Phenotypic spectrum and antialbuminuric response to angiotensin converting enzyme inhibitor and angiotensin receptor blocker therapy in pediatric Dent disease

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Abstract
Background: To characterize the phenotypic spectrum and assess the antialbuminuric response to angiotensin converting enzyme (ACE) inhibitor and/or angiotensin receptor blocker (ARB) therapy in a cohort of children with Dent disease.

Methods: The patients’ clinical findings, renal biopsy results, genetic and follow-up data were analyzed retrospectively. Mutations in CLCN5 or OCRL were detected by next-generation sequencing or Sanger sequencing.

Results: Of 31 Dent disease boys, 24 carried CLCN5 and 7 carried OCRL mutations. Low molecular weight proteinuria and albuminuria were detected in all cases. Nephrotic-range proteinuria and severe albuminuria were identified in 52% and 62% of cases, respectively; by 7 years of age, 6 patients had hematuria and nephrotic-range proteinuria, and 7 patients had hematuria and moderate to severe albuminuria. In addition to disease-related renal features, patients with Dent-1 disease also presented with congenital cataract (1/9) and developmental delay (2/7). Seventeen of 31 patients underwent renal biopsy. Glomerular changes included mild glomerular lesions, mesangial proliferative glomerulonephritis and focal segmental glomerular sclerosis. Thirteen of the 31 patients had follow-up records and received ACE inhibitor and/or ARB treatment for more than 3 months. After a median 1.7 (range 0.3–8.5) years of treatment, a reduction in the urinary microalbumin-to-creatinine ratio was observed in 54% of children.

Conclusions: Hematuria with nephrotic-range proteinuria or moderate to severe albuminuria was common in Dent disease patients. Extrarenal manifestations were observed in Dent-1 patients, which extends the phenotypic spectrum. In addition, ACE inhibitors and ARBs are well tolerated, and they are partially effective in controlling albuminuria.

KEYWORDS
albuminuria, CLCN5, Dent disease, OCRL
Dent disease is a rare X-linked recessive chronic kidney disease (CKD). Proximal renal tubular dysfunction, caused by mutations in the CLCN5 (OMIM# 300008, classified as Dent-1 disease, accounting for approximately 60%) or OCRL (OMIM# 300535, classified as Dent-2 disease, accounting for 15%) genes, leads to the clinical manifestations of this disease (Zhang, Fang, Xu, & Shen, 2017). Dent disease is characterized by low molecular weight (LMW) proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and chronic renal failure (Lieske et al., 1993), and LMW proteinuria is the essential phenotype (Sekine et al., 2014). Affected males usually do not progress to end-stage renal disease before the age of 30 years (Devuyst & Thakker, 2010). Since proteinuria is always the initial symptom, and since nephrotic-level proteinuria can be detected in more than half of children with Dent disease (van Berkel, Ludwig, van Wijk, & Bokenkamp, 2017), it is not unusual for the disease to be misdiagnosed and mistreated (Wang et al., 2016). Therefore, understanding the phenotypic spectrum of Dent disease is helpful to further improve the reorganization of this disorder.

Recently, a study (Wang et al., 2016) showed that, in patients with Dent disease, glomerular pathology, including focal global glomerulosclerosis and foot process effacement, was common and was associated with renal function progression. As a principal biomarker for renal dysfunction, albuminuria increased as well as LMW proteins in the course of Dent disease (Kubo et al., 2016). These results indicated that angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) might be potential interventions for Dent disease. However, there is a scarcity of data for the effectiveness of these drugs on albuminuria in Dent disease.

The aim of the present study was to characterize the phenotypic spectrum and assess the antialbuminuric response to ACE inhibitor and/or ARB therapy in a Chinese cohort of children with Dent disease.

2 | SUBJECTS AND METHODS

2.1 | Ethical compliance

This study was performed in accordance with the Declaration of Helsinki, and the Ethical Committee of Peking University First Hospital approved this project.

2.2 | Patients

Data for this study were collected retrospectively from our hereditary renal diseases registry database initiated in 2012. The following criteria were used to enroll patients: (1) LMW proteinuria; and (2) mutations in CLCN5 or OCRL detected by next-generation sequencing or Sanger sequencing. Patients were excluded if their age at onset of disease was over 18 years, if they were diagnosed with Lowe syndrome, or if their medical records were not available (Figure 1).

2.3 | Research methods

Patient information, including sex, race, age, clinical findings (age at onset of disease, first symptoms, kidney and extrarenal features), renal biopsy results, genotypes and follow-up data, were reviewed.

LMW proteinuria was defined as spot morning urine sample α1-microglobulin (α1-MG) 5 times above the upper limit of normal (Lieske et al., 1993). Mild, moderate and severe albuminuria referred to microalbumin-to-creatinine ratios (mg/g, ACR) <30, 30–300, and ≥300, respectively (Levin & Stevens, 2014). Nephrotic-range proteinuria was defined as 24-hr urine protein > 50 mg/kg. Hypercalciuria was defined as urinary calcium > 0.1 mmol/kg/d or a calcium-to-creatinine ratio above the 95th percentile of reference values in random urine (Lieske et al., 1993). Since the level of serum phosphate in children varies with age, an age-based serum phosphate reference was used to diagnose hypophosphatemia.
(Ruppe, 1993). Nephrocalcinosis was diagnosed by renal ultrasound. The glomerular filtration rate (GFR) was estimated using 24-hr endogenous creatinine clearance (Ccr) (National Kidney, 2002), and the Schwartz formula (Schwartz, Brion, & Spitzer, 1987) was used when 24-hr Ccr was unavailable. Renal dysfunction was defined as a GFR below 75 ml/min/1.73 m² (Pottel, Hoste, & Delanaye, 2015). Short stature was defined as height below the 3rd percentile of the reference for Chinese children. Intellectual disability and developmental delay were evaluated by Gesell developmental schedules, Wechsler intelligence scale or Raven intelligence test. Rickets was diagnosed by bone X-ray or physical examination. When there were more than two measurements of corresponding parameters in a month, we calculated the mean values and used them for subsequent analysis.

Genomic DNA, extracted from peripheral blood leukocytes in all participants, was analyzed by next generation sequencing or Sanger sequencing. Whenever DNA from first-degree relatives of the participants were available, segregation analysis was performed by Sanger sequencing. The interpretation of unpublished variants in \textit{CLCN5} or \textit{OCRL} was performed according to American College of Medical Genetics and Genomics (ACMG) guidelines (Richards et al., 2015).

3 \ RESULTS

As shown in Figure 1, a total of 31 male patients with Dent disease were included in this study. They were from 14 provinces in China and were predominantly of Han Chinese ethnicity (25/29), with the remainder being Hui, Manchu, Bai and Mongolian nationality.

All patients had a genetic diagnosis at the median age of 6.3 (range 1.7–16.8) years old. The median duration from disease onset to genetic diagnosis was 31.2 (range 2.4–187.2) months.

The different \textit{CLCN5} and \textit{OCRL} mutation categories detected in the present study are summarized in Table 1. Almost half of these mutations were not published previously. Table 2 lists the interpretation of these novel mutations according to ACMG guidelines. Among them, 13 variants were classified as pathogenic. Patients 7, 26 and 30 were highly suspected of Dent disease based on LMW proteinuria and hypercalciuria with or without nephrocalcinosis; thus, the novel variants identified in these three patients were classified as clinically pathogenic.

Of 31 patients, 22 patients (71%) were misdiagnosed. Among them, 19 patients received inappropriate steroid and/or immunosuppressive therapy, and genetic diagnosis was made at the median age of 6.9 (range 3.8–16.8) years old. The median duration from onset of disease to genetic diagnosis in these patients was 30 (range 2.4–187.2) months.

### Table 1: Type of mutations in Dent disease cohort

| Types of mutations | Number of patients | Number of novel mutations |
|--------------------|--------------------|--------------------------|
| Dent-1 disease (\textit{CLCN5}) | Missense 5 (20.8%) | 2 |
| Nonsense 8 (33.3%) | 1 |
| Frame shift 9 (37.5%) | 8 |
| Deletion 1 (4.2%) | 1 |
| Splice 1 (4.2%) | 1 |
| Total, n % | 24 | 13 |
| Dent-2 disease (\textit{OCRL}) | Missense 4 (57.1%) | 2 |
| Nonsense 1 (14.3%) | 1 |
| Frame shift 1 (14.3%) | 0 |
| Deletion 1 (14.3%) | 0 |
| Total, n % | 7 | 3 |

3.1 \ Characteristics of onset symptoms

The median age at disease onset was 3.7 (range 0.2–12.3) years old. Manifestations (including abnormal urine color, multiple urine foam, frequent micturition and edema) at disease onset were observed in 13 males (42%) at the median age of 4 (range 0.2–7.3) years, and 18 patients (58%) were asymptomatic; they were found accidentally for other reasons at the median age of 3.4 (range 0.3–12.3) years old.

3.2 \ Renal features

A description of the renal manifestations before treatment is given in Table 3. LMW proteinuria and albuminuria were detected in all cases. The median percentage of urinary albumin detected by urine protein electrophoresis was 36% (range 24.2%–47.6%). Nephrotic-range proteinuria and severe albuminuria were identified in 52% (16/31) and 62% (18/29) of patients, respectively. In patients younger than 7 years old, the percentages of nephrotic-range proteinuria and severe albuminuria were 65% (13/20) and 83% (15/18), respectively. Notably, nephrotic-range proteinuria and severe albuminuria were detected in 83% (5/6) and 100% (5/5) of patients younger than 3 years old, respectively (Figure 2). Microscopic hematuria was detected in 32% (10/31) of patients. Before the age of 7 years, 6 patients had hematuria and nephrotic-range proteinuria, 7 patients had hematuria and moderate to severe albuminuria. Hypercalciuria was detected in 87% (27/31) of patients and occurred at all ages, whereas only 33% (9/27) presented with hematuria. Twenty-seven patients underwent
## TABLE 2 16 CLCN5 and OCRL novel mutations identified

| Pt.ID | Gene | Chromosome position | Location | Nucleotide changes | Amino acid changes | Segregation | ACMG | Interpretation |
|-------|------|---------------------|----------|-------------------|-------------------|-------------|------|----------------|
| 1     | CLCN5| chrX:49840517       | EX 7     | c.483delA         | p.Gly163Aspfs*45  | maternal    | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 3     | CLCN5| chrX:49854931-49854934 | EX 13   | c.1903_1906delAATG | p.Asn635Aspfs*20  | maternal    | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 4     | CLCN5| chrX:49851383-49851384 | EX 11   | c.1413_1414delTG  | p.Cys471X         | maternal    | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 7     | CLCN5| chrX:49853517       | EX 12    | c.1720A>G         | p.Met574Val       | maternal    | PM1 PM2 PP3 PP4     | Likely pathogenic |
| 11    | CLCN5| chrX:49850643       | EX 10    | c.940delT         | p.Ser314Argfs*10  | NA          | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 13    | CLCN5| chrX:49855003       | EX 13    | c.1975delC        | p.Arg659Glyfs*7   | maternal    | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 16    | CLCN5| chrX:49806925-49856876 | EX 4-15 | c.17-2451del      | -                | NA          | PVS1 PM1 PM2 PM4 PP4 | Pathogenic |
| 17    | CLCN5| chrX:49850695-49850696 | EX 10   | c.992-993insAGTATTAT | p.Pro334fs*1     | maternal    | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 18    | CLCN5| chrX:49850692       | EX 10    | c.989G>A          | p.Gly330Asp       | de novo     | PS2 PM1 PM2 PM5 PP3 PP4 | Pathogenic |
| 19    | CLCN5| chrX:49854917-49854918 | EX 13   | c.1889-1990delC  | p.His631Thrfs*25  | maternal    | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 21    | CLCN5| chrX:49854771       | IVS12    | c.1745-2A>G       | Splice defect     | maternal    | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 22    | CLCN5| chrX:49854779       | EX 13    | c.1751delT        | p.Val584Glyfs*4   | maternal    | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 23    | CLCN5| chrX:49851414       | EX 11    | c.1444delG        | p.Gly483Vfs*21    | maternal    | PVS1 PM2 PM4 PP3 PP4 | Pathogenic |
| 26    | OCRL | chrX:128722956      | EX 22    | c.2435T>C         | p.Leu812Pro       | maternal    | PM1 PM2 PP4         | Uncertain significance |
| 30    | OCRL | chrX:128696629      | EX 12    | c.1110C>G         | p.Cys370Trp       | maternal    | PM1 PM2 PP3 PP4     | Likely pathogenic |
| 31    | OCRL | chrX:128691332      | EX 5     | c.269G>A          | p.Trp90X          | maternal    | PVS1 PM2 PM3 PP4    | Pathogenic |

Note: We login Human Gene Mutation Database (HGMD) to check novel mutations in July, 2019. Accession no: CLCN5 (OMIM#300008), NM_001127899.4; OCRL (OMIM#300535), NM_000276.4; NA, not available.

*Agarose gel electrophoresis showed that Patient 16 has a deletion from exon 3 to 15.*
| Pt.ID | Diagnosis | Age at examination before treatment/onset (years) | Nephrotic-range proteinuria | Urinary α1-MG/Cr (mg/g) | Percentage of urinary albumin | Urinary ACR (mg/g) | Nephrocalcinosis (age, years) | Hypercalciuria (age, years) | Hematuria | Hypophosphatemia | Abnormal GFR |
|-------|-----------|--------------------------------------------------|-----------------------------|--------------------------|-------------------------------|-------------------|--------------------------------|--------------------------------|-----------|----------------|----------------|
| D-1   | Dent-1    | 5.3/4.8                                          | Yes                         | 1,125.52                 | 56.2                          | 29                | Severe                         | − (5.3)                        | Yes       | No             | No            |
| D-2   | Dent-1    | 1/0.8                                           | Yes                         | 1,184.68                 | 51.4                          | 40                | Severe                         | + (1)                          | No        | No             | No            |
| D-3   | Dent-1    | 5.6/1                                           | Yes                         | 578.86                   | 54.9                          | 39.8              | Severe                         | − (5.6)                        | No        | No             | No            |
| D-4   | Dent-1    | 16.6/8                                          | No                          | 289.5                    | 55.5                          | 35.1              | Moderate                        | − (16.6)                       | Yes       | No             | No            |
| D-5   | Dent-1    | 3.8/1.5                                         | No                          | 425.55                   | 64.4                          | 32.6              | Moderate                        | − (3.8)                        | No        | No             | No            |
| D-6   | Dent-1    | 4.3/3.2                                         | Yes                         | 583.85                   | 48.2                          | 40.6              | Severe                         | − (4.3)                        | No        | No             | No            |
| D-7   | Dent-1    | 15.4/4                                          | No                          | 456.3                    | 50.1                          | 36.1              | Moderate                        | + (15.4)                       | No        | No             | No            |
| D-8   | Dent-1    | 13.1/3                                          | No                          | 514.23                   | 51.4                          | 38.4              | Moderate                        | + (13.2)                       | Yes       | No             | No            |
| D-9   | Dent-1    | 2.1/2                                           | Yes                         | 1,350.45                 | 47.3                          | 37.8              | Severe                         | + (3.8)                        | Yes       | Yes            | No            |
| D-10  | Dent-1    | 3.8/1.8                                         | Yes                         | 987.55                   | 42.9                          | 45.1              | Severe                         | + (3.7)                        | Yes       | Yes            | No            |
| D-11  | Dent-1    | 6.8/1                                           | Yes                         | 678.37                   | 60.4                          | 29.7              | Moderate                        | − (6.8)                        | Yes       | No             | No            |
| D-12  | Dent-1    | 4.8/4.7                                         | No                          | 485.2                    | 53.6                          | 35                | Moderate                        | − (4.8)                        | No        | No             | No            |
| D-13  | Dent-1    | 13.5/12.3                                       | Yes                         | 667.57                   | 44.4                          | 39.8              | Severe                         | − (12.3)                       | No        | No             | Yes           |
| D-14  | Dent-1    | 9.3/5.9                                         | No                          | 499.92                   | 60.6                          | 26.3              | Moderate                        | NA                             | No        | No             | No            |
| D-15  | Dent-1    | 2.1/2                                           | No                           | Increased                | 62.9                          | 32.8              | NA                             | Yes                           | + (2.1)   | No             | No            |
| D-16  | Dent-1    | 3.7/3.7                                         | Yes                         | 1,053.73                 | 42.9                          | 45.1              | Severe                         | + (3.7)                        | Yes       | Yes            | No            |
| D-17  | Dent-1    | 14/12                                           | Yes                         | 933.97                   | 52.8                          | 36                | Severe                         | − (14)                         | No        | No             | No            |
| D-18  | Dent-1    | 5.4/4.8                                         | No                          | 688.91                   | 61.3                          | 24.2              | Severe                         | − (5.4)                        | No        | No             | No            |
| D-19  | Dent-1    | 2.3/0.3                                         | Yes                         | 1,112.5                  | 60.4                          | NA                | Severe                         | − (1.9)                        | No        | No             | No (1.9 years) |
| D-20  | Dent-1    | 8.3/7.3                                         | No                          | 436.61                   | 52.5                          | 34.4              | Moderate                        | − (8.3)                        | Yes       | No             | No            |
| D-21  | Dent-1    | 5.7/0.6                                         | No                          | 999.6                    | 54.9                          | 38.3              | Severe                         | − (5.7)                        | No        | Yes            | Yes           |
| D-22  | Dent-1    | 2.3/2.1                                         | Yes                         | 1,091.55                 | 53.4                          | 38.8              | Severe                         | + (2.3)                        | No        | No             | No            |
| D-23  | Dent-1    | 3.7/3.6                                         | Yes                         | Increased                | NA                            | NA                | NA                             | Yes                           | − (4)     | No             | No (4 years) |
| D-24  | Dent-1    | 10.3/7.3                                        | No                          | 344.9                    | 53.4                          | 35.7              | Moderate                        | − (8.8)                        | No        | No             | No (9.3 years) |
| D-25  | Dent-2    | 15.6/0.7                                        | No                          | 112.55                   | 57.6                          | 31.9              | Moderate                        | Yes                            | NA        | No             | Yes           |
| D-26  | Dent-2    | 5/3.9                                           | No                          | 904.63                   | 64.4                          | 28.4              | Severe                         | − (4.9)                        | Yes       | No             | No            |
| D-27  | Dent-2    | 5.9/5.8                                         | No                          | 653.88                   | 50.6                          | 36                | Severe                         | − (5.9)                        | No        | No             | No            |
| D-28  | Dent-2    | 1.6/0.2                                         | Yes                         | 1,918.23                 | 51.8                          | 34                | Severe                         | − (1.6)                        | Yes       | No             | No            |
| D-29  | Dent-2    | 5.8/4.2                                         | Yes                         | 774.55                   | 49.3                          | 37                | Severe                         | − (5.8)                        | Yes       | Yes            | Yes           |
| D-30  | Dent-2    | 8.1/7.2                                         | Yes                         | 518.09                   | 62.6                          | 31                | Moderate                        | + (8.1)                        | No        | No             | No            |
| D-31  | Dent-2    | 9.5/3.8                                         | No                          | 567.4                    | 39.6                          | 47.6              | Severe                         | Yes                            | NA        | No             | No            |

**Note:** ACR, microalbumin-to-creatinine ratio; NA, not available; Percentage of LMWP and urinary albumin: analyzed by urinary protein electrophoresis; UPE, urine protein excretion; α1-MG/Cr, urinary α1-microglobulin-to-creatinine ratio.

*Renal function evaluated by Schwartz formula.
renal ultrasound, no nephrolithiasis was identified, whereas nephrocalcinosis was detected in 30% (8/27) of patients. There were only 15 patients who underwent urinary amino acid analysis, and all of them had aminoaciduria. Only four of 27 (15%) patients manifested abnormal GFRs. Among them, one patient (patient 13) presented with nephrotic-level proteinuria (3.6 g/24 hr), bilateral renal cysts, and renal dysfunction (Ccr was 70 ml/min) at the age of 12.9 years. A half years later, he still manifested with nephrotic-level proteinuria (3.02 g/24 hr) while his renal dysfunction progressed (Ccr was 48 ml/min). Bilateral renal cysts may lead to the patient’s renal dysfunction. There were no significant differences in renal features between patients with Dent-1 disease and Dent-2 disease (Table 4).

### 3.3 Extrarenal manifestations

As shown in Table 4, height was affected in 30% (9/30) of patients. Of these patients, 7 patients received steroid therapy, and 5 patients were treated with steroids for more than 6 months. Short stature was more common in patients with Dent-2 disease. In addition to Dent-2 patients, Dent-1 patients also presented with congenital cataract which was diagnosed by ophthalmologist (1/9) and developmental delay (2/7). The patient with Dent-1 disease and congenital cataract had a history of two-week treatment with dexamethasone 10 years ago. He did not receive any corticosteroids before the diagnosis of congenital cataract was made. Rickets was detected in four patients; however, the level of serum 25-hydroxyvitamin D in two of three patients who underwent this examination was deficient, so vitamin D deficiency associated rickets could not be excluded.

### 3.4 Renal pathology

Seventeen of 31 patients (55%) underwent a renal biopsy because of moderate to severe albuminuria or nephrotic-range proteinuria, and 3 patients received a repeated renal biopsy. The histopathological findings are summarized in Table 5. The median interval between the age at disease onset and age at renal biopsy was 1.55 (range 0–11.5) years. Glomerular changes, including mild glomerular lesions, mesangial proliferative glomerulonephritis (MsPGN) and focal segmental glomerular sclerosis (FSGS), were found. Nine patients (53%) had mild
| Pt.ID | Age at onset/renal biopsy (years) | Percentage of urinary albumin closed to renal biopsy | Pathological description                  | Age at treatment/length of treatment (years) | Urinary ACR (mg/g) | Before treatment | After treatment | Decreased percent |
|-------|---------------------------------|--------------------------------------------------|------------------------------------------|---------------------------------------------|-------------------|-----------------|-----------------|------------------|
| 1     | 4.8/5.2                         | 29%                                              | MsPGN                                    | 5.3/1.7                                     | 377               | 192.45          | 49%             |
| 4     | 8/11                            | NA                                               | MsPGN                                    | NA                                          | –                 | –               | –               |
| 6     | None                            | –                                                | –                                        | 4.3/6.7                                     | 477.29            | 474.89          | 0.5%            |
| 7     | 4/15.4                          | 36.1%                                            | Mild glomerular lesions                  | NA                                          | –                 | –               | –               |
| 8     | 3/3.1                           | 21%                                              | MsPGN with tubulointerstitial nephritis  | 13.1/0.6                                    | 205.58            | 322.17          | –57%            |
| 10    | None                            | –                                                | –                                        | 3.8/1.8                                     | 843.59            | 1,484.51        | –76%            |
| 11    | 1/5.5                           | NA                                               | MsPGN                                    | NA                                          | –                 | –               | –               |
| 13    | 12.3/13.1                       | 30.2%                                            | FSGS                                     | –                                           | –                 | –               | –               |
| 13    | 12.3/15.1                       | 32.6%                                            | FSGS with chronic tubulointerstitial damage | 15.4/1.9                                   | 1,087.32          | 893.82          | 45%             |
| 14    | 5.9/7.6                         | NA                                               | Mild glomerular lesions                  | NA                                          | –                 | –               | –               |
| 14    | 5.9/11.4                        | 31.3%                                            | Chronic tubulointerstitial nephropathy with ischemic renal damage | NA | – | – | – |
| 17    | 12/12.1                         | 43.3%                                            | Mild glomerular lesions                  | NA                                          | –                 | –               | –               |
| 18    | 4.8/4.8                         | NA                                               | Mild glomerular lesions                  | 5.4/4.3                                     | 468.42            | 533             | –14%            |
| 18    | 4.8/6.3                         | 39.7%                                            | Tubular injury with mild glomerular lesions | – | – | – | – |
| 19    | None                            | –                                                | –                                        | 2.3/1.5                                     | 504.53            | 500.93          | 0.7%            |
| 21    | 0.6/5.5                         | 38.3%                                            | Tubular interstitial nephritis with mild glomerular lesions | NA | – | – | – |
| 23    | 3.6/3.7                         | 38.5%                                            | Mild glomerular lesions                  | 3.7/1.3                                     | 291.72            | 183.68          | 37%             |
| 24    | 7.3/8.8                         | NA                                               | Mild glomerular lesions                  | 10.7/0.3                                    | 185.5             | 229.84          | –24%            |
| 25    | 0.7/11                          | NA                                               | MsPGN                                    | NA                                          | –                 | –               | –               |
| 26    | 3.9/5.1                         | 28.4%                                            | Mild glomerular lesions                  | 5.7/0.4                                     | 13,302            | 12,874          | 3%              |
| 27    | None                            | –                                                | –                                        | 5.94/5.1                                    | 407.19            | 678.79          | –67%            |
| 28    | None                            | –                                                | –                                        | 1.6/8.5                                     | 1,242             | 408.2           | 67%             |
| 29    | 4.2/5.8                         | 37%                                              | FSGS                                     | NA                                          | –                 | –               | –               |
| 30    | 7.2/7.7                         | 67.9%                                            | MsPGN with tubular injury                | 8.1/0.3                                     | 262.96            | 322.95          | –23%            |
| 31    | 3.8/5.4                         | NA                                               | Mild glomerular lesions                  | NA                                          | –                 | –               | –               |

Note: ACR, microalbumin-to-creatinine ratio; MsPGN, mesangial proliferative glomerulonephritis; FSGS, focal segmental glomerular sclerosis; NA, not available.
glomerular lesions, and the median age at biopsy was 5.5 (3.7–15.4) years old, whereas the mean percentage of urinary albumin examined at the time closest to the renal biopsy was 36.9% ($n = 5$). Among them, patient 14 received a repeated renal biopsy 3.8 years later, and global sclerosis was observed in 7/21 glomeruli, which may be related to chronic tubulointerstitial nephropathy and ischemic renal damage. Patient 18 underwent a repeated renal biopsy 1.5 years later, and tubular injury with mild glomerular lesions was observed. Six patients (35%) had MsPGN, and the median age at biopsy was 6.6 (range 3.1–11) years old, whereas the mean percentage of urinary albumin analyzed at the time closest to the renal biopsy was 39.3% ($n = 3$). The remaining 2 patients (12%), who underwent a renal biopsy at the age of 13.1 and 5.8 years, respectively, had FSGS, and the percentage of urinary albumin detected at the time closest to the renal biopsy was 30.2% and 37%, respectively. In addition to FSGS, chronic tubulointerstitial damage was observed in patient 13’s repeated renal biopsy specimen (biopsy performed 2 years later).

3.5 | Safety and the effects of ACE inhibitors and ARBs on albuminuria

Thirteen of 31 patients had follow-up data and received ACE inhibitor (mainly refers to benazepril, mean dose 0.30 mg/kg/d) and/or ARB (losartan, mean dose 0.79 mg/kg/d) treatment for more than 3 months (Table 5 and Figure 3). The median age at onset of treatment was 5.4 (range 1.6–15.4) years, and the median urinary ACR before treatment was 468.42 (range 185.5–13,302) mg/g. After a median of 1.7 (range 0.3–8.5) years of treatment, a reduction in urinary ACR was observed in 54% (7/13) of children, a slight increase (less than 25%) in urinary ACR was observed in 23% (3/13), whereas a significant increase (more than 25%) was observed in the remainder. Three children (patient 10, 27 and 30) performed urinary protein electrophoresis after treatment. The proportion of urinary albumin before and after treatment were 37.8% versus 35.7%, 36% versus 39.4% and 31% versus 32.9% in these patients, respectively.

The therapy with ACE inhibitors and/or ARBs was well tolerated and did not affect renal function in 12/13 cases. However, one child stopped ARB treatment due to a decline in eGFR. One patient developed hypotension after 6 months of ACE inhibitor therapy, which resolved after taking the medication in the morning.

4 | DISCUSSION

Male patients with a prominent manifestation of LMW proteinuria should be considered for the possibility of Dent disease, which can be diagnosed by genetic testing. However, in clinical practice, an important issue is how to think of this disease. Two clinical characteristics in this cohort are noteworthy. First, hematuria with nephrotic-range proteinuria or moderate to severe albuminuria was common; however, it was not always associated with hypercalciuria. In the absence of a urinary erythrocyte morphology examination and urinary protein component analysis, such patients are usually recommended for renal biopsy (Nammalwar, Vijayakumar, & Prahlad, 2006); unfortunately, it is impossible to obtain a definitive diagnosis, and the patients may receive inappropriate therapy. Therefore, patients with hematuria and nephrotic-range proteinuria or moderate to severe albuminuria should undergo an examination of urinary erythrocyte morphology and urinary protein components.

Second, patients with Dent-1 disease may also present with congenital cataract and developmental delay. The incidence rates of cataract and intellectual impairment in Dent-2 disease were 7% and 27%, respectively (De Matteis, Staiano, Emma, & Devuyst, 2017), whereas there was no report of these two extrarenal abnormalities in patients with Dent-1 disease. However, in our study, patients with congenital cataract or developmental delay had been observed in Dent-1 disease. A study showed CLC proteins (including CLC-5 encoded by the $CLCN5$ gene) could regulate electrical excitability of neurons (Mufson et al., 2018), thus, the relationship between developmental delay and $CLCN5$ mutations may exist. Of course, these abnormalities may also coexist with Dent-1 disease, whereas they need to be differentiated from Lowe syndrome and Dent-2 disease. Thus, patients with suspected Dent disease should undergo ophthalmological examination and intelligence assessment.

Another interesting aspect of the present study is the opportunity to evaluate the percentage of urinary albumin and the effects of ACE inhibitor and/or ARB therapy on albuminuria. The percentage of urinary albumin in the total protein...
ranged from 24.2% to 47.6%; it was usually smaller than that of LMW proteinuria. The use of ACE inhibitors and/or ARBs effectively decreased the albuminuria in half of the children with glomerular pathology, including FSGS, mild glomerular lesions and MsPGN, and this therapy was well tolerated. A similar promising reduction in urine total protein was observed in two boys with less than 1 year of treatment, and the histologic findings were FSGS and extensive mesangial deposition of C1q (Copelovitch, Nash, & Kaplan, 2007; Lim, Yun, Moon, & Cheong, 2007). However, it has also been reported that the level of urine total protein in some patients failed to respond to ACE inhibitor and/or ARB therapy after a maximum of 5 years (Blanchard et al., 2016; Cramer et al., 2014; Frishberg et al., 2009; Vaisbich et al., 2012); unfortunately, data on the efficacy of these drugs on albuminuria were not available. Our results showed that, in half of the children, albuminuria could be controlled with ACE inhibitor and/or ARB treatment. Genotypes, glomerular lesions, the age at the initiation of treatment and the duration of treatment could not explain the difference in antialbuminuric response to ACE inhibitor and/or ARB therapy. In the future, it is worth observing whether patients with Dent disease benefit from long-term albuminuria control.

There are several limitations to this study. First, this was a retrospective study with incomplete medical examinations; for example, not all patients underwent urinary amino acid screening, intelligence assessment and ophthalmological examination. Baseline data of some patients were missing. The second limitation is the absence of urinary protein electrophoresis in most patients during follow-up, and the proportion of urinary albumin after treatment was unknown. The third limitation is the low precision of protein electrophoresis in most patients during follow-up, and the long-term effects of ACE inhibitor/ARB therapy are not clear.

In conclusion, hematuria with nephrotic-range proteinuria or moderate to severe albuminuria was common in Dent disease patients. Extrarenal manifestations could be observed in a small number of Dent-1 patients, which extends the phenotypic spectrum of the disease. In addition, ACE inhibitors/ARBs are well tolerated, and they are partially efficient in controlling albuminuria in Dent disease.

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CONFLICT OF INTEREST
None declared.

DATA AVAILABILITY STATEMENT
All data analyzed during this study are included in this article.

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