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ORIGINAL ARTICLE

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Phenotype and genotype of Dent's disease in three Korean boys

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Abstract Dent's disease is a hereditary renal tubular disorder caused by mutations of the *CLCN5* gene and is clinically characterized by low molecular weight proteinuria, hypercalciuria and nephrocalcinosis. This disease has been reported in several countries. However, there are some phenotypic differences between countries, such as hypophosphatemic rickets, progressive renal failure and hematuria. In this study, phenotypes were analyzed in three Korean boys with Dent's disease, and genetic diagnoses were performed using a new convenient method using peripheral blood RNA. Gene studies revealed two nonsense mutations, R637X in two patients and E609X in one patient. The phenotypes of the two patients with R637X were very similar to those of Japanese patients, i.e., they presented with asymptomatic proteinuria without rickets, renal failure or hematuria. The E609X patient was diagnosed genetically at 3 months of age before the onset of clinical symptoms because of superimposed furosemide-induced nephrolithiasis. This is the first report to characterize mutations in the *CLCN5* gene in Korean patients with Dent's disease, and expands the spectrum of *CLCN5* mutations by reporting a novel mutation, E609X. In addition, the mutational analysis using peripheral blood RNA can be easily applied in the clinical diagnosis.

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Introduction

Dent's disease is a rare hereditary disease characterized by renal tubular dysfunctions, which include low molecular weight (LMW) proteinuria, hypercalciuria and nephrocalcinosis [1, 2]. Nephrolithiasis, hypophospatemic rickets, glomerulosclerosis and chronic renal failure may develop in some patients. Three other hereditary disorders, X-linked recessive nephrolithiasis (XRN) [3], X-linked recessive hypophosphatemic rickets (XLRH) [4] and the familial idiopathic LMW proteinuria (FILMWP) of Japanese children [5], were found to share common clinical features with Dent's disease. During the last decade, it was found that these four disorders share the same genetic defect, namely, loss-of-function mutations of the *CLCN5* gene, which is located in Xp11.22 and encodes a renal-specific voltage-gated chloride channel, ClC-5 [1, 3, 4, 5, 6, 7, 8]. Due to the phenotypic similarities between and the common genetic etiology of these four disorders, they were considered to be variants of one disease and were collectively referred to as Dent's disease [7, 8]. However, there are some phenotypic differences between these disorders that cannot be explained. For example, hypophosphatemic rickets is common in XLRH and in Dent's disease, but not in XRN or FILMWP, and progressive renal failure is prominent in Dent's disease and XRN, but not in FILMWP [2, 3, 4, 5, 9]. Moreover, accompanying hematuria is common in some reports [10, 11] and rare in others, especially reports from Japan.

In this study, phenotype and genotype were analyzed in three Korean boys who were clinically diagnosed with Dent's disease. The present study is the first to characterize mutations in the *CLCN5* gene in Korean patients with Dent's disease, and expands the spectrum of *CLCN5* mutations by reporting a novel mutation, E809X. One of the patients was diagnosed genetically at 3 months of age before the onset of clinical symptoms, because of super-

Table 1 The sequences of the primers used for polymerase chain reactions

Fragment	Direction	Sequence	Covering exons
First round PCR			
Fragment 1+2	Sense	5'-GTGATATGGCTGCAAGTGCC-3'	1–8
	Antisense	5'-GGAGTCCAGAAGGCCACAGT-3'	
Fragment 3+4	Sense	5'-CCATTCATTCTGCTGGGCAT-3'	8–12
	Antisense	5'-CACACTAATGTGACTTCACCATCC-3'	
Second round PCR			
Fragment 1	Sense	5'-AAGTCGTACAATGGTGGAGGA-3'	1–6
	Antisense	5'-TTGAAGCAGTGGCACAGGAT-3'	
Fragment 2	Sense	5'-TTGGGTAAGTGGACTCTGGT-3'	6–8
	Antisense	5'-TGAGCTCACTTGTGCTCATC-3'	
Fragment 3	Sense	5'-GGAGCACTGTTTATCCGCAC-3'	8–10
	Antisense	5'-ATCACATCCATTGCCAGGGT-3'	
Fragment 4	Sense	5'-TCTATGATGCCCACATCCGT-3'	10–12
	Antisense	5'-CCTTCCCGCTTTACATCCAG-3'	

Table 2 Patient phenotypes and genotypes. NC/NL: nephrocalcinosis and/or nephrolithiasis

	Patient 1	Patient 2	Patient 3
Age of onset	33 months	9 years	2 months (?)
Presenting symptom	Foamy urine	Chance proteinuria	Gross hematuria (?)
Hypercalciuria	(+)	(+)	(+)
Beta$_2$-microglobinuria	(+)	(+)	(+)
NC/NL	(+)	(+)	(+)→(−)
Rickets	(−)	(−)	(−)
*CLCN5*mutation	R637X	R637X	E609X
Family history	Not studied	De novo	X-linked recessive

imposed furosemide-induced nephrolithiasis. In addition, we developed a new convenient method of mutational analysis using peripheral blood RNA.

Subjects and methods

Three unrelated Korean boys were included in this study. All met the following three clinical diagnostic criteria for Dent's disease: (1) LMW proteinuria (β_2-microglobinuria), (2) hypercalciuria [spot urine calcium (mg/dl) to creatinine (mg/dl) ratio $\geq$0.25] and (3) nephrocalcinosis and/or nephrolithiasis. Medical records were reviewed retrospectively.

For *CLCN5* gene analysis, total peripheral blood RNAs were reversely transcribed to cDNAs, and four overlapping cDNA fragments covering the entire coding sequences of the *CLCN5* gene were amplified by nested polymerase chain reactions (PCR) and directly sequenced. Mutant sequences were confirmed by direct sequencing of the PCR product of the corresponding exon from peripheral blood total genomic DNA. The sequences of the primers used in the nested PCR are listed in Table 1.

Result

The clinical features and the *CLCN5* gene mutations of the three patients are summarized in Table 2, and the progression of laboratory findings are detailed in Table 3. Other laboratory findings not listed in Table 3, including urine sugar, urine amino acids, serum magnesium, serum calcium and bone X-rays, were normal in all three patients. The laboratory findings of all three patients fulfilled the clinical diagnostic criteria for Dent's disease, β_2-microglobinuria, hypercalciuria and nephrocalcinosis/nephrolithiasis. None of the three patients had rickets or renal failure. A renal biopsy was done in patient 2 only, and revealed areas of focal calcification in the medulla without significant glomerular changes.

Patients 1 and 2 showed almost identical clinical features, except age at onset, and had the same nonsense mutation, 637Arg(CGA) >Stop(TGA), in exon 10 of the *CLCN5* gene. Patient 1 had an additional silent mutation

Table 3 Detailed laboratory findings. *For children, **(mg/dl)/(mg/dl), Ur: urine, TRP: tubular reabsorption of phosphate

Patient	Patient 1		Patient 2	Patient 3		Normal ranges*
Age at examination	3 years	5 years	9 years	3 months	3 years	
Serum phosphate	5.2	4.6	4.7	5.3	5.5	3.7–5.6
Serum HCO_{3-} (mEq/l)	20	26	23	23	21	22–29
Serum K^+ (mEq/l)	4.5	4.4	4.0	3.5	4.2	3.5–5.0
Serum creatinine (mg/dl)	0.3	0.4	0.6	0.4	0.4	0.3–0.7
Hematuria	(−)	(−)	(−)	Gross	Microscopic	(−)
Ur calcium/creatinine**	0.52	0.46	0.25	0.75	0.69	<0.25
Ur beta$_2$-microglobulin (μg/ml)	20.3	21.8	52.2	5.7	9.3	<0.32
Ur total protein (mg/day)	597	570	1,134	275	159	50–80
TRP (%)	94	89	ND	83	86	83–93
Ur uric acid GFR (mg/dl)	0.23	0.30	0.53	0.50	0.72	<0.53

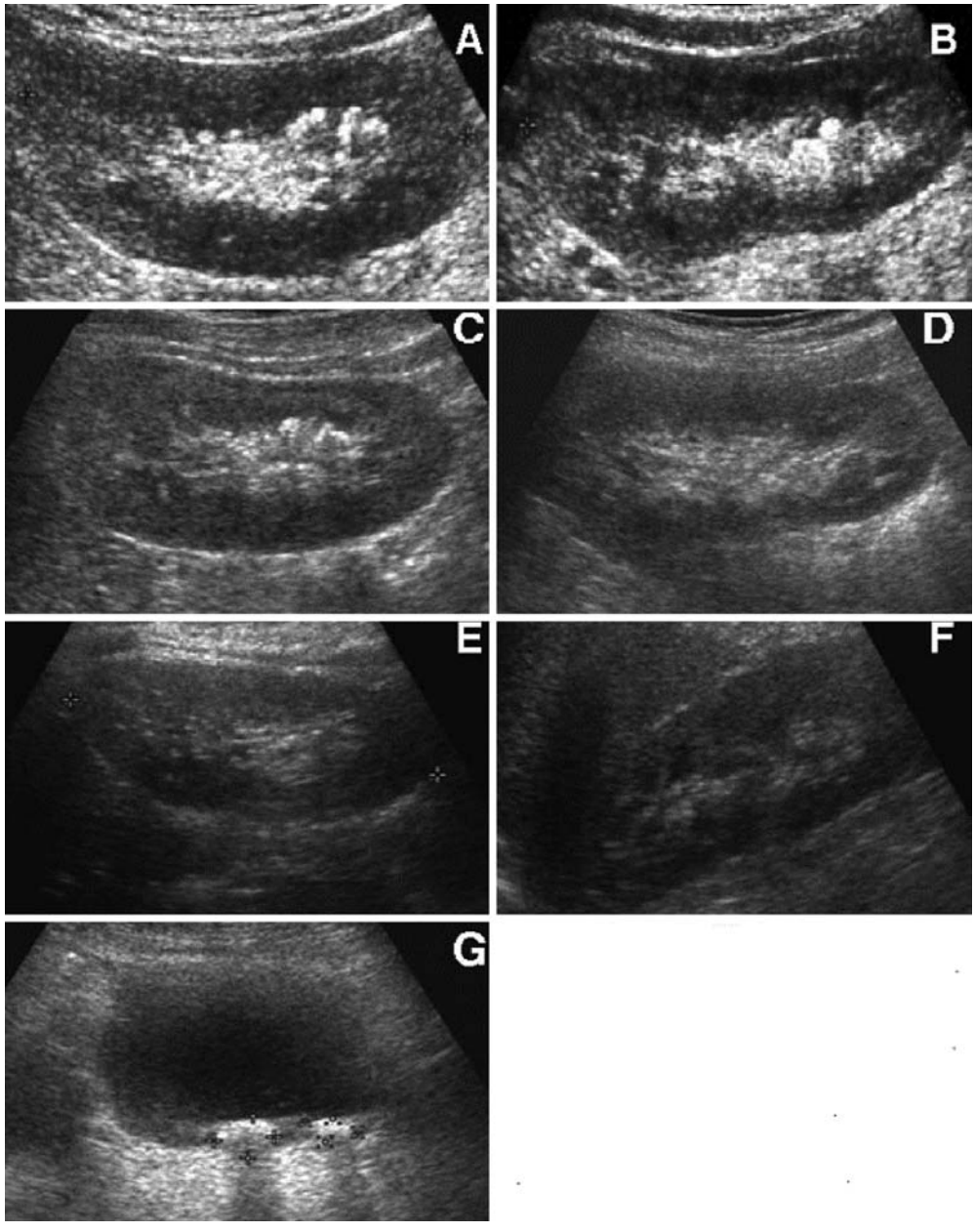

Fig. 1 Serial ultrasonographic findings of patient 3. Multiple renal stones and medullary nephrocalcinosis were detected in the right (**A**) and left (**B**) kidneys at age 3 months. At age 2 years, these renal calcified densities decreased a little in the right kidney (**C**) and nearly disappeared in the left (**D**) at age 2 years, and completely disappeared in the right (**E**) and left (**F**) kidneys at age 3.2 years. At age 2.4 years, two stone densities were detected in the urinary bladder (**G**)

(613Ser(AGT) >Ser(AGC)) in exon 10. Both parents and a sister of patient 1 showed normal urinary calcium and β_2-microglobin excretions, but no genetic study was performed. The mother of patient 2 was heterozygous for this mutation.

The clinical course of patient 3 was complicated by superimposed furosemide-induced nephrolithiasis. He had been medicated with furosemide soon after birth because of congestive heart failure associated with ventricular and atrial septal defect. Intermittent gross hematuria developed at the age of 2 months, and renal ultrasonography revealed multiple renal stones in both kidneys and medullary nephrocalcinosis. Thus, furosemide was replaced by hydrochlorthiazide, and this was continued until open heart surgery at age 3 months. However, the degree of hypercalciuria was unaffected by hydrochlorthiazide treatment. At age 2.4 years, two bladder stones causing acute dysuria and voiding difficulties were detected and removed by cystolitholapaxy. The stones were composed of calcium, phosphate, oxalate, magnesium and carbonate. Serial renal ultrasonography revealed the disappearance of nephrolithiasis/nephrocalcinosis at age 3.2 years (Fig. 1). A retrospective examination of his clinical course showed that nephrolithiasis/nephrocalcinosis and gross hematuria in his early infancy were considered to be furosemide-induced lesions. He had a novel de novo nonsense mutation (609Glu(GAG) >Stop(TAG)) and a silent mutation (613Ser(AGT) >Ser(AGC)) in exon 10 of the *CLCN5* gene.

Discussion

Dent's disease in its broadest sense includes four separate diseases, Dent's disease in the UK [2], XRN in North America [3], XLRH in Europe [4] and FILMWP in Japan [5]. Although these diseases share common clinical features and genetic backgrounds, the reported incidences of some clinical manifestations, such as, rickets, renal failure and hematuria, are different from each other.

Whereas rickets is one of the presenting features in some patients in the UK and Europe [2, 4, 12], it is not associated with the Japanese variant and has been only rarely reported in North America [13]. However, this difference does not correlate with *CLCN5* mutation type [6, 13, 14], and thus may be due to environmental factors, such as dietary calcium intake, the length of exposure to the sun or other genetic factors, such as modifying genes [15]. Interestingly, a recent study performed mainly in North American patients reported a rickets incidence of 5 in 13 patients with *CLCN5* mutations [11]. In this study, none of our patients had rickets or hypophosphatemia.

Severe renal failure is a feature of Dent's disease and XRN [2, 3, 4, 16]. In contrast, earlier reports from Japan reported a milder disease course, i.e., the absence of end-stage renal failure [5, 17]. This may have been due to age differences of the patients involved, as the Japanese patients were mainly children, whereas the British and North American patients were mainly adults. Recently, Igarashi et al. [9] provided indirect evidence of age effect in a report of a Japanese patient who developed end-stage renal failure at 46 years of age. However, Hoopers Jr. et al. [13] reported that 5 of 19 genetically confirmed patients, most of whom were North American, suffered renal failure at a mean age of 11.0±2.0 years. Another possible reason for the different incidences of renal failure may be the different mode of onset in Japanese patients. Whereas most Japanese patients were asymptomatic and identified by a nationwide urinary screening program for school children [15, 17, 18], most of the patients in other countries were detected by clinical symptoms. Patient 2 in this study was detected by a screening program for school children as in Japan.

Gross hematuria is absent or very rare in Dent's disease, and microscopic hematuria is also uncommon. If present, it is usually transient or present at a low degree, at least in Japanese patients [15]. However, several studies in other countries have reported a high incidence of microscopic hematuria [10, 11, 13]. It is difficult to explain the low incidence of hematuria in patients with significant hypercalciuria. Moreover, because gross or microscopic hematuria is common in patients with idiopathic hypercalciuria [19], the mechanism of hypercalciuria in Dent's disease may be different from that in idiopathic hypercalciuria. Although gross hematuria was the presenting symptom in patient 3 in the present study, it is more likely to have been caused by the superimposed furosemide-induced nephrolithiasis. And, after the disappearance of the stones, only a low level of microscopic hematuria remained. Two other patients in the present study had no hematuria.

To date, about 70 different disease-causing mutations have been reported in the *CLCN5* gene, including missense, nonsense, frameshift, splice-site, in-frame insertion and microdeletion mutations [11]. Two nonsense mutations in the *CLCN5* gene were detected in this study, i.e., R637X and E609X, which are predicted to result in a truncated and inactivated protein lacking 110 and 138 amino acids, respectively. R637X has previously been reported in a Japanese patient [20], and E609X was found for the first time in the present study. All mutations are scattered throughout the *CLCN5* gene without a hot spot, and in general, the phenotype-genotype correlations are known to be poor in Dent's disease [6, 13, 14]. Thus, for genetic studies of genomic DNA, all 12 coding exons should be amplified and sequenced separately. In the present study, only four fragments of cDNA covering entire coding sequences were analyzed by using RNA from peripheral blood. Morimoto et al. [21] described a similar method using mRNA in proximal tubular cells. However, in their method, renal tubular cell culture from urine preceded mRNA extraction, and thus, our method is more convenient. Patient 3 in this study was a sporadic case, i.e., by showing the de novo occurrence of E609X. De novo mutation in the *CLCN5* gene is rare, but at least three cases have been reported [7, 20].

Some controversy exists over the effectiveness and safety of thiazide diuretics in Dent's disease [2, 22, 23]. Recently, Raja et al. [24] reported that calcium urinary excretion fell to normal levels after chlorthalidine treatment in seven of eight patients. This intact hypocalciuric response to a thiazide diuretic indicates that calcium transport in the distal convoluted tubule is unaffected in Dent's disease and that thiazides can be usefully used in the management of Dent's disease. However, patient 3 in our study showed no significant reduction in urinary calcium excretion after hydrocholothizide administration.

In conclusion, the phenotype and genotype of the three children in this study are similar to those reported in Japan. Nationwide annual urine screening of school children has been performed in Korea since 1998, as in Japan. However, only one patient in the present study was identified in this manner. Thus, the incidence of Dent's disease in Korea may be lower than that in Japan. The mutational analysis method using peripheral blood RNA developed during the course of this study is more convenient than conventional methods based on genomic DNA, and it can be applied more easily in the clinical field.

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