



# Dent Disease in Chinese Children and Findings from Heterozygous Mothers: Phenotypic Heterogeneity, Fetal Growth, and 10 Novel Mutations

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**Objective** To characterize the phenotypes of Dent disease in Chinese children and their heterozygous mothers and to establish genetic diagnoses.

**Study design** Using a modified protocol, we screened 1288 individuals with proteinuria. A diagnosis of Dent disease was established in 19 boys from 16 families by the presence of loss of function/deleterious mutations in *CLCN5* or *OCRL1*. We also analyzed 16 available patients' mothers and examined their pregnancy records.

**Results** We detected 14 loss of function/deleterious mutations of *CLCN5* in 15 boys and 2 mutations of *OCRL1* in 4 boys. Of the patients, 16 of 19 had been wrongly diagnosed with other diseases and 11 of 19 had incorrect or unnecessary treatment. None of the patients, but 6 of 14 mothers, had nephrocalcinosis or nephrolithiasis at diagnosis. Of the patients, 8 of 14 with Dent disease 1 were large for gestational age (>90th percentile); 8 of 15 (53.3%) had rickets. We also present predicted structural changes for 4 mutant proteins.

**Conclusions** Pediatric Dent disease often is misdiagnosed; genetic testing achieves a correct diagnosis. Nephrocalcinosis or nephrolithiasis may not be sensitive diagnostic criteria. We identified 10 novel mutations in *CLCN5* and *OCRL1*. The possibility that altered *CLCN5* function could affect fetal growth and a possible link between a high rate of rickets and low calcium intake are discussed. (*J Pediatr* 2016;174:204-10).

Dent disease (MIM 300009, 300555) is a rare X-linked recessive disorder. Progressive proximal renal tubulopathy is considered to be fundamental, with impaired tubular reabsorption of proteins that pass through the glomerular filtration barrier.<sup>1,2</sup> Two distinct X-linked genes, chloride voltage-gated channel 5 (*CLCN5*, Entrez Gene ID: 1184) and oculocerebrorenal syndrome of Lowe (*OCRL1*, Entrez Gene ID: 4952), underlie the disease.<sup>3,4</sup> Based on the responsible genes, Dent disease is divided into Dent disease 1 (MIM 300009) for *CLCN5*-related disease and Dent disease 2 (MIM 300555) for *OCRL1*-related disease. *CLCN5* is responsible for ~50%-60% of Dent disease<sup>1</sup> and encodes the H(+)/Cl(-) exchange transporter 5/chloride channel protein 5 (CLC-5, Uniprot ID: P51795). *OCRL1* is responsible for ~15% of Dent disease<sup>1</sup> and encodes an inositol polyphosphate 5-phosphatase OCRL-1/Lowe (oculocerebrorenal) syndrome protein (OCRL1, Uniprot ID: Q01968). Mutations that cause Dent disease 2 mostly are located in the 5' portion of the gene, and mutations that cause Lowe syndrome, which shares some phenotypic similarities with it, are located in the 3' part of the gene.<sup>5</sup> Both genes play important roles in the endocytosis-based reabsorption and processing of low-molecular-weight proteins from the renal tubular brush border.<sup>1,2,6-11</sup> The responsible gene(s) for the remaining ~25%-35% of patients with Dent disease have not yet been identified.<sup>1</sup>

The most common clinical manifestations of Dent disease are low-molecular-weight proteinuria, hypercalcuria, nephrocalcinosis, nephrolithiasis, and progressive renal failure.<sup>1,2</sup> In Dent disease 2, patients may present with subclinical cataract, hypotonia, and mild intellectual disability, which overlap with Lowe syndrome phenotypes.<sup>1</sup> Phenotypic heterogeneity both within and between

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24hUCa	24-hour urine calcium
$\beta_2$ MG	$\beta_2$ -microglobulin
Ca/Cr	Calcium/creatinine
CBS	Cystathionine betasynthase
CLC-5	H(+)/Cl(-) exchange transporter 5/chloride channel protein 5
LGA	Large for gestational age
OCRL1	Inositol polyphosphate 5-phosphatase OCRL-1/Lowe (oculocerebrorenal) syndrome protein
SGA	Small for gestational age

different ethnicities has been reported.<sup>12-15</sup> Data from heterozygous mothers and data concerning intrauterine growth of patients have been lacking in the vast majority of patients described previously. Two patients with Dent disease 1 were large for gestational age (LGA).<sup>16</sup>

## Methods

We screened 1288 individuals with proteinuria, who were referred to us, the major referral center for childhood kidney diseases in Guangdong Province, China, from January 2009 until December 2013, using a protocol modified from that recommended by Edvardsson et al.<sup>17</sup> There was no specific clinical feature except growth abnormalities, such as short stature and/or lower body weight (see Results). Eight patients had polyuria compared with standards for different-aged Chinese children.<sup>18</sup> For patients in whom genetic testing confirmed the diagnosis, we examined retrospectively all available medical records. These records were evaluated according to the criteria for newborns recommended by the Group of Neonatology, Pediatric Society, Chinese Medical Association.<sup>19</sup> Patients' mothers also were studied where available, including any records from pregnancy.

Fifty individuals who were judged completely healthy by our medical examinations were collected as controls and were studied in the same manner. The study was approved by the ethics committee of Sun Yat-sen University. Informed consent was obtained from all participants, their parents, or guardians. Principles outlined in the Declaration of Helsinki were followed.

Laboratory investigations included routine urine testing, the first-line urinary test in Chinese hospitals. This testing includes semiquantitative urinary protein. The routine urinary test also includes urinary gravity, pH, leukocyte esterase, nitrite, glucose, urine occult blood, ketone bodies, urobilinogen, and urine sediments examination on AUTION MAX UF1000i-AX4280 (Sysmex, Kobe, Japan) and Sysmex UF-1000i (Sysmex). Laboratory investigations also included blood and urinary chemistry. Low-molecular-weight proteinuria was determined by increased urinary  $\beta_2$ -microglobulin ( $\beta_2$ MG), analyzed on a BN ProSpec automated analyzer (Siemens, Munich, Germany); hematuria, glucosuria, and aminoaciduria were measured with Sysmex UF-1000i (Sysmex); 24-hour urine calcium (24hUCa) and urinary calcium/creatinine (Ca/Cr) were determined by Vitros Fusion 5.1 (Sysmex). Serum electrolytes, sodium, potassium, calcium, chloride, cholesterol, phosphate, magnesium, blood urinary nitrogen, serum creatinine, and alkaline phosphatase were measured on an ARCHITECT C16000 (Abbott, Abbott Park, Illinois); 25-OH-vitamin D<sub>3</sub> was determined by HITACHI cobas 6000 (Roche, Rotkreuz, Switzerland), parathyroid hormone on the ARCHITECT C16000 (Abbott) and ARCHITECT i4000 (Abbott).

### Renal Pathology

Renal biopsy had been performed in 14 of 19 patients. The slides were examined by light and electron microscopy.

Immunohistochemistry with antibodies against IgA, IgG, IgM, complement 3, complement 1q, and fibrinogen were performed according to the manufacturer's instructions (Dako A/S, Glostrup, Denmark).

### Renal Ultrasound Examination

All patients and their available mothers underwent renal ultrasound examinations. Nephrocalcinosis was diagnosed when there was visible calcification.<sup>20</sup> Nephrolithiasis was considered when the ultrasound examination showed an echogenic focus (preferably with clear acoustic shadowing) in renal pelvis or calyx or hydronephrosis.<sup>21</sup>

### Skeletal Radiographs and Rickets

Of 16 patients who had radiograph examinations for possible bone abnormalities, rickets was diagnosed in 8 patients on the basis of bone deformity on physical examination and the presence of radiographic abnormalities in the wrists (metaphyseal fraying and cupping of the distal radius and ulna), with the support of laboratory testing (elevated serum alkaline phosphatase activity, or hypocalcemia, or hypophosphatemia).<sup>22,23</sup> Osteomalacia was diagnosed when patients had bone pain and tenderness, muscle weakness, and/or signs of tetany in combination with decreased bone mineral density (measured in the forearm, lumbar spine, and hip), supported by laboratory test results as for rickets.<sup>22,24</sup>

### Mutation Detection by Sanger Sequencing

Genomic DNA was extracted from peripheral blood of the 23 patients with clinically diagnosed or suspected Dent disease, 10 available mothers, and 50 control individuals via QIAamp Blood DNA Kits (QIAGEN, Hilden, Germany). Polymerase chain reaction was performed to amplify all exons and exon-intron boundaries of *CLCN5* and *OCRL1* with specific primers designed with Oligo6.0 (<http://www.oligo.net/downloads.html>). Polymerase chain reaction products were sequenced on an ABI 3730XL Automated DNA Sequencer with a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California). The results were compared with GenBank sequences NM\_000084.4 for *CLCN5* and NM\_000276.3 for *OCRL1* retrieved from the UCSC database (<http://genome.ucsc.edu/>). Mutation nomenclature recommended by den Dunnen and Antonarakis (<http://www.hgvs.org/mutnomen/>)<sup>25</sup> was adopted.

### Bioinformatics Analyses

For each of the identified variations we searched the Human Gene Mutation Database (<http://www.hgmd.org/>), the 1000 Genome Project (<http://www.1000genomes.org/>), the dbSNP (<http://www.ncbi.nlm.nih.gov/snp>), and the Exome Variant Server (<http://evs.gs.washington.edu/EVS/>) to determine whether the mutation had been reported. To determine possible biological implications of the variations, we performed cross-species alignment with 5 orthologues (Nomascus, Canine, Mouse, Gallus, and Xenopus) by CLUSTAL X (1.81).<sup>26</sup> We used SIFT (<http://sift.jcvi.org/>) and PolyPhen2 (<http://genetics.bwh.harvard.edu/pph2/>) to predict possible

effects of novel mutations. For frame shifting mutations we used the National Center for Biotechnology Information Open Reading Frame Finder (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>) to predict consequences of the mutations. Domain information for proteins was obtained with Pfam (<http://pfam.xfam.org/>).

### Structural Modeling of CLC-5 and Structure Alignment of OCRL1

Structural modeling of human CLC-5 was performed with the MODELLER module in Discovery Studio 3.0 (Accelrys, San Diego, California) based on the recently reported structure of a eukaryotic chloride channel transporter from *Cyanidioschyzon merolae* (PDB ID: 3ORG).<sup>27</sup> Four extracellular loop regions were deleted because of the lack of sequence homology or missing electron density in the template structure. The refined model was validated by the VERIFY-3D program in Discovery Studio 3.0.

Structural alignment of OCRL1 with homologous proteins: Type II inositol 1,4,5-trisphosphate 5-phosphatase (PDB ID: 4CML) and SH2 domain-containing inositol 5'-phosphatase 2 (PDB ID: 3NR8) were performed using PyMOL.<sup>28</sup>

## Results

### Clinical Diagnosis and Unnecessary Treatment before Reaching the Correct Diagnosis

Of 23 patients provisionally diagnosed with Dent disease on clinical grounds, the diagnosis was confirmed in 19 patients by genetic testing (Table I).<sup>29</sup> The presenting symptom in all of the 19 confirmed patients was urinary abnormalities (Table II); their blood biochemistry results are shown in Table III.<sup>30,31</sup> Thirteen patients were previously diagnosed

with nephrotic syndrome or nephrosis (Table I). Four patients were diagnosed with Bartter syndrome, including P5 from family 5 who were first diagnosed with nephrotic syndrome and then Bartter syndrome (Table I). Eleven of the 19 patients had been treated with prednisolone, methylprednisolone, cyclosporine, cyclophosphamide, or alternative medicine. None of the patients with Dent disease 2 had any Lowe syndrome-like phenotype, subclinical cataract, hypotonia, severe intellectual disability, or dysmorphic features, but all had short stature (Table IV).<sup>32</sup>

### A Diagnosis of Dent Disease Was Established in 19 of 23 Patients by Genetic Testing, and 10 Novel Mutations Were Identified

In the *CLCN5* gene, we identified 14 mutations in 15 patients from 14 families, including 8 novel mutations (Table I). The 2 nonsense mutations and 3 frame shift mutations, c.242G>A (p.Trp81\*), c.813C>G (Tyr271\*), c.746\_749del (p.Ala249Valfs\*4), c.1620dup (Ala541Cysfs\*19), and c.1743\_1765dup (p.Arg590Glnfs\*4), introduce premature stop codon(s) and lead to a predicted truncation of 666, 476, 495, 188, and 154 amino acids at the C terminus of the encoding protein, respectively. The c.242G>A (p.Trp81\*), c.813C>G (Tyr271\*), and c.746\_749del (p.Ala249Valfs\*4) mutations wipe out part of the transmembrane regions and the 2 cystathionine betasynthase (CBS) domains. The c.1620dup (Ala541Cysfs\*19) mutation truncate the 2 CBS domains and c.1743\_1765dup (p.Arg590Glnfs\*4) truncate the part of the first and all the second CBS domain. mRNAs transcribed from the truncated genes are expected to be degraded by nonsense-mediated decay.<sup>33</sup> Tests of the boys' mothers showed that c.746\_749del, c.813C>G, and c.242G>A were inherited from the probands' mothers. The origin of the other 2 mutations c.1620dup and c.1743\_1765dup, however,

**Table I.** Previous diagnoses and mutations of patients with Dent disease

	Previous diagnosis at local hospital	Mutation	Mutation type	Novel/known
<b>Dent disease 1</b>				
P1	Nephrosis	c.1537G>A (p.Gly513Arg)	Missense	Known
P2	Nephrosis	c.833T>C (p.Leu278Ser)	Missense	Novel
P3	?	c.746_749del (p.Ala249Valfs*4)	Deletion	Novel
P4	Bartter syndrome	c.789A>T (p.Leu263Phe)	Missense	Novel
P5	Nephrotic syndrome and then Bartter syndrome	c.793 A>C (p.Ser265Arg)	Missense	Novel
P6	Bartter syndrome	c.2110C>T (p.Arg704*)	Nonsense	Known
P7	Nephrotic syndrome	c.82C>T (p.Arg28*)	Nonsense	Known
P8	?	c.813C>G (p.Tyr271*)	Nonsense	Novel
P9	Nephrotic syndrome	c.813C>G (p.Tyr271*)	Nonsense	Novel
P10	Nephrosis	c.242G>A (p.Trp81*)	Nonsense	Novel
P11	Nephrotic syndrome	c.1620dup (p.Ala541Cysfs*19)	Duplication	Novel
P12	Nephrotic syndrome	c.720_721del (p.Glu241Glyfs*26)	Deletion	Known
P13*	Nephrosis	ChrX: 49,780,222 - 49,840,741 del	Deletion	Known
P14	Nephrosis	c.117del (p.Ser40Alafs*8)	Deletion	Known
P15	Nephrosis	c.1743_1765dup (p.Arg590Glnfs*4)	Duplication	Novel
<b>Dent disease 2</b>				
P16	Nephrotic syndrome	c.833_838del (p.Glu278_Leu279del)	Deletion	Novel
P17	?	c.833_838del (p.Glu278_Leu279del)	Deletion	Novel
P18	Bartter syndrome	c.523del (p.Arg175Glyfs*10)	Deletion	Novel
P19	Nephrosis	c.523del (p.Arg175Glyfs*10)	Deletion	Novel

P8 and P9 from family 8; P16 and P17 from family 15; P18 and P19 from family 16; the other patients are each from independent families.

\*This patient has been reported in *Human Genetics*.<sup>29</sup>

**Table II.** Urinary tests of patients with Dent disease

	24-hour urine protein, g/d*	Albumin, mg/L <sup>†</sup>	$\alpha_1$ -microglobulin, mg/L <sup>‡</sup>	$\beta_2$ MG, mg/L <sup>§</sup>	Low-molecular-weight proteins/total protein, %	24hUCa, mg/kg/24h <sup>¶</sup>	Ca/Cr, mg/mg**
Dent disease 1, mean $\pm$ SD	1.3 $\pm$ 0.9	231.9 $\pm$ 175.2	239.4 $\pm$ 163.9	121.1 $\pm$ 93.9	59.6 $\pm$ 7.8	6.9 $\pm$ 1.5	0.39 $\pm$ 0.19
Dent disease 2, mean $\pm$ SD	1.3 $\pm$ 0.5	396.5 $\pm$ 72.9	364.7 $\pm$ 144.4	287.3 $\pm$ 232.3	55.2 $\pm$ 9.8	6.5 $\pm$ 2.2	0.41 $\pm$ 0.14

Hematuria was found in P1, P2, P7, P10, P13, P15, P16, P17, P18, and P19. Glucosuria was found in P3, P4, P7, and P11. Aminoaciduria was found in P3, P4, P5, P7, P8, and P9.

\*Normal value: 0-0.1 g/day.

<sup>†</sup>Normal value: 0-30 mg/L.

<sup>‡</sup>Normal value: 0-12 mg/L.

<sup>§</sup>Normal value: 0-0.206 mg/L.

<sup>¶</sup>Normal value <4 mg/kg/24h.

\*\*Normal value <0.2 mg/mg.

was uncertain as the probands' mothers were unavailable for analysis. We also identified 3 missense mutations, c.789A>T (p.Leu263phe), c.793A>C (p.Ser265Arg), and c.833T>C (p.Leu278Ser), in conserved amino acids that are predicted to be damaging or probably damaging by SIFT and PolyPhen2. The c.789A>T (p.Leu263phe) and the c.793A>C (p.Ser265Arg) occurred in the fifth transmembrane region; the c.833T>C (p.Leu278Ser) occurred in the sixth transmembrane region and is predicted to replace a hydrophobic leucine with hydrophilic serine.

We identified 2 novel mutations in the *OCRL1* gene, c.833\_838del (Glu278\_Leu279del) and c.523del (Arg175-Glyfs\*10) in 4 patients from 2 families (Table I). The

c.833\_838del (Glu278\_Leu279del), an in-frame deletion, is predicted to delete the glutamic acid and leucine at positions 278 and 279, respectively. These 2 amino acids are conserved between human and the 5 orthologs analyzed and are located in the endonuclease/exonuclease/phosphatase family domain, the patient's mother carried the mutation. c.523del (Arg175Glyfs\*10) is a frameshift mutation introducing a premature stop codon and removing 718 amino acids from the C terminus of the wild-type protein, which harbors the endonuclease/exonuclease/phosphatase family domain and the GTPase-activator protein towards Rho/Rac/Cdc42-like small GTPases domains; the patients' mother also carried the

**Table III.** Blood biochemistry tests of patients with Dent disease

	Hyponatremia	Hypokalemia	Hypochloremia	Hypocalcemia	Hypophosphatemia	Hypomagnesemia	Acidosis/alkalosis
Dent disease 1							
P1	—	—	—	—	—	—	—/—
P2	—	—	—	—	—	+	—/—
P3	—	—	—	—	—	—	—/—
P4	+	+	+	—	+	+	—/+
P5	+	+	+	+	+	+	—/+
P6	+	+	+	+	+	+	—/+
P7	—	+	—	—	+	—	—/—
P8	—	+	—	+	—	+	+/—
P9	—	+	—	—	+	—	—/—
P10	—	—	—	—	—	—	—/—
P11	—	—	—	—	—	ND	—/—
P12	—	—	—	—	—	—	—/—
P13	—	—	—	—	—	—	—/—
P14	—	—	—	—	—	—	—/—
P15	—	—	—	—	—	—	—/—
Abnormality	3/15	6/15	3/15	3/15	5/15	5/14	1/15 3/15
Dent disease 2							
P16	—	—	—	—	—	—	—/—
P17	—	—	—	—	—	—	—/—
P18	—	+	+	—	—	—	—/+
P19	—	—	—	—	—	—	—/—
Abnormality	0/4	1/4	1/4	0/4	0/4	0/4	0/4 1/4
	Blood urine nitrogen, mmol/L*	Serum creatinine, mg/dL <sup>†</sup>	Estimated glomerular filtration rate, mL/min/1.73 m <sup>2</sup> <sup>‡</sup>		25-OH-vitamin D <sub>3</sub> , ng/mL <sup>§</sup>	Alkaline phosphatase, U/L <sup>¶</sup>	Intact parathyroid hormone, pmol/L**
Dent disease 1, mean $\pm$ SD	6.2 $\pm$ 6.4	1.0 $\pm$ 2.2	110.7 $\pm$ 48.6		18.5 $\pm$ 8.4	316.6 $\pm$ 145.7	81.7 $\pm$ 113.6
Dent disease 2, mean $\pm$ SD	5.9 $\pm$ 2.4	1.0 $\pm$ 0.8	82.6 $\pm$ 49.1		29.5 $\pm$ 11.3	398.5 $\pm$ 166.7	62.5 $\pm$ 71.4

ND, not detected.

P8 and P9 from family 8; P16 and P17 from family 15; P18 and P19 from family 16; the other patients are each from independent families.

\*Normal value: 1.8-6.4 mmol/L (<1 month), 2.5-6.4 mmol/L (1 month to <12 years), 2.9-7.5 mmol/L (12-18 years), 2.9-8.6 mmol/L (>18 years).<sup>30</sup>

<sup>†</sup>Normal value: 0.45-0.81 mg/dL (<7 days), 0.29-0.40 mg/dL (7 days to <1 month), 0.25-0.38 mg/dL (1 month to <6 years), 0.43-0.61 mg/dL (6 years to <14 years), 0.62-0.71 mg/dL (14-18 years), 0.66-0.96 mg/dL (>18 years).<sup>31</sup>

<sup>‡</sup>Normal value >90 mL/min/1.73m<sup>2</sup>.

<sup>§</sup>Normal value: 13-25 ng/mL.

<sup>¶</sup>Normal value: 1-200 U/L.

\*\*Normal value: 15-68.3 pmol/L.

**Table IV.** Growth data and clinical features of patients with Dent disease

	Gestational age (week + day)	Age, y	Age at presentation, y	Ages at diagnosis, y	Birth weight, kg (percentile)*	At diagnosis		Nephrocalcinosis/ nephrolithiasis <sup>‡</sup>	Rickets
						Height, cm (varied) <sup>†</sup>	Weight, kg (varied) <sup>†</sup>		
<b>Dent disease 1</b>									
P1	40	7.8	2.3	4.3	4.1 (>P97)	112.3 (+1.4 SD)	19 (+0.9 SD)	No/No	Classical
P2	37 + 3	7.8	2.6	3.7	3.7 (>P97)	102 (+0.0 SD)	19 (-1.4 SD)	No/No	Mild
P3	40 + 3	3.4	1.1	1.3	3.6 (P76)	75 (-2.0 SD)	9.0 (-1.8 SD)	No/No	Mild
P4	38	10.9	3.8	8.5	3.9 (>P97)	110 (-3.8 SD)	21.5 (-1.5 SD)	No/No	Mild
P5	42 + 3	15.3	1.2	13.2	4.25 (>P97)	126 (-4.7 SD)	24.5 (-3.5 SD)	No/No	Mild
P6	38	14.8	3.7	10.7	3.5 (P92)	101 (-6.5 SD)	18.5 (-3.5 SD)	No/No	Mild
P7	41	7.9	4.5	4.8	3.8 (P88)	105 (-1.1 SD)	19 (+0.15 SD)	Yes/No	Mild
P8	ND	30.6	12	28	ND	150 (-3.8 SD)	36 (-3.9 SD)	No/No	No
P9	37 + 3	14.6	9	12.5	3.3 (P87)	146 (-1.2 SD)	35 (-0.9 SD)	No/No	No
P10	39 + 3	4.5	1.8	2.1	3.5 (P77)	87 (-1.0 SD)	12 (-0.55 SD)	No/No	Mild
P11	39 + 3	6.6	3.3	4.2	4.3 (>P97)	106 (+0.1 SD)	17 (+0.0 SD)	No/No	No
P12	39 + 6	11.8	3	8	4.4 (>P97)	125 (-0.9 SD)	22.5 (-1.2 SD)	No/No	No
P13	41	14.8	1	12	3.4 (P53)	125.6 (-2.65 SD)	44 (+0.1 SD)	No/No	No
P14	38	12.8	4.5	11	2.4 (P4)	134 (-1.1 SD)	25 (-1.8 SD)	No/No	No
P15	41	9.9	4.6	5.1	3.9 (P94)	111 (-0.1 SD)	19 (+0.0 SD)	No/No	No
Mean ± SD		11.5 ± 6.5	3.9 ± 3.0	8.6 ± 6.6					
Abnormality					9/14	5/15	3/15	1/15	8/15
<b>Dent disease 2</b>									
P16	39 + 5	8.1	4.4	4.9	2.7 (P6)	96.6 (-3.5 SD)	14.5 (-2.2 SD)	No/No	No
P17	40 + 3	5.6	2.5	3.3	3.0 (P22)	87.5 (-2.2 SD)	10.5 (-3.2 SD)	No/No	No
P18	40	30.5	1	30	3.6 (P80)	156 (-2.7 SD)	56 (-0.7 SD)	No/No	No
P19	39 + 1	17.7	0.7	15	3.4 (P71)	146 (-3.7 SD)	50 (-0.8 SD)	No/No	No
Mean ± SD		15.4 ± 11.2	2.2 ± 1.7	13.3 ± 12.2					
Abnormality					1/4	4/4	2/4	0/4	0/4

ND, records not available.

P8 and P9 from family 8; P16 and P17 from family 15; P18 and P19 from family 16; the other patients are each from an independent families.

\*Normal range: (P10-P90).<sup>32</sup>

†Varied when compared with mean.

‡Nephrocalcinosis and nephrolithiasis were absent in all patients at diagnosis, P7 was found to have nephrocalcinosis at follow-up at the age of 6 years and 9 months.

mutation. Consistent with previous findings, the mutations identified in the *OCRL1* gene were located at the 5' portion of the gene.

### Growth Abnormality in the 2 Types of Patients/ Fetuses with Dent Disease

Retrospective study of the maternal records of the mothers of patients showed that 8 of 14 patients with Dent disease 1 were LGA, and one was small for gestational age (SGA) who had intrauterine growth restriction by ultrasound compared with Chinese reference standards (Table IV).<sup>34</sup> One patient with Dent disease 2 was SGA (Table IV). There were no evident maternal conditions that are known to cause fetal enlargement or growth restriction, such as diabetes or placental problems. The pregnancies were uneventful. These patients were born by vaginal delivery with no medical complications. At the time of diagnosis, reduced weight for age was found in 3 of 15 patients with Dent disease 1 and 2 of 4 patients with Dent disease 2, and 5 of 15 patients with Dent disease 1 and 4 of 4 patients with Dent disease 2 had height below the second SD (Table IV). No birth defects were evident pre- or postneonatally.

### Nephrocalcinosis and Nephrolithiasis Were Not Observed at Diagnosis in All Patients but Were Common in Heterozygous Mothers

Nephrocalcinosis and nephrolithiasis were not present in any of the 19 confirmed patients at diagnosis, although 1 (P7) was

found to have nephrocalcinosis at follow-up at the age of 6 years and 9 months (Table IV). In contrast, nephrolithiasis or nephrocalcinosis was present in 6 of 14 (42.8%) of the mothers (mothers of P5, P11, P12, P14, P15, and P16 from 6 families). The age at which nephrolithiasis or nephrocalcinosis was identified was 21 to 40 years. Urinary Ca/Cr and 24hUCA were normal in mothers, except the mother of 2 siblings (P18, P19, family 16), whose 24hUCA was 7.2 mg/kg/24h and Ca/Cr ratio was 0.343 mg/mg. Routine urine testing also was normal in all except 2 tested mothers (P3, P5 from family 3 and 5, respectively) who were pregnant at the time of our investigation and had occasional mild proteinuria and increased urinary  $\alpha_1$ -microglobulin and  $\beta_2$ MG. Urinary  $\beta_2$ MG was increased slightly in 11 of 13 of carrier mothers, including the 2 pregnant mothers. Estimated glomerular filtration rate was normal in all mothers whose data were available.

### Greater Rate of Rickets in Patients with Dent Disease 1

At diagnosis, we observed rickets in 8 of 15 (53.3%) patients with Dent disease 1 (Table IV). Among them 7 showed mild skeletal changes, mild pigeon chest (P4, P5, P6), pectus excavatum (P5, P10), rachitic rosary (P3, P5, P6), Harrison groove (P2, P4, P5, P6, P10), O-shaped (P4, P5, P7) or X-shaped lower limbs with genu varus (P6), caput quadratum (P3, P4), decreased bone density and/or delayed bone age

(P2-P7, P10), and 1 (P1) exhibited a “pigeon chest” and extensive hypodensity in the ulna, radius, carpal bones, metacarpal bones, and phalanges of left wrist.

### Structural Modeling and Bioinformatics Analyses of the Mutant Proteins

Structural modeling and bioinformatics analyses were performed to study the missense mutations c.789A>T (p.Leu263Phe), c.793A>C (p.Ser265Arg), and c.833T>C (p.Leu278Ser) in *CLCN5* and the impact of the in-frame deletion mutation c.833\_838del (p.Glu278\_Leu279) found in *OCRL1*.

Consistent with previous human chloride channel studies based on crystal structures of prokaryotic homologues,<sup>35,36</sup> our homology model predicted that the mutation of Leu263-Phe, Ser265Arg, and Leu278Ser are located in the 2 helices (Figure, A and B; available at [www.jpeds.com](http://www.jpeds.com)), which are proposed to be involved in the formation of the dimer interface. Both sequence and structure alignments show that the Leu263 site is conserved in chloride channels and was located at the dimer interface (Figure, C and D). The closest distance between the side chains of the Leu residues from the 2 monomers is 3.7 Å in the chloride channel transporter from *Cyanidioschyzon merolae* and 3 Å in chloride channel transporter from *Escherichia coli*. Therefore, substitution with a larger phenylalanine residue at Leu263 site may disrupt the assembly of the dimer. In the mutation p.Ser265Arg, the small wild-type hydrophilic Ser265 residue was oriented towards a hydrophobic pocket, so a large hydrophilic arginine substitution at this location might disrupt protein folding. In the mutation p.Leu278Ser, it has been reported that replacing Leu278 with phenylalanine was associated with a marked chloride current reduction in a functional assessment in *Xenopus* oocytes.<sup>37</sup> This site is in a hydrophobic environment (Figure, A and B). Changing leucine to a hydrophilic serine could have impact on the activity or stability of the dimeric form of the protein. OCRL1 belongs to the type II inositol polyphosphate-5-phosphatases, which react with substrate in an Mg<sup>2+</sup>-dependent manner.<sup>38</sup> It has been suggested that residue Glu278 in OCRL1 is responsible for Mg<sup>2+</sup> binding (Figure, E) and is involved in the interaction with phosphatidylinositol, hence critical for the enzymatic activity of OCRL1. Structure alignment shows that Glu278 was orientated towards the catalytic pocket with a highly conserved conformation (Figure, E). The Leu279 residue, altered in the same mutation, is not as conserved as Glu278, but is involved in hydrophobic interactions within the normal structure of OCRL1 (Figure, E). Thus deletion of Glu278 and Leu279 may abolish the enzymatic activity or stability of the protein.

## Discussion

We performed detailed clinical and genetic investigations in 19 confirmed patients from 16 families. Onset of disease was

insidious in all patients, and misdiagnosis occurred in 16 patients; 11 patients were treated unnecessarily before the correct diagnosis was reached, which illustrates the difficulties in reaching the correct diagnosis in early childhood, where the clinical manifestations are not typical of those described in adults. In particular, nephrocalcinosis or nephrolithiasis, which are classic features in adults, were not present before the correct diagnosis was reached, although nearly all of the patients had elevated 24hUCa. In contrast to this low frequency, there was a high rate of nephrolithiasis and nephrocalcinosis in the mothers. The low frequency of nephrocalcinosis or nephrolithiasis in our patients may well be because these conditions need time to develop. This result underlines that nephrocalcinosis or nephrolithiasis should not be required for the diagnosis of Dent disease in children.

It has been reported that rickets or osteomalacia occur in a minority patients<sup>2</sup>; however, we identified it in 8 of 15 (53.3%) cases of rickets in Dent disease 1, which is much greater than the reported frequency in Japanese patients.<sup>13</sup> A similar rickets rate also was reported in Italian patients with Dent disease.<sup>39</sup> It is known that rickets and osteomalacia in Dent disease can be corrected by vitamin D supplementation. The greater rate of rickets in our cases may have been exacerbated by the generally lower calcium intake, including lower milk consumption by Chinese children.

In reviewing the maternal records and the history of our patients, we found that 8 of 14 patients with Dent disease 1 were LGA. Overall, 1 patient with Dent disease 1 was SGA with intrauterine growth restriction, and 1 patient with Dent disease 2 was SGA. Reviewing the literature, we only found 1 relevant paper that reported 2 patients with Dent disease 1 who were LGA, who subsequently had growth restriction after birth.<sup>16</sup> Intrauterine growth of these patients seems to be a neglected area, probably because of the fact that the disease is only recognized later in life. Our study, combined with the previous report, implies that Dent disease 1 may increase intrauterine growth, but this implication requires independent validation. It is known that *CLCN5* is expressed in the placenta,<sup>40</sup> providing a potential link between fetal growth and Dent disease 1.

Our investigation shows the rate of genetic testing in establishing a definitive diagnosis of Dent disease 1 and 2, particularly in early childhood, and underlines that this would avoid inappropriate treatment. We observed a high rate of rickets in our patients. We also identified a high rate of nephrocalcinosis or nephrolithiasis in carrier mothers but none in the patients at diagnosis. Finally, the possibility exists that Dent disease could be associated with aberrant intrauterine growth. Environmental effects on Dent disease phenotypes, including rickets, merit further investigation. ■

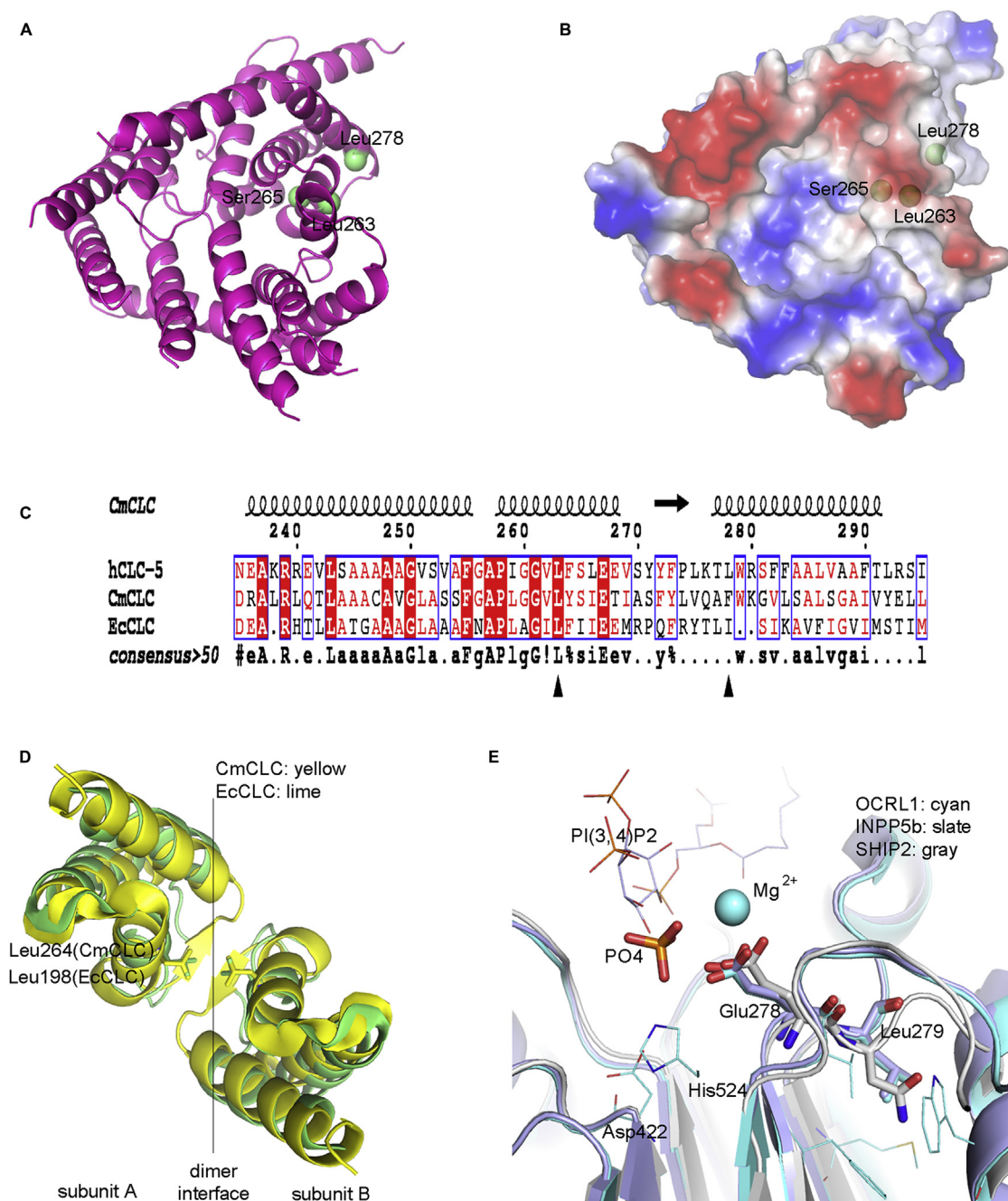
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**Figure.** **A** and **B**, Ribbon and surface representation of the model of the human CLC-5 monomer. The mutant sites are marked and colored in *green*. **C**, Sequence alignment between human CLC-5 and its homologues: CmCLC (chloride channel transporter from *Cyanidioschyzon merolae*) and EcCLC (chloride channel transporter from *Escherichia coli*). **D**, Structure alignment of the part identical to **C** between CmCLC (*yellow*, PDB ID: 3ORG) and EcCLC (*lime*, PDB ID: 1KPK) dimer from the same view of **A**. **E**, Structure alignment between OCRL1 (*cyan*, PDB ID: 4CMN) and homologous proteins: Type II inositol 1,4,5-trisphosphate 5-phosphatase (INPP5b, *slate*, PDB ID: 4CML) in complex with phosphatidylinositol 3,4-bisphosphate (PI(3,4)P<sub>2</sub>), SH2 domain-containing inositol 5'-phosphatase 2 (SHIP2, *gray*, PDB ID: 3NR8). The mutant residues Glu278, Leu279, and the corresponding wild-type residues in the homologues and the phosphate group from OCRL1 structure are presented as sticks. Catalytic residues His524, Asp422, and residues involved in the hydrophobic interactions with Leu279 in OCRL1 and the PI(3,4)P<sub>2</sub> substrate from INPP5b structure are all presented as thin lines. Mg<sup>2+</sup> in the OCRL1 structure is shown as a cyan sphere.