuria decreased in parallel with CKD progression (4). Similar to previous reports (8,22,32), incomplete Fanconi syndrome was also observed in our series with aminoaciduria found in 45.9% of patients and glycosuria in 20.6% (34), and to less extend 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 (35). Lack of vitamin D reabsorption due to downregulation of megalin/cubilin could be involved as well (36,37), but further research is needed to confirm that.

Comparison of adult vs. pediatric patients revealed that the ratio calcium/creatinine in urine and proteinuria decreased accordingly to eGFR and age. Likewise, adults presented more frequently with nephrocalcinosis and nephrolithiasis than younger patients. Indeed, we did not find the rate of hypokalemia 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 use of phosphate supplementation) was more common in adult patients. However, both children and adults remained with proteinuria; and low albumin/creatinine ratio was observed despite CKD progression. Therefore, though DD1 manifestation was characterized by proximal tubular dysfunction in pediatrics and by CKD in affected adults, detection of LMWP or low albuminuria in male patients with proteinuria remained the disease hallmark, reassuring its diagnostic specific value (29,30). Further, one major contribution of our study is that even large inter-individual variability, grouped analysis showed that CKD progression in DD1 was age-dependent and older patients had worse kidney function despite similar therapeutic approach.

Interestingly, median age at diagnosis was four years earlier than other European cohorts (4,26), possibly related to expertise and genetic availability in reference centers. Few patients were identified after family screening despite positive history what confirms the need of increasing DD1 awareness. Similarly to other authors, we suggest that males with non-nephrotic proteinuria should be checked for LMWP and potentially for *CLCN5* variants (28), to avoid delayed diagnosis and poor management (30,31).

CLCN5 variants

CLCN5 nonsense variants were the 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 on patients with severe *CLCN5* gene variants to reach late CKD stages at a younger age than those with moderate variants. Besides, patients with severe variants presented less hypophosphatemia than patients with moderate variants. Similarly, patients with truncating variants also reported less episodes of nephrolithiasis than those with non-truncating *CLCN5* variants. This could be due to CKD phenotype prevailing over 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 at 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 CKD1 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 (for example, affected pore) is more dangerous for proximal tubule cells than a partial or non-functioning protein, which cells 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 history of nephrolithiasis at last follow-up than those 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, 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 (38). Potassium citrate was also used, since high-citrate diet showed to slow progression of CKD in *Clcn5* knockout mice (39). Some patients were treated with ACEI/ARB. Although they are not effective for tubular proteinuria, they have proved effective in few DD1 cases hypothetically because glomerular damage was present in these patients (4,31,40). We did not find any major difference in the treatment between adult and pediatric patients, which supports that phenotypic variability between children and adults are better explain by the 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 phenotype, *CLCN5* gene variants and progression to CKD.

Limitations

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 data variability and differences in the number of

cases/country, it was not possible to calculate DD1 European prevalence. On the other hand, we cannot discard that a selection bias may have occurred due to participation of nephrology expert centers and larger proportion of pediatric 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 pediatric and adult DD1 patients. Further, in this study, 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 to accomplish a 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 an heterogenous presentation and as patients grow older, there is a blurring of main phenotypic traits, yet it progresses to CKD, which makes diagnosis challenging unless LMWP or low albuminuria in male patients with proteinuria are 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 lay on other factors such as functional disparities among variants that deserve further research.

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 conflict of interest.

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APPENDIX

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Table 1: Clinical manifestation in patients with Dent's Disease 1.

	Dent's Disease type 1 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
Hypokalemia 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
HTN (n, %)	17 (11.2%)	152

Abbreviations. IQR: Interquartile range; LMWP: Low molecular weight proteinuria; HTN: Hypertension.

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

	Dent's Disease type 1 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[a], 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, mg/g (median, [IQR])	1415 [500-2353]	103
Albumin/creatinine, mg/g (median, [IQR])	223 [80-400]	59
$U_{Calcium/creatinine}$, mg/mg (median, [IQR])	0.26 [0.17-0.46]	93
CKD		
CKD2	42 of 144 (29.2%)	144
Age CKD2 (years; median, [IQR])	12.5 [7-18]	144
CKD3	19 of 144 (13.2%)	144
Age CKD3 (years; median, [IQR])	17 [13-21]	144
CKD4	11 of 144 (7.6%)	144
Age CKD4 (years; median, [IQR])	33 [26-45]	144
CKD5 or KRT	16 of 144 (11.1%)	144
Age CKD5 (years; median, [IQR])	51.5 [43-63.2]	144

Abbreviations. IQR: Interquartile range; eGFR: Estimated glomerular filtration rate; SD: standard deviation; CKD: Chronic Kidney Disease; KRT: kidney replacement therapy.

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

	<18 years old (n=94)	>18 years old (n=56)	p
Age at diagnosis (years; median, [IQR])	4 [1-7]	12 [6.7-18.5]	*** <0.001**
History of nephrolithiasis (n, %)	12/91 (13.1%)	26/57 (45.6%)	*** <0.001**
Nephrocalcinosis (n, %)	50/82 (60.9%)	41/52 (78.8%)	*** 0.04**
History of rickets (n, %)	15/85 (17.6%)	9/59 (15.2%)	0.06
History of fractures (n, %)	5/89 (5.6%)	4/56 (7.1%)	0.29
Hypertension (n, %)	4/90 (4.4%)	11/55 (20%)	*** 0.01**
Age at last follow-up, years (median, [IQR])	8 [5-12]	29 [20-33.5]	*** <0.001**
Serum creatinine, mg/dL (mean ± SD)	0.69 ± 0.38	1.83 ± 1.11	*** <0.001**
Hypophosphatemia or use of phosphate supplementation (n, %)	18/83 (21.6%)	21/37 (56.7%)	*** <0.001**
Hypokalemia or use of K^+ supplementation (n, %)	21/89 (23.5%)	7/48 (14.5%)	0.31
Protein/creatinine, mg/g (median, [IQR])	1563 [690-2530]	540 [239-1712]	*** 0.002**
Albumin/creatinine, mg/g (median, [IQR])	270 [82-502]	170 [72.8-353]	0.53
Hypercalciuria (n, %)	36/69 (73.4%)	4/21 (19.0%)	*** <0.001**
Aminoaciduria (n, %)	21/40 (52.1%)	6/16 (37.5%)	0.22
Glycosuria (n, %)	8/74 (10.8%)	15/45 (33.3%)	*** 0.039**
CKD 2-5 (n, %)	44/88 (50%)	44/56 (78.5%)	*** 0.003**

Abbreviations. IQR: Interquartile range; SD: standard deviation; CKD: Chronic Kidney Disease, K^+: potassium.

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

	Moderate variants (n=42)	Severe variants (n=70)	p
Age at diagnosis, years (median, [IQR])	6.5 [1-11]	7 [3-12]	0.29
History of nephrolithiasis (n, %)	11/40 (27.5%)	12/68 (17.6%)	0.42
Nephrocalcinosis (n, %)	22/33 (66.6%)	41/60 (68.3%)	0.61
History of rickets (n, %)	5/40 (11.1%)	12/68 (20.8%)	0.67
History of fractures (n, %)	2/40 (5%)	5/67 (7.4%)	0.87
HTN (n, %)	4/38 (10.5%)	9/67 (13.2%)	0.49
Age at last follow-up, years (median, [IQR])	14.5 [7.7-21]	15 [8-21.7]	0.64
Serum creatinine, mg/dL (mean ± SD)	1.2 ± 0.8	1.01 ± 0.75	0.29
Hypophosphatemia (n, %)	14/23 (60.8%)	12/49 (24.2%)	***0.004**
Hypokalemia or use of K^+ supplements (n, %)	9/36 (25%)	14/57 (24.5%)	0.81
Protein/creatinine, mg/g (median, [IQR])	703.1 [445-1712]	1465 [500-2572]	0.15
Albumin/creatinine, mg/g (median, [IQR])	246 [77.7-404]	108.3 [43.5-302.5]	0.09
Hypercalciuria (n, %)	7/21 (33.3%)	20/40 (50%)	0.35
Aminoaciduria (n, %)	6/8 (75%)	14/31 (45.1%)	0.13
Glycosuria (n, %)	5/32 (15.6%)	12/56 (21.4%)	0.62
CKD2 (n, %)	14 of 42 (33.3%)	19 of 70 (27.1%)	
Age at CKD2 (median, [IQR])	18.5 [6.7-25]	9 [6.2-15.7]	0.12
CKD3 (n, %)	8 of 42 (19.1%)	8 of 70 (11.4%)	
Age at CKD3 (years; median, [IQR])	17.5 [12.5-33]	20 [14.5-27.5]	0.81
CKD4 (n, %)	3 of 42 (7.1%)	5 of 70 (7.1%)	
Age at CKD4 (years; median, [IQR])	33 [30-39]	26 [19-32]	0.39
CKD5 (n, %)	3 of 42 (7.1%)	9 of 70 (12.9%)	
Age at CKD5 (years; median, [IQR])	55 [49-62]	53 [45.5-67.5]	0.91

Abbreviations. IQR: Interquartile range; SD: standard deviation; CKD: Chronic Kidney Disease; HTN: Hypertension; K^+: potassium.

FIGURE 1

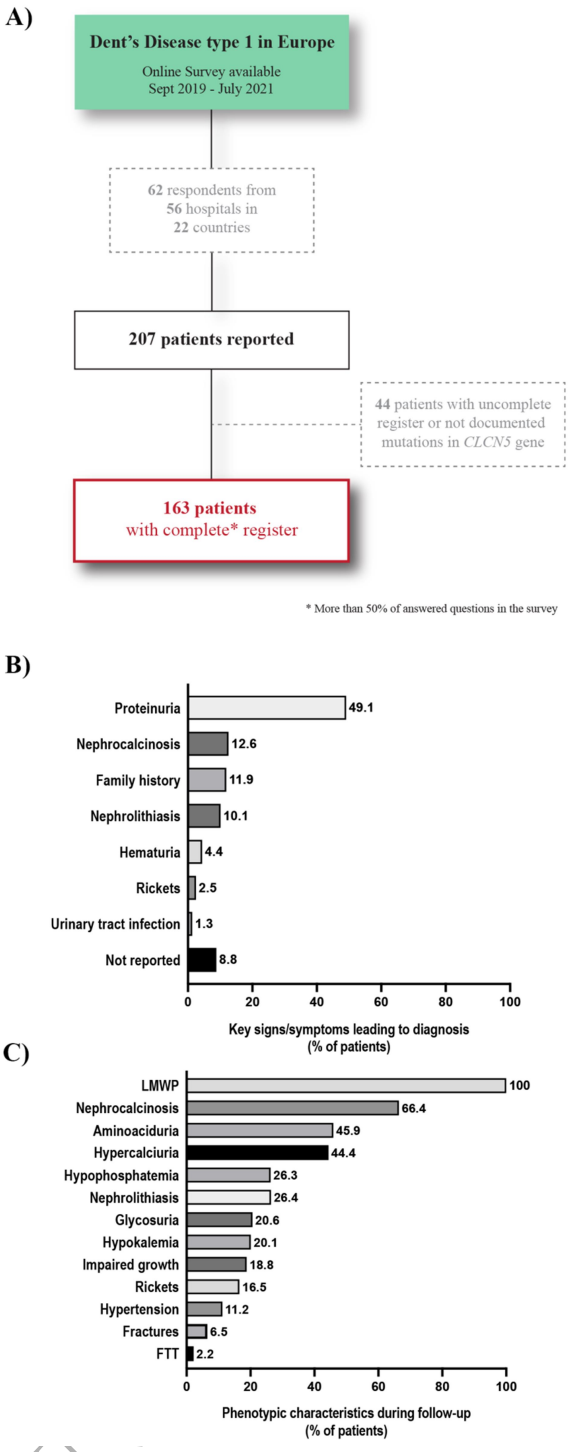

Figure 1. Description of the survey and main patients' characteristics. A) Scheme of the survey to study Dent's Disease type 1 in Europe. B) Key signs/symptoms leading to DD1 diagnosis. C) Graphical representation of the phenotypic characteristics during follow-up. Abbreviations: HTN: Hypertension, LMWP: Low Molecular Weight Proteinuria, FTT: Failure to thrive.

FIGURE 2

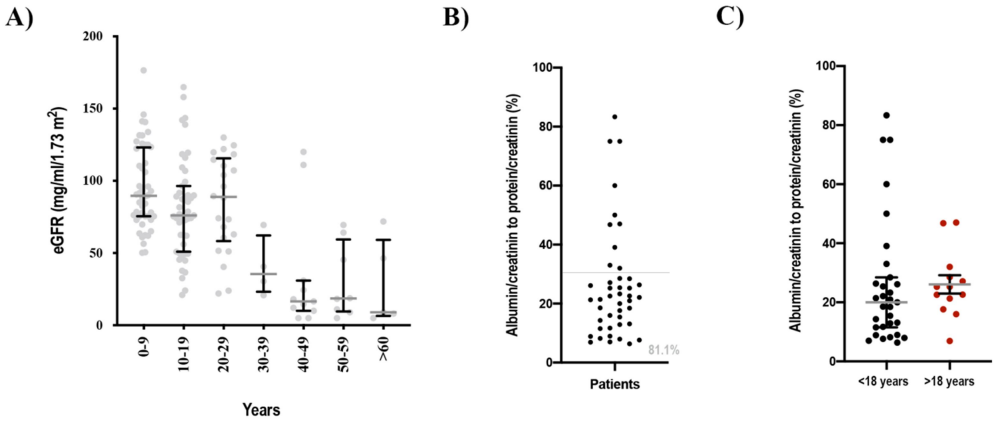

Figure 2. Phenotypic characteristics at last follow-up. A) Dot plot of eGFR (y axis) according to age (x axis) at last follow-up. Patients were divided into groups according to their age. Each point represents a single patient. B) Albumin/creatinine to protein/creatinine levels (%). Each point represents a single patient. C) Albumin/creatinine to protein/creatinine levels (%) in pediatric (>18 years, black dots) and adult (>18 year, red dots) patients. Each point represents a single patient. * $p<0.01$. Abbreviations: CKD: Chronic Kidney Disease, eGFR: estimated glomerular filtration rate.

FIGURE 3

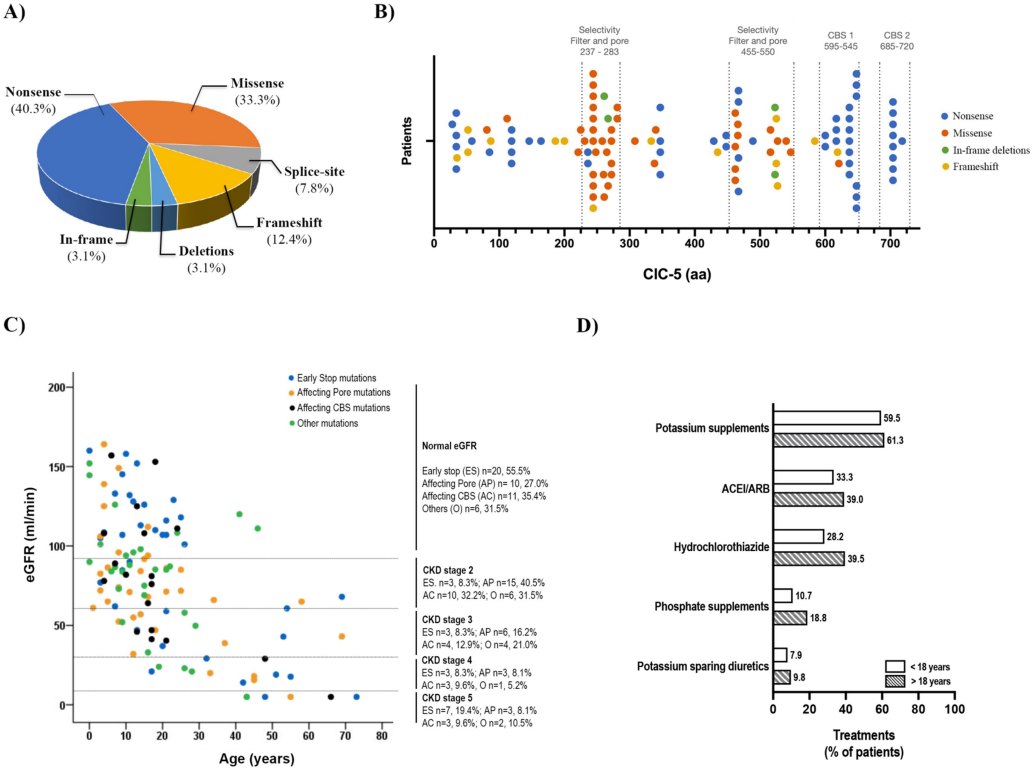

Figure 3. Variants on *CLCN5* gene and genotype-phenotype correlation. A) Classification of variants on *CLCN5*. B) Distribution of variants on ClC-5 aminoacidic sequence (Transcript 3 was used). C) eGFR according to age at last follow-up and variant distribution. Each point represents a single patient. 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 of our cohort. Abbreviations: CKD: Chronic Kidney Disease, HTN: hypertension, mut.: mutation, ES: Early Stop, AP: Affecting Pore, AC: Affecting CBS domains, O: Others.