Original Article

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Diagnosis and treatment of Dent disease in 10 Chinese boys

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Summary **Dent disease is a rare X-linked recessive proximal tubular disorder that affects mostly male patients in childhood or early adult life. Dent disease is clinically characterized by the presence of low molecular weight proteinuria (LMWP), hypercalciuria, medullary nephrocalcinosis, nephrolithiasis, and progressive renal failure. The clinical features, diagnosis, and treatment of Dent disease were examined in 10 Chinese boys. All 10 childhood cases of Dent disease in China presented with tubular proteinuria in the nephrotic range and hypercalciuria. The ratio of α1-microglobulinuria to microalbuminuria, if close to or above 1, can be used as a diagnostic criterion for tubuloproteinuria. Lotensin was ineffective at treating proteinuria while dihydrochlorothiazide reduced urine calcium excretion.**

Keywords: Dent disease, diagnosis, treatment, childhood, China

1. Introduction

Dent disease is a rare X-linked recessive proximal tubulopathy that presents with hypercalciuria, low molecular weight proteinuria (LMWP), nephrolithiasis, nephrocalcinosis. and progressive renal failure. Based on its phenotype, Dent disease is divided into two types, Dent disease 1 (OMIM #300009) and Dent disease 2 (OMIM#300555). The former is caused by mutations in the *CLCN5* gene on chromosome Xp11.22 and the latter is caused by mutations in the *OCRL* gene on chromosome Xq25. Dent disease mainly affects males, whereas female carriers may exhibit a milder phenotype. Patients are usually diagnosed in childhood or in young adulthood. LMWP is the most consistent feature, occurring in 99% of affected males (*1,2*).

This study has focused on analyzing the clinical features, diagnosis, and treatment of childhood Dent disease in China.

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2. Subjects and Methods

2.1. *Participants*

This study was approved by the ethics committee of the Peking University First Hospital and was conducted in accordance with the guidelines of the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008. Consent was obtained from all patients and their family members.

Data on 10 Chinese patients with childhood Dent disease were collected from January 1, 2014 to December 31, 2015 and retrospectively analyzed. The patients in question belonged to 9 families (Patients 6 and 7 were brothers).

The clinical diagnosis of Dent disease is based on the presence of all three of the following criteria: *i*) LMWP (elevation of urinary excretion of α1-microglobulin at least 100-fold above the upper limit of normality, or LMWP above 50 percent in urine protein electrophoresis); *ii*) hypercalciuria (> 0.1 mmol/kg in a 24-hour urine collection or > 0.21 mg/mg calcium to creatinine ratio in a spot sample); and *iii*) at least one of the following: nephrocalcinosis, kidney stones, hematuria, hypophosphatemia, or renal insufficiency. The identification of a mutation in either *CLCN5* or *OCRL1* confirms the diagnosis (*3,4*).

2.2. *Clinic and laboratory examinations*

Table 1. Clinical data on 10 cases of Dent disease

Patient	Age of onset	Age at diagnosis	Nephrocalcinosis	Renal function	Renal biopsy	Drugs used	Other
No.1	5.7 y	9.8 y	Y	normal	FSGS	Pre, CsA, CTX	N
No.2	4.7 y	5.8 y	N	normal	MCD	Pre, CTX, Tac	N
No.3	4.8 y	5.2 y	N	normal	N	Pre, CsA	N
No.4	3.2 y	4.5 y	N	normal	MsPGN	Pre, CsA, TWP	growth deficiency
No.5	3.2 y	5.7 y	Y	normal	N	Pre, Tac, MMF	N
No.6*	4.1 y	5.8 y	N	normal	FSGS	Pre, CTX, CsA	rickets
No.7*	3.5 y	3.5 y	N	normal	N	N	N
No.8	1.2 y	1.5 y	N	normal	N	N	N
No.9	3.8 y	5.1 y	N	normal	N	Pre, CTX, MMF	growth deficiency
No.10	3.4 y	5.2 y	N	normal	MCD	Pre, CTX, CsA	congenital cataract and growth deficiency

*Patients 6 and 7 are brothers; N: no; Y: yes; CsA: Cyclosporin A; CTX: Cyclophosphamide; FSGS: Focal segmental glomerulosclerosis; MCD: Minimal change disease; MMF: Mycophenolic acid; MsPGN: Mesangial proliferative glomerulonephritis; Pre: Prednisone; Tac: Tacrolimus; TWP: Tripterygium Glycosides.

General information such as age of onset and age at diagnosis and medications used was obtained from all subjects. The ratio of urinary calcium to creatinine (UC/Ucr) was calculated and 24-h urinary calcium (UCE) and protein were quantitatively determined. α1-microglobinuria and microalbuminuria, renal function, and electrolytes were monitored and urine protein electrophoresis and renal ultrasound were performed.

2.3. *Detection of mutations in CLCN5 and OCRL1*

Mutations in *CLCN5* (MIM:300008) and *OCRL1* (MIM:300535) were detected by amplifying the exons of both genes with PCR (*5-7*).

2.4. *Treatment*

Once diagnosis was confirmed, all 10 patients were orally given dihydrochlorothiazide (HCTZ) 0.5-1 mg/kg and potassium citrate 2-3 mmol/kg twice daily, and patients were also instructed to consume a calcium-free diet. Seven patients over the age of 5 and weighing over 20 kg were orally given Lotensin 5 mg once daily, while the remaining 3 patients (Nos. 4, 7, and 8) were not since they were under the age of 5 and weighed less than 20 kg.

2.5. *Statistics*

Statistical analysis was performed with the software SPSS 12.0. Every index was measured three times and expressed as the mean ± SD. Differences before and after treatment were analyzed using a paired-samples *t* test. The relationship between the ratio of α1-microglobulinuria to microalbuminuria and LMWP in urine protein electrophoresis was analyzed using Pearson's correlation coefficient. *p* values less than 0.05 were considered statistically significant.

3. Results

All 10 patients were boys, with an age of onset from 1.2 to 5.7 years and an age at diagnosis from 1.5 to 9.8 years. The median time to clinical diagnosis was a year or longer in 9 patients, and up to 4.1 years in 1. Eight of the patients were administered glucocorticoids and more than one immunosuppressive agent. Five patients underwent a renal biopsy. The biopsy revealed focal segmental glomerulosclerosis (FSGS) in 2 patients, minimal change disease (MCD) in 2, and mesangial proliferative glomerulonephritis (MsPGN) in 1. Nephrocalcinosis was noted in 2 patients (Nos. 2 and 5), growth deficiency was noted in 3 (Nos. 4, 9, and 10), and rickets was noted in 1 (No.10), but renal impairment was not noted. The patient with a *OCRL* gene mutation (No.10) also presented with congenital cataracts (zonular). No other relevant findings such as hypophosphatemia, hyperphosphaturia, hypokalemia, or microscopic hematuria were noted, as shown in Table 1.

All of the patients had proteinuria in the nephrotic range (51-64 mg/kg/24 h), hypercalciuria (UC/Ucr 0.31-0.56, UCE 0.16-0.28 mmol/kg/24h), and aminoaciduria. α1-microglobinuria and microalbuminuria markedly increased, with α1-microglobinuria 100-300-fold above the upper limit of normality. In all of the patients, LMWP was above 50 percent in urine protein electrophoresis and the ratio of α1-microglobulinuria to microalbuminuria was above 1. Those 2 findings were closely correlated ($r = 0.972$, $p < 0.001$). Nine patients carried a mutation in the *CLCN5* gene while one carried a mutation in the *OCRL* gene. The mutations included 8 missense mutations and 1 deletion. Six mutations were noted for the first time, as shown in Table 2.

After 2 weeks of therapy with dihydrochlorothiazide and potassium citrate, the ratio of urinary calcium to creatinine and urinary calcium excretion decreased to a median excretion of 0.16 (range: 0.15-0.25, $n = 10$) and 0.09 mmol/kg/24 h (range: 0.07-0.13, $n = 10$) compared to 0.35 (range: 0.31-0.56, $n = 10$) and 0.19 mmol/kg/24 h (range: 0.16-0.28, $n = 10$) at initial measurement ($p < 0.001$). Eight patients became normocalciuric. Marked hypokalemia was not noted in a follow-up at 6-15 months. The 2 patients who had nephrocalcinosis

Table 2. Laboratory data on 10 cases of Dent disease

Patient	Alb (g/L)	UPE (mg/kg/24 h)	α1MG (mg/L)	MAmg/L	α1MG/MA	LMWP (%)	UC/Ucr	UCE (mmol/kg/24 h)	Aminoaciduria	Gene mutation	
No.1	32 ± 8	64 ± 18	402 ± 113	285 ± 89	1.5 ± 0.12	61.3 ± 9.3	0.31 ± 0.07	0.19 ± 0.02	Y	CLCN5 c.1633A>C	p.S545R
No.2	36 ± 6	58 ± 17	349 ± 125	301 ± 81	1.3 ± 0.14	53.1 ± 7.5	0.56 ± 0.12	0.28 ± 0.03	Y	CLCN5 c.779G>A	p.G260D
No.3	36 ± 8	55 ± 15	305 ± 108	257 ± 76	1.3 ± 0.11	53.5 ± 8.4	0.48 ± 0.13	0.27 ± 0.01	Y	CLCN5 c.259G>T	p.E87X
No.4	32 ± 6	51 ± 16	346 ± 101	273 ± 86	1.3 ± 0.12	51.3 ± 8.1	0.35 ± 0.09	0.19 ± 0.01	Y	CLCN5 c.1744G>C	p.G582R
No.5	35 ± 7	53 ± 14	375 ± 134	268 ± 79	1.4 ± 0.10	56.5 ± 7.2	0.38 ± 0.10	0.24 ± 0.02	Y	CLCN5 c.2110C>T	p.R704X
No.6*	33 ± 8	53 ± 13	382 ± 120	258 ± 82	1.5 ± 0.16	59.5 ± 7.6	0.33 ± 0.09	0.23 ± 0.02	Y	CLCN5 c.731C>T	p.S244L
No.7*	36 ± 7	62 ± 19	353 ± 131	249 ± 73	1.3 ± 0.15	52.8 ± 9.0	0.32 ± 0.10	0.18 ± 0.01	Y	CLCN5 c.731C>T	p.S244L
No.8	36 ± 5	59 ± 17	258 ± 102	197 ± 68	1.4 ± 0.13	56.3 ± 6.5	0.31 ± 0.09	0.19 ± 0.02	Y	CLCN5 del whole gene	p. del whole
No.9	35 ± 6	56 ± 11	341 ± 116	238 ± 62	1.4 ± 0.12	56.1 ± 7.5	0.35 ± 0.09	0.16 ± 0.02	Y	CLCN5 c.1677G>A	p.W559X
No.10	36 ± 5	57 ± 10	355 ± 124	248 ± 61	1.4 ± 0.13	55.9 ± 6.3	0.35 ± 0.12	0.18 ± 0.03	Y	OCRL c.2435T>C	p.L812P

*Patients 6 and 7 are brothers; Y: yes; α1MG: α1-microglobinuria; Alb: Albumin; LMWP: Low molecular weight proteinuria; MA: Microalbuminuria; UC: Urine calcium; UCE: Urine calcium excretion; Ucr: Urine creatinine; UPE: Urinary protein excretion.

(No.2 and No.5) had no changes on renal ultrasound in a follow-up at 8 months and 13 months, respectively. The 7 patients treated with Lotensin had no change in proteinuria in a follow-up at 3-6 months ($p > 0.05$), as shown in Tables 3 and 4.

4. Discussion

Dent disease is an X-linked recessive renal tubulopathy, and LMWP is its most consistent feature. Dent disease mainly affects male children, and female carriers are generally asymptomatic. In two-thirds of patients, the disease is caused by mutations in the *CLCN5* gene, which encodes the electrogenic chloride/proton exchanger ClC-5. A few patients have mutations in *OCRL*, which encodes a phosphatidylinositol-4, 5-biphosphate-5-phosphatase (OCRL). Both ClC-5 and OCRL1 are involved in the endocytic pathway for reabsorption of low molecular weight proteins in the proximal tubules (*8,9*).

There are few reports on mutations in *CNCL5* in Chinese cases of childhood Dent disease (*7*). The clinical characteristics of Dent disease in China are unclear. The current study has explored the clinical features, diagnosis, and treatment of 10 cases of childhood Dent disease in China.

First, all of the current patients had LMWP and hypercalciuria, although proteinuria was in the nephrotic range (51-64 mg/kg/24 h). This finding was similar to the results of other studies, which found that patients with childhood Dent disease all presented with proteinuria in the nephrotic range (*7,10*). This is presumably because Dent disease is incorrectly diagnosed in China. The median time to clinical diagnosis was a year or longer in 9 patients and up to 4.1 years in 1 patient. Eight patients were administered glucocorticoids and more than one immunosuppressive agent. This finding was consistent with the results of other studies, which reported that many patients with Dent disease presented with nephrotic-range proteinuria and some were treated for nephrotic syndrome (*11-13*). The current results suggested that Dent disease should be considered in all male patients with nephrotic-range proteinuria without hypoalbuminemia or edema, and especially in young or adolescent patients, in order to prevent adverse reactions to unnecessarily administered immunosuppressive agents (*14,15*).

Second, there are many factors that may contribute to the underdiagnosis of Dent disease, including incomplete penetrance, variable expressivity, and occurrence in individuals with no family history. To improve detection, urinary low molecular weight proteins should be measured as a practical screening test for male patients with unexplained proteinuria. LMWP was defined by excessive urinary loss of α1-microglobulin or β2-microglobulin or urinary protein electrophoresis. The current results indicated

Table 3. Changes in urine calcium excretion in 10 cases of Dent disease before and after treatment with dihydrochlorothiazide and potassium citrate

Patient	UC/Ucr (g/gcr)			Urine calcium excretion (mmol/kg/24 h)		
	Before	After	Decreased percent	Before	After	Decreased percent
No.1	0.31 ± 0.07	0.15 ± 0.02	51.61%	0.19 ± 0.02	0.09 ± 0.01	52.63%
No.2	0.56 ± 0.12	0.25 ± 0.08	55.36%	0.28 ± 0.03	0.13 ± 0.02	53.57%
No.3	0.48 ± 0.13	0.23 ± 0.06	50.08%	0.27 ± 0.01	0.13 ± 0.01	51.85%
No.4	0.35 ± 0.09	0.17 ± 0.03	51.43%	0.19 ± 0.01	0.08 ± 0.01	57.89%
No.5	0.38 ± 0.10	0.17 ± 0.03	55.26%	0.24 ± 0.02	0.10 ± 0.01	58.33%
No.6*	0.33 ± 0.09	0.16 ± 0.04	51.52%	0.23 ± 0.02	0.10 ± 0.01	56.52%
No.7*	0.32 ± 0.10	0.15 ± 0.03	53.13%	0.18 ± 0.01	0.09 ± 0.01	50.00%
No.8	0.31 ± 0.09	0.15 ± 0.04	51.61%	0.19 ± 0.02	0.08 ± 0.01	57.89%
No.9	0.35 ± 0.09	0.16 ± 0.05	54.29%	0.16 ± 0.02	0.07 ± 0.02	56.25%
No.10	0.35 ± 0.12	0.16 ± 0.08	54.29%	0.18 ± 0.03	0.08 ± 0.02	55.56%
Statistics	$t = 13.254$	$p < 0.001$		$t = 16.515$	$p < 0.001$	

*No.6 and No.7 are brothers; UC: Urine calcium; UCE: Urine calcium excretion; Ucr: Urine creatinine.

Table 4. Changes in urine protein excretion before and after treatment with Lotensin in 7 cases of Dent disease

Patient	α1MG (mg/L)		MA (mg/L)		UPE (mg/kg/24 h)		LMWP (%)	
	Before	After	Before	After	Before	After	Before	After
No.1	402 ± 113	389 ± 135	285 ± 89	276 ± 84	64 ± 18	58 ± 17	61.3 ± 9.3	58.7 ± 6.8
No.2	349 ± 125	354 ± 133	301 ± 81	315 ± 89	58 ± 17	55 ± 18	53.1 ± 7.5	57.4 ± 8.3
No.3	305 ± 108	312 ± 112	257 ± 76	248 ± 78	55 ± 15	58 ± 13	53.5 ± 8.4	51.7 ± 9.6
No.5	375 ± 134	368 ± 129	268 ± 79	271 ± 75	53 ± 14	50 ± 16	56.5 ± 7.2	55.6 ± 9.2
No.6	382 ± 120	371 ± 127	256 ± 82	267 ± 85	53 ± 13	55 ± 15	59.5 ± 7.6	58.7 ± 8.2
No.9	341 ± 116	338 ± 107	239 ± 62	227 ± 58	56 ± 11	54 ± 12	56.1 ± 7.5	54.2 ± 6.7
No.10	355 ± 124	351 ± 115	248 ± 61	219 ± 63	57 ± 10	55 ± 14	55.9 ± 6.3	51.6 ± 6.8
Statistics	$t = 1.303$	$p = 0.240$	$t = 0.785$	$p = 0.462$	$t = 1.341$	$p = 0.229$	$t = 1.131$	$p = 0.301$

α1MG: α1-microglobinuria; LMWP: Low molecular weight proteinuria; MA: Microalbuminuria; UPE: Urinary protein excretion.

that urinary α1-microglobinuria increased markedly (*i.e.*100-300-fold above the upper limit of normality) in all 10 patients. The ratio of α1-microglobulinuria to microalbuminuria was above 1, and this finding was closely correlated with the percentage of low molecular weight proteins in urinary protein electrophoresis ($r = 0.972$, $p < 0.001$).

Third, patients with Dent disease are currently given supportive care in order to prevent nephrolithiasis. The current results indicated that the ratio of urinary calcium to creatinine and urinary calcium excretion decreased after 2 weeks of therapy with dihydrochlorothiazide and potassium citrate. Eight of the patients became normocalciuric. This finding is similar to that reported in other studies (*12,16,17*). Angiotensin-converting enzyme inhibitors (ACEI) have been suggested as an option to control proteinuria in Dent disease, but the current results indicated that Lotensin was ineffective at treating proteinuria in 7 patients according to a follow-up at 3-6 months ($p > 0.05$). This finding is similar to the results of other studies (*12,13,18*). However, Copelovitch *et al*. (*19*) noted moderate improvement in proteinuria in a patient with Dent disease after initiation of enalapril. Further studies need to be conducted with larger cohorts in order to establish treatment guidelines for Dent disease.

The current study has reported the clinical features, diagnosis, and treatment of 10 cases of childhood Dent disease in China. All of the cases presented with proteinuria in the nephrotic range, hampering diagnosis. Results suggested that the ratio of α1-microglobulinuria to microalbuminuria, if close to or above 1, can be used as a diagnostic criterion for tubuloproteinuria. Dihydrochlorothiazide and potassium citrate effectively controlled hypercalciuria while Lotensin was ineffective at treating proteinuria.

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