Brief Report

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Dent disease: Same *CLCN5* mutation but different phenotypes in two brothers in China

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Summary **Dent disease is an X-linked recessive proximal tubular disorder that affects mostly male patients in childhood or early adult life, caused by mutations in *CLCN5* (Dent disease 1) or *OCRL* (Dent disease 2) genes, respectively. It presents mainly with hypercalciuria, low-molecular-weight proteinuria, nephrocalcinosis and progressive renal failure. We report here the same *CLCN5* mutation but different phenotypes in two Chinese brothers, and speculate on the possible reasons for the variability of the genotype-phenotype correlations.**

Keywords: Dent disease, *CLCN5*, phenotpye, China

1. Introduction

Dent disease (OMIM #300009) is a rare X-linked renal proximal tubulopathy clinically defined by low molecular weight proteinuria (LMWP) associated with hypercalciuria and/or its complications (nephrocalcinosis or nephrolithiasis) and progressive renal failure. Dent disease may also be associated with aminoaciduria, phosphaturia, glycosuria, uricosuria, kaliuresis, hematuria, impaired urinary acidification, and rickets or osteomalacia (*1,2*). According to the differences in phenotype, it is divided into two groups, Dent disease 1 (OMIM #300008), which is caused by mutations of the *CLCN5* genes located in chromosome Xp 11.22, and Dent disease 2 (OMIM#300555), which is caused by mutations in *OCRL* genes localized on chromosome Xq 25 (*3*). Overall, 60% of individuals with Dent disease are found to have a *CLCN5* mutation, 15% have an OCRL mutation, and in the remaining 25% a mutation cannot be identified (*4*). The prevalence of Dent disease is unknown and it may be underdiagnosed. Dent disease mainly affects males, whereas female carriers may show a milder phenotype (*5,6*). Patients are usually diagnosed in childhood or in young adult years. LMWP is the most consistent feature, occurring in 99% of affected male patients. Proteinuria is usually subnephrotic but may reach a nephrotic level (*2,7,8*).

Fanconi syndrome (FS) is a generalized dysfunction of the renal proximal tubules leading to excessive urinary wasting of amino acids, glucose, phosphate, uric acid, bicarbonate, and other solutes. The patients develop failure to thrive, polyuria, polydipsia, dehydration, and rickets in children, and osteoporosis and osteomalacia in adults. The patients also manifest renal salt wasting, hypokalemia, metabolic acidosis, hypercalciuria, and LMWP. The causes of FS are divided into three main categories; hereditary, acquired, and exogenous substances (*9,10*).

The effects of Dent disease 1 are variable both within and between affected families (*11*). It is well known that a *CLCN5* mutation could be a rare cause of inherited forms of induced Fanconi syndrome (*12-16*). Moreover, a considerable intra and between familial variability in disease severity has been observed and no genotype-phenotype correlation has been described (*11,17*).

We report here two brothers with the same *CLCN5* mutation but different phenotypes, the elder was consistent with Fanconi syndrome while the younger was consistent with Dent disease. We speculate on the possible reasons for the variability of genotype-phenotype correlations.

2. Materials and Methods

2.1. *Participants*

This work was carried out with human research ethics approval from the Peking University First Hospital

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and followed the guidelines of the 2000 Declaration of Helsinki and the Declaration of Istanbul 2008. All patients and their family members gave their consent for inclusion in this study.

2.2. *Methods*

Genomic DNA was extracted from the peripheral blood of the patients and their parents. Genetic analysis was performed in the Genetics laboratory of Biotechnology companies in China, using "the hereditary renal tubular disease" which covers about 50 genes strongly correlated with this disorder.

The expected segregation of putative mutations was confirmed in families, whenever possible, and their absence was confirmed in SNPs databases of common benign variants (*http://exac.broadinstitute.org/*, *http://www.ncbi.nlm.nih.gov/projects/SNP/*), and *http://www.1000genomes.org/*. Human Splicing Finder (*http://www.umd.be/HSF/*) and Mutation tasting (*http://mutationtaster.org/*) were used for analysis on splicing mutations and other mutations, respectively.

2.3. *Case presentation*

Case 1: A 10.9-years boy (Birth date 29/10, 2004) was hospitalized to our hospital (14/08, 2015) with a complaint of proteinuria for 5.3 years, polydipsia and polyuria for 4 years. He was found to present with proteinuria (78 mg/kg) but without edema, hypoalbuminemia and hypercholesterolemia 5.3 years ago (28/05, 2010), renal biopsy was done in a local hospital. Immunofluorescence (IM) was negative. The light microscope (LM) showed 13 glomeruli with podocyte swelling, minimal proliferation of mescenteric cells and matrix, segmental glomeruli base membrane thickening, tubular atrophy (15%) and interstitial fibrosis. Electron microscopy (EM) found no obvious abnormal podocytes, except for tubular atrophy and interstitial fibrosis. With a presumptive diagnosis of minimal change disease (MCD), he was treated as nephrotic syndrome with a full steroid dose for 4 weeks, then combined with cyclophosphamide (CTX) for 7 doses (one dose per month), but no apparent change of his proteinuria (55~83 mg/kg) was seen. Further investigation revealed a normal creatine (29.4 umol/L), an estimated glomerular filtration rate (eGFR) of 125 mL/min/1.73 m^2, serum albumin level 46 mg/L, cholesterol 4.18 mmol/L, autoimmune profile including C3 and C4 compliments within normal range; Urine protein 64 mg/kg, urine α1-microglobin 161 mg/L, urine microalbumin 64 mg/L, LMWP 61.3%, hypophosphatemia (0.62~0.89 mmol/L), hypokalemia (2.9~3.2 mmol/L), acidosis (HCO_3- 15.6~20.7 mmol/L), hypomagnesemia (0.75~0.84 mmol/L), hypochloremia (91.8~95.4 mmol/L), aminoaciduria and rickets, but no hypercalciuria (0.05~0.08 mmol/kg/24 h). Renal ultra-sonography showed no obvious abnormality. He was diagnosed with Fanconi syndrome, treated initially with oral potassium citrate (2 mmol/kg/d) and sodium bicarbonate (3 mmol/kg/d), but the therapy was discontinued and no regular monitoring was carried out. Polydipsia and polyuria appeared 4 years ago, and intermittent apyretic tetanus appeared two years ago.

At admission, his height was 110 cm, weight was 21 kg, both below the 1st centile. Investigation in our hospital revealed a normal creatine (54.2 umol/L) and estimated glomerular filtration rate (eGFR) of 102 mL/min/1.73 m^2, serum albumin level 46 mg/L, cholesterol 4.12 mmol/L, urine protein 44~94 mg/kg, urine α1-microglobin 161~186 mg/L, urine microalbumin 70~124 mg/L, LMWP 63.7%, hypokalemia (1.76 mmol/L), hypomagnesemia (0.76 mmol/L), hypophosphatemia (0.57 mmol/L), hypochloremia (91.3 mmol/L), hyposthenuria (specific gravity 1.005~1.008), aminoaciduria and rickets, but without acidosis (HCO_3- 23.6 mmol/L) and still no hypercalciuria (0.11 mmol/kg/24h). Renal ultra-sonography showed nephrocalcinosis. He fulfilled the criteria for Fanconi syndrome. Because his brother also had low-molecular-weight proteinuria (see Case 2), hereditary renal tubular disease was suspected. See Table 1, 2, and 3.

Case 2: A 2.3-years boy (Birth date 13/04, 2013) was hospitalized in our hospital (17/08, 2015) with a complaint of proteinuria for 8 months. He was found to present with proteinuria (2+ ~ 3+) but without edema, hypoalbuminemia and hypercholesterolemia about 8 months ago (28/12, 2014). There was no proteinuria change and other signs after observation for 8 months.

At admission, his height was 88 cm, weight was 12 kg. Investigation in our hospital revealed a normal creatine (25.8 umol/L) and estimated glomerular filtration rate (eGFR) of 116 mL/min/1.73 m^2, serum albumin level 50 mg/L, cholesterol 3.26 mmol/L, urine protein 85~98 mg/kg, urine α1-microglobin 413~708 mg/L, urine microalbumin 318~470 mg/L, LMWP 56%, hypercalciuria (0.23~0.25 mmol/kg/24 h, urine calcium to creatine = 0.26 g/g) and aminoaciduria, but without any other electrolyte disturbances and rickets. Renal ultra-sonography showed no abnormality. He was suspected for Dent disease, and also hereditary renal tubular disease same as his elder brother (see Case 1). See Table 1, 2, and 3.

3. Results and Discussion

Our two brothers both presented with a nephrotic level of LMWP and aminoaciduria, the elder brother also had polydipsia, polyuria, nephrocalcinosis, hypophosphatemia, hypokalemia, acidosis, hyposthenuria and rickets but without hypercalciuria, while the younger brother also had hypercalciuria but without other symptoms, they fulfilled the diagnostic criteria of Fanconi syndrome and Dent disease, respectively. Genetic analysis showed they both carried

Table 1. General information of the two brothers

Items	Case 1	Case 2
Gender	Male	Male
Onset age	5.6 years	1.7 years
Diagnosis age	10.9 years	2.3 years
Body weight	21 kg	12 kg
Body height	110 cm	88 cm
Chief complaint	Proteinuria, polydipsia and polyuria	Proteinuria

Table 2. Clinical characters of the two brothers

Items	Case 1	Case 2
Proteinuria	Yes	Yes
Hypercalciuria	No	Yes
Hematuria	No	No
Aminoaciduria	Yes	Yes
Nephrocalcinosis	Yes	No
Renal function	Normal	Normal
Renal biopsy	MCD	Not Done
CLCN5 mutation	c.731C>T, p.S244L	c.731C>T, p.S244L
Others	Hypophosphatemia, hypokalemia, acidosis, hyposthenuria and rickets	None

Table 3. Laboratory data of the two brothers

Items	Case 1	Case 2
Alb (g/L)	50 ± 2	50 ± 1
UPE (mg/kg/24h)	68 ± 16	99 ± 10
α1MG (mg/L)	174 ± 13	535 ± 98
MA (mg/L)	101 ± 21	394 ± 86
α1MG/MA	1.7 ± 0.6	1.4 ± 0.1
LMWP (%)	52.3 ± 9.1	55.9 ± 4.5
UC/Ucr	0.11 ± 0.05	0.26 ± 0.08
UCE (mmol/kg/24h)	0.07 ± 0.02	0.25 ± 0.02

Alb: Albumin; UPE: Urinary protein excretion; α1MG: α1-microglobinuria; MA: Microalbuminuria; LMWP: Low molecular weight proteinuria; UC: Urine calcium; Ucr: Urine creatinine; UCE: Urine calcium excretion.

the homozygous mutation c.731C>T (p.S244L) in exon 7 in the *CLCN5* gene, transmitted from their mother. The mother carried the same mutation but father was normal. Mother showed no proteinuria, hematuria, hypercalciuria, nephrolithiasis or nephrocalcinosis, and her renal function was normal. No variations were found in inherited Fanconi syndrome related genes in both cases, such as *OCRL1*, *COQ2*, *CNTS*, *GALT1*, *ALDOB*, *SLC2A2*, *SLC5A2*, *ATP7B*, or *NDUFAF6*. The mutation has been reported (*20,21*).

Case 1 was treated continuously with potassium citrate (2~3 mmol/kg/d) and a mixture of phosphate (disodium hydrogen phosphate and sodium dihydrogen phosphate, 3~5 mmol/kg/d), with a follow up of 10 months, his electrolyte disturbance recovered and remained normal. Case 2 was treated with hydrochlorothiazide (1 mg/kg/d) and potassium citrate (2~3 mmol/kg/d), with a follow up of 10 months, his urine calcium decreased and remained 0.08~0.09 mmol/kg/d, but with no change of urine protein (58~89 mg/kg/d).

Fanconi syndrome represents a generalized dysfunction of the proximal tubular resorption of glucose, phosphate, bicarbonate and amino acids in the kidney. Dent disease is one form of inherited renal Fanconi syndrome (*14*). It was confusing why the same *CLCN5* mutation in our two brothers induced different phenotypes.

First, the course of disease maybe had some effects. The elder brother was 5.6 years old while the younger brother was only 1.7 years old when proteinuria was found. As a genetic disease, the LMWP of Dent disease should be presented after birth, it is well known that persistent proteinuria itself is a damaging factor for the renal system. For example, polydipsia, polyuria and hyposthenuria appeared in the younger brother a year after the finding of proteinuria. It was similar to another report of Dent disease in China. Jian *et al*. reported four cases of Dent disease, three of which were diagnosed Fanconi syndrome aged 12, 10 and 8 years old, respectively (*15*). We suggested that the renal tubulopathy might be worsening as the course progresses, but this needs further observation, especially for our younger brother. However, Hodgin *et al*. also reported two Dent disease brothers both presenting as partial Fanconi syndrome, 6 years old and 4 years old, respectively (*12*), which suggested excepting the course of disease, there might be other factors influencing the phenotype of Dent disease.

Second, the genotype maybe had some effects on phenotype. As a rare hereditary renal tubular disorder, there are no genotype-phenotype correlations because various mutations are associated with different clinical phenotypes, even within the same family (*22*). The mutation c.731C>T (p.S244L) has been previously reported, the missense mutation occurs within transmembrane domains, and none of the cases presented

as Fanconi syndrome (*20,21*). Our two brothers have the same mutation but presented a different phenotype, one Dent disease while the other Fanconi syndrome. There was no mutation found on other Fanconi syndrome related genes in the elder brother, and it seemed that the genotype could not explain the variance. However, because the younger brother is perhaps too young, his phenotype should be monitored to see if any changes take place when he reaches his elder brother's age. A considerable intrafamilial variability in disease severity has been observed. Dent disease patients have a variable phenotype ranging from renal Fanconi syndrome with or without rickets to the association of LMWP and hypercalciuria (with or without nephrocalcinosis or nephrolithiasis) (*11*). The severity of renal disease varies greatly among individuals within a family, and there is no relationship between the degree of LMW proteinuria and the severity of chronic kidney disease (CKD) or hypercalciuria (*17*).

Third, the renal pathology may have some effects on the phenotype. Renal biopsy of our elder brother showed no obvious abnormality on podocytes, except tubular atrophy (15%) and interstitial fibrosis. Also, he had no history of drugs used which might damage the renal tubule and interstitium. However, it was a pity that our younger brother did not have a renal biopsy, and therefore the severity of his tubular atrophy was unknown. There was a report on renal pathology and renal function, which showed that higher percentages of globally sclerotic glomeruli, foot process effacement, and interstitial inflammation were associated with a lower estimate of glomerular filtration rate (eGFR) at biopsy, whereas foot process effacement was associated with steeper annual eGFR decline (*23*). However, no correlation between phenotype and tubular atrophy has been reported in Dent disease.

Finally, how *CLCN5* mutations can cause a lot of clinical manifestations is not very clear. As a vesicular chloride/proton exchanger, it has been proposed that CLC-5 allows maximal acidification of the endosome by providing an electrical shunt to dissipate the positive charge created by the proton-ATPase pump. LMWP are freely filtered by the glomerulus and reabsorbed by the proximal tubular epithelium. Thus, the LMWP seen with CLC-5 dysfunction, results from impaired proximal tubular endocytosis. Consequent abnormalities in membrane recycling could explain other defects in proximal tubular function such as phosphaturia, aminoaciduria, and glycosuria. However, the mechanisms of hypercalciuria and nephrocalcinosis remain unclear. It is known that in patients with proven *CLCN5* mutations, as many as 30% do not demonstrate hypercalciuria on repeated urine analysis, even though some of these do exhibit nephrocalcinosis, such as our elder brother, who had nephrocalcinosis but without hypercalciuria (*24*). It is hypothesized that the functional loss of ClC-5 is essentially reflected by manifestations of proximal tubular dysfunction and may contribute to the genesis of kidney stones in different ways, reflecting its involvement in specific tubular functions (*25*).

In conclusion, we report two brothers in China with the same *CLCN5* mutation of S244L but different phenotypes, one presented with a nephrotic level of LMWP, aminoaciduria, polydipsia, polyuria, nephrocalcinosis, hypophosphatemia, hypokalemia, acidosis, hyposthenuria and rickets but without hypercalciuria, while the other presented with a nephrotic level of LMWP, hypercalciuria and aminoaciduria, they fulfilled the diagnosis criteria of Fanconi syndrome and Dent disease, respectively. The possible reasons for the variability of genotype-phenotype correlations remain unclear.

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