Nephrol Dial Transplant (2014) 29: 376–384
doi: 10.1093/ndt/gft394
Advance Access publication 29 September 2013

Japanese Dent disease has a wider clinical spectrum than Dent disease in Europe/USA: genetic and clinical studies of 86 unrelated patients with low-molecular-weight proteinuria

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ORIGINAL ARTICLE

ABSTRACT

Dent disease is an X-linked disorder characterized by low-molecular-weight (LMW) proteinuria, hypercalciuria, nephrocalcinosis, urolithiasis and renal dysfunction. Dent disease is caused by mutations in at least two genes, i.e. *CLCN5* and *OCRL1*, and its genetic background and phenotypes are common among European countries and the USA. However, only few studies on Dent disease in Japan, which was originally called 'low-molecular-weight proteinuric disease', have been reported thus far. In this study, we analysed genetic background and clinical phenotype and laboratory data of 86 unrelated Japanese Dent disease patients. The results demonstrated that the genetic basis of Japanese Dent disease was nearly identical to those of Dent disease in other countries. Of 86 unrelated Japanese Dent patients, 61 possessed mutations in *CLCN5* (Dent-1), of which 27 were novel mutations; 11 showed mutations in *OCRL1* (Dent-2), six of which were novel, and the remaining 14 patients showed no mutations in *CLCN5* or *OCRL1* (Dent-NI). Despite the similarity in genetic background, hypercalciuria was detected in only 51%, rickets in 2% and nephrocalcinosis in 35%. Although the patients were relatively young, six patients (8%) showed apparent renal dysfunction. Japanese Dent disease has a wider clinical spectrum than Dent disease in Europe and the USA.

Keywords: *CLCN5*, dent disease, Japanese Dent disease, low-molecular-weight proteinuria, Lowe syndrome, *OCRL1*

INTRODUCTION

Dent disease is an X-linked disorder characterized by low-molecular-weight (LMW) proteinuria, hypercalciuria, nephrocalcinosis, urolithiasis and renal insufficiency [1–4]. In 1964, Dent and Friedman described the first two cases with hypercalciuric rickets associated with renal tubular dysfunction [1]. In the early 1990s, Wrong *et al.* reported detailed data of similar cases including Dent's report, and designated it as Dent disease [2]. Other than Dent disease, several similar disorders, such as 'X-linked recessive nephroliasis with renal failure' in North America and 'X-linked recessive hypophosphatamic rickets' in Italy, had been reported [3]. After the identification of the most common responsible gene, *CLCN5*, these disorders have been collectively called 'Dent disease' [4, 5]. As described below, the same is true for Japanese Dent disease. Thus far, two genes responsible for Dent disease have been identified [4, 6]. The first is *CLCN5,* which encodes voltage-dependent chloride channel 5 (CLC-5) [4]. CLC-5 is localized in the proximal tubular cells, thick ascending limb of Henle and collecting duct cells; CLC-5 is supposed to play critical roles in the acidification of intraendosomal compartments and endosomal recycling [7]. *CLCN5* knockout mice manifest phenotypes nearly identical to those in Dent disease [8, 9]. In 2005, Hoopes *et al.* identified *OCRL1* as the second causative gene for Dent disease [6]. Thereafter, we also identified mutations in *OCRL1* in Japanese Dent disease patients [10]. *OCRL1* was originally identified as the causative gene for Lowe syndrome, which is characterized by congenital cataract,

moderate-to-severe mental retardation and generalized solute transport dysfunction of proximal tubular cells [11].

Other than Dent and Wrong's studies, Suzuki and Okada proposed a disease entity named 'low-molecular-weight (LMW) proteinuria disease' in 1985 and 1990 [12, 13]. In Japan, annual urinary screening in school has been routinely conducted for a long time, and markedly high levels of LMW protein urine were identified in some children. In these children, renal biopsy examination did not show any glomerular or tubular lesions, and they showed no further increase in urinary protein level or renal function deterioration. Thus, the prognosis of LMW proteinuria disease in Japan had been considered benign.

In 1995, Igarashi *et al.* speculated that LMW proteinuria disease in Japan is the same as Dent disease [14]. Thereafter, Igarashi and Thakker identified mutations in *CLCN5* in Japanese patients with LMW proteinuria, and concluded that these two disorders are genetically identical [15–17]. Other groups also identified mutations in *CLCN5* in Japanese Dent disease patients [18, 19].

Although the genetic basis of patients with LMW proteinuria disease in Japan and Dent disease may be similar in limited patients, there are few actual genetic data of Japanese patients. Moreover, differences in the phenotype of Dent disease between patients in Europe/the USA and Japanese patients still remain unclear. In European countries and the USA, most of the patients with Dent disease showed hypercalciuria, renal calcification or renal stone, which appears to be the cause of the deterioration of renal function [2–5]. In particular, hypercalciuria is an essential clinical symptom in Dent disease as proposed by Hoopes *et al.* [20], although the frequency of hypercalciuria in LMW proteinuria disease is unknown. Furthermore, the prognosis of the renal function of patients with LMW proteinuria disease is also not known: in England, between the third and fifth decades of life, more than two-thirds of the affected males developed end-stage renal failure after 40–50 years of age [2].

In the present study, we conducted genetic and clinical analyses of 86 unrelated patients with LMW proteinuria. All of the patients suspected of having LMW proteinuria disease were subjected to the genetic analysis of *CLCN5*/*OCRL1*. As many clinical data as possible, including those of family members, were collected by the main attending physicians, and we carefully analysed them. The present study revealed the similarities and differences between Dent disease and LMW proteinuria disease in Japan. Hereafter, we will refer to 'LMW proteinuria disease in Japan' as Japanese Dent disease to eliminate confusion.

PATIENTS AND METHODS

Patient inclusion criteria

The criteria for inclusion of patients in this study were as follows:

(i) Extremely high-LMW proteinuria determined on the basis of the urinary level by either β2- microglobulin (β2-MG) or α1-microglobulin (α1-MG).

(ii) Absence of histories or clinical data indicative of underlying renal diseases that cause proximal tubular dysfunction, such as mitochondrial disorders, ingestion of nephrotoxic substances and other tubular diseases.

In Hoopes's criteria [20], hypercalciuria is essential for the clinical diagnosis of Dent diseases. However, because Japanese Dent disease patients with mutations in *CLCN5* or *OCRL1* are not necessarily accompanied by hypercalciura or nephrocalcinosis, we did not include hypercalciuria as an inclusion criterion.

When OCRL1 mutations were identified, the following were confirmed to exclude Lowe syndrome patients:

(i) Absence of cataract, which was confirmed by slit lamp examination by ophthalmologists.

(ii) No apparent neurological or mental abnormalities indicative of Lowe syndrome, such as mental retardation, muscular hypotonus and behavioral abnormalities.

All of the patients in this study were clinically diagnosed as having Japanese Dent disease by pediatric nephrologists, and referred to our institute for genetic examination.

Genetic analyses

Informed consent for participation in this study was obtained from the patients and their family members after the purpose, methods and potential risks were thoroughly explained to them. This study was approved by the Ethics Committee for Analysis of the Human Genome of Tokyo University Hospital. For each patient, direct sequencing of *CLCN5* was first conducted as described previously [15, 16]; when no mutation was identified, *OCRL1* was directly sequenced. The conditions of genetic analysis of *CLCN5* and *OCRL1* were the same as those described previously [10].

Clinical data

The most recent clinical data were collected from the attending pediatric nephrologists.

The clinical data including urinary β2-microgloburin, Ca/Cr ratio and urinary protein excretion are the mean of multiple measurements or single data. When the attending doctors informed us of the data of multiple measurements, we calculated the mean values, and used them for subsequent analyses. If the data of each patient were of single measurements, we used it. Approximately, two thirds of the data are the mean of multiple measurements.

RESULTS

Except for D-88 and D-48, all of the patients are males. The Patients, ages ranged from 0.3 to 66 years. The average age of patients was 12.8 years, which reflects that most Japanese Dent patients are diagnosed by the annual school urinary screening test.

Table 1 shows a summary of the mutations in the patients enrolled in this study. In 86 unrelated patients with Japanese

Table 1. Genetic background of 86 unrelated Japanese Dent Patients. (see also Tables 2–5)

	Types of mutations	Number of patients	Number of novel mutations
Dent 1 (*CLCN5* mutation)	Deletion	4	
	Frame shift	7	7
	Missense	27	15
	Nonsense	16	5
	Spice site	7	
	Total number (%)	61(71%)	
Dent 2 (*OCRL1* mutation)	Frame shift	1	
	Missense	9	4
	Nonsense	1	1
	Total number (%)	11(13%)	
Dent NI (mutation not identified)	Total number (%)	14(16%)	

Dent NI; Dent-NOT identified mutations in either *CLCN5* or *OCRL1*.

Dent disease, 61 (71%) possessed mutations in *CLCN5* (Dent-1) and 11 (13%) in *OCRL1* (Dent-2). In the remaining 14 patients (16%), no mutations in *CLCN5* or *OCRL1* were identified (Dent-NI: Dent-not identified). The mutations in each gene were divided according to the mutational type (Table 1). As is evident in Table 1, mutations in *CLCN5* (Dent-1) are diverse. In contrast, 9 of 11 *OCRL1* mutations are missense mutations, and their sites of mutations are concentrated in several codons (see also Table 3).

Table 2 shows description of the mutations identified in *CLCN5* in this study. The novel mutations that have not been reported elsewhere are indicated by #. Nearly half of the mutations identified in this study in *CLCN5* are novel (29 mutations: 48%). Mutations in *CLCN5* are scattered throughout the gene, whereas there are small numbers of hot spots of mutations in *CLCN5*, i.e. p.Arg34X, p.Arg637X, p.Arg704X, p.Ser244Leu and p.Arg516Trp. These mutations are detected in other countries and Japan.

Table 3 shows the genotypes and phenotypes of Dent-2 patients. Most mutations in *OCRL1* are missense mutations (9 out of 11, 80%). In this study, four mutations were found to be located at p.Arg318. Except p.Arg318Cys/His and p.Arg493Trp, all of the other mutations in *OCRL1*, namely, c.46G>C, c.265266insGG, p.Leu136X, p.Lys293Glu, p.Arg493Trp, p.Phe689Ser and p.Pro829Leu are novel. Regarding p.Arg318Cys mutation in *OCRL1*, we examined the genotype of mothers of D-47 and D-111, and revealed that they do not possess this mutation. This result indicates that p.Arg318Cys mutation is *de novo* in these cases, and denies the founder effect of p.Arg318Cys. Moreover, pArg318 mutation is also reported in other countries. Taken together, p.Arg318 is the unique hot spot, whose missense mutation leads to Dent-2 phenotype.

Figure 1 illustrates the scheme of the primary structure of *OCRL1* gene, and mutations identified in Dent-2 diseases. The mutations depicted in box are those identified in the present study, and grey colored ones are novel mutations. As will be discussed later, most mutations in Dent-2 exist between exons 5 and 15. Thus far, only two missense mutations after exon 15, i.e. p.Glu737Asp and p.Pro799Leu, have been identified as the causative mutations in Dent-2. In the present study, we identified two more missense mutations at the 3′ side of exon 15; i.e. p.Phe680Ser and p.Pro829Leu, which exist in exons 18 and 23, respectively.

Table 3 shows the laboratory data and clinical phenotype of Dent-2 patients. In previous studies, it was shown that the levels of the serum muscle enzymes, creatinine phosphokinase (CPK) and lactate dehydrogenase (LDH) are relatively high. In this study, however, the level of serum CPK was not necessarily high; for example, the levels of serum CPK were very low in D-88 and D-105. Also, LDH level ranges from 205 IU to 578 IU. Thus, even in Dent-2 laboratory findings were not common, which might also reflect the phenotypical variance among *OCRL1* mutations.

Figure 2A shows the relationship between urinary excretion of β2-microglobulin (β 2-MG), and Figure 2B shows those between urinary β 2-MG and urinary Ca/Cr ratios of the patients of the present study. Since all of the clinical data, except for β2-microglobulin and α1-microglobulin, could not be collected in this study, we plotted the data of patients in whom both of these data could be analysed. As depicted in Figure 1, the urinary β2-microglobulin level is extremely high (more than several thousands, and mostly ten thousands to one hundred thousands). As shown in Figure 2B, patients with extremely high levels of LMW proteinuria do not necessarily show high calciuria. Figure 3 shows the data of urinary β2-MG among different mutations in *CLCN5* (Dent 1). We found no statistical difference among the mutational types (ANOVA, $P = 0.212$)

Table 4 shows the data of patients with renal impairment. Six patients (7%) developed definite renal dysfunction. Among these six patients, four had mutations in *CLCN5*. In the remaining two patients, D-79 and D-115, no mutations were identified in *CLCN5* or *OCRL1*. However, the extremely high level of LMW protein in the urine, and the exclusion of other renal diseases in these two patients are compatible with Dent disease.

Table 5 summarizes the important clinical characteristics of the Japanese Dent disease patients. The data are presented for genotype including the total counts. The data are compared with those previously reported in a review article [22]. Regarding hypercalciuria, the number of patients with hypercalciuria (urinary Ca/Cr mg/mg >0.2) was 37 out of 73 patients (51%). The urinary Ca/Cr ratio ranged from 0.016 to 0.192 in patients without hypercalciuria, and from 0.2 to 0.75 in those with hypercalciuria. Among mutational types, calciuria is present, 46% in Dent-1, 70% in Dent-2 and 56% in Dent-NI. If we define hypercalciuria as having a Ca/Cr ratio of more than 0.25, hypercalciuria was present in only 42% of Dent-1 patients. The correlation of ages of patients and the levels of urinary β2-MG were also analysed using linear regression analysis, and the results show that the relation of these two values is not significant ($r = 0.356$). The percentage of patients with hypercalciuria was apparently lower in Japan than in other countries. Nephrocalcinosis was only identified in 35% of the patients. As has been expected, the percentage of patients with renal failure was relatively low in Japan, which is partly due to the early diagnosis

Table 2. Each mutation of CLCN5. Novel mutations are depicted as asterisk (#)

Pt. ID	Mutation type	Exon (intron)	Nucleotide changes	Amino acid changes	Codon, site or portion of mutation
D-56	del	7	c.801_803del	p.Glu267del	267
D-83	del	10	c.1566_1568del	p.Val522del	522
D-96	del large	4, 5	Deletion of exons 4 and 5	Deletion of exons 4 and exon 5	Exons 4–5
D-51	del large	1_12	CLCN5 total del	CLCN5 total del	total del
D-124	fs	3	c.165_169del	# p.Phe55Leufs*41	55
D-18	fs	3	c.191del	# p.Ile64Metfs*7	64
D-50	fs	7	c.746_752del	# p.Ala249Aspfs*3	249
D-58	fs	9	c.1526del	# p.Ala509Alafs*3	509
D-85	fs	10	c.1537del	# p.Gly513Glyfs*2	513
D-98	fs	10	c.1668del	# p.Gly556Glyfs*30	556
D-37	fs	11	c.2081_2082insC	# p.Thr694Thrfs*48	694
D-70	mis	4	c.263G>T	# p.Gly88Val	88
D-109	mis	4	c.270C>G	# p.Cys90Trp	90
D-60	mis	4	c.307T>C	# p.Trp103Arg	103
D-125	mis	6	c.527T>A	# p.Ile176Asn	176
D-81	mis	6	c.608C>T	p.Ser203Leu	202
D-106	mis	6	c.631G>C	# p.Glu211Gln	211
D-104	mis	6	c.634G>A	# p.Gly212Ser	212
D-43	mis	6	c.638C>T	# p.Pro213Leu	213
D-63	mis	7	c.731C>T	p.Ser244Leu	244
D-55	mis	7	c.731C>T	p.Ser244Leu	244
D-36	mis	7	c.796C>G	# p.Leu266Val	266
D-117	mis	8	c.814T>A	# p.Tyr272Asn	272
D-103	mis	8	c.815A>G	p.Tyr272Cys	272
D-13	mis	8	c.834G>C	p.Leu278Phe	278
D-66	mis	9	c.1403T>C	# p.Leu468Pro	468
D-100	mis	9	c.1505A>G	# p.Tyr502Cys	502
D-42	mis	9	c.1516G>A	# p.Gly506Arg	506
D-69	mis	10	c.1537G>A	p.Gly513Arg	513
D-24	mis	10	c.1546C>T	p.Arg516Trp	516
D-39	mis	10	c.1546C>T	p.Arg516Trp	516
D-102	mis	10	c.1546C>T	p.Arg516Trp	516
D-112	mis	10	c.1547G>A	p.Arg516Gln	516
D-34	mis	10	c.1571T>A	p.Ile524Lys	524
D-68	mis	11	c.2108T>C	# p.Phe703Ser	703
D-27	mis	11	c.2117T>C	# p.Leu706Pro	706
D-44	mis	11	c.2133C>G	# p.Cys711Trp	711
D-119	mis	12	c.2173A>G	p.Lys725Glu	725
D-40	non	2	c.82C>T	p.Arg28*	28
D-64	non	2	c.100C>T	p.Arg34*	34
D-99	non	2	c.100C>T	p.Arg34*	34
D-61	non	4	c.277G>T	# p.Gly93*	93
D-54	non	4	c.370C>T	# p.Gln124*	124
D-65	non	6	c.566G>A	# p.Trp189*	189
D-38	non	8	c.836G>A	p.Trp279*	279
D-74	non	8	c.1039C>T	p.Arg347*	347
D-97	non	9	c.1467G>A	# p.Trp489*	489
D-77	non	10	c.1885C>T	# p.Gln629*	629
D-35	non	10	c.1909C>T	p.Arg637*	637
D-95	non	10	c.1909C>T	p.Arg637*	637
D-12	non	11	c.1942C>T	p.Arg648*	648
D-16	non	11	c.2110C>T	p.Arg704*	704
D-33	non	11	c.2110C>T	p.Arg704*	704
D-26	non	11	c.2110C>T	p.Arg704*	704
D-86	splice	(int 3)	c.206G-1G>A		int 3
D-67	splice	(int 4)	c.393+1G>A	#	int 4
D-15	splice	(int 4)	c.394-2A>C		int 4
D-101	splice	(int 4)	c.394-2A>G		int 4
D-46	splice	(int 5)	c.516+1G>A		int 5
D-45	splice	(int 8)	c.1347+1G>T		int 8
D-17	splice	(int 10)	c.1933+2_1933+3insTGGT	#	int 10

Del, deletion mutation; fs, frame shift mutation; mis, missense mutation; non, nonsense mutation; splice, splice donor site mutation.

Table 3. Each mutation of OCRL1. Novel mutations are depicted as asterisk (#)

Pt. ID	Age (years)	Mutation type	Exon	Nucleotide changes	Amino acid changes	CPK[a] (IU/L)	LDH[a] (IU/L)	Cataract	Intelligence	Behavior
D-108	4.2	fs	5	# c.265_266insGG	p.Asp89Glyfs[a]18	298	390	None	Normal	Normal
D-88	9.1	mis	2	# c.46G>C	p.Glu16Gln	62	357		Normal	Normal
D-105	0.3	mis	10	# c.877A>G	p.Lys293Glu	39	319	None		
D-21	24.8	mis	11	c.953G>A	p.Arg318His	116	205	None	Normal	Normal
D-28	9.5	mis	11	c.952C>T	p.Arg318Cys		210	None	Normal	Normal
D-47	9.6	mis	11	c.952C>T	p.Arg318Cys		578			
D-111	15.3	mis	11	c.952C>T	p.Arg318Cys	342	257			
D-120	3.4	mis	15	c.1477C>T	p.Arg493Trp					
D-30	17.4	mis	18	# c.2039T>C	p.Phe680Ser	299	224	(+)	Normal	Normal
D-49	9.0	mis	23	# c.2486C>T	p.Pro829Leu	255	268		Normal	Normal
D-118	4.1	non	6	# c.[407T>A; 408A>G]	p.Leu136[a]	192	274	None	Normal	Normal

Abbreviations are the same as in Table 3.
[a]Normal value of CPK and LDH.
CPK < 30∼ 350 IU/L (more than 1 year).
LDH < 150∼380 IU/L (more than 1 year).

and young age of patients, whereas in Europe and the USA, renal insufficiency develops after middle age. One of the most distinct sets of data that differs from the European countries and USA are the rate of rickets. Only two patients (2%) showed rickets in Japanese Dent patients, whereas in other countries it is 33%. In addition, in the present study, no apparent patients with Fanconi syndrome were identified (glucosuria, aminoaciduria, metabolic acidosis and phosphaturia).

DISCUSSION

The present study is the first large-scale comprehensive analysis of Japanese Dent disease in terms of both genetic background and clinical phenotype. The first important finding of this study is that the genetic background of patients with Japanese Dent disease is nearly identical to those in Europe and the USA. In other countries, *CLCN5* mutations were identified in ∼60% in patients with Dent disease, and 15% in *OCRL1*. Among 86 unrelated Japanese Dent patients, 71% (61 patients) possess mutations in *CLCN5* (Dent-1), and 48% (29 patients) are novel; 13% possesses mutations in *OCRL1* (Dent-2) (Tables 1–3). In the remaining 16%, no mutations in *CLCN5* or *OCRL1* were identified (Dent-NI). The second critical finding is that there are large clinical differences, such as the incidence of hypercalciuria, nephrocalcinosis, urolithiasis and rickets, between patients in Japanese Dent disease and those in Europe/USA, despite the similarity of genetic background. For example, hypercalciuria, which is usually more prominent in children, was observed only in 51% of Japanese Dent disease patients, whereas in other countries it is nearly 90% (Table 5) [22]. Only two patients, both with Dent-2, developed rickets. Furthermore, apparent Fanconi syndrome was not identified in this Japanese cohort. The third is that several Japanese Dent disease patients developed renal impairment. This is somehow unexpected because most of Japanese patients are young, and did not necessarily have the complication of nephrocalcinosis or urolithiasis.

Regarding the *CLCN5* mutations, many novel missense, nonsense and frameshift mutations were identified in this study. As reported previously, mutations in *CLCN5* exist throughout the gene and are specific to each pedigree or individual. One of the critical concerns for these mutations, especially missense mutation, is the mechanism(s) by which each mutation disrupts the function of CLC-5. Recently, Smith *et al.* classified CLC-5 mutants into three classes [23]. Class 1 mutation, including p.Ser270Arg, p.Gly513Glu, p.Arg516Trp and p.Ile524Lys, results in the retention of CLC-5 in the endoplasmic reticulum; Class 2 comprises the functionally defective mutations, such as p.Glu527Asp and Class 3 mutations, p.Gly57Val and p.Arg280Pro, result in altered subcellular distribution of CLC-5. Grand *et al.* also examined several CLC-5 missense mutations identified in the patients, and divided the mutations into two types. Type I mutations depress chloride current, while they are actually expressed in the plasma membrane. Type II includes mutants that could not traffic to the membrane. At the moment, CLC-5 is considered to function as an chloride/proton antiporter and its role in endosomal acidification is unclear [24, 25]. Besides *in vitro* expression study, analyses using bioinformatics methods are useful in speculating the significance of each residue and the impact of its mutation. Wu *et al.* [26] reported that most missense mutations of CLC-5 are located in helices forming the subunit interface using a three-dimensional homology model of human CLC-5. Smith *et al.* [23] also reported that all of the examined mutants are located in the dimer interface.

In order to examine the impact of the missense mutation identified in the present study, we first made the alignment of CLC-5 protein among different mammalian species (see supplementary data S1). As expected, all of the residues, where missense mutations are identified, are conserved among all species tested. In parallel, we are examining several mutants by an *in vitro* expression system, and started to perform a bioinformatics analysis. Preliminary data indicate that novel mutations identified in this study are not necessarily located in the dimer interface of CLC-5. One characteristic mutation identified exclusively in this study is located in a narrow region at the C-terminus of CLC-5. This type of mutation includes p.Phe703Ser, p.Leu706Pro, p.Cys711Trp, p.Lys725Glu, p.Arg704X and p.Thr694Thrfs*48. The C-terminus is

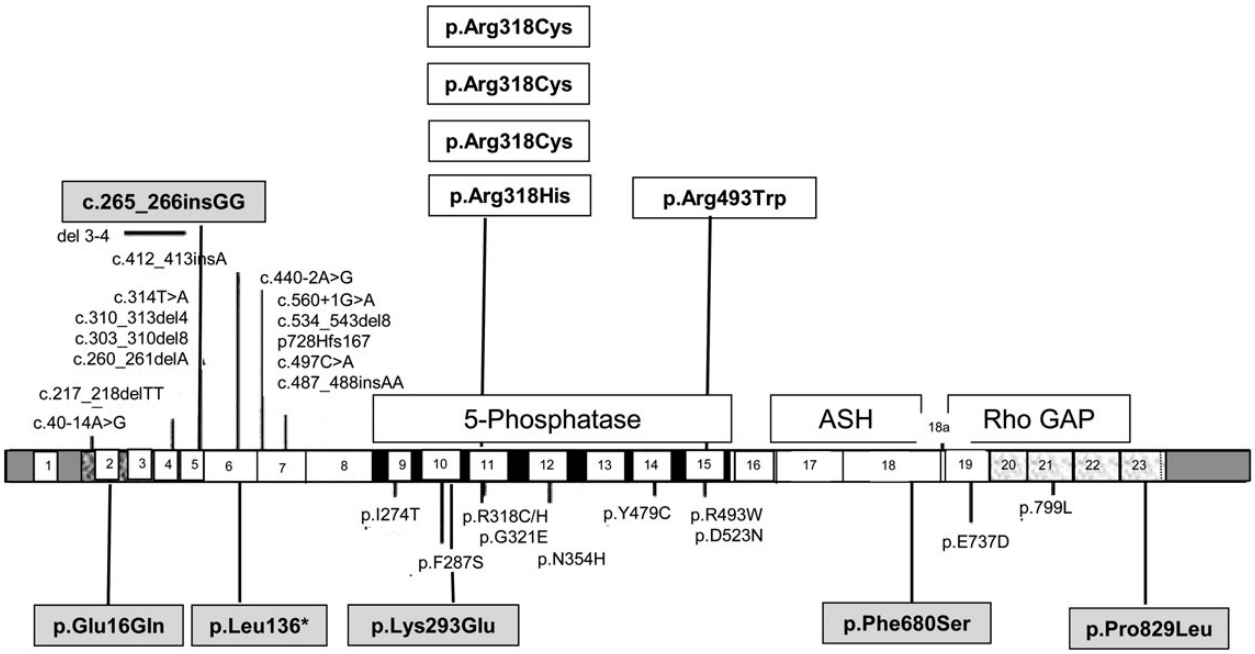

FIGURE 1: *OCRL1* mutations manifesting Dent-2 phenotype. Mutations in closed boxes are those identified in the present study. Mutations in white boxes are those previously reported, and those in gray boxes are novel mutations. The original figure in the report by Hichri [21] was modified and newly identified in this study mutations are added.

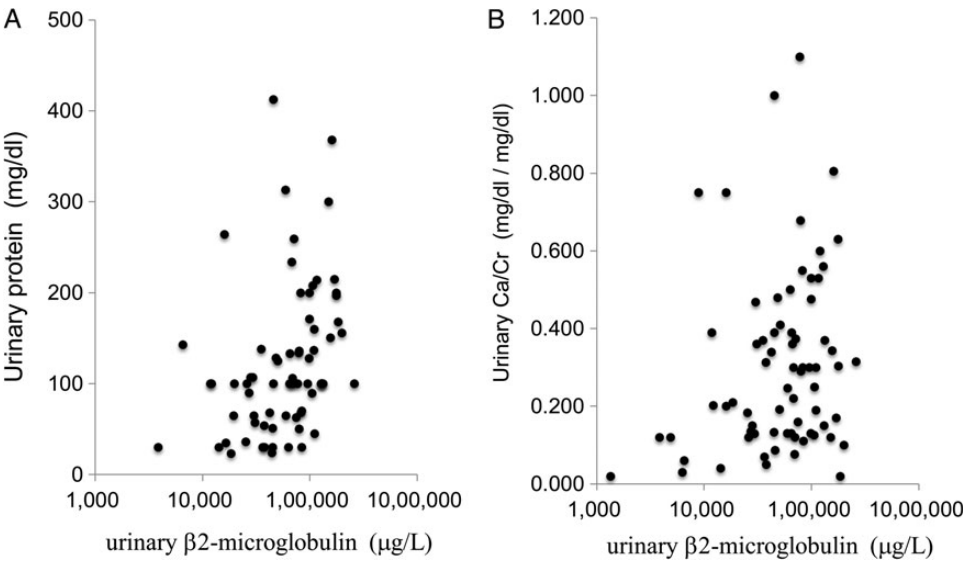

FIGURE 2: (**A**) Relationship between urinary β2-MG and urinary excretion of total protein. (B) Relationship between urinary β2-MG and urinary Ca/Cr ratio.

considered to bind ATP; as a result, conformational change in CLC-5 protein occurs and functions as proton/chloride channel. Thus, these mutations in the C-terminus would result in disruption of ATP binding, and abolish CLC-5 function. In the present study, we identified 15 novel missense mutations including C-terminus in *CLCN5*. In the next study, we would obtain further clues to elucidate the structure–functional relationship of CLC-5.

Regarding *OCRL1* mutations, six, i.e. p.Asp89Glyfs*18, p.Glu16Gln, p.Lys293Glu, p.Phe680Ser, p.Pro829Leu and p.Leu136*, are novel mutations in Dent-2. The reason why mutations in *OCRL1* lead to Dent disease or Lowe syndrome still remains unclear. Two hypotheses have been proposed. The first is that all of the frameshift mutations or nonsense mutations identified in Dent-2 are clustered in the 5′ region of *OCRL1* (from exons 1 to 7); missense mutation identified in Dent-2 are all found in exons 9–23, mostly between 9 and 15, which comprise a catalytic phosphatase domain [27]. Hichri *et al.* [27] also indicate the same hypothesis [21]. Using bioinformatics analysis, Shrimpton *et al.* proposed an alternative

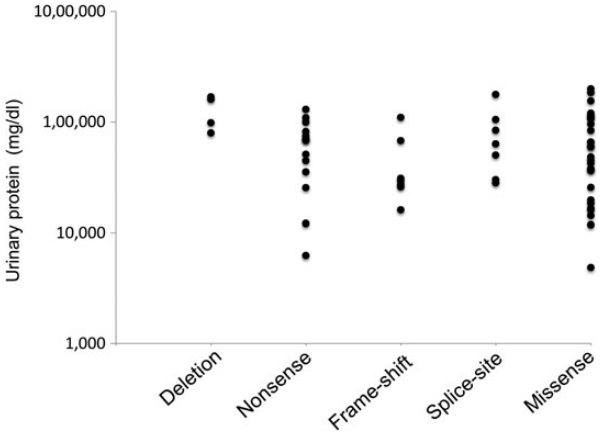

FIGURE 3: The level of urinary β2-MG among the mutational types of *CLCN5*. There are no statistical difference among the mutational types (ANOVA, P = 0.212).

splicing variant of OCRL1 whose translation methionine is Met189. All the six novel *OCRL1* mutations identified in this study follow this rule, and reinforce this hypothesis. In particular, p.Phe680Ser and p.Pro829Leu, which are located outside the catalytic domain (exons 8–15), might be useful for solving this question. The second hypothesis is that Dent-2 is a variant and a mild phenotype of Lowe syndrome, and there are some modifying molecules (e.g. compensatory phosphatase and interacting proteins) whose expression and function depend on the genetic background of other molecules, such as INPP5B [28]. Indeed, Hichri *et al.* reported that one patient with a p.Ile274Thr missense mutation developed bilateral congenital cataract, while his elder brother with the same mutation showed no ocular abnormalities [21]. Pasternack *et al.* also reported a 13-year-old patient with a nonsense mutation (p.Gln199X) in exon 8 of OCRL1, who developed a typical cerebro-renal phenotype of Lowe syndrome, but without any ocular involvement [29]. These results indicate selective organ involvement among *OCRL1* mutations [30]. Except for Dent-2 and Lowe phenotype, there are large clinical variabilities in clinical manifestations of patients with *OCRL1* mutations [28]. We recently experienced several patients with congenital cataract and mental retardation, whereas there is no or only extremely mild renal manifestation. In these patients, we did not identify mutations in *OCRL1*. Thus, as Dent-2 and renal manifestation in Lowe syndrome, other molecules might participate in the proximal function.

In Dent-2, the characteristic laboratory findings are elevated levels of serum CK and LDH. Bökenkamp *et al.* reported that the levels of serum CK and/or LDH are elevated in 31 patients with Dent-2 and Lowe syndrome. However, D-88, D-105, D-21 and D-118 showed normal levels of serum CK and LDH when considering their ages [27]. Thus, the elevation of serum CK and/or LDH levels does not necessarily occur in patients with Dent-2.

Whether Japanese Dent disease patients would develop renal dysfunction is one of the important issues. Igarashi *et al.* [14] reported a case of a 51-year-old Japanese Dent disease patient with a splice donor site mutation in *CLCN5* who

Table 4. Clinical data of the patients who develop renal dysfunction

Dent No.	Dent type	CLCN5 mutation	Patients' age (years)	Serum Cr	BUN	Ca	IP	Urinary Ca/Cr (mg/mg)	Renal calcification	Urolithiasis	Urinary-B2MG (<270 μg/L)
D-38	Dent-1	p.Trp279*	24.3	1.58	13	9.7	3.4	0.12	Presence	None	70 036
D-34	Dent-1	p.Ile524Lys	20.7	1.63	14	10	3.8	0.39	None	None	11 791
D-37	Dent-1	p.Thr694Thrfs*48	19.4	5.33	44.1	9.7	3.8	0.12	Presence		25 800
D-16	Dent-1	p.Arg704*	20.2	1.61	16	9.8	1.4	0.183	Presence	None	25 347
D-115	Dent-NI		24.0	12.8	101.7			0.087	Presence	None	45 800
D-79	Dent-NI		59.0	2.36	14			0.34	None	Presence	α1-MG: 165

Table 5. Comparison of main clinical data of Japanese Dent patients and those reported so far

Clinical/biochemical characteristics	Previously reported data: Dent-1*	Dent disease in Japan (incidence/determined)			
		Total	Dent-1	Dent-2	Dent-NI
LWMP	100 (%)	100% (86/86)	100% (61/61)	100% (11/11)	100% (14/14)
Hypercalciuria	89 (%)	51% (37/73)	46% (25/54)	70% (7/10)	56% (5/9)
Nephrocalcinosis	76 (%)	35% (26/75)	38% (20/53)	10% (1/10)	42% (5/12)
Renal failure	42 (%)	8% (6/74)	8% (4/53)	0% (0/10)	18% (2/11)
Rickets/osteomalacia	33 (%)	2% (2/86)	0% (0/61)	9% (1/11)	7% (1/14)

developed end-stage renal failure at 46 years of age. To date, this is the sole case of apparent renal dysfunction in Japanese Dent disease. In the present study, at least 6 (7%) out of 86 patients developed definite renal dysfunction. Among these six cases, four patients possessed mutations in *CLCN5*. Although the remaining two patients, D-79 and D-115, showed no mutations in *CLCN5* or *OCRL1*, clinical findings were compatible with Dent disease. In addition, D-18, D-22 and D-20 might have subclinical levels of renal impairment. Thus, the present findings clearly demonstrate that Japanese Dent disease patients have the risk of developing renal impairment. Except D-79, all of the patients with renal dysfunction were younger than 25 years. In addition, it should be noted that among these six patients with renal impairment, two (D-34 and D-34) showed hypercalciuria (urinary Ca/Cr mg/mg >0.2) and four showed nephrocalcinosis. Thus, hypercalciuria and/or nephrocalcinosis is not necessarily essential for the development of renal insufficiency in Japanese Dent disease patients. Just recently, Frishberg *et al.* reported three unrelated familial cases of Dent-1. Renal biopsy specimens of two unrelated boys revealed focal segmental glomerulosclerosis and/or focal global glomerulosclerosis; tubulo-interstitial changes were minimal [31]. In other reports, Copelovitch *et al.* described precise renal pathological findings in two Dent disease patients with *CLCN5* mutation [32]. In both patients, despite the minimal tubulo-interstitial alterations, FGS was prominent. In one patient, two to three glomeruli out of six showed segmental sclerosis; only one glomerulus was completely normal. Thus, the mechanisms by which patients with Dent disease develop renal dysfunction do not only owe to nephrocalcinosis, but some direct effects of mutations in *CLCN5* or *OCRL1* on renal function might exist. This hypothesis should be challenged in a future study.

The criteria proposed by Hoopes are generally accepted for the clinical diagnosis of Dent disease. Hoopes's criteria include two essential data, i.e. LMW proteinuria and hypercalciuria [20]. In addition, symptoms such as nephrocalcinosis, urolithiasis, hematuria and renal impairment would strengthen the diagnosis [20]. Indeed, accumulated clinical and biochemical data on Dent disease are compatible with Hoopes's criteria. In contrast, hypercalciuria is observed only in 51% of Japanese Dent patients. As has been reported, hypercalciuria is more prominent in young patients than in elderly patients. In Japan, most patients are diagnosed with Dent disease before 15 years of age. Thus, in this population, hypercalciuria should exist in most of the patients. Why did this difference in the clinical spectrum arise? The most probable explanation is the diagnosis of Japanese Dent disease by the annual school urinary screening test. Considering the common molecular basis of Dent disease between patients in Japan and other countries, and the high sensitivity Japanese urinary screening system, 'LMW proteinuria in Japan' has a wider clinical spectrum than Dent disease. In other words, patients with only LMW proteinuria and mutations in *CLCN5* or *OCRL1* might be missed in other countries. In addition, the degree and incidence of hypercalciuria, nephrocalcinosis, urolithiasis and renal impairment in Japanese Dent disease patients are lower than those in Europe/USA and this might be partially owing to the content of the water. In Japan, drinking water contains very low levels of minerals, especially calcium. This is true throughout Japan, except Okinawa prefecture, and the reference data for calciuria are urinary Ca/Cr ratio and normal level, these data are <0.2 in Japan.

In conclusion, the present study demonstrated the genetic and clinical backgrounds of Japanese Dent disease. Japanese Dent disease, in other words 'LMW proteinuria disease', appears to include a wider clinical spectrum than Dent disease in Europe and the USA, although both diseases showed the same genetic background. In addition, many novel mutations in *CLCN5* and *OCRL1* identified in this study would provide clues to the molecular mechanisms underlying Dent disease and the mechanisms of renal impairment.

SUPPLEMENTARY DATA

Supplementary data are available online at http://ndt.oxfordjournals.org.

ACKNOWLEDEGMENTS

The authors deeply thank Ms Masayo Matsumura, Naoko Hoshino, Emiko Shimiura-Matsui, Fumiko Tozawa and Yoko Sawada for the skillful technical assistance and help in the preparation of this manuscript. The authors also thank Dr Masato Takeuchi for statistical analysis.

FUNDING

This work was supported by grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology (grant number 13671101) and from Japanese Kidney Foundation.

CONFLICT OF INTEREST STATEMENT

On behalf of all authors, T.S. declares no competing interests that should be disclosed.

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Received for publication: 9.2.2013; Accepted in revised form: 16.8.2013