Characterization of Renal Chloride Channel (CLCN5) Mutations in Dent’s Disease

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Abstract. Dent’s disease is an X-linked renal tubular disorder characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and renal failure. The disease is caused by mutations in a renal chloride channel gene, CLCN5, which encodes a 746 amino acid protein (CLC-5), with 12 to 13 transmembrane domains. In this study, an additional six unrelated patients with Dent’s disease were identified and investigated for CLCN5 mutations by DNA sequence analysis of the 11 coding exons of CLCN5. This revealed six mutations: four frameshift deletions involving codons 392, 394, 658, and 728, one nonsense mutation (Tyr617Stop), and an A to T transversion at codon 601 that would result in either a missense mutation (Asp601Val) or creation of a novel donor splice site. These mutations were confirmed by restriction endonuclease or sequence-specific oligonucleotide hybridization analysis and were not common polymorphisms. The frameshift deletions and nonsense mutation predict truncated and inactivated CLC-5. The effects of the putative missense Asp601Val mutant CLC-5 were assessed by heterologous expression in Xenopus oocytes, and this revealed a chloride conductance that was similar to that observed for wild-type CLC-5. However, an analysis of the mutant CLCN5 transcripts revealed utilization of the novel donor splice site, resulting in a truncated CLC-5. Thus, all of the six mutations are likely to result in truncated CLC-5 and a loss of function, and these findings expand the spectrum of CLCN5 mutations associated with Dent’s disease.

Dent’s disease is a renal tubular disorder characterized by low molecular weight proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and progressive renal failure (1,2). The disease also may be associated with aminoaciduria, phosphaturia, glycosuria, kaliuresis, uricosuria, and impaired urinary acidification, and is complicated by rickets or osteomalacia in some patients. Thus, Dent’s disease may be considered a form of the renal Fanconi syndrome (2,3). The etiology of Dent’s disease, and three other phenotypically similar disorders referred to as X-linked recessive nephrolithiasis (4–6), X-linked recessive hypophosphatemic rickets (7), and the idiopathic low molecular weight proteinuria of Japanese children (8–12), has been established to be due to inactivating mutations of a renal chloride channel gene designated CLCN5 (11,13,14).

The human CLCN5 gene, which is located on chromosome Xp11.22, has a 2238-bp coding sequence that consists of 11 exons that span 25 to 30 kb of genomic DNA and encode a 746 amino acid protein (Figure 1) (15,16). CLCN5 belongs to the family of voltage-gated chloride channel genes (CLCN1-CLCN7, and CLCKa and CLCKb) that have approximately 12 transmembrane domains (17,18). These chloride channels have an important role in the control of membrane excitability, transepithelial transport, and possibly cell volume (17,18). Heterologous expression studies of wild-type CLCN5 in Xenopus oocytes have revealed that the channel, CLC-5, conducts chloride currents that are outwardly rectifying and time-inde-
pendent (19), and similar expression of disease-associated CLC-5 mutants has demonstrated markedly reduced or absent currents (9,11–14). The expression of CLC-5 in the human nephron has been shown to be in the proximal tubule, the thick ascending limb, and the intercalated cells of the collecting duct (20). Furthermore, CLC-5 has been localized intracellularly to the subapical endosomes and with the vacuolar H\(^{+}\)-ATPase, thereby suggesting that it may have a role in the counterion transport mechanism that facilitates acidification within endosomes (20,21). These endosomes form part of the receptor-mediated endocytic pathway that transports proteins such as albumin (20–22), and thus CLC-5 dysfunction in this pathway may help provide an explanation for the observed low molecular weight proteinuria in Dent’s disease (20,21). However, the mechanisms by which CLC-5 dysfunction results in hypercalciuria and the other features of Dent’s disease remain to be elucidated. The identification of additional CLCN5 mutations may help in these studies, and we have pursued such studies in patients with Dent’s disease.

Materials and Methods

Patients

Six probands who suffered from Dent’s disease (Table 1) were investigated after giving informed consent. All six of the probands had low molecular weight proteinuria, five had hypercalciuria, four had nephrocalcinosis and/or nephrolithiasis, and three had renal impairment. None of the probands suffered from rickets. Five of the six probands were of Northern European origin and one proband (26/97, Table 1) was of Japanese origin. A family history of Dent’s disease could be established in four of the probands, while in the remaining

Figure 1. Schematic representation of CLCN5 mutations within the framework of the predicted topology of CLC-5, which consists of 746 amino acids (13,16). The correct topology of the CLC-5 putative transmembrane domains (D1 to D13) is not established, and this representation is based on a model reported previously (Inset) (13). The consensus phosphorylation and glycosylation sites are indicated by the asterisks and branch sites, respectively. The locations of the six mutations detected by the present study (Table 2) are indicated by the filled arrowheads, and the mutations are illustrated in bold and within boxes. These six mutations consist of four deletional frameshifts (392delGT, 394delT, 658delC, and 728del123bp), one nonsense (Tyr617Stop), and the loss of codons 601 to 645 (filled arrowheads, marked 601 and 645) due to the novel donor splice-site mutation (gacagt to gcag). Of the 52 CLCN5 mutations reported from previous studies (6,9–14,26,37–39), 31 are shown and eight of these have been observed to occur more than once; in addition, two large deletions encompassing the entire CLCN5 gene (10,13), two small intragenic deletions (12,13), and four splice-site mutations (two donor and two acceptor splice mutations) (11,13,39), which are not shown, have been reported. The two intragenic deletions would result in a loss of codons 132 to 241 (12,13) and codons 132 to 449 (12). The two donor splice sites would both result in a loss of codons 132 to 172 (13), and of the two acceptor splice sites, one was shown to result in a loss of codons 173 to 241 (39) and the effects of the other one were not characterized (11).
two probands family members were not available for study to establish an inherited basis for the disease. Venous blood samples were obtained from these six probands and from three affected and six unaffected family members for mutational analysis of the CLCN5 gene.

**DNA Sequence Analysis of the CLCN5 Gene**

Leukocyte DNA was extracted and used with CLCN5-specific primers (9) for PCR amplification, using methods described previously (9). The DNA sequence of PCR products was determined by Taq polymerase cycle sequencing, using a semiautomated detection system (ABI 373XL sequencer; Applied Biosystems, Foster City, CA) (23). DNA sequence abnormalities were confirmed by either restriction endonuclease analysis or sequence-specific oligonucleotide (SSO) hybridization analysis of the appropriate genomic PCR products (23,24). The DNA sequence abnormalities were demonstrated to be absent as common polymorphisms in the DNA obtained from 74 unrelated normal individuals (34 males, 40 females), and to cosegregate with the disorder in the available members from three of the families.

**CLCN5 mRNA Analysis**

RNA was extracted from Epstein-Barr virus (EBV)-transformed lymphoblastoid cell lines established from peripheral blood cells of proband 15/97 (Table 1), and from three unrelated normal individuals. Reverse transcription (RT)-PCR was performed using pairs of nested CLCN5-specific primers (outer primers: forward 5’-CTTGGAGGAGTC-CAGAAGGCC-3’; reverse 5’-GGTACCAGTTAATACAA-CATATCC-3’; inner primers: forward 5’-ATTCAGGGGAGCTT-GCATCAC-3’, and reverse 5’-CATATCCATGTTCTGAAATGTC-3’), at an annealing temperature of 65°C for both the first and second rounds, which consisted of 20 and 30 cycles, respectively. The PCR products were gel-purified, and the DNA sequences of both strands were determined as described (23).

**Functional Expression in Xenopus Oocytes**

Wild-type and mutant CLC-5 were expressed in *Xenopus* oocytes as described previously (9,11,13,14). A cDNA encoding the human CLC-5 protein was inserted into the expression vector PTNL (25). Mutations were introduced by recombinant PCR. Capped cRNA was synthesized using SP6-RNA polymerase after linearization of the construct. About 10 ng of cRNA was injected into manually defolliculated *Xenopus* oocytes and incubated for 2 d at 17°C. Currents were measured by standard two-electrode voltage-clamp techniques, using a Turbo TEC-05 amplifier (NPI, Tamm, Germany) and pCLAMP software (Axon Instruments, Foster City, CA). Measurements were carried out in ND96 (96 mM NaCl, 2 mM KCl, 1.8 mM CaCl2, 1 mM MgCl2, and 5 mM Hepes, pH 7.4). The oocyte membrane was held at the resting potential and stepped for 500 ms to potentials ranging from −100 to +80 mV in 20-mV steps. Currents were determined at a membrane potential of +80 mV. Results were expressed as mean values ± SEM.

**Determination of Protein Expression in Xenopus Oocytes**

Oocytes were pooled after measurement and stored at −20°C. After homogenization in 5 mM ethylenediaminetetra-acetic acid, 10 mM Tris-HCl, and a protease inhibitor mix (Complete; Boehringer Mannheim, Mannheim, Germany) on ice, the yolk platelets were removed by three low-speed centrifugations. From the supernatant, the equivalent of two oocytes was dissolved in sodium dodecyl sulfate (SDS)-Laemmli buffer and loaded onto a 6% SDS-polyacrylamide gel electrophoresis. After separation, the proteins were blotted on polyvinylidene difluoride membranes, and CLC-5 protein was detected using a rabbit polyclonal antiserum raised against a peptide encompassing the 13 carboxy-terminal amino acid residues of hCLC-5 (11,21). Detection was carried out using a Protein A-peroxidase-based kit (Renaissance; DuPont, Boston, MA).

**Results**

DNA sequence analysis of the entire 2238-bp coding region and exon-intron boundaries of the CLCN5 gene from the six probands (Table 1) with Dent’s disease revealed six novel mutations that consisted of four deletional frameshifts (Figure 2), one nonsense mutation, and an A to T transversion in the second base of codon 601 (Figure 3, Table 2). The deletional frameshifts occurred in exons 8, 11, and 12, and the nonsense mutation (Tyr617Stop) and A to T transversion both occurred in exon 10 (Table 2). Three of these mutations (Tyr617Stop, 392delGT, and 728del23bp) resulted in an alteration of a restriction enzyme site (Table 2) that facilitated their confirmation (Figure 1). The other three mutations (394delT, 658delC, and the A to T transversion in codon 601) were not

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**Table 1. Clinical and biochemical abnormalities in six male probands with Dent’s disease**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Proband’s Family</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6/97</td>
</tr>
<tr>
<td>Age (yr) at first symptoms or diagnosis</td>
<td>43</td>
</tr>
<tr>
<td>Urinary abnormalities</td>
<td></td>
</tr>
<tr>
<td>low molecular weight proteinuria (^b)</td>
<td>+</td>
</tr>
<tr>
<td>hypercalciuria</td>
<td>+</td>
</tr>
<tr>
<td>Renal impairment (^c)</td>
<td>+</td>
</tr>
<tr>
<td>Nephrocalcinosis and nephrolithiasis (^d)</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\) +, present; −, absent.

\(^b\) Low molecular weight proteinuria refers to excessive urinary loss of one or more of the following: α1 microglobulin, β2 microglobulin, retinol-binding protein, and lysozyme; and/or the presence of albuminuria (2).

\(^c\) Renal failure: creatinine clearance <80 ml/min per 1.73 m\(^2\) or serum creatinine >130 μmol/L. No patient had end-stage renal failure.

\(^d\) Nephrocalcinosis and nephrolithiasis detected by ultrasonography or radiology.
associated with altered restriction enzyme sites, and the method of SSO hybridization analysis (Figure 3) was used (Table 2). The absence of each of these six DNA sequence abnormalities in 110 alleles from 74 unrelated normal individuals established that these abnormalities were not sequence polymorphisms that would be expected to occur in $1\%$ of the population.

The four deletional frameshift mutations and the nonsense mutation are predicted to result in truncated CLC-5 that lack from 14 to 346 amino acids (Table 2). The functional effect of such truncated CLC-5, which has been assessed previously (9,11,13,14), is likely to be a loss of function, and this was confirmed by the expression of the CLC-5 mutant due to the 23-bp deletional frameshift (Figure 2) at codon 728 (Figure 4). The effects of the A to T transversion in codon 601 were more difficult to predict. This transversion may result in either a missense mutation, Asp601Val, or a novel donor splice site. The functional effects of such missense mutations may be to abolish or markedly reduce CLC-5 chloride conductance (9,11–14). The heterologous expression, in *Xenopus* oocytes, of the Asp601Val CLC-5 mutant revealed that Cl$^-$ conductance was unaffected and remained similar to that of the wild-type CLC-5 (Figure 4); translation of both the wild-type and mutant CLC-5 proteins was confirmed by Western blot analysis (data not shown) of the *Xenopus* oocyte membranes, using an antibody directed against the carboxy terminus (21). However, an analysis of CLCN5 transcripts resulting from this A to T transversion revealed that the mutation was associated with a novel donor splice site (Figure 5). This novel donor splice site led to skipping of the 3’ portion of exon 10 that

Figure 2. Detection of mutation in exon 12 by restriction enzyme analysis. DNA sequence analysis of the proband 26/97 (Table 1) revealed a 23-bp deletion commencing at codon 728. This led to a frameshift that resulted in five missense amino acids (Gly, Glu, Pro shown) followed by a termination signal (Stop) at codon 733 (A). This deletion also resulted in the loss of an *MseI* restriction enzyme site (T/TAAA). PCR amplification and *MseI* digestion (B) would result in four fragments of 116, 82, 46 (not shown), and 10 bp (not shown) from the normal sequence, but only three products of 175, 46 (not shown), and 10 bp (not shown) from the mutant sequence, as illustrated in the restriction map (C). This deletion was not present in 74 normal individuals (N1 to N3 shown), indicating that it is not a common DNA sequence polymorphism. The standard size marker (S in Panel B) in the form of a 1-kb ladder is indicated. Similar restriction enzyme analysis was used to confirm the 392delGT and Tyr617Stop mutations (Table 2). The symbols denoting the phenotype in the individual are as described in Figure 3.
encoded codons 601 to 645. Thus, the mutant CLCN-5 transcript consisted of the 5' portion of exon 10 (encoding codons 512 to 600) spliced to exon 11. If translated, this abnormal CLCN5 transcript would result in 32 missense amino acids from codons 601 to 632, followed by a termination signal (Stop) (Figure 5, Table 2). Such a truncated CLC-5 is likely to result in a loss of function that is similar to those observed by heterologous expression of the 23-bp deletion commencing at codon 728 (Figure 4). Thus, all of the six mutations detected in this study of patients with Dent’s disease are likely to be associated with a loss of CLC-5 function.

**Discussion**

Our results, which have identified six novel CLCN5 mutations (Table 2), expand the spectrum of mutations that are associated with Dent’s disease. All of the six CLCN5 mutations predict structural alterations of CLC-5 that are likely to result in a loss of function. The total number of CLCN5 mutations now reported, including the results of our present study, is 58, and these are scattered throughout the channel (Figure 1) with no evidence for mutational hot spots. Furthermore, there appears to be no correlation between the mutations and phenotypes (13,26). Of the total 58 CLCN5 mutations, approximately 32% are nonsense mutations, 22% are frameshift deletions or insertions, 2% are in-frame insertions, 4% are donor splice-site mutations, 3% are acceptor splice-site mutations, 27% are missense mutations, 8% are intragenic deletions, and 2% are complete deletions of the gene. The majority (>70%) are predicted to result in truncated or absent CLC-5, which would lead to a complete loss of channel function. Only four of the mutations (30:His insertion, Gly57Val, Leu278Phe, and Arg280Pro), which are predicted not to result in truncated CLC-5 and which are all located outside the predicted transmembrane domains, have been reported to be associated with reduced, but not abolished, Cl− currents (Figure 1). Indeed, the clustering of such missense mutations (Leu278Phe, Arg280Pro), which are associated with residual channel activity (9,11) in the putative loop between D5 and D6 (Figure 1),
is of interest and suggests that this loop may have a regulatory role in CLC-5 function (11). In relation to this, our finding of the A to T transversion in codon 601, which predicted a possible missense mutation Asp601Val, was intriguing. The Asp601 is evolutionarily conserved in mouse (27), rat (19), and Xenopus (28) and in other CLC members, e.g., CLC-3 and CLC-4 (29,30). Thus, the replacement of this negatively charged conserved Asp residue with the nonpolar uncharged Val residue would be expected to abolish or reduce Cl\(^{-}\) currents, and yet our results revealed no alteration in Cl\(^{-}\) conductance (Figure 4). This suggested that the A to T transversion was likely to be altering CLC-5 structure and function by a mechanism other than that of a missense mutation. An examination of the DNA sequence of codons 600, 601, and 602 (Figure 3) indicated that the A to T transversion may have resulted in a novel donor splice site (g\(^{\text{a}}\)cagt to g\(^{\text{t}}\)cagt) in exon 10. The first two bases (gt) of donor splice sites are invariant (31), and the effects of this mutation leading to a putative novel donor splice site were demonstrated by an analysis of RNA obtained from the patient’s EBV-transformed lymphoblastoids (Figure 5). This novel donor splice site, which has not been previously reported in Dent’s disease, led to skipping of the 3\(^{9}\)portion of exon 10 and a truncated, and likely inactivated, CLC-5 (Table 2). The utilization of such novel donor splice sites, which may be used preferentially to the wild-type, has been previously observed to occur in association with the Laron and Lesch-Nyhan syndromes (32,33).

The mechanisms by which a functional loss of this renal chloride channel (CLC-5) leads to a generalized proximal renal tubular defect with low molecular weight proteinuria and hypocalciuria remain to be elucidated. Studies of CLC-5 expression in the human nephron have suggested some possibilities, as CLC-5 is expressed at multiple sites, including the proximal tubule, thick ascending limb of Henle, and intercalated cells of the collecting duct (20). CLC-5 is located intracellularly and in the early endosomes, which contain the vacuolar H\(^{+}\)-ATPase and which form part of the receptor-mediated endocytic pathway (20). This suggests a role for CLC-5 as a counterion transport mechanism allowing the action of the electrogenic H\(^{+}\)-ATPase in the acidification of the endosomes (20,21). Indeed, CLC-5 has been shown to have a suitable pH dependence for its activity (34), and endosomal

Table 2. CLC-5 mutations found in patients with Dent’s disease

<table>
<thead>
<tr>
<th>Family</th>
<th>Exon</th>
<th>Nucleotide Number and Base Change(^{a})</th>
<th>Codon</th>
<th>Amino Acid Change(^{b})</th>
<th>Restriction Enzyme Change/SSO(^{b})</th>
<th>Predicted Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nonsense mutation</td>
<td>8/97</td>
<td>10 1851 C&gt;A</td>
<td>617</td>
<td>Tyr → Stop (X)</td>
<td>MseI</td>
<td>Loss of 130 aa</td>
</tr>
<tr>
<td>Deletions</td>
<td>6/97</td>
<td>8 1175–1176delGT</td>
<td>392</td>
<td>fs, 9 missense aa, Stop (X)</td>
<td>BsrI</td>
<td>Missense peptide from aa 392–400 and loss of 346 aa</td>
</tr>
<tr>
<td></td>
<td>12/97</td>
<td>8 1182delT</td>
<td>394</td>
<td>fs, 38 missense aa, Stop (X)</td>
<td>SSO</td>
<td>Missense peptide from aa 395–432 and loss of 314 aa</td>
</tr>
<tr>
<td></td>
<td>9/97</td>
<td>11 1974delC</td>
<td>658</td>
<td>fs, 18 missense aa, Stop (X)</td>
<td>SSO</td>
<td>Missense peptide from aa 659–676 and loss of 70 aa</td>
</tr>
<tr>
<td></td>
<td>26/97</td>
<td>12 2183–2205del23bp</td>
<td>728</td>
<td>fs, 5 missense aa, Stop (X)</td>
<td>MseI</td>
<td>Missense peptide from aa 728–732 and loss of 14 aa</td>
</tr>
<tr>
<td>Novel donor splice site mutation</td>
<td>15/97</td>
<td>10 1802 A&gt;T</td>
<td>601</td>
<td>fs, 32 missense aa, Stop (X)</td>
<td>SSO</td>
<td>Missense peptide from aa 601–632 and loss of 114 aa</td>
</tr>
</tbody>
</table>

\(^{a}\) Nomenclature as recommended (40).

\(^{b}\) SSO, sequence-specific oligonucleotide; fs, frameshift; aa, amino acid.

Figure 4. Electrophysiologic analysis of Xenopus oocytes expressing human wild-type (WT) CLC-5, the putative mutant Asp601Val (Figure 3), and 728del23bp (Figure 2) channels. The Cl\(^{-}\) currents were measured as described previously (11,13), and the averaged (mean ± SEM) whole cell currents measured at +80 mV in the Xenopus oocytes injected with water control (0.34 ± 0.067), WT CLC-5 (2.54 ± 0.45), the putative Asp601Val mutant (2.61 ± 0.44), and the 728del23bp mutant (0.33 ± 0.09) are shown. The Asp601Val mutant channel had currents that were similar to that of the WT channel, whereas the deletional mutant resulted in abolished Cl\(^{-}\) currents. Western blot analysis of the Xenopus oocyte membranes using an antibody directed against a carboxy-terminal fragment (21) identified translated CLC-5 proteins (data not shown).
acidification is inhibited by nonspecific chloride channel blockers. A possible explanation for the proximal tubular reabsorptive defects seen in Dent’s disease may be as follows: Low molecular weight proteinuria may be due to defective endocytosis of these proteins, which are known to be absorbed through the receptor-mediated endocytic pathway, and the other features (e.g., glycosuria and aminoaciduria) may be due to defective endosomal recycling of plasma membrane transporters responsible for the sodium-coupled reabsorption of these solutes (20,21,35). The hypercalcuria, which is not a typical feature of proximal tubular disorders, may partly be of the absorptive type and may be due to the associated high normal to elevated serum concentrations of 1,25-dihydroxyvitamin D₃ that are observed in patients with Dent’s disease (36). However, the localization of CLC-5 to the thick ascending limb of Henle, where 60% of calcium reabsorption occurs, suggests another interesting mechanism: The possible changes in endosomal recycling stemming from CLC-5 dysfunction in this segment may lead to altered recycling and activities of the surface transporters (18), e.g., the sodium-potassium-chloride cotransporter (NKCCT), the chloride channel (CLC-Kb), and the renal outer medullary potassium channel (ROMK), which are involved in generating the transepithelial potential difference that is necessary for paracellular calcium uptake. The role of the Dent’s disease-associated CLC-5 mutants (Figure 1) in these physiologic mechanisms of calcium homeostasis still remains to be elucidated.

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**References**


8. Igarashi T, Hayakawa H, Shiraga H, Kawato H, Yan K, Kawagu-


35. Kelleher CL, Buckalew VM, Frederickson ED, Rhodes DJ, Conner DA, Seidman JG, Seidman CE: CLCN5 mutation Ser244Leu
