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PDF issue: 2025-12-04

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(Citation)
Pediatric Nephrology,35(12):2319-2326

(Issue Date)
2020-12

(Resource Type)
journal article

(Version)
Accepted Manuscript

(Rights)
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(URL)
https://hdl.handle.net/20.500.14094/90008357

Comparison of clinical and genetic characteristics between Dent disease-1 and Dent disease-2

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Abstract

Background

Dent disease is associated with low-molecular-weight proteinuria and hypercalciuria, and caused by pathogenic variants in either of two genes: *CLCN5* (Dent disease-1) and *OCRL* (Dent disease-2). It is generally not accompanied by extrarenal manifestations and it is always difficult to distinguish Dent disease-1 from Dent disease-2 without a gene test. We retrospectively compared the characteristics of these two diseases using one of the largest cohorts to date.

Methods

We performed gene tests for clinically suspected Dent disease, leading to the genetic diagnosis of 85 males: 72 with Dent disease-1 and 13 with Dent disease-2. A retrospective review of the clinical findings and laboratory data obtained from questionnaires submitted in association with the gene tests was conducted for these cases.

Results

The following variables had significantly higher levels in Dent disease-2 than in Dent disease-1: height standard deviation score (height SDS), serum creatinine-based estimated GFR (Cr-eGFR) (median: 84 vs. 127 mL/min/1.73 m^2, $p<0.01$), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), serum lactate dehydrogenase (LDH), serum creatinine phosphokinase (CK), serum potassium, serum inorganic phosphorus, serum uric acid, urine protein/creatinine ratio (median: 3.5 vs. 1.6 mg/mg, $p<0.01$), and urine calcium/creatinine ratio. There were no

significant differences in serum sodium, serum calcium, alkaline phosphatase (ALP), urine β2-microglobulin, incidence of nephrocalcinosis, and prevalence of intellectual disability or autism spectrum disorder.

Conclusion

The clinical and laboratory features of Dent disease-1 and -2 were shown in this study. Notably, patients with Dent disease-2 showed kidney dysfunction at a younger age, which should provide a clue for the differential diagnosis of these diseases.

Keywords

Dent disease-1, Dent disease-2, *CLCN5*, *OCRL*, Kidney dysfunction

Declarations

Funding

This study was supported by Grants-in-Aid for Scientific Research (KAKENHI) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (subject ID: 19K17297 to Nana Sakakibara, 17H04189 to Kazumoto Iijima, and 19K08726 to Kandai Nozu).

Conflicts of interest/Competing interests

The authors have nothing to disclose.

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the Institutional Review Board of Kobe University Graduate School of Medicine (IRB approval number 301) and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individuals participating in this study.

Authors' contributions

NS and KN: study conception and design, interpretation of the data, and drafting of the manuscript. NS: data acquisition and management. All authors: critical revision of the manuscript. All authors gave final approval of the version to be published, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Introduction

Dent disease [1] is an X-linked disorder characterized by low-molecular-weight proteinuria, hypercalciuria, nephrolithiasis, and nephrocalcinosis [2, 3]. It can be divided into two types: Dent disease-1 (OMIM #300009), which is caused by pathogenic variants of the *CLCN5* gene; and Dent disease-2 (OMIM #300555), caused by pathogenic variants of the *OCRL* gene. Approximately 60% of patients with clinically diagnosed Dent disease have Dent disease-1, while 15% have Dent disease-2. In the remaining 25%, the causative gene has not been identified [4, 5].

Dent disease-1 is caused by dysfunction of the ClC-5 chloride channel due to an abnormality in the *CLCN5* gene located on the X chromosome at Xp11.22; it is mainly expressed in early endosomes of the proximal tubule [6, 7]. In contrast, Dent disease-2 is caused by an abnormality of *OCRL* encoding phosphatidylinositol 4,5-bisphosphate 5-phosphatase, also located on the X chromosome at Xq25 [5, 8]. This protein is also expressed in endosomes of the proximal tubule like *CLCN5*, but is also widely expressed elsewhere, including in the brain [9].

OCRL mutations were originally described in patients with Lowe syndrome [10], which almost always comprises Fanconi syndrome, congenital cataracts, hypotonia, and severe developmental delay [11]. In addition, this condition often leads to chronic kidney disease (CKD) stage 5 [12, 13]. Despite having the same causative gene as Lowe syndrome, symptoms in Dent disease-2 are usually restricted to the kidney and are less severe than in Lowe syndrome. The reason for this difference remains unclear, but previous

reports have shown that patients with any type of mutations in exons 1-7 were diagnosed with Dent disease-2, and those with truncating mutations in exons 8-24 were diagnosed with Lowe syndrome [14]. It has also been reported that Dent disease-2 is a mild form of Lowe syndrome because some patients diagnosed with Dent disease-2 have mild cataracts and/or developmental delay [15].

Although the renal manifestations of Dent disease-1 and Dent disease-2 are quite similar, some cases of Dent disease-2 with mild symptoms of Lowe syndrome suggest that there may be differences in the renal and extra-renal manifestations between Dent disease-1 and Dent disease-2, as reported previously [15, 16].

Against this background, the purpose of this study is to clarify the differences in clinical symptoms and laboratory data between pediatric patients with Dent disease-1 and Dent disease-2 who underwent gene testing at a single institution.

Methods

Subjects

We have been performing gene tests for patients clinically suspected of having Dent disease since September 2014. Among them, all male cases clinically and genetically diagnosed with Dent disease were studied. Patients with cataracts were excluded as having Lowe syndrome, even when the diagnosis by their primary doctor was Dent disease. As a result, a total of 72 cases in 64 families with Dent disease-1 and 13 cases in 10 families with Dent

disease-2 were recruited. We conducted a retrospective review of these patients regarding the clinical findings and laboratory data collected from questionnaires submitted at the timing of application for a gene test. Details regarding the clinical features were obtained from the referring clinician or the patient's hospital records by the local doctors. This report does not include cases from a previous cohort of Dent disease in Japan [17].

Assessment of clinical findings

The presence or absence of intellectual disability, autism spectrum disorder, nephrocalcinosis, metabolic acidosis, or glycosuria was determined according to the questionnaire completed by each clinician. The presence or absence of intellectual disability in this study was basically based on developmental tests, but for patients without developmental tests, the decision was made by their clinician. The height SDS was calculated from the standard data for the Japanese. Cr-eGFR was calculated using the formula for Japanese children and adolescents under 18 years old [18, 19]. For patients over 18 years old, the Cr-eGFR calculation formula adjusted for the Japanese was also used [20]. CKD was defined as Cr-eGFR of <90 ml/min/1.73 m^2. The presence or absence of hypercalciuria was determined using previously reported age-specific reference values [21].

Gene test

Gene test was performed after informed consent was received. Genomic DNA was isolated from the peripheral blood leukocytes of patients and their family

members using the QuickGene-Mini80 system (Wako Pure Chemical Industries, Ltd., Tokyo, Japan), in accordance with the manufacturer's instructions. Direct sequencing or targeted sequencing using next-generation sequencing (NGS) was conducted on genes responsible for inherited kidney diseases. NGS samples were prepared using a HaloPlex target enrichment system kit (Agilent Technologies, Santa Clara, CA, USA), in accordance with the manufacturer's instructions. Briefly, 225 ng of genomic DNA was used for a restriction reaction and hybridized at 54°C for 16 h with NGS probes. All indexed DNA samples were amplified by polymerase chain reaction (PCR) and sequenced using the MiSeq platform (Illumina, San Diego, CA, USA). We analyzed the data using SureCall 4.0, which is a desktop application combining algorithms for end-to-end NGS data analysis, from alignment to the categorization of mutations (Agilent Technologies). The cDNA reference number of *CLCN5* in this study is NM_000084.4 and that of *OCRL* is NM_000276.3. Pathogenicity predictions were performed in accordance with the American College of Medical Genetics guidelines [22]. Several online predictive tools, including SIFT (https://sift.bii.a-star.edu.sg/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), Mutation Taster (http://www.pathogenic varianttaster.org/), Human Splicing Finder (http://www.umd.be/HSF/), MaxEntScan (http://genes.mit.edu/burgelab/maxent/Xmaxentscan_ scoreseq.html), and NNSplice (http://www.fruitfly.org/seq_tools/splice.html), were used to predict the pathogenicity of the variants.

Statistical analysis

Data are shown as the median and interquartile range (IQR). Clinical findings and laboratory test results of the patients were compared using the Mann–Whitney U test and Fisher's exact test, as appropriate. Factors considered to be related to age were adjusted for age using analysis of covariance. Statistical analysis was performed using standard statistical software (JMP version 10 for Windows; SAS Institute, Cary, NC, USA). $p<0.05$ was considered statistically significant.

Results

We examined differences in clinical and laboratory data between Dent disease-1 and Dent disease-2, the results of which are shown in Table 1. The median age at which gene test was performed was significantly lower in Dent disease-2 than in Dent disease-1. The height SDS were significantly lower in Dent disease-2 than in Dent disease-1 (−2.2 SD vs. −0.2 SD; Fig. 1a). There was no significant difference in the prevalence of intellectual disability or autism spectrum disorder.

Patients with Dent disease-2 had significantly lower Cr-eGFR than patients with Dent disease-1 (median 87 vs. 127 ml/min/1.73 m^2) (Fig. 1b). When Cr-eGFR under 90 ml/min/1.73 m^2 was defined as CKD, the prevalence of CKD was only 8% (6 out of 71) in Dent disease-1, but 58% (7 out of 11) in Dent disease-2. The six patients with CKD in Dent disease-1 consisted of five with CKD stage 2 (60–89 ml/min/1.73 m^2) and one with CKD stage 3 (30–59

ml/min/1.73 m^2). All seven patients with CKD in Dent disease-2 were at CKD stage 2. There were no patients with CKD stage 5 in either group.

Serum levels of CK, LDH, AST, and ALT were significantly higher in Dent disease-2 (Fig. 2). None of the patients had metabolic acidosis. Serum potassium and phosphate levels were significantly lower in Dent disease-1, but there were no significant differences for serum sodium, calcium, uric acid, and alkaline phosphatase. After adjustment for age, serum level of uric acid was significantly lower in Dent disease-1.

The findings from comparing the urinalysis results are shown in Fig. 3. Urine protein/creatinine ratio and urine calcium/creatinine ratio were significantly higher in Dent disease-2. However, there was no significant difference in urine β2-microglobulin and the prevalence of hypercalciuria in relation to the age-specific baseline values for children. The number of patients with glycosuria was low and there was no significant difference in this regard between the two groups. Nephrocalcinosis was observed only in Dent disease-1, but the difference did not reach significance due to the small number of Dent disease-2 cases.

The frequency of major renal manifestations of Dent disease is summarized in Table 2, along with the results of previous studies comparing Dent disease-1 and Dent disease-2.

All genetic variants detected in this study are shown in Supplementary Table S1. Evaluation of novel missense mutations using in silico analysis and population database are shown in Supplementary Table S2. We detected 64 families with *CLCN5* mutation, comprising 32 missense, 17 nonsense, and 10

splice-site mutations, 4 small deletions, as well as 1 small insertion. Of these, 28 were novel mutations. We also identified *OCRL* mutations in 10 families, 5 of which were novel. The mutations included 5 missense, 1 nonsense, and 2 splice-site mutations, as well as 2 small deletions.

Discussion

Dent disease does not usually cause extrarenal symptoms, and it is difficult to distinguish between Dent disease-1 and Dent disease-2 based on clinical symptoms alone. A gene test is required to distinguish them. However, several reports have been published showing differences in the clinical features of Dent disease-1 and Dent disease-2 [15, 16].

In Japan, Dent disease is usually detected as asymptomatic proteinuria at the time of urine screening, such as urinalysis routinely performed at the age of 3 or at school. In other words, the age group differs from that in cohort studies in other countries because it is often detected much earlier in Japan than in Europe and the US. Therefore, it is common for there to be no extrarenal symptoms at the time of diagnosis, and there are no patients with CKD stage 5 among the subjects. However, it is unclear how many patients will eventually develop CKD stage 5. As pointed out in a report on a large study in Japan [17], Japanese cases may have milder symptoms of Dent disease.

Table 2 compares the frequency of major renal manifestations of Dent disease in this study with those in previous large cohorts; some symptoms in this study are similar to those in previous reports, while others are not. Although subtle differences in the definitions of hypercalciuria and kidney

dysfunction in each study may have contributed to this, our reported findings are relatively similar to those in a previous Japanese cohort [17], so the renal manifestations of Dent disease may vary from country to country.

A previous large French cohort showed no difference in GFR decline between Dent disease-1 and Dent disease-2 [12], and another report showed a trend toward lower eGFR in Dent disease-2, but the difference was not significant [15]. Another Chinese cohort, without statistical analysis, noted a mean eGFR of 82.6 mL/min/1.73 m^2 in Dent disease-2 versus 110.7 mL/min/1.73 m^2 in Dent disease-1 [23].

In our study, Dent disease-2 had significantly lower eGFR than Dent disease-1 and it was proven that kidney dysfunction was shown from an earlier age in Dent disease-2.

There may also be differences in other renal manifestations characteristic of Dent disease. In this study, although there was no significant difference in urine β2-microglobulin, there was a significantly higher urine protein/creatinine ratio reflecting the amount of low-molecular-weight proteinuria in Dent disease-2.

Hypercalciuria is a common symptom in both disorders. In a previous Japanese report, the incidence of hypercalciuria was significantly higher in Dent disease-2 than in Dent disease-1 [17]; however, this is not always the case in other reports. Hypercalciuria in Dent disease is thought to be caused by increased absorption of calcium from the gastrointestinal tract [24]. The activity of TRPV6, an intestinal calcium channel, is suppressed by OCRL protein, and mutations in the *OCRL* gene alleviate this suppression [25]. Thus,

intestinal calcium absorption may be enhanced in cases of Dent disease-2. In addition, in our study, urine calcium/creatinine ratio was significantly higher in patients with Dent disease-2, even after adjusting for age. However, there was no significant difference in the prevalence of hypercalciuria. Urine calcium/creatinine ratio varies widely in younger children, so the result for this variable would have been influenced by age differences between the two groups.

The incidence of nephrocalcinosis was 0% in Dent disease-2 and 22% in Dent disease-1, although there was no significant difference in this study. In five previous reports [12, 15-17, 26], the incidence of nephrocalcinosis appears to have been lower in patients with Dent disease-2. This was contrary to the expected result from our study that the calcium creatinine ratio was significantly higher for Dent disease-2 than for Dent disease-1. In other words, hypercalciuria may not necessarily be a direct risk for nephrocalcinosis.

Some of the symptoms of Fanconi syndrome are observed in Dent disease [12]. Abnormalities observed in Fanconi syndrome such as hypokalemia, hypouricemia, and hypophosphatemia were also examined in this study. None of the patients in our study had hypophosphatemia or hypokalemia that required medication, but serum inorganic phosphorus and potassium levels were significantly lower in patients with Dent disease-1 than in those with Dent disease-2. After adjustment for age, in addition to these two parameters, serum uric acid levels were lower in patients with Dent disease-1. Although Fanconi syndrome is rare in patients with Dent disease and often only some of its symptoms are exhibited, it is possible that electrolyte abnormalities

associated with Fanconi syndrome are more observed in patients with Dent disease-1.

In a previous Korean cohort, it has also been reported that CK, LDH, and AST are higher in Dent disease-2 than in Dent disease-1 [16]. This was again confirmed in our investigation. In Lowe syndrome, which is caused by the same *OCRL* gene abnormality as Dent disease-2, muscle weakness is one of the major symptoms and the levels of muscle enzymes are elevated. If Dent disease-2 can be categorized as a mild form of Lowe syndrome, it is reasonable to assume that muscle enzyme elevation is also observed in Dent disease-2. However, the precise reason for the muscle enzyme elevation in Lowe syndrome is unknown.

As in our study, patients with Dent disease-2 were previously reported to be significantly shorter than those with Dent disease-1 [15]. In addition, the lack of growth hormone in patients with Dent disease-2 has been demonstrated elsewhere [27]. It remains unclear whether growth hormone should be given to patients with Dent disease, but we will need to evaluate laboratory data such as growth hormone and insulin-like growth factor-1 levels to determine this.

There were no significant differences in intellectual disability or autism spectrum disorder between the two groups in this study, but those with Dent disease-2 tended to show a higher prevalence of these abnormalities. In this study, the rate of intellectual disability or autism spectrum disorder was also high in Dent disease-1: 5 out of 69 patients (7%). Two of them were twins and another two had mild delays with developmental quotients (DQ) of 78 and 82.

However, this high rate of intellectual disability may have been coincidental. To obtain more definitive findings, it is desirable to increase the number of cases and to collect detailed scoring data such as on DQ or intelligence quotient (IQ).

In this study, we were able to identify a number of novel mutations in addition to those previously reported. Mutations in *CLCN5* were detected at various points across the whole gene. Missense mutations accounted for 50%, nonsense mutations 26%, and splice-site mutations 15%. In our study, the rate of missense mutations was higher and the rate of frameshift mutations was lower than in two previous reports [26, 28], but they were similar to those in a previous cohort from the Japanese population [17]. Among *OCRL* mutations, missense mutations were also the most common at 50%, which is similar to the rates in some previous reports [17, 26, 29]. Regarding the other types of mutation in *OCRL*, comparison was difficult since the number of cases was small. However, as indicated previously [14, 30], frameshift and nonsense mutations in Dent disease-2 were also specifically identified in exons 1-7.

As a limitation of this study, data may initially have been collected from questionnaires by the patient's local doctor. In other words, there may have been a lack of objectivity regarding the classification of nephrocalcinosis and intellectual disability. Another problem is that the distinction between Dent disease and Lowe syndrome is unclear. In this study, patients with cataracts were ruled out from this study of Dent disease as having Lowe syndrome because, in our experience, most patients without cataracts do not have

representative symptoms of Lowe syndrome, such as Fanconi syndrome and severe developmental delay. However, this definition includes patients without Fanconi syndrome or cataracts but with mild mental or developmental disability, such as children who are unable to keep up with their academic studies at school. As mentioned earlier, it is necessary to clearly differentiate the presence or absence of intellectual disability based on objective indicators such as IQ.

Despite these limitations, this work provides important findings regarding the differences in clinical manifestations, especially kidney function, between patients with Dent disease-1 and Dent disease-2 at a single institution.

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Figure legends

Fig. 1

Comparison of height SDSand Cr-eGFR between patients with Dent disease-1 and Dent disease-2.

(a) Patients with Dent disease-2 were shorter than those with Dent disease-1 (median: −2.2 SD vs. −0.2 SD, $p<0.01$). (b) Cr-eGFR levels were significantly lower in patients with Dent disease-2 than in those with Dent disease-1 (median: 87 vs. 127 ml/min/1.73 m^2, $p<0.01$).

Fig. 2

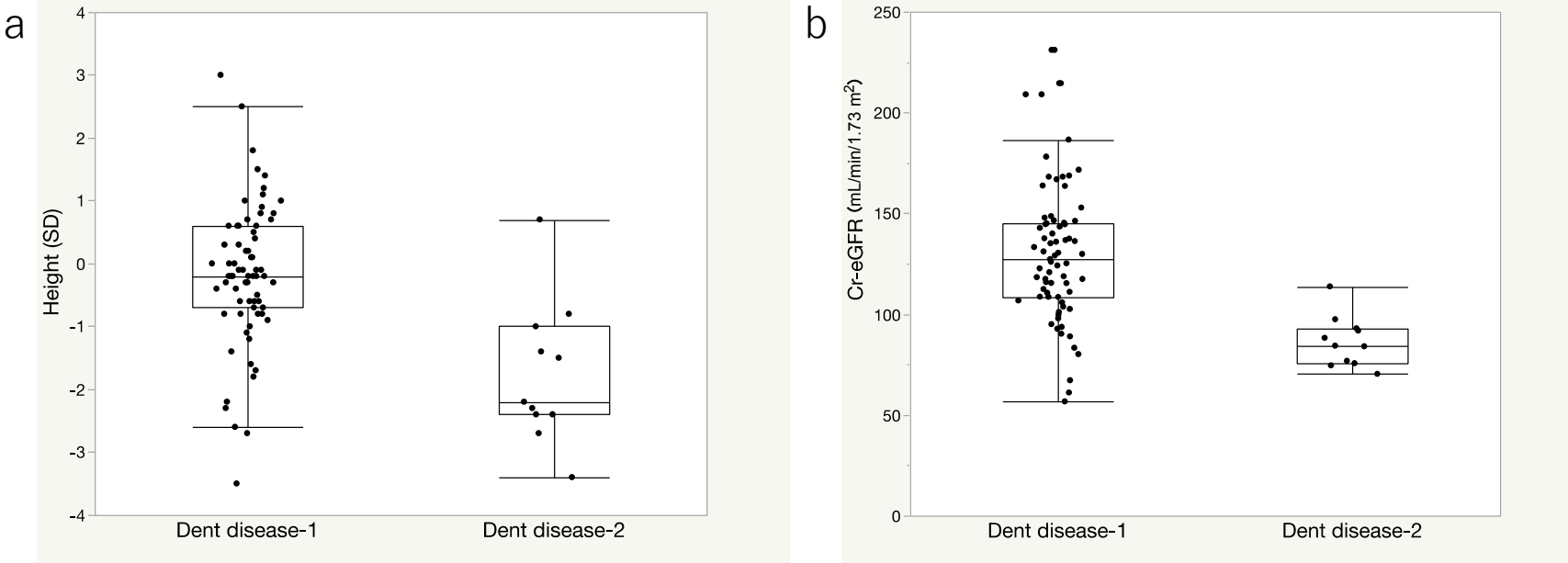
Comparison of (a) CK, (b) LDH, (c) AST, and (d) ALT between patients with Dent disease-1 and Dent disease-2.

All parameters were significantly higher in patients with Dent disease-2 than in those with Dent disease-1.

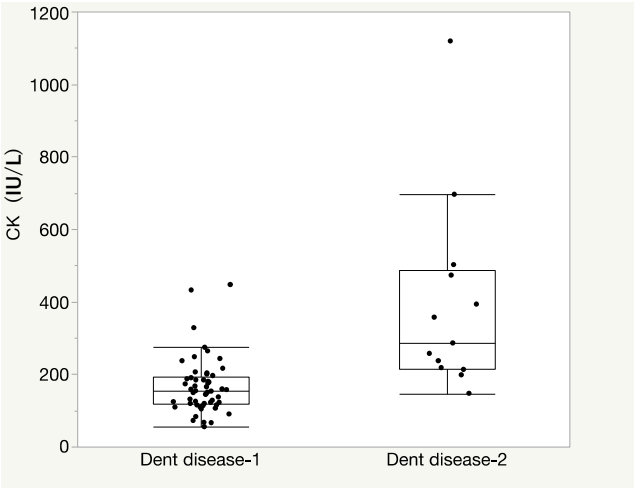
Fig. 3

Comparison of urine protein/creatinine ratio (urine Pro/Cre), urine β2-microglobulin (urine β2MG), and urine calcium/creatinine ratio (urine Ca/Cre) between patients with Dent disease-1 and Dent disease-2.

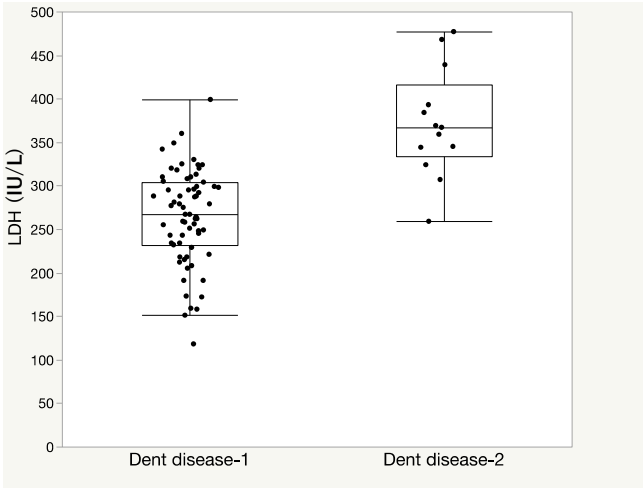
(a) Urine protein/creatinine ratio was significantly higher in patients with Dent disease-2 (median: 3.5 vs. 1.6 mg/mg, $p=0.01$). (b) However, there was no significant difference in urine β2-microglobulin. (c) Urine calcium/creatinine ratio was significantly higher in patients with Dent disease-2 (median: 0.69 vs. 0.25 mg/mg, $p<0.01$).


a
Height (SD)
4
3
2
1
0
-1
-2
-3
-4
Dent disease-1
Dent disease-2
b
Cr-eGFR (mL/min/1.73 m2)
250
200
150
100
50
0
Dent disease-1
Dent disease-2

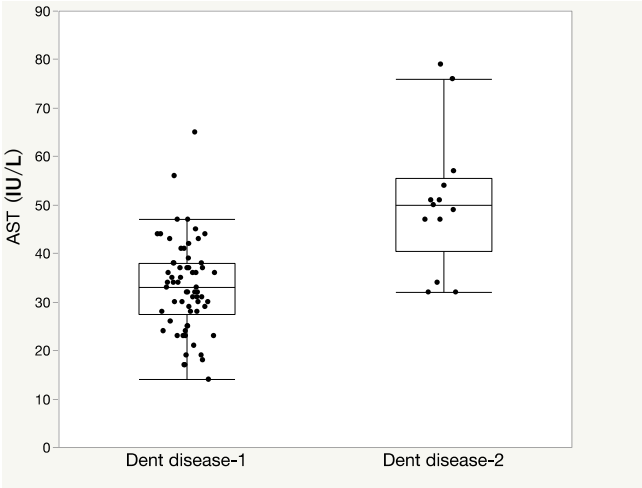
a


CK (IU/L)
1200
1000
800
600
400
200
0
Dent disease-1
Dent disease-2

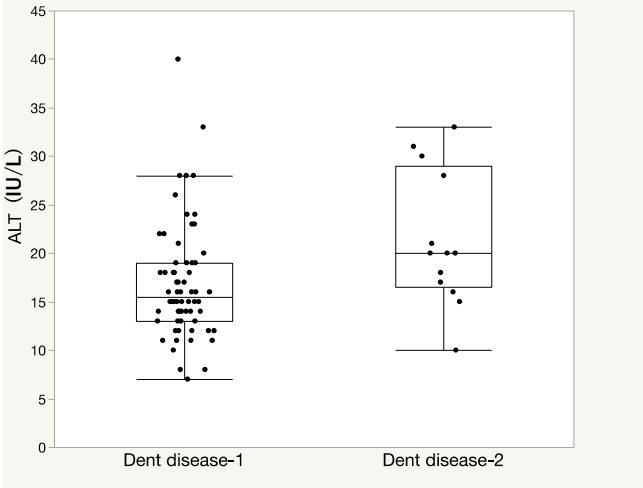
b


LDH (IU/L)
500
450
400
350
300
250
200
150
100
50
0
Dent disease-1
Dent disease-2

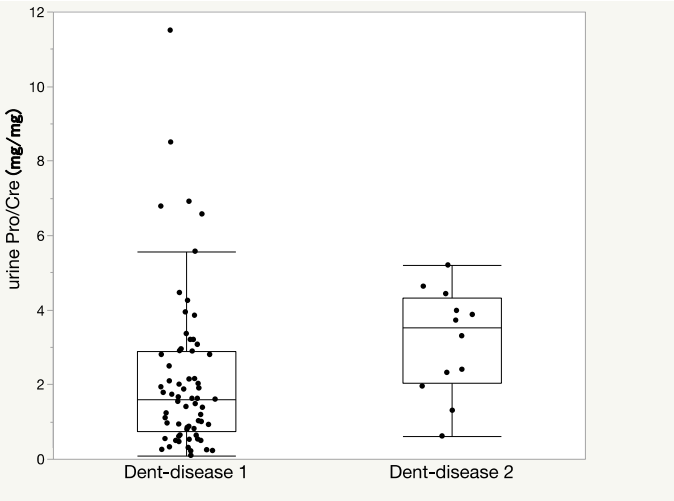
c


AST (IU/L)
90
80
70
60
50
40
30
20
10
0
Dent disease-1
Dent disease-2

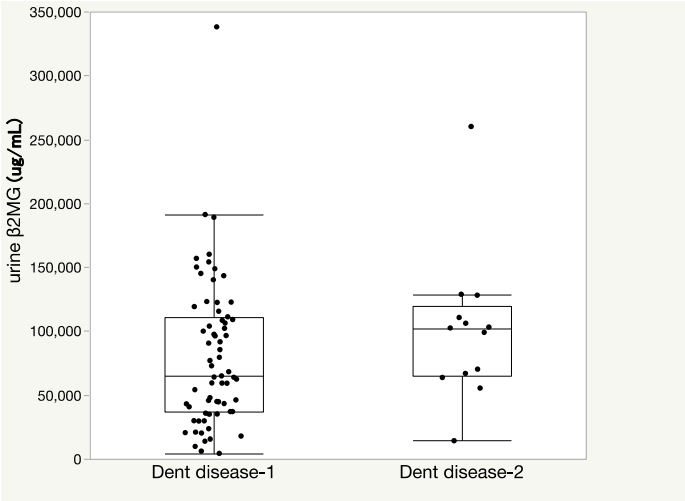
d


ALT (IU/L)
45
40
35
30
25
20
15
10
5
0
Dent disease-1
Dent disease-2

a


urine Pro/Cre (mg/mg)
12
10
8
6
4
2
0
Dent-disease 1
Dent-disease 2

b


urine β2MG (ug/mL)
350,000
300,000
250,000
200,000
150,000
100,000
50,000
0
Dent disease-1
Dent disease-2

c

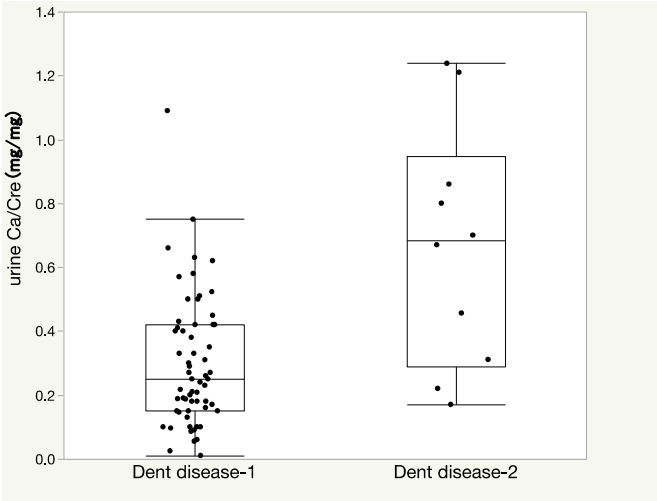

urine Ca/Cre (mg/mg)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Dent disease-1
Dent disease-2

Table 1　Comparison of clinical findings between patients with Dent disease-1 and Dent disease-2

	Dent disease-1	Dent disease-2	p-value	
			Unadjusted	Age-adjusted
*Age (years)	5.0 [3.0–9.0]	3.0 [1.5–5.5]	0.03	
*Height (SD)	-0.2 [-0.7–-0.6]	-2.2 [-2.4–-1.0]	<0.01	
Intellectual disability/autism spectrum disorder (%, n)	7% (5/69)	23% (3/13)	0.11	
CKD　(Cr-eGFR <90 ml/min/1.73 m^2)	8% (6/71)	58% (7/12)	<0.01	
60-89 ml/min/1.73 m^2	7% (5/71)	58% (7/12)		
30-59 ml/min/1.73 m^2	1% (1/71)	0% (0/12)		
*Cr-eGFR (mL/min/1.73 m^2)	127 [109–169]	84 [76–111]	<0.01	<0.01
Nephrocalcinosis (%, n)	22% (14/63)	0% (0/12)	0.11	
*CK (IU/L)	154 [118–192]	286 [216–488]	<0.01	
*LDH (IU/L)	267 [232–304]	367 [334–416]	<0.01	
*AST (IU/L)	33 [28–38]	50 [41–56]	<0.01	<0.01
*ALT (IU/L)	16 [13–19]	20 [17–29]	0.01	0.03
Metabolic acidosis (%, n)	0% (0/65)	0% (0/13)	1.0	
*Na (mEq/L)	139 [137–140]	138 [137–140]	0.61	
*K (mEq/L)	4.0 [3.9–4.2]	4.2 [4.1–4.4]	0.02	0.03
*Ca (mg/dL)	9.7 [9.4–10.0]	9.8 [9.5–10.3]	0.48	0.95
*P (mg/dL)	4.7 [4.2–5.0]	5.3 [4.7–5.5]	<0.01	0.01

*UA (mg/dL)	3.6 [3.0–4.2]	4.0 [3.7–4.7]	0.10	0.02
*ALP (IU/L)	896 [730–995]	996 [779–1107]	0.18	
*Urine protein/creatinine (mg/mg)	1.6 [0.8–2.9]	3.5 [2.0–4.3]	0.01	
*Urine β2-microgloblin (μg/mL)	64,900 [36,900–110,400]	102,300 [65,200–119,200]	0.17	
*Urine calcium/creatinine (mg/mg)	0.25 [0.15–0.42]	0.69 [0.29–0.95]	<0.01	0.01
Hypercalciuria (%, n)	39% (24/62)	70% (7/10)	0.09	
Glycosuria (%, n)	3% (2/68)	0% (0/13)	1.0	

*Median (IQR)

Table 2 Comparison of major renal manifestations of Dent disease-1 and Dent disease-2 in previous and our reports

	Bokenkamp et al. (2009) [15] Europe and Korea		Sekine et al. (2014) [17] Japan		Park et al. (2014) [16] Korea		Blanchard et al. (2016) [12] France		Ye et al. (2019) [26] China		Our cases	
	Dent-1 vs Dent-2		Dent-1 vs Dent-2		Dent-1 vs Dent-2		Dent-1 vs Dent-2		Dent-1 vs Dent-2		Dent-1 vs Dent-2	
Low-molecular-weight proteinuria	100% (212/212)	100% (28/28)	100% (61/61)	100% (11/11)	No data		100% (93/93)	100% (7/7)	100% (32/32)	100% (13/13)	100% (85/85)	100% (13/13)
Hypercalciuria	90% (180/200)	86% (24/28)	46% (25/54)	70% (7/10)	No data		92% (81/88)	100% (3/3)	66% (21/32)	92.3% (12/13)	39% (24/62)	70% (7/10)
Nephrocalcinosis	75% (137/182)	39% (11/28)	38% (20/53)	10% (1/10)	65% (15/23)	20% (1/5)	42% (44/104)	11% (1/9)	44% (14/32)	23% (3/13)	22% (14/63)	0% (0/12)
Kidney dysfunction	30% (60/203)	32% (8/25)	8% (4/53)	0% (0/10)	0% (0/23)	0% (0/5)	No data		13% (4/32)	8% (1/13)	8% (6/71)	58% (7/12)

Supplementary Table S1 *CLCN5* and *OCRL* mutations detected in this study

Disease type	Patient	Causative gene	Location	Nucleotide change	Amino acid change	ACMG hazard level	ACMG evidence	Reference
Dent disease-1	D3	CLCN5	intron5	c.516+1G>A	-	pathogenic	PVS1+PM2+PP3+PP5	[1, 2]
	D4		exon10	c.1546C>T	p.(Arg516Trp)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[3]
	D5		intron5	c.516+1G>A	-	pathogenic	PVS1+PM2+PP3+PP5	[1, 2]
	D7		exon7	c.731C>T	p.(Ser244Leu)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[4]
	D10		exon7	*c.724G>T	p.(Val242Phe)	likely pathogenic	PM1+PM2+PP2+PP3	
	D13 D13 relative		exon6	*c.530T>G	p.(Leu177Trp)	likely pathogenic	PM1+PM2+PP1+PP2+PP3	
	D16		exon11	c.1942C>T	p.(Arg648*)	pathogenic	PVS1+PM2+PP5	[1, 4]
	D18		exon11	c.2110C>T	p.(Arg704*)	pathogenic	PVS1+PM2+PP5	[4, 5]
	D19 D19 brother		exon6	c.608C>T	p.(Ser203Leu)	likely pathogenic	PM1+PM2+PP1+PP2+PP3+PP5	[6]
	D20		exon9	c.1516G>A	p.(Gly506Arg)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[7]
	D23		exon6	*c.692A>G	p.(Lys231Arg)	likely pathogenic	PM1+PM2+PM5+PP2+PP3	
	D24		exon2	c.82C>T	p.(Arg28*)	pathogenic	PVS1+PM2+PP5	[1, 8]
	D26		exon5	c.1537G>A	p.(Gly513Arg)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[9]
	D29		exon6	*c.703A>C	p.(Asn235His)	likely pathogenic	PM1+PM2+PP2+PP3	
	D30		exon11	c.2110C>T	p.(Arg704*)	pathogenic	PVS1+PM2+PP5	[4, 5]

D33	exon3	*c.194G>A	p.(Gly65Glu)	likely pathogenic	PM1+PM2+PM5+PP2+PP3	
D34	exon5	*c.633-634insA	p.(Gly212Argfs*25)	likely pathogenic	PVS1+PM2	
D38	exon8	*c.988G>C	p.(Gly330Arg)	likely pathogenic	PM1+PM2+PP2+PP3	
D39	exon10	c.1701C>A	p.(Try567*)	pathogenic	PVS1+PM2+PP5	[10]
D41	intron6	*c.723+2 T>A	-	pathogenic	PVS1+PM2+PP3	
D42	intron2	*c.106-11 T>G	-	pathogenic	PVS1+PM2+PP3	
D46	exon4	*c.365G>C	p.(Trp122Ser)	likely pathogenic	PM1+PM2+PP2+PP3	
D47 D47 brother	exon10	*c.1572_74del	p.(Ile524del)	likely pathogenic	PM1+PM2+PM4+PP1	
D48	exon10	c.1909C<T	p.(Arg637*)	pathogenic	PVS1+PM2+PP5	[11]
D49	exon11	*c.953C>T	p.(Glu318Val)	likely pathogenic	PM1+PM2+PP2+PP3	
D50	intron2	*c.105+5G>A	-	pathogenic	PVS1+PM2+PP3	
D51	exon6	*c.632A>C	p.(Glu211Ala)	likely pathogenic	PM1+PM2+PM5+PP2+PP3	
D54 D54 brother	exon9	c.1397G>A	p.(Gly466Asp)	likely pathogenic	PM1+PM2+PM5+PP1+PP2+PP3 +PP5	[12]
D55 D55 brother	exon4	c.263G>A	p.(Gly88Asp)	likely pathogenic	PM1+PM2+PP1+PP2+PP3+PP5	[13]
D56	exon2	*c.66G>T	p.(Trp22Cys)	likely pathogenic	PM2+PM5+PP2+PP3	
D57	exon5	*c.411_414del	p.(Val138Ilefs*35)	likely pathogenic	PVS1+PM2	
D58	exon6	*c.712A>T	p.(Lys238*)	likely pathogenic	PVS1+PM2	

D60	exon8	c.1039C>T	p.(Arg347*)	pathogenic	PVS1+PM2+PP5	[3]
D61	exon11	c.1942C>T	p.(Arg648*)	pathogenic	PVS1+PM2+PP5	[1, 4]
D63	exon8	c.815A>G	p.(Tyr272Cys)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[14]
D64 D64 brother	exon10	*c.1562_64del	p.(Val523del)	likely pathogenic	PM1+PM2+PM4+PP1	
D65 D65 brother	exon7	*c.784G>T	p.(Val262Leu)	likely pathogenic	PM1+PM2+PP1+PP2+PP3	
D66	exon6	*c.664G>A	p.(Gly222Arg)	likely pathogenic	PM1+PM2+PP2+PP3	
D68	exon10	c.1546C>T	p.(Arg516Trp)	likely pathogenic	PM1+PM2+PM5+PP2+PP3+PP5	[3]
D69	intron11	c.2150+1G>T	-	pathogenic	PVS1+PM2+PP3+PP5	[1, 14]
D71	exon2	c.82C>T	p.(Arg28*)	pathogenic	PVS1+PM2+PP5	[1, 8]
D72	exon3	*c.203C>A	p.(Ser68*)	likely pathogenic	PVS1+PM2	
D73	exon7	c.793A>C	p.(Ser265Arg)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[15]
D74	exon2	c.100C>T	p.(Arg34*)	pathogenic	PVS1+PM2+PP5	[1, 8]
D75 D75 brother	exon4	c.263G>A	p.(Gly88Asp)	likely pathogenic	PM1+PM2+PP1+PP2+PP3+PP5	[13]
D82	exon10	c.1701C>A	p.(Tyr567*)	pathogenic	PVS1+PM2+PP5	[10]
D85	intron2	*c.106-2A>C	-	pathogenic	PVS1+PM2+PP3	
D86	intron8	*c.1347+1G>A	-	pathogenic	PVS1+PM2+PP3	
D91	exon2	c.82C>T	p.(Arg28*)	pathogenic	PVS1+PM2+PP5	[1, 8]

	D92		exon10	c.1547G>A	p.(Arg516Gln)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[7]
	D96		exon7	c.731C>T	p.(Ser244Leu)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[4]
	D99		exon2	c.100C>T	p.(Arg34*)	pathogenic	PVS1+PM2+PP5	[1, 8]
	D103		exon9	c.1505A>G	p.(Tyr502Cys)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[7]
	D106		exon4	c.389A>G	p.(Asp130Gly)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[16]
	D107		exon4	c.346delT	p.(Cys116Valfs*22)	pathogenic	PVS1+PM2+PP5	[5]
	D109		exon11	*c.2131T>C	p.(Cys711Arg)	likely pathogenic	PM1+PM2+PM5+PP2+PP3	
	D111		exon3	*c.205+1G>A	-	pathogenic	PVS1+PM2+PP3	
	D112		exon4	*c.254T>G	p.(Leu85*)	likely pathogenic	PVS1+PM2	
	D114		intron4	c.394-2A>G	-	pathogenic	PVS1+PM2+PP3+PP5	[1, 17]
	D115		exon5	*c.512C>T	p.(Pro171Leu)	likely pathogenic	PM1+PM2+PP2+PP3	
	D116		exon10	*c.1585A>C	p.(Thr529Pro)	likely pathogenic	PM1+PM2+PP2+PP3	
	D117		exon9	c.1399C>T	p.(Arg467*)	pathogenic	PVS1+PM2+PP5	[1, 18]
	D118		exon9	c.1537G>A	p.(Gly513Arg)	likely pathogenic	PM1+PM2+PP3+PP2+PP5	[9]
	D120		exon7	c.731C>T	p.(Ser244Leu)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[4]
Dent disease-2	D6	OCRL	intron3	*c.199+1G>T	-	pathogenic	PVS1+PM2+PP3	
	D31		intron3	c.199+1G>A	-	pathogenic	PVS1+PM2+PP3+PP5	[19]
	D36 D36 brother 1		exon14	*c.1430A>T	p.(Tyr477Phe)	likely pathogenic	PM1+PM2+PP1+PP2+PP3	

D36 brother 2						
D52	exon9	c.821T>C	p.(Ile274Thr)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[20]
D53	exon3	*c.175_178del	p.(Asn59Alafs*14)	likely pathogenic	PVS1+PM2	
D70	exon15	c.1477C>T	p.(Arg493Trp)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[21]
D77	exon11	c.953G>A	p.(Arg318His)	likely pathogenic	PM1+PM2+PP2+PP3+PP5	[22]
D78	exon20	*c.2206A>G	p.(Lys736Gln)	likely pathogenic	PM1+PM2+PP2+PP3	
D89	exon5	c.304_311del	p.(Glu102Phefs*27)	pathogenic	PVS1+PS1+PM2+PP5	[23]
KC311 KC311 brother	exon4	*c.208G>T	p.(Glu70*)	pathogenic	PVS1+PM2+PP1	

*Novel mutation

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Supplementary Table S2 Evaluation of novel missense variants using in silico analysis and population data base

patient	causative gene	location	nucleotide change	amino acid change	in silico analysis			population data base		
					SIFT	Mutation Taster	PolyPhen-2	gnomAD	dbSNP	HGVD
D10	CLCN5	exon7	*c.724G>T	p.(Val242Phe)	Deleterious	Disease Causing	Benign	-	-	-
D13		exon6	*c.530T>G	p.(Leu177Trp)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D23		exon6	*c.692A>G	p.(Lys231Arg)	Tolerated	Disease Causing	Possibly Damaging	0.0011%	rs782733541	-
D29		exon6	*c.703A>C	p.(Asn235His)	Deleterious	Disease Causing	Possibly Damaging	-	-	-
D33		exon3	*c.194G>A	p.(Gly65Glu)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D38		exon8	*c.988G>C	p.(Gly330Arg)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D46		exon4	*c.365G>C	p.(Trp122Ser)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D49		exon11	*c.953C>T	p.(Glu318Val)	Tolerated	Disease Causing	Probably Damaging	-	-	-

D51		exon6	*c.632A>C	p.(Glu211Ala)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D56		exon2	*c.66G>T	p.(Trp22Cys)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D65		exon7	*c.784G>T	p.(Val262Leu)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D66		exon6	*c.664G>A	p.(Gly222Arg)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D109		exon11	*c.2131T>C	p.(Cys711Arg)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D115		exon5	*c.512C>T	p.(Pro171Leu)	Deleterious	Disease Causing	Possibly Damaging	-	-	-
D116		exon10	*c.1585A>C	p.(Thr529Pro)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D36	OCRL	exon14	*c.1430A>T	p.(Tyr477Phe)	Deleterious	Disease Causing	Probably Damaging	-	-	-
D78		exon20	*c.2206A>G	p.(Lys736Gln)	Tolerated	Disease Causing	Probably Damaging	-	-	-