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BRIEF REPORT

Expanding the phenotype of proteinuria in Dent disease. A case series

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Abstract
Background Dent disease is an X-linked recessive renal tubular disorder characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and progressive renal failure (MIM 300009). A recent case series identified four patients with *CLCN5* mutations who presented with nephrotic-range proteinuria, histologic evidence of focal segmental and/or global sclerosis, and low molecular weight proteinuria.
Case-Diagnosis/Treatment We characterize the clinical, genetic, and histopathological features of seven unrelated adolescent males with nephrotic-range proteinuria and *CLCN5* mutations. Six patients underwent renal biopsy prior to assessing tubular proteinuria. All biopsied patients had either segmental sclerosis (3/6) or segmental increase in mesangial matrix (3/6). Five patients revealed some degree of foot process effacement, but only one patient biopsy revealed >50 % foot process effacement. The attenuated foot process effacement suggests the glomerulosclerosis is not due to a primary podocytopathy.
Conclusions These data suggest that clinicians should consider a diagnostic evaluation for Dent disease in young males presenting with high-grade proteinuria.

Keywords Dent disease · Nephrotic-range proteinuria · Glomerulosclerosis

Introduction

Dent disease (MIM 300009) is an X-linked renal tubular disorder characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis or nephrolithiasis, and progressive renal failure. Individuals with Dent disease classically present with a proximal tubulopathy that includes low molecular weight proteinuria as the most constant feature. Atypical presentations have recently been reported. Copelvitch et al. and Frishberg et al. each described young males who presented with nephrotic-range proteinuria and histologic findings of focal and segmental sclerosis (FSGS) on renal biopsy [1, 2]. We have corroborated this atypical presentation in seven boys with *CLCN5* mutations who presented with high-grade proteinuria and episodic evidence of isolated proximal tubular dysfunction.

Cases

The clinical characteristics of seven boys with Dent disease are outlined in Table 1. Serum creatinine was measured using

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Table 1 Clinical parameters

Patient	1	2	3	4	5	6	7	Normal
Age (years)	3	6	7	9	6	4	8	–
Height (cm)	98.4	119.7	120.4	109.9	122.9	99.6	126.6	–
Creatinine (mg/dl)	0.5	0.5	0.8	0.5	0.5	0.9	1.1	–
CrCl (ml/min/1.73 m^2)	81	98	62	90	135	61	47	90–140
Potassium (mEq/l)	4.3	4.0	4	3.8	3.2	3.8	3.2	3.3–4.6
Phosphorus (mg/dl)	5.2	4.2	4.3	4.0	4.5	3.2	3.1	3.7–5.6
Albumin (g/dl)	4.9	4.5	4.4	3.9	4.9	5.2	4.9	3.5–5.2
Urine pr:cr ratio (mg/mg)	2.8	3.4	2.1	5.2	2.6	4	2.9	< 0.2
Urine ca:cr ratio (mg/mg)	0.57	0.18	0.22	0.21	0.10	–	0.41	< 0.2
Urine Ca^+ 24 h (mg/kg/day)	–	3.5;5.2	–	9.7	3.8	–	–	< 4
Urine TRP[a] (%)	–	85	–	86	–	–	–	82–95
Urine β2M[b] (mcg/g cr)	159,583	166,111	125,918	223,037	–	–	–	< 132
RBP (mcg/g cr)	235,714	195,333	78,900	–	–	–	–	< 130
Nephrocalcinosis[c]	No	No	No	No	Yes	No	Yes	–
CLCN5 mutation	IVS9-1 G>A	R704X	IVS6+1 G>A	R28X	L521F	V363fs	–	
Urine protein electrophoresis								
Albumin (%)	–	25	21	24	27	11.5	–	–
Alpha-1 globulin (%)	–	1	3	4	18.2	3.3	–	–
Alpha-2 globulin (%)	–	38	38	35	22.6	44.7	–	–
Beta-globulin (%)	–	20	17	21	18.3	17.3	–	–
Gamma-globulin (%)	–	16	21	17	13.5	23.3	–	–

[a] Tubular reabsorption of phosphorus

[b] β2-microglobulin

[c] As determined by ultrasound

an enzymatic method and renal function was calculated using the modified Schwartz formula: 0.413 × height(cm) / creatinine (mg/dl) in patients 1–4 and patient 7. Creatinine was measured using the Jaffe method in patients 5 and 6 and renal function was calculated using the original Schwartz formula: 0.55 (height in cm) / creatinine (mg/dl). Family history was negative for renal disease, bone deformity, or rickets in five patients. Patient 6 had a maternal aunt who had developed ESRD at age 60 years for unknown reasons and a maternal uncle who was reported to have proteinuria. The mother and maternal aunt of patient 7 had a history of premature tooth loss with rapid progression to loss of all maxillary teeth. By report, his grandfather also had had a tooth loss problem and required calcium supplementation as a child. His maternal great uncle is currently on dialysis and, by report, a renal imaging study revealed "kidneys that looked as if they had been shot through with BBs". It is not clear whether this observation relates to multiple small foci of nephrolithiasis, nephrocalcinosis, or renal cystic disease.

All seven patients presented with urinary dipsticks positive for protein. Each patient also demonstrated nephrotic-range proteinuria with urine protein/creatinine ratios greater than 2.0 mg/mg. Serum albumin levels were normal in all cases. Five patients had normal serum phosphorus levels at presentation, one of whom developed intermittent hypophosphatemia. Four patients had decreased renal function at presentation.

Five patients had their vitamin A levels measured and all were found to be deficient. They were asymptomatic without ophthalmologic signs. In one patient, vitamin A levels returned to normal after supplementation of 10,000 U per day. Three patients continue vitamin A therapy but follow-up levels are not available and one patient reported non-compliance with supplementation.

Renal biopsies were performed on patients 1–6 due to nephrotic-range proteinuria, prior to assessing tubular proteinuria (Supplementary Figure 1). Patient 7 did not undergo a renal biopsy. All the renal biopsies were reviewed by an experienced renal pathologist (Agnes Fogo, Vanderbilt University). Tissue from five patients was available for electron microscopy and in all samples there was some degree of foot process effacement, but only one biopsy revealed greater than 50 % effacement. All six biopsied patients had either segmental sclerosis (3/6) or segmental increase in mesangial matrix (3/6). Two patients demonstrated more typical findings of Dent disease with presence of calcium phosphate crystals in

the interstitium, while two patients had more unusual findings of intratubular proteinaceous casts which were fragmented, metachromatic, and were occasionally surrounded by mononuclear inflammatory cells. In two patients, there was evidence of mild medial thickening of the interlobular arteries and intimal proliferation of arterioles, findings not previously reported in Dent disease patients. Importantly, neither patient had hypertension. Finally, one patient had findings suggestive of tubular hypoplasia, which also has not been reported in this disorder. After renal biopsy was performed, patient 5 was treated with a 4-week course of oral steroids for a diagnosis of minimal change nephrotic syndrome. He had no reduction in proteinuria.

Based on the previous case reports [1, 2], the findings of focal global and/or segmental glomerulosclerosis with paucity of foot process effacement and/or presence of calcium phosphate crystals prompted our analysis of low molecular weight proteinuria in these patients. Sequence analysis revealed seven distinct *CLCN5* variants, including 2/7 previously reported mutations and 5/7 novel, likely pathogenic variants.

Discussion

We describe seven unrelated boys with *CLCN5* mutations who presented with nephrotic-range proteinuria without nephrotic syndrome. Our data support two recent reports describing young males with *CLCN5* mutations whose predominant presenting feature was nephrotic-range proteinuria and whose renal histopathology was suggestive of (although not entirely consistent with) FSGS [1, 2].

In classic Dent disease, low molecular weight proteins constitute 50–75 % of urinary proteinuria consisting mainly of beta-2 microglobulin, alpha-1 microglobuin, and retinol-binding protein [3]. However, urine protein electrophoresis in our cohort revealed non-selective proteinuria, with low molecular weight proteins and albumin detected.

Historically, specific renal histopathological abnormalities were not reported in Dent disease patients. Renal histopathology in Dent disease patients was first described by Frymoyer et al. and included tubular atrophy, interstitial fibrosis, and glomerulosclerosis [4]. Calcium phosphate deposition can be observed in the cortex and medulla. However, no significant basement membrane abnormalities have been described [3]. More recent studies have reported histopathological features suggestive of focal global and/or segmental glomerulosclerosis [1, 2].

In our cohort, global sclerosis involving a minority of glomeruli was observed in each patient, not beyond what may be seen in the normal population. The observed segmental increase in mesangial matrix and segmental sclerosis could suggest idiopathic FSGS, especially in the face of high-grade proteinuria. However, the absence of extensive foot process effacement is not compatible with this diagnosis, and the additional finding of periglomerular fibrosis suggests a secondary sclerosing process. The attenuated foot process effacement further suggests that the sclerosis is not due to a primary podocytopathy. The previous case studies also reported minimal foot process effacement, leading the authors to conclude that segmental glomerulosclerosis combined with a paucity of foot process effacement may differentiate between primary FSGS and a secondary cause associated with a primary tubulopathy [1, 2]. In addition to these findings, our cohort reveals that the presence of unusual intratubular casts, which may warrant additional consideration of Dent disease. While two patients had medial arterial thickening and intimal proliferation of arterioles, this finding is non-specific and has been observed in patients with scarring.

Three of our patients had significant renal failure with creatinine clearance 47–62 ml/min/1.73 m^2, which is unusual in this age group. This seemed to correlate with the degree of interstitial fibrosis on renal histopathology. All three individuals harbored novel *CLCN5* mutations.

CLCN5 belongs to a family of voltage-gated chloride-channel genes and encodes CLC-5, a H+/Cl- antiporter protein predominantly expressed in the proximal tubule, thick ascending limb and the alpha-intercalated cells of the collecting duct [5–8]. Studies in animal models have demonstrated a role for CLC-5 in several different pathways involved in endocytosis [6]. Although disruption of receptor-mediated endocytosis has been described in CLC-5 deficiency, the mechanism of sclerotic glomerular lesions and variable degree of foot process effacement in our Dent disease cohort is unclear. It is also unclear whether the urinary excretion of albumin in our patients reflects glomerular disease or rather failure to reabsorb filtered albumin in the proximal tubule. However, we note that Ovunc et al. [9] described two siblings with nephrotic-range proteinuria and a novel homozygous frameshift mutation in the cubilin gene, supporting proximal tubulopathy as the etiology for this proteinuria.

At present, there are no targeted therapies for Dent disease. Current management strategies aim to delay renal disease progression through blood pressure control and reduction of nephrocalcinosis and proteinuria. Vitamin A levels should be assessed and supplementation initiated as indicated. In theory, patients with tubular proteinuria would not significantly benefit from renin-angiotensin system inhibition. Copelvitch et al. reported moderate improvement in proteinuria in a Dent disease patient after initiation of enalapril [2]. In our cohort, two patients were treated with high-dose angiotensin-converting enzyme inhibitor (ACEI) and angiotensin receptor blocker (ARB) therapy and had no reduction in proteinuria. A prospective study in a larger cohort of patients with this proteinuric *CLCN5*-related disorder would be required to assess treatment efficacy. Other treatment strategies have targeted

the reduction of urinary calcium excretion. However, the use of thiazide diuretics in these patients has been controversial due to adverse metabolic effects [10].

The identification of *CLCN5* mutations in our cohort corroborates recent case reports of a proteinuric variant and expands the phenotypic spectrum of Dent disease. Renal biopsy results also provide additional insight into the histopathology associated with this disorder. More importantly, these data suggest that clinicians should consider Dent disease in young males who present with high-grade proteinuria in the absence of nephrotic syndrome and evaluate them for low molecular weight proteinuria, hypercalciuria, and nephrocalcinosis. Findings of global or segmental glomerulosclerosis with paucity of foot process effacement and/or presence of calcium phosphate crystals and unusual casts may suggest Dent disease, and thus, spare these boys unnecessary, and likely ineffective, immunosuppressive therapy.

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