

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

Running title: Dent's Disease 1 in Europe

CARLA BURBALLA¹, GERARD CANTERO-RECASENS¹, LARISA PRIKHODINA², FRANCESCA LUGANI³, KARLPETER SCHLINGMANN⁴, PETR V ANANIN⁵, MARTINE BESOUW⁶, DETLEF BOCKENHAUER⁷, LEIRE MADARIAGA⁸, AURELIA BERTHOLET-THOMAS⁹, FRANCESCA TARONI¹⁰, MATTIA PAROLIN¹¹, PETER CONLON¹², FRANCESCO EMMA¹³, DORELLA DELPRETE¹⁴, DOMINIQUE CHAUVEAU¹⁵, LINDA KOSTER-KAMPHUIS¹⁶, MARC FILA¹⁷, ANDREA PASINI¹⁸, ISABEL CASTRO¹⁹, GIACOMO COLUSSI²⁰, MARTA GIL²¹, BARIAN MOHIDIN²², TANJA WLODKOWSKI²³, FRANZ SCHAEFER²⁴ & GEMA ARICETA¹ & for the DENT study group[#]

Affiliations:

- 1- Vall d'Hebron University Hospital (HUVH) - Vall d'Hebron Research Institute (VHIR), Barcelona, Spain
- 2- Veltishev Research & Clinical Institute for Pediatrics, Pirogov Russian National Research Medical University, Moscow, Russia
- 3- IRCCS Istituto G. Gaslini, Genova, Italy
- 4- University Childrens Hospital, Münster, Germany
- 5- Scientific Centre of Children Health 2, Moscow, Russia
- 6- University Medical Center, Groningen, the Netherlands
- 7- UCL Division of Medicine and Great Ormond Street Hospital, London, United Kingdom
- 8- Hospital Universitario Cruces, Biocruces-Bizkaia, UPV/EHU, Barakaldo, Spain
- 9- Hopital Femme Mere Enfant, Lyon, France
- 10- Pediatric Nephrology Dialysis and Transplant Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
- 11- Nefrologia Pediatrica, Dialisi e Trapianto, Università di Padova, Italy
- 12- Beaumont Hospital, Dublin, Ireland
- 13- Division of Nephrology, Bambino Gesù Children's Hospital—IRCCS, Rome, Italy
- 14- Azienda Ospedale-Universita, Padova, Italy
- 15- Hopital Rangueil, Toulouse, France
- 16- Amalia Children's Hospital, Radboud University Medical Center, Nijmegen, The Netherlands.
- 17- Arnaud de Villeneuve, Montpellier, France
- 18- Azienda Ospedaliero-Universitaria Santorsola-Malpighi Bologna, Italy
- 19- Complejo Hospitalario de Pontevedra, Pontevedra, Spain
- 20- Niguarda Hospital, Milan, Italy
- 21- Hospital Universitario Santiago de Compostela, Santiago de Compostela, Spain
- 22- Royal Free Hospital, London, UK
- 23- Heidelberg University Hospital, Germany

& Correspondence to:

Dr. Gema Ariceta; Hospital Universitari Vall d'Hebron (HUVH), Pediatric nephrology service, 08035 Barcelona, Spain; T: +34-934893082; gariceta@vhebron.net.

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The DENT study group

The following persons and institutions participated in the DENT Study Group:

1. *Hopital Femme Mère Enfant (Lyon, France)*: JUSTINE BACCHETTA
2. *Pediatric Nephrology Dialysis and Transplant Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico (Milan, Italy)*: FABIO PAGLIALONGA
3. *Nefrologia Pediatrica, Dialisi e Trapianto, Università di Padova (Padova, Italy)*: LUISA MURER
4. *Children's Clinical University Hospital (Riga, Latvia)*: ILZE ANDERSONE
5. *Renal Services Centre, The Newcastle upon Tyne Hospitals NHS Foundation Trust (Newcastle, UK)*: JOHN A. SAYER
6. *Necker Hospital (Paris, France)*: OLIVIA BOYER
7. *University Medical Centre Ljubljana (Ljubljana, Slovenia)*: TANJA KERSNIK LEVART, RINA RUS
8. *University Children's Hospital (Belgrade, Serbia)*: DUŠAN PARIPOVIĆ
9. *Hospital Universitario Puerta de Hierro Majadahonda (Madrid, Spain)*: ESTHER RUBIO GONZALEZ
10. *Hospital Materno Infantil de Málaga (Málaga, Spain)*: FRANCISCO NIETO
11. *University Hospital Motol (Prague, Czech Republic)*: JAKUB ZIEG
12. *Hospital Virgen del Rocío (Sevilla, Spain)*: JOSÉ ÁNGEL CABALLERO
13. *Hospital 12 de octubre (Madrid, Spain)*: JULIA VARA
14. *UMCU Wilhelmina Children's Hospital (Utrecht, Netherlands)*: MANDY KEIJZER-VEEN
15. *Fondazione Policlinico Universitario A. Gemelli IRCCS (Roma, Italy)*: PIETRO MANUEL FERRARO
16. *Complejo Hospitalario Navarra (Pamplona, Spain)*: RAMON GONZALEZ
17. *Hospital Universitario de Álava (Vitoria, Spain)*: RAMON MARIA SARACHO ROTAECHE
18. *San Giovanni Bosco Hospital (Turin, Italy)*: ROBERTA FENOGLIO
19. *Hospital Clínico Universitario Valladolid (Valladolid, Spain)*: SANDRA SANZ BALLESTEROS
20. *Hospital Universitario Guadalajara (Guadalajara, Spain)*: SERAFIN TALLON LOBO
21. *Clinique CHC MontLégia (Liege, Belgium)*: MARIE SOPHIE GHUYSEN
22. *Hospital Universitario Central de Asturias (Oviedo, Spain)*: FLOR A. ORDÓÑEZ ÁLVAREZ
23. *University Hospitals Leuven (Leuven, Belgium)*: MARIA VANDYCK
24. *Department of Internal Medicine, University of Tartu and Tartu University Hospital (Tartu, Estonia)*: MAI ROSENBERG
25. *Oslo University Hospital (Oslo, Norway)*: HJØRDIS THORSTEINSDOTTIR
26. *University Children's Hospital (Skopje, North Macedonia)*: VELIBOR TASIC
27. *Dokuz Eylul University (Izmir, Turkey)*: MERAL TORUN BAYRAM
28. *Ege University (Izmir, Turkey)*: SEVGI MIR
29. *Fundeni Clinical Institute (Bucharest, Romania)*: GEORGE CLAUDIU COSTEA
30. *Marmara University Medical School (Istanbul, Turkey)*: NURDAN YILDIZ
31. *Hospital Universitari Son Espases (Palma de Mallorca, Spain)*: JAVIER LUMBRERAS
32. *Erciyes University (Kayseri, Turkey)*: SIBEL YEL
33. *Clinic of pediatrics, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University (Vilnius, Lithuania)*: RIMANTE CERKAUSKIENE
34. *Università degli Studi della Campania (Naples, Italy)*: ANGELA LA MANNA
35. *Beaumont Hospital (Dublin, Ireland)*: ELHUSSEIN ELHASSAN, FRANCESCA CIURLI
36. *Vall d'Hebron Research Institute (Barcelona, Spain)*: ANNA MESEGUER, MONICA DURAN

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:

- 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 origin associated with non-nephrotic proteinuria, or nephrocalcinosis/nephrolithiasis.

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

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 \pm 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 m² (92.2±30.8 ml/min/1.73 m²). 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 m²), 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 m² vs. 59.2 ± 44.1 mg/ml/1.73 m², $p < 0.0001$; 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 CIC-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 \geq 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 \geq 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 CIC-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 CIC 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 *Cln5* 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.

DISCLOSURES

The authors declare no conflict of interest.

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REFERENCES

1. Dent CE, Friedman M: Hypercalcuric Rickets Associated with Renal Tubular Damage. *Arch Dis Child* [Internet] 39: 240–249, 1964 Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2019188/> [cited 2020 Oct 27]
2. Thakker RV: Pathogenesis of Dent's disease and related syndromes of X-linked nephrolithiasis. *Kidney Int* 57: 787–793, 2000
3. Ludwig M, Utsch B, Monnens LAH: Recent advances in understanding the clinical and genetic heterogeneity of Dent's disease. *Nephrol Dial Transplant* 21: 2708–2717, 2006
4. Blanchard A, Curis E, Guyon-Roger T, Kahila D, Treard C, Baudouin V, Bérard E, Champion G, Cochat P, Dubourg J, de la Faille R, Devuyst O, Deschenes G, Fischbach M, Harambat J, Houillier P, Karras A, Knebelmann B, Lavocat M-P, Loirat C, Merieau E, Niaudet P, Nobili F, Novo R, Salomon R, Ulinski T, Jeunemaître X, Vargas-Poussou R: Observations of a large Dent disease cohort. *Kidney Int* 90: 430–439, 2016
5. Giancesello L, Del Prete D, Anglani F, Calò LA: Genetics and phenotypic heterogeneity of Dent disease: the dark side of the moon. *Hum Genet* 140: 401–421, 2021
6. Mansour-Hendili L, Blanchard A, Le Pottier N, Roncelin I, Lourdel S, Treard C, González W, Vergara-Jaque A, Morin G, Colin E, Holder-Espinasse M, Bacchetta J, Baudouin V, Benoit S, Bérard E, Bourdat-Michel G, Bouchireb K, Burtsey S, Cailliez M, Cardon G, Cartery C, Champion G, Chauveau D, Cochat P, Dahan K, De la Faille R, Debray F-G, Dehoux L, Deschenes G, Desport E, Devuyst O, Dieguez S, Emma F, Fischbach M, Fouque D, Fourcade J, François H, Gilbert-Dussardier B, Hannedouche T, Houillier P, Izzedine H, Janner M, Karras A, Knebelmann B, Lavocat M-P, Lemoine S, Leroy V, Loirat C, Macher M-A, Martin-Coignard D, Morin D, Niaudet P, Nivet H, Nobili F, Novo R, Faivre L, Rigothier C, Roussey-Kesler G, Salomon R, Schleich A, Sellier-Leclerc A-L, Soulami K, Tiple A, Ulinski T, Vanhille P, Van Regemorter N, Jeunemaître X, Vargas-Poussou R: Mutation Update of the CLCN5 Gene Responsible for Dent Disease 1. *Hum Mutat* 36: 743–752, 2015
7. Reinhart SC, Norden AG, Lapsley M, Thakker RV, Pang J, Moses AM, Frymoyer PA, Favus MJ, Hoepner JA, Scheinman SJ: Characterization of carrier females and affected males with X-linked recessive nephrolithiasis. *J Am Soc Nephrol* 5: 1451–1461, 1995
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* 87: 473–493, 1994
9. Smith AJ, Reed AAC, Loh NY, Thakker RV, Lippiat JD: 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* 296: F390-397, 2009

10. Lourdel S, Grand T, Burgos J, González W, Sepúlveda FV, Teulon J: CLC-5 mutations associated with Dent's disease: a major role of the dimer interface. *Pflugers Arch* 463: 247–256, 2012
11. Devuyst O, Christie PT, Courtoy PJ, Beauwens R, Thakker RV: Intra-renal and subcellular distribution of the human chloride channel, CLC-5, reveals a pathophysiological basis for Dent's disease. *Hum Mol Genet* 8: 247–257, 1999
12. Carr G, Simmons N, Sayer J: A role for CBS domain 2 in trafficking of chloride channel CLC-5. *Biochemical and Biophysical Research Communications* [Internet] 310: 600–605, 2003 Available from: <https://www.sciencedirect.com/science/article/pii/S0006291X03018217> [cited 2022 May 9]
13. Devuyst O, Luciani A: Chloride transporters and receptor-mediated endocytosis in the renal proximal tubule. *J Physiol* 593: 4151–4164, 2015
14. Sekine T, Komoda F, Miura K, Takita J, Shimadzu M, Matsuyama T, Ashida A, Igarashi T: 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* 29: 376–384, 2014
15. Bhardwaj S, Thergaonkar R, Sinha A, Hari P, Hi C, Bagga A: Phenotype of Dent Disease in a Cohort of Indian Children. *Indian Pediatr* 53: 977–982, 2016
16. Mejía N, Santos F, Claverie-Martín F, García-Nieto V, Ariceta G, Castaño L, RenalTube group: RenalTube: a network tool for clinical and genetic diagnosis of primary tubulopathies. *Eur J Pediatr* 172: 775–780, 2013
17. WHO 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 [Internet]. Available from: <https://www.who.int/publications-detail-redirect/924154693X> [cited 2022 May 9]
18. Matos V, van Melle G, Boulat O, Markert M, Bachmann C, Guignard JP: Urinary phosphate/creatinine, calcium/creatinine, and magnesium/creatinine ratios in a healthy pediatric population. *J Pediatr* 131: 252–257, 1997
19. Habbig S, Beck BB, Hoppe B: Nephrocalcinosis and urolithiasis in children. *Kidney Int* 80: 1278–1291, 2011
20. Pottel H, Hoste L, Martens F: A simple height-independent equation for estimating glomerular filtration rate in children. *Pediatr Nephrol* 27: 973–979, 2012
21. Levey AS, Stevens LA, Schmid CH, Zhang Y (Lucy), Castro AF, Feldman HI, Kusek JW, Eggers P, Van Lente F, Greene T, Coresh J: A New Equation to Estimate Glomerular Filtration Rate. *Ann Intern Med* [Internet] 150: 604–612, 2009 Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2763564/> [cited 2022 May 9]
22. Ramos-Trujillo E, Claverie-Martin F, Garcia-Nieto V, Ariceta G, Vara J, Gonzalez-Acosta H, Garcia-Ramirez M, Fons J, Cordoba-Lanus E, Gonzalez-Paredes J, Valenciano B, Ramos L, Muley R, Caggiani M, Alvarez-Estrada P, Madrid A, RenalTube Group: Dent's disease: Identification of seven new pathogenic mutations in the CLCN5 gene. *J Pediatr Genet* 2: 133–140, 2013
23. Giancesello L, Ceol M, Bertoldi L, Terrin L, Priante G, Murer L, Peruzzi L, Giordano M, Paglialonga F, Cantaluppi V, Musetti C, Valle G, Del Prete D, Anglani F: Genetic Analyses in Dent Disease and Characterization of CLCN5 Mutations in Kidney Biopsies. *Int J Mol Sci* [Internet] 21: 516, 2020 Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7014080/> [cited 2022 May 9]

24. Worobey CC, Singh AK: Proteinuria [Internet]. In: *Decision Making in Medicine (Third Edition)*, edited by Mushlin SB, Greene HL, pp 358–361, 2010 Available from: <https://www.sciencedirect.com/science/article/pii/B9780323041072501265> [cited 2022 May 24]
25. Hogg RJ, Portman RJ, Milliner D, Lemley KV, Eddy A, Ingelfinger J: 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* 105: 1242–1249, 2000
26. Tosetto E, Ghiggeri GM, Emma F, Barbano G, Carrea A, Vezzoli G, Torregrossa R, Cara M, Ripanti G, Ammenti A, Peruzzi L, Murer L, Ratsch IM, Citron L, Gambaro G, D'angelo A, Anglani F: Phenotypic and genetic heterogeneity in Dent's disease--the results of an Italian collaborative study. *Nephrol Dial Transplant* 21: 2452–2463, 2006
27. Ye Q, Shen Q, Rao J, Zhang A, Zheng B, Liu X, Shen Y, Chen Z, Wu Y, Hou L, Jian S, Wei M, Ma M, Sun S, Li Q, Dang X, Wang Y, Xu H, Mao J, for Chinese Children Genetic Kidney Disease Database (CCGKDD) "Internet Plus" Nephrology Alliance of National Center for Children's Care: Multicenter study of the clinical features and mutation gene spectrum of Chinese children with Dent disease. *Clinical Genetics* [Internet] 97: 407–417, 2020 Available from: <https://onlinelibrary.wiley.com/doi/abs/10.1111/cge.13663> [cited 2022 May 9]
28. Beara-Lasic L, Cogal A, Mara K, Enders F, Mehta RA, Haskic Z, Furth SL, Trachtman H, Scheinman SJ, Milliner DS, Goldfarb DS, Harris PC, Lieske JC, investigators of the Rare Kidney Stone Consortium: Prevalence of low molecular weight proteinuria and Dent disease 1 CLCN5 mutations in proteinuric cohorts. *Pediatr Nephrol* 35: 633–640, 2020
29. Devuyst O, Thakker RV: Dent's disease. *Orphanet Journal of Rare Diseases* [Internet] 5: 28, 2010 Available from: <https://doi.org/10.1186/1750-1172-5-28> [cited 2022 May 9]
30. van Berkel Y, Ludwig M, van Wijk JAE, Bökenkamp A: Proteinuria in Dent disease: a review of the literature. *Pediatr Nephrol* 32: 1851–1859, 2017
31. Wang X, Anglani F, Beara-Lasic L, Mehta AJ, Vaughan LE, Herrera Hernandez L, Cogal A, Scheinman SJ, Ariceta G, Isom R, Copelovitch L, Enders FT, Del Prete D, Vezzoli G, Paglialonga F, Harris PC, Lieske JC: Glomerular Pathology in Dent Disease and Its Association with Kidney Function. *Clin J Am Soc Nephrol* [Internet] 11: 2168–2176, 2016 Available from: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5142066/> [cited 2022 May 9]
32. Hodgin JB, Corey HE, Kaplan BS, D'Agati VD: Dent disease presenting as partial Fanconi syndrome and hypercalciuria. *Kidney Int* 73: 1320–1323, 2008
33. De Matteis MA, Staiano L, Emma F, Devuyst O: The 5-phosphatase OCRL in Lowe syndrome and Dent disease 2. *Nat Rev Nephrol* 13: 455–470, 2017
34. Pereira-Moreira R, Muscelli E: Effect of Insulin on Proximal Tubules Handling of Glucose: A Systematic Review. *Journal of Diabetes Research* [Internet] 2020: e8492467, 2020 Available from: <https://www.hindawi.com/journals/jdr/2020/8492467/> [cited 2022 May 9]
35. Hoopes RR, Raja KM, Koich A, Hueber P, Reid R, Knohl SJ, Scheinman SJ: Evidence for genetic heterogeneity in Dent's disease. *Kidney Int* 65: 1615–1620, 2004
36. Nielsen R, Christensen EI, Birn H: Megalin and cubilin in proximal tubule protein reabsorption: from experimental models to human disease. *Kidney Int* 89: 58–67, 2016

37. Anglani F, D'Angelo A, Bertizzolo LM, Toso E, Ceol M, Cremasco D, Bonfante L, Addis MA, Del Prete D, Dent Disease Italian Network: Nephrolithiasis, kidney failure and bone disorders in Dent disease patients with and without CLCN5 mutations. *Springerplus* 4: 492, 2015
38. Raja KA, Schurman S, D'Mello RG, Blowey D, Goodyer P, Why SV, Plutz-Snyder RJ, Asplin J, Scheinman SJ: Responsiveness of Hypercalciuria to Thiazide in Dent's Disease. *JASN* [Internet] 13: 2938–2944, 2002 Available from: <https://jasn.asnjournals.org/content/13/12/2938> [cited 2022 May 9]
39. Cebotaru V, Kaul S, Devuyst O, Cai H, Racusen L, Guggino WB, Guggino SE: High citrate diet delays progression of renal insufficiency in the CIC-5 knockout mouse model of Dent's disease. *Kidney Int* 68: 642–652, 2005
40. Zaniew M, Mizerska-Wasiak M, Załuska-Leśniewska I, Adamczyk P, Kiliś-Pstrusińska K, Haliński A, Zawadzki J, Lipska-Ziętkiewicz BS, Pawlaczyk K, Sikora P, Ludwig M, Szczepańska M: Dent disease in Poland: what we have learned so far? *Int Urol Nephrol* [Internet] 49: 2005–2017, 2017 Available from: <https://doi.org/10.1007/s11255-017-1676-x> [cited 2022 May 9]

TABLES

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 \pm SD)	1.2 \pm 0.8	1.01 \pm 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 LEGENDS

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. 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. Variants on *CLCN5* gene and genotype-phenotype correlation. A) Classification of variants on *CLCN5*. B) Distribution of variants on CIC-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.

SUPPLEMENTAL MATERIAL

Supplementary Figure 1. European map with the geographic distribution of the centers participating in this study.

Supplementary material 1. On-line survey.

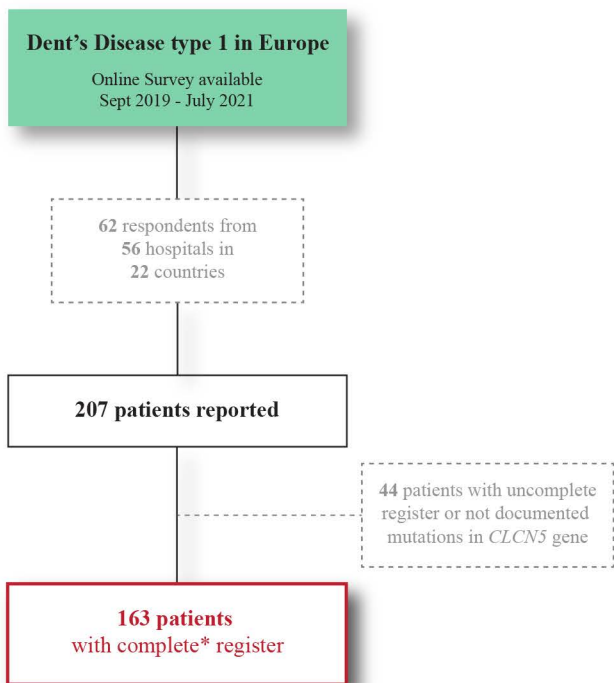
Supplementary table 1. *CLCN5* variants.

Supplementary table 2. Kidney function at last follow-up in DD1 patients carrying truncating or non-truncating *CLCN5* variants.

Supplementary table 3. Kidney function at last follow-up according to variants' distribution on CIC-5 sequence.

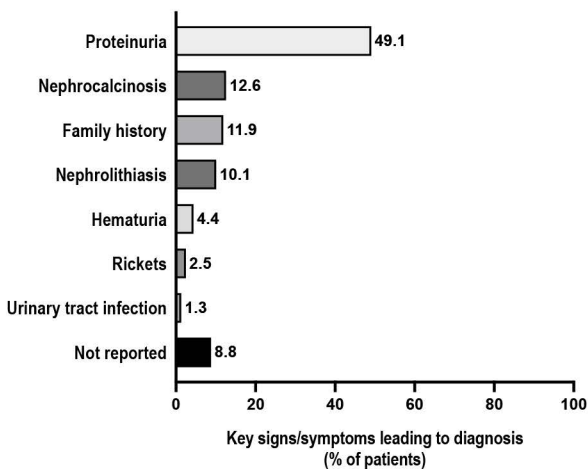
FIGURE 1

A)



* More than 50% of answered questions in the survey

B)



C)

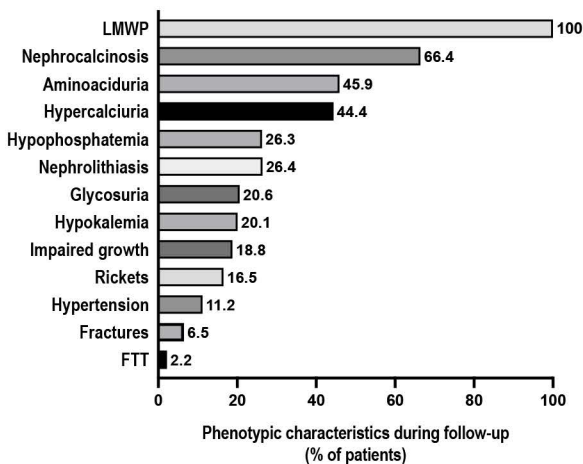
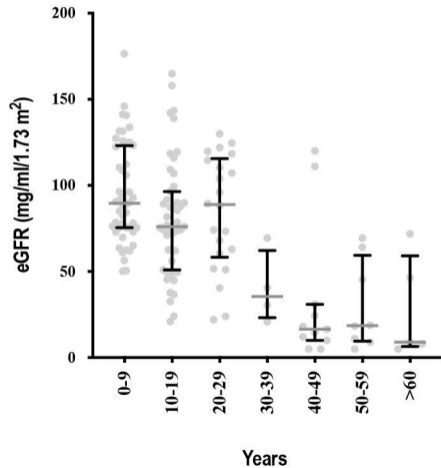
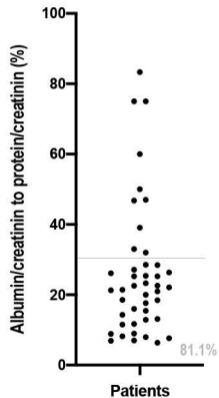


FIGURE 2

A)



B)



C)

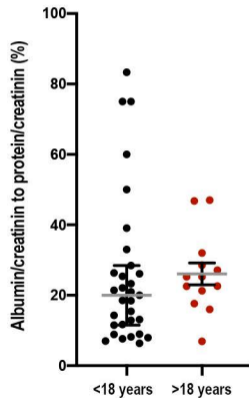
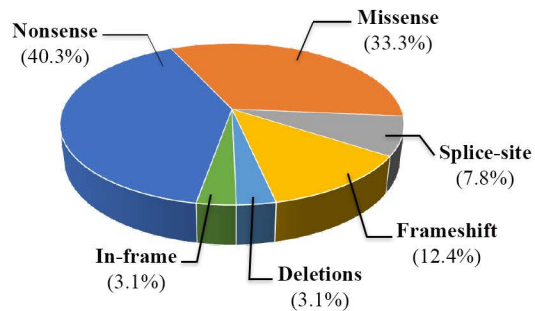
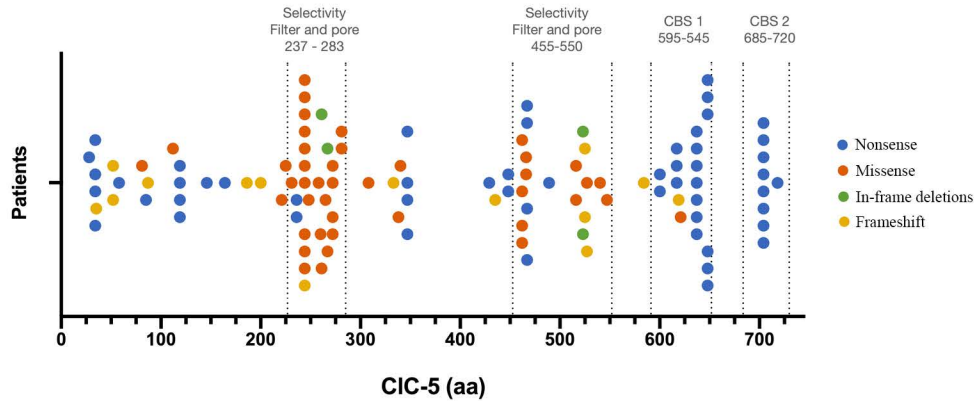
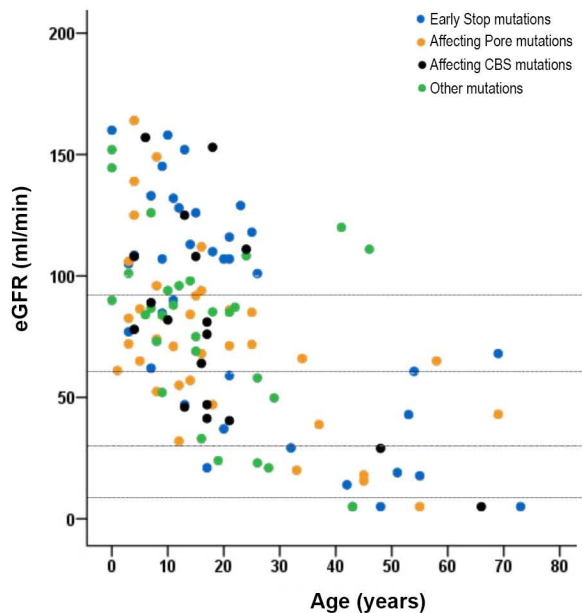


FIGURE 3**A)****B)****C)****Normal eGFR**

Early stop (ES) n=20, 55.5%
 Affecting Pore (AP) n= 10, 27.0%
 Affecting CBS (AC) n=11, 35.4%
 Others (O) n=6, 31.5%

CKD stage 2

ES: n=3, 8.3%; AP n=15, 40.5%
 AC n=10, 32.2%; O n=6, 31.5%

CKD stage 3

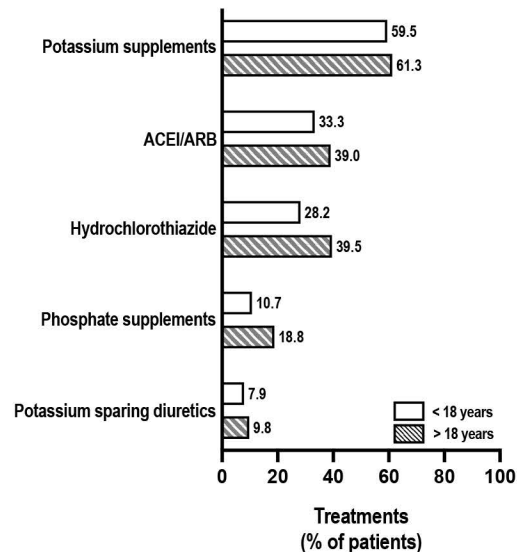
ES n=3, 8.3%; AP n=6, 16.2%
 AC n=4, 12.9%; O n=4, 21.0%

CKD stage 4

ES n=3, 8.3%; AP n=3, 8.1%
 AC n=3, 9.6%; O n=1, 5.2%

CKD stage 5

ES n=7, 19.4%; AP n=3, 8.1%
 AC n=3, 9.6%; O n=2, 10.5%

D)

SUPPLEMENTAL MATERIAL

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

Running title: Dent's Disease 1 in Europe

CARLA BURBALLA¹, GERARD CANTERO-RECASENS¹, LARISA PRIKHODINA², FRANCESCA LUGANI³, KARLPETER SCHLINGMANN⁴, PETR V ANANIN⁵, MARTINE BESOUW⁶, DETLEF BOCKENHAUER⁷, LEIRE MADARIAGA⁸, AURELIA BERTHOLET-THOMAS⁹, FRANCESCA TARONI¹⁰, MATTIA PAROLIN¹¹, PETER CONLON¹², DORELLA DELPRETE¹³, DOMINIQUE CHAUVEAU¹⁴, LINDA KOSTER-KAMPHUIS¹⁵, MARC FILA¹⁶, ANDREA PASINI¹⁷, ISABEL CASTRO¹⁸, GIACOMO COLUSSI¹⁹, MARTA GIL²⁰, BARIAN MOHIDIN²¹, TANJA WLODKOWSKI²², FRANZ SCHAEFER²² & GEMA ARICETA^{1.&} for the DENT study group[#]

Affiliations:

- 1- Vall d'Hebron University Hospital (HUVH) - Vall d'Hebron Research Institute (VHIR), Barcelona, Spain
- 2- Veltishev Research & Clinical Institute for Pediatrics, Pirogov Russian National Research Medical University, Moscow, Russia
- 3- IRCCS Istituto G. Gaslini, Genova, Italy
- 4- University Childrens Hospital, Münster, Germany
- 5- Scientific Centre of Children Health 2, Moscow, Russia
- 6- University Medical Center, Groningen, the Netherlands
- 7- UCL Division of Medicine and Great Ormond Street Hospital, London, United Kingdom
- 8- Hospital Universitario Cruces, Biocruces-Bizkaia, UPV/EHU, Barakaldo, Spain
- 9- Hopital Femme Mere Enfant, Lyon, France
- 10- Pediatric Nephrology Dialysis and Transplant Unit, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Milan, Italy
- 11- Nefrologia Pediatrica, Dialisi e Trapianto, Università di Padova, Italy
- 12- Beaumont Hospital, Dublin, Ireland
- 13- Azienda Ospedale-Universita, Padova, Italy
- 14- Hopital Rangueil, Toulouse, France
- 15- Amalia Children's Hospital, Radboud University Medical Center, Nijmegen, The Netherlands.
- 16- Arnaud de Villeneuve, Montpellier, France
- 17- Azienda Ospedaliero-Universitaria Santorsola-Malpighi Bologna, Italy
- 18- Complejo Hospitalario de Pontevedra, Pontevedra, Spain
- 19- Niguarda Hospital, Milan, Italy
- 20- Hospital Universitario Santiago de Compostela, Santiago de Compostela, Spain
- 21- Royal Free Hospital, London, UK
- 22- Heidelberg University Hospital, Germany

& Correspondence to:

Dr. Gema Ariceta; Hospital Universitari Vall d'Hebron (HUVH), Pediatric nephrology service, 08035 Barcelona, Spain; T: +34-934893082; gariceta@vhebron.net.

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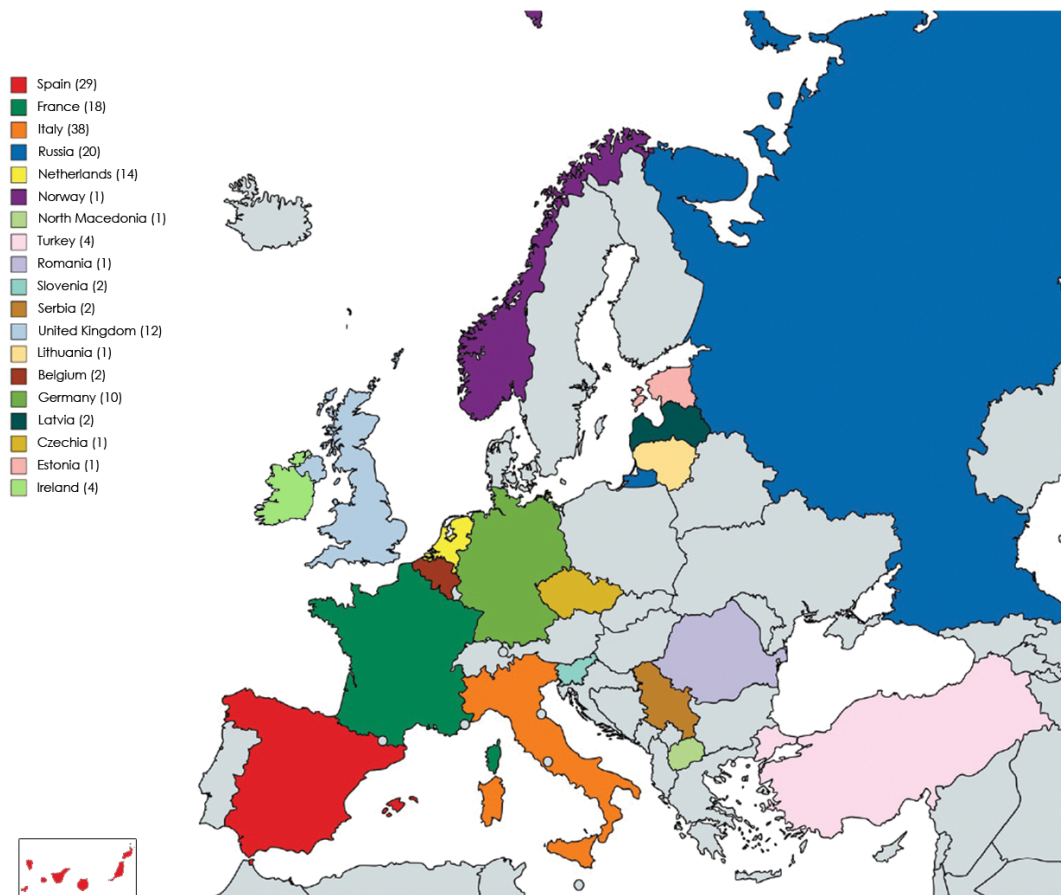
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SUPPLEMENTARY FIGURE 1

SUPPLEMENTARY FIGURE 1

A)



Supplementary Figure 1. European map with the geographic distribution of the centers participating in this study. Number of patients from each country are included between brackets.

SUPPLEMENTARY MATERIAL 1: ON-LINE SURVEY

Dent's Disease Type 1 in Europe

Welcome to this Survey on Dent's Disease type 1 in Europe. The survey will take approximately 12 minutes.

We are launching this survey aimed at gathering information about Dent's Disease type 1 in Europe. By these few questions we mean to investigate the prevalence, the clinical phenotype and evolution as well as genetics variability and practice patterns of centers involved in the management and study of Dent patients around Europe.

In case you are interested, please complete the survey by selecting "Agree".

Results of the survey will be published and those participating with complete data will be considered as contributors.

Thank you for your consideration and best regards

Agree

Please do not forward this email as its survey link is unique to you.
[Privacy](#) | [Unsubscribe](#)

Powered by  SurveyMonkey

Dent's Disease Type 1 in Europe

Treating physician

What is your **country of practice**?

Please enter your **center** (name, city)

Please enter your **email address**

Do you follow **Dent type 1 patients**?

- Yes
- No

If yes, **how many**?

Is it the first time you fill in this survey?

If you have already completed the survey for other patients, please tell us how many times.

Patient's basic information

Patient's **gender**

- Male
- Female

Please write down the month and year the patient **was born**

Was the patient a **premature baby**?

- Yes
- No

How much did the patient **weight** (g) at birth? (if available)

How **long** was the patient (cm) at birth? (if available)

3. Diagnosis

Is there any **family history** of Dent's Disease or kidney disease?

Please list the affected members of the family below and the medical signs they presented with.

When was the patient **clinically diagnosed**?

Date

Did genetic test find a **mutated gene**?

(If mutated gene is other than CLCN5, please go to the end page and finish the survey)

- CLCN5
- OCRL
- Unknown
- Genetic test could not be performed
- Other (please specify)

Which are the details of the **mutation found**? (exon, nucleotide change. for example V523del, Y272C...)

Which was the main **symptom/sign** which determined the **diagnosis**?

Patient's current situation

Which is the **patient's current age**?

Which is the patient **current weight** (kg)?

Which is the patient's **current height** (cm)?

Does the patient have **aminoaciduria** currently?

- Yes
- No
- Unknown

Does the patient have **glycosuria** currently?

- Yes
- No
- Unknown

Does the patient have **nephrocalcinosis**?

- Yes
- No
- Unknown

Has the patient had any **renal lithiasis/renal colic episode**?

- Yes
- No
- Unknown

At what **age** (if known) was the **first renal lithiasis/renal colic episode**?

Has the patient developed **rickets**?

- Yes
- No
- Unknown

Has the patient suffered any **fracture**?

- Yes
- No
- Unknown
- If yes, location and type of fracture

At what **age** did the patient suffer the **first fracture**?

Does the patient have **hypertension**?

- Yes
- No
- Unknown

At what **age** was the patient diagnosed with **hypertension**?

Has the patient needed **renal replacement therapy**?

(Current situation)

- No
- Dialysis
- Kidney transplant

At what **age** did the patient **start renal replacement therapy**?

Patient's current blood/urine tests

If you don't have these data available, please leave the field blank

Date

Current serum creatinine (mg/dl)
($\mu\text{mol/L} = \text{mg/dl} * 88.4$)

Current serum potassium (mmol/L)

Current serum calcium (mg/dl)

Current serum phosphate (mg/dl)

Current urine Ca/Cr (mg/mg)

Current urine protein/creatinine ratio (mg/g)

Current urine albumin/creatinine ratio (mg/g)

Which low molecular weight protein do you

- A1-microalbuminuria
- B2-microalbuminuria
- Retinol-binding protein

Current low molecular weight Protein (mg/mol creat)

Management

Does the patient currently receive any **treatment**?

- Potassium-sparing diuretics
- Angiotensin-converting enzyme or angiotensin receptor blockers
- Thiazides
- Potassium citrate
- Growth hormone
- None

- Does the patient get any other treatment?

End of the Survey

Thank you very much to fill up the survey!!

If you have any doubt, please do not hesitate to contact us to carla.burballa@vhir.org.

If you have more than one followed-up patient it would be great if you could please fill-up the survey for each of them. If you do not, you can leave the page.

Thank you very much!

SUPPLEMENTARY TABLE 1

Supplementary table 1. *CLCN5* variants.

Patients	Type of mutation		Nucleotide	Protein	Exon/Intron	Reference
DD1_1	Large deletion	Others	c.429-? ?_c.294+?del	NA	NA	Not reported
DD1_111	Missense	Others	c.-38 C>A	3'- UTR altered	1	Not reported
DD1_2	Large deletion	Early stop	c.(?- 30) (*220_?)de	Whole gene deletion	1-12	(Akuta et al., 1997; Mansour-Hendili et al., 2015)
DD1_3	Frameshift	Early stop	c.100_101insG	p.Glu35Glyfs*4	2	(Gianesello et al., 2020)
DD1_4, DD1_5, DD1_76, DD1_82	Nonsense	Early stop	c.100C>T	p.Arg34*	2	(Ramos-Trujillo et al., 2013; Sekine et al., 2014)
DD1_6	Missense	Others	c.1012 C>T	p.Arg338Cys	8	Not reported
DD1_7	Missense	Others	c.1019 A>G	p.Asn340Ser	8	Not reported
DD1_10, DD1_11, DD1_12, DD1_13	Nonsense	Early stop	c.1039C>T	p.Arg347*	8	(Akuta et al., 1997; Hoopes et al., 2004a; Mansour-Hendili et al., 2015; Tosetto et al., 2006; Wu et al., 2009)
DD1_16	Nonsense	Early stop	c.1287 G>A	p.Trp429*	8	(Gianesello et al., 2020)
DD1_31	Frameshift	Early stop	c.1304- 1305delTC	p.Leu435Hisfs*25	8	Not reported
DD1_17, DD1_18	Frameshift	Early stop	c.1341delC	p.Met448*	8	Not reported
DD1_19	Splice-site	Affecting CBS	c.1348-1G>T	skipping 26 bases exon 9, truncated CBS domains	Intron 9	(Gianesello et al., 2020; Matsumoto et al., 2018)
DD1_20, DD1_21, DD1_22, DD1_23	Missense	Affecting pore	c.1384G >A	p.Gly462Ser	9	(Grand et al., 2011; Mansour-Hendili et al., 2015; Ramos-Trujillo et al., 2013)
DD1_24, DD1_25	Missense	Affecting pore	c.1397G >A	p.Gly466Asp	9	(Mansour-Hendili et al., 2015; Ramos-Trujillo et al., 2013)
DD1_26, DD1_27, DD1_28, DD1_29	Nonsense	Early stop	c.1399C>T	p.Arg467*	9	(Tosetto et al., 2006)
DD1_30	Nonsense	Early stop	c.1467G>A	p.Trp489*	9	(Mansour-Hendili et al., 2015; Sekine et al., 2014)
DD1_32, DD1_33	Missense	Affecting pore	c.1546C>T	p.Arg516Trp	10	(Mansour-Hendili et al., 2015; Smith, Reed, Loh, Thakker, & Lippiat, 2008)
DD1_79, DD1_80	Frameshift	Early stop	c.155delA	p.Ser52Valfs*8	4	Not reported
DD1_34, DD1_35	Inframe	Affecting pore	c.1566_c.1568 delTGT	p.Val523del	10	(Ramos-Trujillo et al., 2013; Wu et al., 2009)
DD1_36	Frameshift	Early stop	c.1571dupT	p.Met525Asnfs*3	10	(Mansour-Hendili et al., 2015)
DD1_37	Frameshift	Early stop	c.1574_1575de ITT	p.Met525Serfs*4	10	(Mansour-Hendili et al., 2015)
DD1_38	Frameshift	Early stop	c.1576- 1577dup	p.Glu527Leufs*1	10	Not reported
DD1_42	Missense	Affecting pore	c.1581A>T	p.Glu527Asp	10	Lloyd et al 1997; Smith et al, 2009
DD1_39	Missense	Affecting pore	c.1619C>T	p.Ala540Val	10	(Mansour-Hendili et al., 2015; Wong et al., 2017)
DD1_40	Missense	Affecting pore	c.1639T>G	p.Trp547Gly	10	(Mansour-Hendili et al., 2015; Ramos-Trujillo et al., 2013)
DD1_81	Nonsense	Early stop	c.174 G>A	p.Trp58*	3	Not reported
DD1_41	Frameshift	Affecting CBS	c.1751delA	p.Asp584Valfs*2	10	(Mansour-Hendili et al., 2015)
DD1_62, DD1_63	Nonsense	Affecting CBS	c.1798C>T	p.Gln600*	10	(Mansour-Hendili et al., 2015; Tosetto et al., 2006)

DD1_53	Frameshift	Affecting CBS	c.1914delA?	p.Asp619Ile*fs38	10	Not reported
DD1_54, DD1_55, DD1_56, DD1_57, DD1_58, DD1_59	Nonsense	Affecting CBS	c.1942C>T	p.Arg648*	11	(Blanchard et al., 2016; Mansour-Hendili et al., 2015; Tosetto et al., 2006)
DD1_60	Splice-site	Others	c.206-2 A>G	p.?	Intron 3	(Bitsori, Vergadi, & Galanakis, 2019)
DD1_61	Splice-site	Others	c.206-8 C>G	p.?	Intron 3	Not reported
DD1_64, DD1_65, DD1_66, DD1_67, DD1_68, DD1_69, DD1_72, DD1_73	Nonsense	Affecting CBS	c.2110C>T	p.Arg704*	11	(Lloyd et al., 1997; Mansour-Hendili et al., 2015; Sekine et al., 2014)
DD1_71	Nonsense	Affecting CBS	c.2152C>T	p.Arg718*	12	(Grand et al., 2009; Hoopes et al., 2004b; Mansour-Hendili et al., 2015)
DD1_74	Missense	Others	c.243G>T	p.Trp81Cys	4	Not reported
DD1_75	Nonsense	Early stop	c.254 T>G	p.Leu85*	4	Not reported
DD1_86	Frameshift	Early stop	c.259del?	p.Glu87Lysfs*51	4	Cox et al., 1999, Dinour et al., 2009
DD1_77, DD1_78, DD1_91, DD1_92	Nonsense	Early stop	c.357 G>A	p.Trp119*	4	Not reported
DD1_84	Unknown	Others	c.426del	p.?	NA	NA
DD1_85	Nonsense	Early stop	c.438del	p.Trp146*	5	(Mansour-Hendili et al., 2015)
DD1_87	Nonsense	Early stop	c.492T>G	p.Tyr164*	5	(Mansour-Hendili et al., 2015; Ramos-Trujillo et al., 2013)
DD1_89	Missense	Others	c.544 G>A	p.Glu112Lys	4	Not reported
DD1_90	Frameshift	Early stop	c.558insT	p.Lys186Phefs*2	6	Not reported
DD1_93	Frameshift	Early stop	c.597_598dup	p.Leu200Argfs*8	6	(Mansour-Hendili et al., 2015)
DD1_94	Missense	Others	c.661 T>C	p.Cys221Arg	6	(Hoopes et al., 2004; Grand et al., 2009)
DD1_95	Missense	Others	c.674T>C	p.Leu225Pro	6	(Grand et al., 2011; Mansour-Hendili et al., 2015; Ramos-Trujillo et al., 2013)
DD1_117	Missense	Affecting pore	c.693A>T	p.Lys231Asn	8	Not reported
DD1_96, DD1_97	Nonsense	Early stop	c.706G>T	p.Glu236*	6	Not reported
DD1_98, DD1_99, DD1_100, DD1_101, DD1_102, DD1_103, DD1_104, DD1_118, DD1_119, DD1_120	Missense	Affecting pore	c.731C>T	p.Ser244Leu	7	Lloyd 1996 or others
DD1_88	Frameshift	Early stop	c.731delC	p.Ser244Trpfs*9	7	Not reported
DD1_105	Missense	Affecting pore	c.743 C>A	p.Ala248Glu	7	Not reported
DD1_121	Missense	Affecting pore	c.773 C>G	p.Pro258Arg	7	Not reported
DD1_14	Missense	Affecting pore	c.779G>T	p.Gly260Val	7	(Mansour-Hendili et al., 2015; Tosetto et al., 2006)
DD1_106	Inframe	Affecting pore	c.781G>A	p.Gly261Arg	7	(Mansour-Hendili et al., 2015; Tosetto et al., 2006)
DD1_107	Inframe	Affecting pore	c.782_784	p.Gly261del	7	Tosetto et al., 2009
DD1_108	Missense	Affecting pore	c.794 G>A	p.Ser265Asn	?	Not reported
DD1_109	Inframe	Affecting pore	c.801_803del	p.Glu267del	7	(Sekine et al., 2014)
DD1_110	Missense	Affecting pore	c.801A>C	p.Glu267Asp	7	(Mansour-Hendili et al., 2015)
DD1_112, DD1_113, DD1_8, DD1_9	Missense	Affecting pore	c.815A>G	p.Tyr272Cys	8	(Mansour-Hendili et al., 2015; Tosetto et al., 2006)
DD1_114	Nonsense	Early stop	c.82 C>T	p.Arg28*	2	Hoopes et al., 1998
DD1_115, DD1_116	Missense	Affecting pore	c.842C>T	p.Ser281Leu	8	Prikhodina et al., 2018
DD1_15	Missense	Others	c.922 G>A	p.Val308Met	8	Gianesello, 2020

DD1_122	Frameshift	Early stop	c.998del	p.Gly333Glufs*25	8	Not reported
DD1_124	Large deletion	Affecting pore	Deletion exon 8-9	p.?	8-9	Not reported
DD1_123	Large deletion	Affecting CBS	Deletion exon 9-12	p.?	9-12	Not reported
DD1_125	Splice-site	Others	Intron 8 mutation?	p.?	Intron 8	Not reported
DD1_130	Splice-site	Others	IVS3 -1	p.?	Intron 3	(Tosetto et al., 2006)
DD1_83	Splice-site	Others	IVS3+3 A>G	p.?	Intron 3	Not reported
DD1_126, DD1_127	Splice-site	Others	IVS5+5 G>T	p.?	Intron 5	(Tosetto et al., 2006)
DD1_128	Splice-site	Others	IVS7+1 G>T	p.?	Intron 7	Not reported
DD1_129	Splice-site	Others	IVS8+1G>T	p.?	Intron 8	(Tosetto et al., 2009)

*Numbering is according to the cDNA sequence (GenBank entry NM 00084.5, Ensembl ENST00000307367.2). The A of the ATG of the Methionine initiation codon is defines as nucleotide 1

SUPPLEMENTARY TABLE 2

Supplementary table 2. Kidney function at last follow-up in DD1 patients carrying truncating or non-truncating *CLCN5* variants.

	Truncating (n=47)	Non-truncating (n=76)	p
Age at diagnosis, years (median, [IQR])	7 [2-12.5]	6.5 [3-10.5]	0.95
History of nephrolithiasis (n, %)	5 of 47 (10.6%)	18 of 60 (30.0%)	*0.018
History of nephrocalcinosis (n, %)	28 of 40 (70.0%)	35 of 52 (67.3%)	0.82
History of rickets (n, %)	8 of 48 (16.6%)	9 of 59 (15.2%)	0.99
History of fractures (n, %)	5 of 49 (10.0%)	2 of 57 (3.5%)	0.25
HTN (n, %)	5 of 46 (10.8%)	8 of 58 (13.7%)	0.76
Age at last follow-up, years (median, [IQR])	19 [7.5-25.5]	15 [10-21.7]	0.671
Serum creatinine, mg/dL (mean ± SD)	0.97 ± 0.6	1.2 ± 0.83	0.4
Hypophosphatemia (n, %)	10 of 39 (25.6%)	22 of 48 (45.8%)	0.07
Hypokalemia or use of K ⁺ supplements (n, %)	13 of 45 (28.8%)	11 of 55 (20.0%)	0.35
Protein/creatinine, mg/g (median, [IQR])	1500 [500-2565]	1140 [500-1923]	0.5
Hypercalciuria (n, %)	14 of 27 (51.8%)	13 of 34 (38.2%)	0.31
Aminoaciduria (n, %)	11 of 21 (52.3%)	9 of 17 (52.9%)	0.99
Glycosuria (n, %)	8 of 38 (21.0%)	8 of 48 (16.6%)	0.78
CKD2 (n, %)	13 of 46 (28.6%)	21 of 59 (35.3%)	0.53
Age at CKD2 (median, [IQR])	10 [6.5-20.5]	14 [6-24]	0.7
CKD3 (n, %)	7 of 46 (15.2%)	10 of 59 (16.9%)	0.99
Age at CKD3 (years; median, [IQR])	28 [22-29]	21 [13-28.7]	0.9
CKD4 (n, %)	3 of 46 (6.5%)	5 of 59 (8.4%)	0.99
Age at CKD4 (years; median, [IQR])	20 [19-23.5]	34 [30-50]	0.1
CKD5 (n, %)	6 of 46 (12.7%)	6 of 59 (10.0%)	0.76
Age at CKD5 (years; median, [IQR])	50.5 [39.2-58.5]	61 [44.5-72.25]	0.6

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

SUPPLEMENTARY TABLE 3

Supplementary table 3. Kidney function at last follow-up according to mutation distribution on CIC-5 sequence.

	Early stop mutations (n=36)	Affecting pore mutations (n=37)	Affecting CBS mutations (n=31)	p
CKD1 (n, %)	20/36 (55.5%)	10/37 (27.0%)	11/31 (35.4%)	*0.039
Age at CKD1 (years; median, [IQR])	13.6 [10.1-17.1]	8.7 [4.4-12.9]	14.3 [3.4-25.2]	0.42
CKD2 (n, %)	3/36 (8.3%)	15/37 (40.5%)	10/31 (32.2%)	
Age at CKD2 (years; median, [IQR])	6.3 [0.0 -13.9]	16.7 [8.3-25.0]	13.2 [9.0-17.4]	0.38
CKD3 (n, %)	3/36 (8.3%)	6/37 (16.2%)	4/31 (12.9%)	
Age at CKD3 (years; median, [IQR])	18.0 [7.2-28.8]	16.8 [5.9-27.8]	20.0 [5.4-34.6]	0.87
CKD4 (n, %)	3/36 (8.3%)	3/37 (8.1%)	3/31 (9.6%)	
Age at CKD4 (years; median, [IQR])	34.7 [0.0-82.0]	41.0 [23.8-58.2]	24.3 [12.6-36.1]	0.31
CKD5 (n, %)	7/36 (19.4%)	3/37 (8.1%)	3/31 (9.6%)	
Age at CKD5 (years; median, [IQR])	55.7 [45.3-66.1]	55.7 [23.3-88.0]	43.0 [43.0-43.0]	0.37

Abbreviations. IQR: Interquartile range; CKD: Chronic Kidney Disease.

MATERIALS AND METHODS

Study design and settings

A 46-item web-based cross-sectional survey was developed using the online tool SurveyMonkey (SurveyMonkey.com). The sampling frame was first the reference centers of the ERKNet, which was later extended to the membership list of the ESPN, the ERA, and the Spanish Societies for Nephrology - Sociedad Española de Nefrología- (SEN) and Pediatric Nephrology -Sociedad Española de Nefrología Pediátrica- (AENP). Part of the data was also obtained from RenalTube after patients/parents signed appropriate consent (16). Potential respondents were contacted repeatedly by mail and the questionnaire link was available online from September 2019 to July 2021. Survey responses were anonymously collected and stored electronically by the survey service until further analysis.

The survey contained multiple-choice and open-ended, non-mandatory questions, divided into five sections: patient socio-demographic and anthropometric data; diagnosis; patient updated clinical evaluation; patient updated blood/urinary biochemistry parameters, genetic diagnosis and management. The list of all questions is provided in as supplementary material (**Supplementary material 1**). Data were deemed adequate for analysis if more than 50% of the items were completed for each single 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.

Patient growth was assessed and adjusted to age and gender Z-score estimation, using reference growth data (17). Hypercalciuria was defined as a urinary calcium/creatinine ratio value above upper limit of normality for age range (18,19). Information regarding presence of nephrocalcinosis and nephrolithiasis was provided by the clinicians in charge and reflect the local imaging protocols and interpretation.

Estimated glomerular filtration rates could not be calculated because height data were not always available.

Since height data were not always available, estimated glomerular filtration rate (eGFR) was calculated using the Pottel formula for children (<18 years) (20) and the Chronic Kidney Disease - Epidemiology Collaboration (CKD-EPI) formula for adults (>18 years) (21). For patients who received kidney replacement therapy (KRT), last follow-up point time was determined the previous one before KRT. Limited data from some patients had been previously published (22,23). These data are accordingly referenced in the text. Proteinuria was defined as the presence of >150 mg/day of protein in the urine in adults (24), and >100 mg/m²/day in children, or by the random urine protein-to-creatinine ratio <0.2 (Upr/cr; in mg/mg) (25). The ratio albumin/ total protein was calculated by dividing the albumin level by the total protein minus albumin. Hypertension was defined by the clinician/researcher.

Statistical analysis

Data are shown as frequencies (percentages) for categorical variables. Continuous variables are presented as means \pm 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. In the assessment of the impact of mutations on the development of CKD, univariate analysis was performed to correct data by age. Because not every participant answered all questions, the percentage of respondents providing a given answer was calculated individually for each item, using the number of respondents to that question in the denominator. A p value <0.05 was considered statistically significant. Statistical analysis and graphs were performed and generated using SPSS V 21.0 (SPSS Inc., Chicago, Illinois, USA) or GraphPad Prism 8 (GraphPad Software, San Diego, California USA).