

CLINICAL STUDY



Clinical features and genetic analysis of 15 Chinese children with dent disease

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ABSTRACT

Objective: The clinical characteristics, genetic mutation spectrum, treatment strategies and prognoses of 15 children with Dent disease were retrospectively analyzed to improve pediatricians' awareness of and attention to this disease.

Methods: We analyzed the clinical and laboratory data of 15 Chinese children with Dent disease who were diagnosed and treated at our hospital between January 2017 and May 2023 and evaluated the expression of the *CLCN5* and *OCRL1* genes.

Results: All 15 patients were male and complained of proteinuria, and the incidence of low-molecular-weight proteinuria (LMWP) was 100.0% in both Dent disease 1 (DD1) and Dent disease 2 (DD2) patients. The incidence of hypercalciuria was 58.3% (7/12) and 66.7% (2/3) in DD1 and DD2 patients, respectively. Nephrocalcinosis and nephrolithiasis were found in 16.7% (2/12) and 8.3% (1/12) of DD1 patients, respectively. Renal biopsy revealed focal segmental glomerulosclerosis (FSGS) in 1 patient, minimal change lesion in 5 patients, and small focal acute tubular injury in 1 patient. A total of 11 mutations in the *CLCN5* gene were detected, including 3 missense mutations (25.0%, c.1756C>T, c.1166T>G, and c.1618G>A), 5 frameshift mutations (41.7%, c.407delT, c.1702_c.1703insC, c.137delC, c.665_666delGGinsC, and c.2200delG), and 3 nonsense mutations (25.0%, c.776G>A, c.1609C>T, and c.1152G>A). There was no significant difference in age or clinical phenotype among patients with different mutation types ($p > 0.05$). All three mutations in the *OCRL1* gene were missense mutations (c.1477C>T, c.952C>T, and c.198A>G).

Conclusion: Pediatric Dent disease is often misdiagnosed. Protein electrophoresis and genetic testing can help to provide an early and correct diagnosis.

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Dent disease is a rare hereditary X-linked recessive proximal tubular disease. It is characterized by low-molecular-weight proteinuria (LMWP), hypercalciuria, nephrocalcinosis/nephrolithiasis, and progressive renal failure [1]. Some patients might present with incomplete Fanconi syndrome, hypophosphatemic rickets or abnormal electrolyte balance [2]. It is rare for Dent disease to develop into CKD in childhood, and only a few cases have been reported to date [3]. Between 30 and 50 years of age, 30–80% of patients with Dent disease can develop end-stage renal disease (ESRD) [4]. At present, there are no more than 1,000 families of reported cases worldwide. Dent disease was first reported in China in 2008, and most articles belong to case report or series case reports. There is only

one multicenter study including 45 Chinese patients with Dent disease.

Dent disease is divided into Dent disease 1 (DD1) and Dent disease 2 (DD2) based on the mutated gene on the X chromosome [5]. The *CLCN5* gene is located at Xp11.22-p11.23 and encodes the protein CIC-5, a chloride channel protein. CIC-5 is mainly expressed in the kidney and participates in the endocytic reabsorption of LMWP in proximal tubular cells [6]. The *OCRL1* gene is located on chromosome Xq25 and has 24 exons. It is widely distributed in human tissues, especially the eyes, kidneys and brain. It encodes phosphatidylinositol 4,5-diphosphate 5-phosphatase, which hydrolyzes phosphatidylinositol 4,5-diphosphate (PIP₂), which is a second

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messenger involved in vesicle transport [7]. DD1 with mutations in the *CLCN5* gene accounts for approximately 60% of patients with Dent disease, and DD2 with mutations in the *OCRL1* gene accounts for approximately 15% of patients. The genetic origin of the remaining cases (25% of all cases) remains unknown [8]. Dent disease almost exclusively affects hemizygous males in childhood. A small minority of affected females might exhibit a mild phenotype, such as LMWP or hypercalciuria, probably due to the random inactivation of one of the two X chromosomes [9].

In the present study, the clinical characteristics, genetic mutation spectrum, treatment strategies and prognosis of 15 children with Dent disease were retrospectively analyzed to improve pediatricians' awareness of and attention to this disease.

Subjects and methods

Subjects

The clinical data of 15 children with Dent disease (12 DD1 patients and 3 DD2 patients) who were admitted to the Department of Pediatric Nephrology and Rheumatology of Shandong Provincial Hospital from January 2017 to May 2023 were retrospectively analyzed. This study was approved by the Medical Ethics Committee of Shandong Provincial Hospital.

Methods

Clinical data collection

We used the electronic medical records system of the hospital to collect clinical data from the children's medical records. The collected information included sex, age at diagnosis, clinical manifestations, routine blood test results, routine urine test results, the ratio of urinary calcium to creatinine (UC/Ucr), 24-h urinary calcium and protein content, the urinary protein to creatinine ratio (UP/Ucr), retinol-binding protein levels, α 1 microglobulin levels, β 2 microglobulin levels, microalbuminuria, hepatic and renal function test results, electrolyte levels, renal ultrasound, kidney biopsy, gene variations, treatment and prognosis.

The clinical diagnosis of Dent disease is based on the presence of at least two of the following criteria: i) LMWP: early renal injury index indicates that the low-molecular-weight protein concentrations in urine are at least 5 times greater than expected. The low-molecular-weight proteins used for monitoring include retinol-binding protein, α 1 microglobulin, and β 2 microglobulin. Urine protein electrophoresis shows that low-molecular-weight proteins account for more than 50% of urinary proteins. ii) Hypercalciuria: the 24-h urinary calcium value > 0.1 mmol/kg or a random increase in the UC/Ucr ratio, which varies greatly with age. iii) One of the following conditions: nephrocalcinosis, nephrolithiasis, hematuria, hypophosphatemia, or renal insufficiency. The identification of a mutation in either *CLCN5* or *OCRL1* confirms the diagnosis.

Kidney histopathology

Kidney biopsy was performed on six of 15 patients. Kidney tissue samples were obtained using a spring-loaded needle biopsy kit under the real-time guidance of a b-ultrasound detection system. After a percutaneous kidney puncture biopsy, the kidney tissue was stained [hematoxylin-eosin (HE) staining, periodic acid-Schiff (PAS) staining, hexamine silver (PASM) staining and Masson staining], and electron microscopy and immunofluorescence were used to detect IgA, IgG, IgM, Fib, C1q, and C3.

Genetic testing

Whole-exome sequencing (WES) was performed on the patients. Whole blood samples (2–5 mL) were collected from the affected children and their parents. DNA extraction was performed, and the whole exome was captured and sequenced with the IDT xGen Exome Research Panel v2.0 Full Exon Capture Chip. Variants were classified according to the three-factor classification system and the American College of Medical Genetics and Genomics (ACMG) gene variation classification system.

Statistical tests

The statistical software SPSS version 26.0 (IBM Corp., Armonk, NY, USA) was used for the statistical analyses. Quantitative data are expressed as the median with interquartile range (IQR). The Mann-Whitney test and Kruskal-Wallis H test were used for comparisons of two or three groups of nonnormally distributed sample data, respectively. The chi-square test was used to compare the rates between groups. $p < 0.05$ was considered to indicate statistical significance.

Results

Clinical phenotypes of DD1 and DD2

All 12 patients with DD1 were boys, with an age of onset ranging from 1.75 to 10.4 years (median 3.76 years) and an age at diagnosis ranging from 2.1 to 10.57 years (median 6.12 years). Three patients with DD2 were also boys, with an age of onset ranging from 2.50 to 6.29 years (median 5.8 years) and an age at diagnosis ranging from 2.67 to 12.8 years (median 6.87 years). The time from onset to clinical diagnosis was 0.25~48 months (median 24 months) and 2~84 months (median 7 months) in the DD1 and DD2 groups, respectively. There was no significant difference between the two groups ($p > 0.05$) (Table 1).

In the DD1 group, one patient was born prematurely at 32 weeks of gestation for unknown reasons and presented with delayed language development after birth (Patient 3). One patient was born prematurely at 35 weeks plus 5 days due to placenta previa (Patient 12). Three patients had a family history of kidney disease. One patient's grandfather was diagnosed with ESRD, and his mother and elder brother had proteinuria (Patient 8). A younger brother of one patient

Table 1. Clinical features and gene mutations of patients with dent disease.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Group	DD1	DD1	DD1	DD1	DD1	DD1	DD1	DD1	DD1	DD1	DD1	DD1	DD1	DD2	DD2
Sex	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male	Male
Age at onset (years)	8.46	10.4	2.97	4.65	3.66	1.94	3.85	2.9	7.47	1.75	2.99	4.77	5.8	2.5	6.29
Age at diagnosis (years)	9.46	10.57	4.97	8.15	6.5	2.11	7.43	2.93	9.8	5.75	3.49	4.79	12.8	2.67	6.87
Time from onset to diagnosis (months)	12	2	24	42	34	2	43	ND	28	48	6	0.25	84	2	7
24-hour urine protein (mg/kg weight)	40.9	42	51.1	20.37	23.91	13.33	35.8	-	25.96	53.66	34.29	16.9	32.05	65.83	67.39
Urine protein/creatinine ratio	1.77	4.18	3.02	1.1	2.02	2.28	1.46	5.35	1.59	3.87	2.85	0.82	2.48	4.97	3.46
Urine immunoglobulin (mg/L)	47.47	59.34	55.12	48.98	21.24	22.84	52.33	-	17.21	35.95	31.4	16.52	67.74	66.31	102.45
Urine microalbuminuria (mg/L)	160.97	192.71	252.11	104.3	84.07	137.2	188.29	-	104.63	124	129.73	37.86	230	258.7	267.45
Urine transferrin (mg/L)	20.91	19.92	31.63	15.03	11.68	14.02	21.53	-	14.43	9.68	16.06	5.36	25.12	31.32	36.07
Urine α1 microglobulin (mg/L)	286	272	413	303	133	166	190	-	139	275	188	56.6	331	388	415
Urine β2 microglobulin (mg/L)	34.8	210.8	95.5	35.96	77	56.7	115	-	24.34	72.48	52.47	17.8	102.5	196.5	188
Urine retinol-binding protein (mg/L)	5.84	6.01	5.79	6.6	7.12	8.18	8.13	-	9.91	133.98	8.27	9.59	4.95	4.37	3.51
Ratio of small-molecule proteinuria	0.587	0.643	0.603	0.672	0.65	0.57	0.544	-	0.56	0.739	0.584	0.584	0.576	0.623	0.599
Urine calcium/urinary creatinine ratio (mg/mg)	0.49	-	0.539	0.35	0.288	0.087	0.11	0.078	0.69	0.53	0.46	0.46	0.14	0.58	0.49
24-hour urinary calcium (mmol/kg)	0.25	-	0.19	0.14	0.04	-	-	-	0.29	-	0.17	0.2	0.13	0.13	0.11
Hypophosphatemia	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y
Metabolic acidosis	Y	N	N	Y	Y	N	N	N	N	Y	N	Y	Y	Y	Y
Growth deficiency	N	N	N	N	N	N	N	N	N	N	N	N	Y	N	N
Glucosuria	N	N	N	N	N	N	N	N	N	Y	N	N	Y	N	N
Language development delay	N	N	Y	N	N	N	N	N	N	N	N	N	N	N	N
Renal ultrasound	Normal	Normal	Normal	Normal	Normal	Normal	Enhanced renal cone echo	Enhanced renal cone echo	Normal	Normal	Normal	Medullary sponge kidney	Normal	Normal	Normal

(Continued)

Table 1. Continued.

Patient	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Renal pathology	FSGS	MCL	-	-	MCL	-	MCL	-	MCL with small focal acute tubular injury	-	-	-	MCL	-	-
Gene with mutation	CLCN5	CLCN5	CLCN5	CLCN5	CLCN5	CLCN5	CLCN5	CLCN5	CLCN5	CLCN5	CLCN5	CLCN5	OCRL1	OCRL1	OCRL1
Type of mutation	Missense	Missense	Missense	Missense	Frameshift	Frameshift	Frameshift	Frameshift	Frameshift	Nonsense	Nonsense	Nonsense	Missense	Missense	Missense
Nucleotide	c.1756C>T	c.1166T>G	c.1756C>T	c.1618G>A	c.407delT	c.1702_c.1703insC	c.137delC	c.665_666delGGinsC	c.2200delG	c.776G>A	c.1609C>T	c.1152G>A	c.1477C>T	c.952C>T	c.198A>G
Protein	p.Arg586Trp	p.L389R	p.Arg586Trp	p.Gly540Arg	p.L136fs	p.T568Tfs*30	p.A46fs	p.G222Afs*32	p.E734fs*14	p.W259*	p.R537*	p.W384*	p.R493W	p.R318C	p.Q66=
Segregation	de novo	Maternal	Maternal	Maternal	Maternal	Maternal	Maternal	Maternal	Maternal	Maternal	Maternal	Maternal	-	de novo	Maternal
ACMG hazard level	Pathogenic	Uncertain	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Likely pathogenic	Pathogenic	Pathogenic	Likely pathogenic	Pathogenic	Pathogenic	Uncertain
Novel/recurrent mutation	Recurrent	Novel	Recurrent	Recurrent	Novel	Novel	Novel	Novel	Novel	Recurrent	Recurrent	Recurrent	Recurrent	Recurrent	Novel
References	[5,16,22–24,31–34]		[5,16,22–24,31–34]	[21]						[16]	[5,23,35,36]	[37]	[5,11,16,38]	[16,17,26,39,40]	

DD: Dent disease; N: No; Y: Yes; FSGS: focal segmental glomerulosclerosis; MCL: minimal change lesion.

presented with proteinuria (Patient 2). Pyelectasia was found in one patient's father (Patient 7).

The main complaint of all patients with DD1 and DD2 was proteinuria. One patient was misdiagnosed with interstitial nephritis and was treated with prednisone for 1.5 years (Patient 7). One patient was misdiagnosed with glomerular nephritis and was given mycophenolate mofetil (MMF) for 2 months (Patient 9). One patient was diagnosed with nephrotic syndrome, and renal pathology revealed minimal change lesion. He was subsequently treated with prednisone for 2 months at a local hospital (Patient 13).

Proteinuria was observed in all DD1 and DD2 patients. Albustix testing yielded results of 1~3+ and 2~3+ in the DD1 and DD2 groups, respectively. Fourteen patients underwent urinary protein electrophoresis, and the results suggested LMWP, with low-molecular-weight proteins accounting for 54.4~73.9% and 57.6~62.3% of the total urinary protein in the DD1 and DD2 groups, respectively ($p > 0.05$). The 24-h urinary protein concentrations were 13.3~53.66 mg/kg (median 34.29 mg/kg) in the DD1 group and 32.05~67.39 mg/kg (median 65.83 mg/kg) in the DD2 group ($p > 0.05$). The UP/Ucr ratio ranged from 0.82 mg/mg to 5.35 mg/mg (mean 2.15) and 2.48 to 4.97 mg/mg (mean 3.46) in the two groups, respectively ($p > 0.05$) (Table 1).

The UC/Ucr ratios were 0.078~0.69 mg/mg and 0.14~0.58 mg/mg in the DD1 and DD2 groups, respectively ($p > 0.05$). The 24-h urinary calcium concentration ranged from 0.04 to 0.29 mmol/kg and 0.11 to 0.13 mmol/kg in the two groups ($p > 0.05$). Hypercalciuria was observed in 58.3% (7/12) and 66.7% (2/3) of DD1 and DD2 patients, respectively ($p > 0.05$) (Table 1).

Metabolic acidosis was observed in 5 patients (41.7%), glucosuria in 1 patient (8.3%), backward language development in 1 patient (8.3%) and enuresis in 1 patient (8.3%) in the DD1 group. Hypophosphatemia was observed in 2 patients (66.7%), metabolic acidosis in 3 patients (100%), growth deficiency in 1 patient (33.3%), and glucosuria in 1 patient (33.3%) in the DD2 group. The incidence of hypophosphatemia, metabolic acidosis, and growth deficiency was lower in the DD1 group than in the DD2 group ($p < 0.05$). Renal ultrasound revealed slightly enhanced renal cone echo in 2 patients (16.7%) and double renal medullary sponge kidney in 1 patient (8.3%) in the DD1 group (Table 1).

Five patients with DD1 and one patient with DD2 underwent renal biopsy. Biopsy revealed focal segmental glomerulosclerosis (FSGS) in 1 patient, minimal change lesion in 5 patients, and small focal acute tubular injury in 1 patient (Table 1).

Novel and recurrent mutations

DNA sequence analysis revealed that seven of the *CLCN5* and *OCRL1* mutations have not previously been described (c.1166T>G, c.407delT, c.1702_c.1703insC, c.137delC, c.665_666delGGinsC, and c.2200delG in the *CLCN5* gene and c.198A>G in the *OCRL1* gene). The other seven mutation sites were previously described (Table 1).

Of the 11 mutations in the *CLCN5* gene, 3 were missense mutations (25.0%, c.1756C>T, c.1166T>G, and c.1618G>A), 5 were frameshift mutations (41.7%, c.407delT, c.1702_c.1703insC, c.137delC, c.665_666delGGinsC, and c.2200delG), and 3 were nonsense mutations (25.0%, c.776G>A, c.1609C>T, and c.1152G>A). There was no significant difference in age of onset, age of diagnosis or clinical phenotype among patients with different mutation types ($p > 0.05$). We identified 3 mutations in the *OCRL1* gene, all of which were missense mutations (c.1477C>T, c.952C>T, and c.198A>G) (Table 1).

Bioinformatics analysis

The three missense variants of *CLCN5* in this cohort were p.Leu389Arg, p.Gly540Arg, and p.Arg586Trp. These amino acid sequences are highly conserved according to multiple-species sequence alignment with MEGA 7 software. The two CLC-5 monomers form dimers, and all three missense mutations are located on helices involved in the formation of the dimer interface. There was no significant change in hydrogen bonds for p.Leu389Arg and p.Gly540Arg. The proximity of p.Gly540Arg to the transporter interface affects the stability of the helix and thus prevents proper folding of the monomer. p.Arg586Trp leads to the

disappearance of hydrogen bond interactions with Gly583, Ala619, Asp620, and Gly623, forming a new hydrogen bond with Glu625; a mutation in this position may have an effect on the protein structure and thus affect protein function (Figure 1).

The two missense variants of *OCRL1* in this cohort were p.Arg318Cys and p.Arg493Trp. These amino acid sequences are highly conserved according to multiple-species sequence alignment with MEGA 7 software. Arg318Cys has one hydrogen bond with Glu294 and Val320 and two hydrogen bonds with Phe289. After mutation to Cys, these four hydrogen bond interactions disappear, which may have affect the protein structure and thus the protein function. p.Arg318Cys affects the 5-phosphatase domain of *OCRL1*. Arg493Trp has one hydrogen bond with Asp487, and no hydrogen bond changes occur after mutation to Trp (Figure 2).

Treatment and prognosis

All 15 patients received oral benazepril or perindopril. Six patients were given oral potassium citrate, and two patients were treated with dihydrochlorothiazide (HCTZ). Two children were lost to follow-up, and the follow-up time of the remaining 13 patients ranged from 0.75 to 111.2 months (median 46.9 months). At the last follow-up visit, the patients' urinary

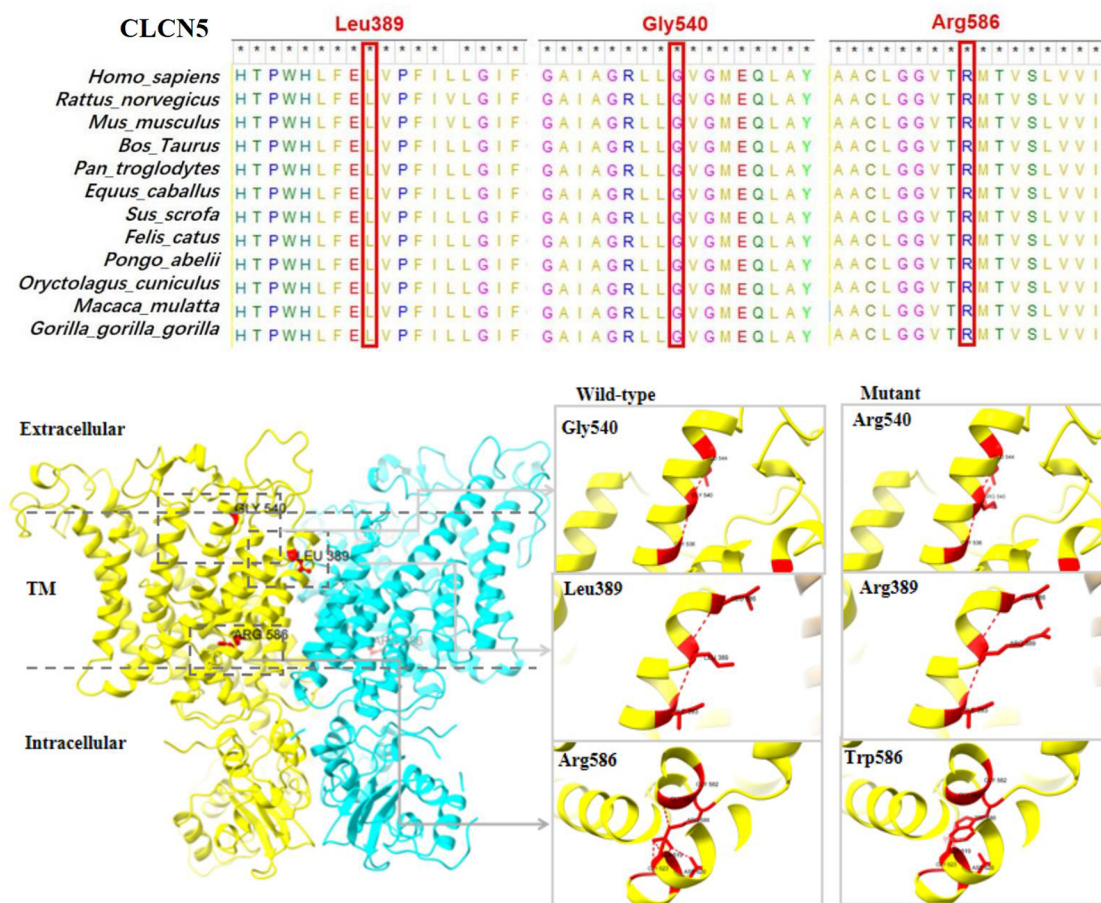


Figure 1. Structural diagram of the protein structure of three missense variants of the *CLCN5* gene (p.Gly540Arg, p.Leu389Arg, and p.Arg586Trp).

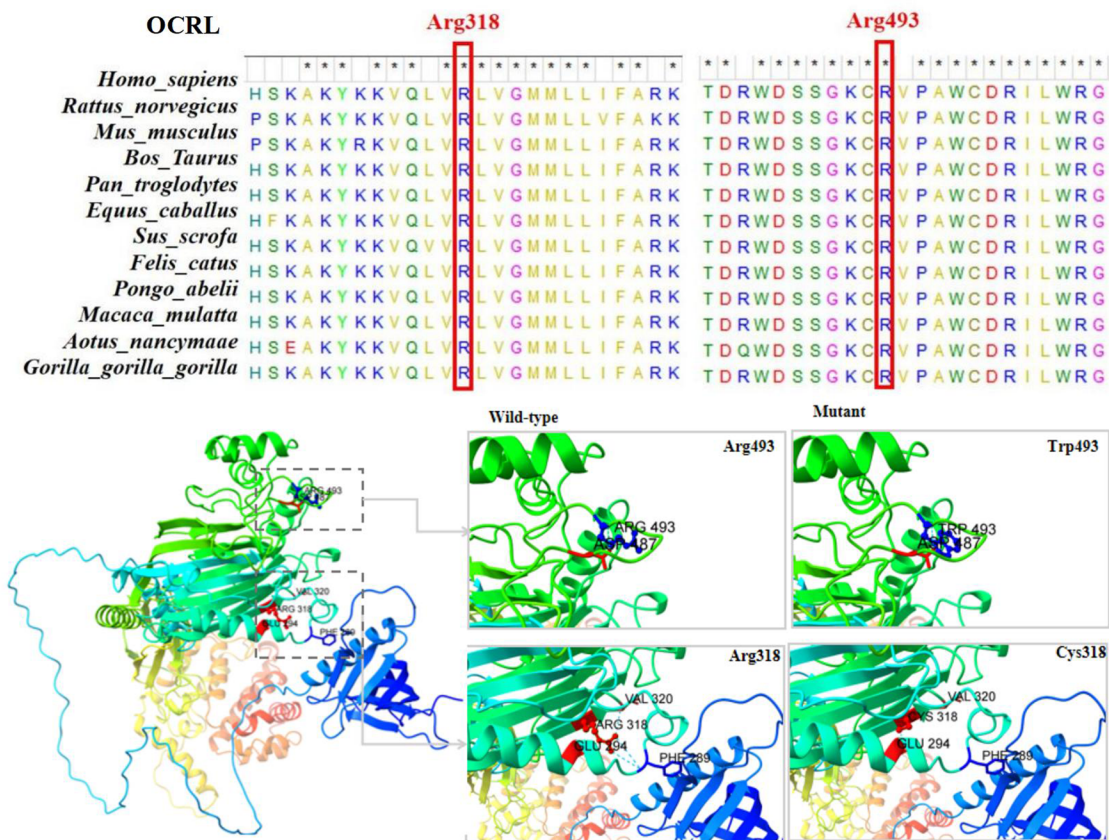


Figure 2. Structural diagram of the protein structure of two missense variants of the OCRL1 gene (p.Arg493Trp, p.Arg318Cys).

protein levels remained positive. Renal function was normal, and serum creatinine levels were within the normal range. Hypercalciuria was observed in three patients with DD1 (Patients 1, 4, 9). Three patients were found to have medullary calcium deposition (Patients 1, 3, 5); one child developed a left kidney stone (Patient 5), and one child had a stroke with moyamoya disease (Patient 9) in the DD1 group.

Discussion

Dent disease is a rare hereditary X-linked recessive renal tubular disease characterized by LMWP and hypercalciuria with or without nephrocalcinosis. Other signs include kidney stones, hematuria, hypophosphatemia, and chronic kidney disease (CKD), which occur at variable frequencies. Occasionally, rickets, osteomalacia, and short stature are observed [10]. Dent disease mainly occurs in male patients, with relatively mild manifestations in female patients.

In our study, all 15 patients were male and complained of proteinuria, and the incidence of LMWP was 100.0% in both DD1 and DD2 patients. The incidence of hypercalciuria was 58.3% (7/12) and 66.7% (2/3) in DD1 and DD2 patients, respectively. Nephrocalcinosis and nephrolithiasis were observed in 16.7% (2/12) and 8.3% (1/12) of DD1 patients, respectively. Hypophosphatemia, metabolic acidosis, glucosuria, growth deficiency, delayed language development, and enuresis were also observed in this study. However, no

rickets, microscopic hematuria, or renal impairments were observed in DD1 or DD2 patients. In one national multicenter study in China enrolling 32 DD1 and 13 DD2 patients [11], the incidence of LMWP was 100.0% in both the DD1 and DD2 populations; the incidence of hypercalciuria was 65.6% (21/32) and 92.3% (12/13), that of nephrocalcinosis was 43.8% (14/32) and 23.1% (3/13), that of abnormal renal function was 12.5% (4/32) and 7.7% (1/13), that of nephrolithiasis was 9.4% (3/32) and 15.4% (2/13), and that of rickets was 9.4% (3/32) and 15.4% (2/13) in DD1 and DD2 patients, respectively. The incidences of LMWP, hypercalciuria, nephrocalcinosis, abnormal renal function, nephrolithiasis and rickets were not significantly different from those in our study ($p > 0.05$). A 46-item web-based cross-sectional survey from the European Rare Kidney Disease Reference Network (ERKNet) identified 163 male patients with genetically confirmed DD1 [12]. During follow-up, all patients had LMWP, 66.4% had nephrocalcinosis, 44.4% had hypercalciuria, and 26.4% had nephrolithiasis. These findings were consistent with those of previous studies of European and American populations and Asian populations [13]. However, there was significant intra- and interfamilial variability in clinical presentations [14]. An increasing number of reports have described subjects carrying *CLCN5* or *OCRL1* mutations who exhibit an incomplete phenotype. This diversity of phenotypes can lead to missed diagnoses, misdiagnosis, inappropriate treatment, or even unnecessary kidney biopsies.

In our study, DNA sequence analysis revealed 11 different mutations in the *CLCN5* gene and three mutations in the *OCRL1* gene. These mutations were classified as frameshift mutations (8 patients), nonsense mutations (3 patients), or missense mutations (3 patients). We compared the clinical data of DD1 patients according to *CLCN5* mutation type and did not observe obvious differences in age of onset, age of diagnosis or clinical phenotype. Previous reports have shown that the distribution of *CLCN5* mutation types is consistent in European and American populations and Asian populations. There was no obvious relationship between mutation types and clinical phenotypes in different populations [13].

Although Dent disease is a proximal tubular disease, steroid-resistant nephrotic syndrome (SRNS), FSGS, and minimal change lesion are the most common clinical misdiagnoses before a molecular diagnosis of Dent disease is made in children [15]. In our study, three patients were misdiagnosed with interstitial nephritis, glomerular nephritis or nephrotic syndrome and were treated with prednisone and/or immunosuppressive agents.

The main reason for misdiagnosis was the lack of urine protein electrophoresis performed to detect the composition of urinary protein. The ratios of low-molecular-weight proteins, including retinol-binding protein, α 1 microglobulin, and β 2 microglobulin, are among the most important criteria for the diagnosis of Dent disease. Nephrotic-range proteinuria or mixed proteinuria in Dent disease patients is often secondary to renal tubular lesions, so the early detection of Dent disease is particularly important. Dent disease is usually detected by asymptomatic proteinuria during urine screening, such as that routinely performed at age 3 or at school in Japan [16]. Therefore, it is suggested that urine screening can aid in early detection of urinary protein concentrations and analysis of the composition of urinary protein, which can identify Dent disease early and reduce the occurrence of misdiagnosis.

A variety of histopathological findings have been reported in renal biopsies from DD1 and DD2 patients. Patients may have normal glomerular and/or tubular compartments or glomerulosclerosis with or without tubular atrophy or interstitial fibrosis [17,18]. In our study, six patients underwent renal biopsy. FSGS was found in 1 patient, minimal change lesion was found in 5 patients, and small focal acute tubular injury was found in one patient. In recent years, glomerular involvement in Dent disease has been hypothesized. Since the discovery that *CLCN5* and *OCRL1* are expressed in the glomerular compartment, a new theory has emerged suggesting that the loss of function of these two proteins leads to primary glomerular cell damage [19]. Glomerular injury is responsible for nephrotic-range proteinuria in more than 30% of patients with Dent disease. However, no correlations have emerged between the type of *CLCN5/OCRL1* mutations and histopathological findings. Renal biopsy is not helpful in the diagnosis of Dent disease because the histological findings are nonspecific; six of our patients underwent a kidney biopsy, but the results did not help to establish a definitive diagnosis.

The screening of patients with Dent disease by next-generation sequencing (NGS) may be a good method for identifying unknown, atypical or mixed phenotypes of other known genetic nephropathies. Genetic screening of males with LMWP and at least one typical associated clinical sign (hypercalciuria, nephrocalcinosis, renal stones, hematuria, and/or hypophosphatemia) led to a Dent disease detection rate ranging from 66 to 92% [20]. To date, approximately 200 *CLCN5* mutations that cause Dent disease have been reported [21]. The main mutation types include missense mutations and frameshift mutations; the other mutation types are nonsense mutations, splicing mutations and large fragment deletions. The frameshift deletion-insertion, donor splice site and nonsense mutations are predicted to result in truncated or absent CIC-5 proteins, which would lead to complete loss of antiporter function and chloride conductance [22]. Missense and synonymous variants can also have severe effects on the function and expression of a protein. The three missense variants of *CLCN5* in this cohort were p.Leu389Arg, p.Gly540Arg, and p.Arg586Trp, which are located on helices involved in the formation of the dimer interface. p.Leu389Arg is located on helix J, and the nearby Leu394Arg mutation has been reported to interfere with channel function and prevent transport to the plasma membrane [23]. The p.Gly540Arg mutation close to the transporter interface has been reported to affect the stability of the helix and thus prevent proper folding of the monomer [21]. In addition, p.Arg586Trp has been reported to cause CIC-5 retention in the endoplasmic reticulum [24].

More than 140 mutations in the *OCRL1* gene, including insertions, deletions, splicing and missense mutations, have been reported to cause Dent disease. Most DD2 mutations are deletions in exons, while the proportions of truncating variants (nonsense and frameshift) and nontruncating variants (missense) are similar. Truncating variants were found in the PH and linker domains, while missense variants were found in the inositol-5-phosphatase domain and only occasionally in the ASH-RhoGAP module [25]. The two missense variants of *OCRL1* in this cohort were p.Arg318Cys and p.Arg493Trp. These 2 variants are recurrent variants and have been reported several times. p.Arg318Cys affects the 5-phosphatase domain of *OCRL1* and is considered a mutational hotspot in the *OCRL1* gene [26]. The Arg493Trp mutation may have an effect on protein structure and thus protein function. Another variant at the same position of Arg493, R493A, has been reported to eliminate 5-phosphatase activity [27].

The clinical management of patients with Dent disease is focused on symptoms rather than any disease-specific treatment [28]. Proteinuria is a key feature of the DD1 and DD2 phenotypes. Angiotensin-converting enzyme inhibitors (ACEis) and angiotensin II receptor blockers (ARBs) have been shown to be effective in reducing proteinuria in Dent disease patients [29]. ACEis/ARBs are well tolerated in children with Dent disease. Thiazide diuretics are commonly used to reduce hypercalciuria in patients with Dent disease. Kidney stones are usually controlled by citrate

supplementation (mainly potassium citrate). Citrates are thought to improve acidosis and slow the progression of CKD [30]. In our study, all 15 patients received benazepril or perindopril orally. Six patients received potassium citrate orally, and two patients were treated with HCTZ. Only 13 patients were followed up from 0.75 to 111.2 months (median 46.9 months). At the last follow-up visit, the urinary protein levels remained positive. Three patients developed hypercalciuria. Three patients were found to have medullary calcium deposition, and one child presented with a left kidney stone. The incidence of nephrolithiasis and nephrocalcinosis increases with increasing age. A survey by ERKNet revealed that hypercalciuria was more common in pediatric patients than in adults, while nephrolithiasis, nephrocalcinosis and renal dysfunction were more common in adults [12].

In summary, pediatric Dent disease is often misdiagnosed. Protein electrophoresis and genetic testing can help to achieve an early and correct diagnosis.

Disclosure statement

No potential conflicts of interest are reported by the authors.

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Data availability statement

The data used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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