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.[1,2] 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.[3,4] 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[1] 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[1] 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.[5] Both genes play important roles in the endocytosis-based reabsorption and processing of low-molecular-weight proteins from the renal tubular brush border.[1,2,6-11] The responsible gene(s) for the remaining ~25%-35% of patients with Dent disease have not yet been identified.[1]

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

24hUCa	24-hour urine calcium
β_2MG	β_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

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different ethnicities has been reported.[12-15] 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).[16]

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.[17] 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.[18] 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.[19] 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 semiqualitative 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 β_2-microglobulin (β_2MG), 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_3 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.[20] Nephrolithiasis was considered when the ultrasound examination showed an echogenic focus (preferably with clear acoustic shadowing) in renal pelvis or calyx or hydronephrosis.[21]

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).[22,23] 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.[22,24]

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/)[25] 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).[26] We used SIFT (http://sift.jcvi.org/) and PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/) to predict possible