Inherited Hypercalciuric Syndromes: Dent’s Disease (CLC-5) and Familial Hypomagnesemia With Hypercalciuria (Paracellin-1)

By Stephen J. Knohl and Steven J. Scheinman

Dent’s disease and familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) are inherited diseases in which hypercalciuria, nephrocalcinosis, and renal failure are prominent features. Dent’s disease resembles a Fanconi syndrome, with impaired reabsorption in the proximal tubule; FHHNC, with urinary loss of magnesium and calcium, is associated with impaired cation transport in the thick ascending limb of Henle’s loop. Gene mapping in families and positional cloning led in both cases to identification of the responsible gene. Dent’s disease is associated with mutations that disrupt function of a voltage-gated chloride channel, CLC-5, expressed in subapical endosomes of the proximal tubule and in other nephron segments. Impaired function of this channel disturbs reabsorption of filtered proteins, as well as other transport functions of the proximal tubule, and leads, apparently indirectly, to hypercalciuria and renal failure. FHHNC results from mutations in paracellin-1, a tight-junction protein that appears to be important in conducting or regulating paracellular cation transport. Impaired function of paracellin-1 leads specifically to urinary losses of magnesium and calcium, but because transcellular transport is intact these patients do not have hypokalemia or salt wasting. Identification of both genes represent triumphs of a genetic approach to solving problems of pathophysiology.

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ADVANCES IN MOLECULAR genetics have made it possible to unravel the physiology of syndromes that had seemed unsolvable puzzles. Two diseases of renal transport, both with hypercalciuria, represent striking successes of this approach. Physiologic observations in patients with Dent’s disease (X-linked nephrolithiasis) and familial hypomagnesemia with hypercalciuria and nephrocalcinosis (FHHNC) pointed to a primary defect in the proximal tubule in the former and in the thick ascending limb of Henle’s loop in the latter, but it was only with cloning of the responsible genes and identification of the gene products that the physiology of these disorders has begun to be understood. Table 1 summarizes the clinical differences between these 2 syndromes.

DENT’S DISEASE

The hallmarks of this disease are hypercalciuria and low-molecular weight proteinuria. The fully expressed syndrome includes proximal tubular reabsorptive failure resembling a Fanconi syndrome, nephrolithiasis, nephrocalcinosis, deterioration in renal function, and rickets. Different clinical characteristics predominated in initial descriptions of the disease from different geographic areas, prompting a variety of descriptive names such as X-linked nephrolithiasis, but this is now recognized as a single disease with a single name, Dent’s disease, in honor of Charles Dent who in 1964 described 2 unrelated male children with hypercalciuria, rickets, and proximal tubular dysfunction. Because this is an X-linked recessive trait, all daughters of an affected male are heterozygous (carriers) for the condition, 50% of sons from a female carrier will be affected, and male-to-male transmission is impossible.1,2

Mutations in the CLCN5 gene on the X chromosome are associated consistently with this disease.3 CLCN5 was identified through gene mapping and positional cloning in affected families.3,4 It encodes a voltage-gated chloride channel, CLC-5, that had not been known previously. CLC-5 is a member of a channel family that also includes CLC-0, the major chloride channel in human muscle that is mutated in congenital myotonia; CLC-Kb, the basolateral chloride channel in the renal medullary thick ascending limb that is mutated in a large subset of patients with Bartter’s syndrome; and CLC-7, expressed at the ruffled surface of osteoclasts that facilitates acidification of the mineralization front of bone and that is mutated in congenital malignant osteopetrosis.5 CLC-5 is expressed predominantly in kidney, although low levels of expression can be found in other tissues including intestine. Along the nephron, CLC-5 is expressed in the subapical endosomes of the prox-
imal tubule, and in endosomes in the cells of the medullary thick ascending limb of Henle’s loop. It also is expressed in apical and subapical regions of α-intercalated cells of the collecting duct.6,7 Although its function in these distal segments remains poorly understood, the role of CLC-5 in proximal tubules has come into clearer focus.

In the proximal tubule, CLC-5 colocalizes with the proton-adenosine triphosphatase in subapical endosomes that are a critical part of the reabsorptive apparatus for proteins.6,7 Filtered proteins (including those of low-molecular weight as well as albumin) adsorb to the receptor megalin on the apical surface of the proximal tubular epithelial cells. When endosomes bud off this surface, the first step in degrading the adsorbed proteins requires acidification of the endosomal lumen, and this is achieved through the activity of the vacuolar proton adenosine triphosphatase. It has been known for a decade that acidification of the endosomes requires dissipation of the charge by a protein kinase A–regulated chloride conductance,8 and this appears to be the function of CLC-5, although regulation by protein kinase A has not been documented.

Impaired function of megalin-dependent protein uptake would lead to urinary loss of proteins. About 50% to 70% of the urinary protein in patients with Dent’s disease is comprised of low-molecular weight proteins, and the remainder is albumin.9 This pattern is present from infancy (A. Norden, personal communication), indicating that the albuminuria is not a nonspecific consequence of glomerular sclerosis because renal insufficiency does not begin in the first decade of life. Thus, Norden et al9 used measurements of urinary excretion of proteins in patients with Dent’s disease without renal insufficiency to derive an in vivo estimate of glomerular sieving coefficients. Patients with Dent’s disease excrete 0.5 to 2.0 g of protein per day, and are not clinically nephrotic. The most useful screening tests for Dent’s disease are measurement of β2-microglobulin levels, which are widely available in clinical laboratories, and of retinal-binding globulin, which is available less often but is more sensitive and specific.10

Further evidence for proximal tubular dysfunction in Dent’s disease is the wasting of glucose, amino acids, and phosphate, which have in common that they are reabsorbed through the activities

Table 1. Clinical Differences Between Dent’s Disease and FHHNC

<table>
<thead>
<tr>
<th></th>
<th>Dent’s Disease</th>
<th>FHHNC</th>
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<tbody>
<tr>
<td>Inheritance pattern</td>
<td>X-linked recessive</td>
<td>Autosomal recessive</td>
</tr>
<tr>
<td>Mutated gene (protein)</td>
<td>CLCN5 (CLC-5)</td>
<td>PCLN1 (paracellin-1)</td>
</tr>
<tr>
<td>Age at presentation</td>
<td>Infancy or childhood</td>
<td>Infancy</td>
</tr>
<tr>
<td>Major clinical features</td>
<td>LMW proteinuria and other proximal solute wasting</td>
<td>Hypomagnesemia</td>
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<tr>
<td></td>
<td>Poluria</td>
<td>Polyuria</td>
</tr>
<tr>
<td></td>
<td>Microscopic hematuria</td>
<td>Infantile seizures</td>
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<tr>
<td></td>
<td>Hypercalciuria</td>
<td>Hypercalciuria</td>
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<tr>
<td></td>
<td>Nephrocalcinosis</td>
<td>Nephrocalcinosis</td>
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<tr>
<td></td>
<td>Nephrolithiasis</td>
<td>Nephrolithias</td>
</tr>
<tr>
<td></td>
<td>Renal failure</td>
<td>Renal failure</td>
</tr>
<tr>
<td></td>
<td>Rickets/osteomalacia</td>
<td>Ocular abnormalities</td>
</tr>
<tr>
<td>Onset of renal failure</td>
<td>Adolescence; end-stage renal disease by 4th decade</td>
<td>Early childhood; end-stage renal disease often by late adolescence</td>
</tr>
</tbody>
</table>

Laboratory abnormalities

<table>
<thead>
<tr>
<th></th>
<th>Serum</th>
<th>Dent’s Disease</th>
<th>FHHNC</th>
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<tbody>
<tr>
<td></td>
<td>Calcium</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Magnesium</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Uric acid</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td></td>
<td>Intact PTH</td>
<td>Normal or low</td>
<td>Normal or low</td>
</tr>
<tr>
<td></td>
<td>1,25(OH)2D</td>
<td>Normal or high</td>
<td>High</td>
</tr>
<tr>
<td></td>
<td>Hypercalciuria</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Hypermagnesuria</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td></td>
<td>Low-molecular weight proteinuria</td>
<td>Always present</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Nephrocalcinosis</td>
<td>Present</td>
<td>Present</td>
</tr>
</tbody>
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of sodium-dependent transporters. Physiologic regulation of the activities of these transporters involves recycling between endosomes and the apical cell surface,11,12 and it has been shown that when acidification of subapical endosomes is inhibited, membrane trafficking is impaired.13 In 2 mouse models in which CLCN5 expression is prevented by targeted disruption of the gene, proximal tubular endocytosis is disturbed,12,14 and in one of these knockouts trafficking of the sodium-dependent phosphate cotransporter NaPi2 is altered.12 Glycosuria, aminoaciduria, and phosphaturia are variable among patients with Dent’s disease, and can be intermittent. It is plausible to speculate that these defects may vary with fluctuations in the state of endosomal congestion, perhaps with variations in the load of proteins presented for reabsorption.

As further evidence of abnormal endosomal function, Piwon et al12 have shown that their CLC-5 knockout mice have reduced expression of megalin in proximal tubular cells, and very low levels of megalin in the urine. Urinary megalin excretion also is decreased in patients with Dent’s disease.15

Hypercalciuria is a hallmark of Dent’s disease, and is the major risk factor for stone formation and nephrocalcinosis because these patients excrete normal quantities of oxalate, citrate, uric acid, and other stone risk determinants.10,16 The degree of hypercalciuria in adolescents and adults resembles that in patients with idiopathic hypercalciuria, approximating 4 to 6 mg/kg body weight. In patients with Dent’s disease at these ages, hypercalciuria is diet-dependent and often can normalize with dietary calcium restriction (which is not recommended because it could exacerbate bone demineralization). Infants and young children, however, exhibit heavier degrees of hypercalciuria in the range of 10 to 12 mg/kg, and the hypercalciuria may persist on fasting.10

In 2 mouse models of CLCN5 inactivation, hypercalciuria is diet-dependent,17,18 as in the patients. However, in one mouse model in which CLCN5 expression is eliminated completely through targeted disruption of the gene, a small degree of hypercalciuria persists on a very low calcium intake. This latter model resembles the persistent hypercalciuria seen in young children. It is not clear whether this persistent hypercalciuria results from a renal leak or a primary tendency to impaired bone mineralization. In patients with Dent’s disease, hypercalciuria10 responds to a thiazide diuretic, as in idiopathic hypercalciuria.19 This is consistent with the fact that the CLC-5 chloride channel is not expressed in the thiazide-sensitive cortical distal tubule.6,7

Serum calcium levels are normal, and in some patients tend toward the upper limit of the normal range.10,16,20,21 In patients with normal creatinine clearances, measurements of intact parathyroid hormone tend to be low, and levels of 1,25 dihydroxyvitamin D often are increased.1 This is more consistent with a pattern of absorptive hypercalciuria rather than a primary renal calcium leak. It is not yet known whether the relatively high levels of 1,25-dihydroxyvitamin D result from hypophosphatemia or represent a dysregulation of activity of the 1α-hydroxylase as a consequence of CLC-5 inactivation.

Parathyroid hormone is among the filtered low-molecular weight proteins that are found in great excess in the urine of knockout mice12 and humans with Dent’s disease.9 Jentsch and colleagues have speculated that the inhibition of phosphate transport and the stimulation of 1-hydroxylation of vitamin D are consequences of activation of parathyroid hormone receptors on the apical surface of cells of the late proximal tubule.12

Renal failure occurs in about two thirds of patients with Dent’s disease. Renal function may begin to decline in the teenage years and reach end stage by the fourth decade of life, but it is not clear why some patients are spared renal failure even into old age. Prominent histologic features include tubular atrophy and interstitial fibrosis, and there are varying degrees of glomerular sclerosis. Basement membranes appear normal on electron microscopy and immune deposits have not been identified on immunofluorescence.16,20,21 The etiology of the dysfunction is unclear. Sayer et al22 showed, in cultured collecting duct cells in which CLC-5 expression is prevented, that exposure to calcium oxalate crystals results in cellular engorgement with crystals. However, in anecdotal series, the severity of nephrocalcinosis does not appear to correlate with the degree of renal failure.1,16 Norden et al9 have documented increased urinary excretion of bioactive peptides including hormones and cytokines including parathyroid hormone, insulin-like growth factor 1, and monocyte chemoattractant protein 1, and proposed that high lumenal
levels of cytokines may promote tubulointerstitial fibrosis. In a preliminary report, Cebotaru et al. recently described that renal failure can be seen if CLC-5 knockout mice are followed-up to a sufficient age, with histologic evidence of interstitial inflammation and fibrosis, and tubular atrophy. In this report, the knockout mice also had increased tissue levels of messenger RNA encoding transforming growth factor β1, a profibrogenic cytokine.

About one fourth of patients with Dent’s disease have rickets, which often presents in infancy. As with renal failure, this feature does not correlate with the nature of the mutation, and can vary even within families in which multiple affected males all share the same mutation. It is not clear that the occurrence of rickets can be explained by the degree of hypophosphatemia, which usually is mild. The role, if any, of CLC-5 in bone, and of any possible modifying genes, is not known.

**Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis**

FHHNC was first described in 1972, and identified as the Michelis-Castillo syndrome. FHHNC is a rare entity transmitted in an autosomal-recessive fashion presenting at birth. Prominent features include renal wasting of magnesium and calcium associated with development of nephrocalcinosis by early childhood. As would be expected in patients with a renal calcium leak (and in contrast to Dent’s disease), serum levels of parathyroid hormone are high in patients with FHHNC. Hypomagnesemia can be severe, with neonatal seizures. Polyuria or urinary infections often are presenting complaints. Renal function declines progressively and many patients reach end-stage by the teenage or young adult years. Most patients have hyperuricemia. In addition to these renal problems, a range of ocular abnormalities are seen in some patients.

In elegant work from Lifton’s laboratory, the disease gene was mapped in 12 families to a region on the long arm of chromosome 3 (3q27) (as is often helpful in mapping autosomal-recessive diseases, most of these families exhibited consanguinity). Through positional cloning, a gene (PCLN1) was identified that encoded a protein with homology to the claudin family of tight-junction proteins; the protein was named paracellin-1 (also known as claudin 16). Claudins are membrane proteins with 4 transmembrane domains and 2 extracellular loops that are presumed to play an important role in the integrity of the tight junction. Paracellin-1 is expressed in the tight junctions of the thick ascending limb of Henle’s loop in humans; in the rat its expression has been detected in the thick ascending limb as well as the distal convoluted tubule and collecting duct. Functional studies have not been performed, but it is speculated that paracellin might be a paracellular ion channel, or might be involved in regulating paracellular cation conductance. In either case, paracellin-1 would represent the first protein shown to participate in renal paracellular transport.

Mutations in PCLN1 segregated with disease in these families and were not polymorphisms; this has now been confirmed by others. Consistent with the recessive nature of inheritance of this disease, most of the reported patients have been homozygous for a mutation or compound heterozygotes with 2 different mutations in PCLN1. Heterozygotes have been reported with mild, asymptomatic hypomagnesemia, hypercalciuria, or even clinically overt disease.

Most of the reported mutations in patients with FHHNC are missense mutations that predict substitution of a single amino acid, and these occur within the transmembrane domains and the 2 extracellular domains of the protein. Thus, it is plausible that these mutations would interfere with the function of the paracellular barrier and lead to abnormalities of transport in the thick ascending limb, a major site for reabsorption of calcium and magnesium. Unlike the distal convoluted tubule where the bulk of calcium and magnesium reabsorption occurs across the epithelial cell, cation transport in the thick limb is largely paracellular, driven by the lumen-positive electrical potential.

Maintenance of the positive charge in the lumen depends on proper functioning of the transcellular transport pathway in the cells of the thick ascending limb, including the bumetanide-sensitive Na-K-2Cl cotransporter, the apical potassium channel (ROMK), and the basolateral chloride channel CLC-Kb with its associated protein barttin. Defective function of genes encoding these transport proteins result in Bartter’s syndrome, in which hypercalciuria is a universal but secondary consequence and hypomagnesemia, when it occurs, usually is mild.
It is instructive to contrast the findings in patients with FHHNC, in whom transeellular transport is normal but paracellin is mutated, with those in patients with Bartter’s syndrome, whose inherited defect impairs transeellular transport. Hypokalemic metabolic alkalosis, the hallmark of Bartter’s and Gitelman’s syndromes, is absent in patients with FHHNC. Both syndromes can present in infancy, but whereas children with Bartter’s syndrome often have salt wasting and volume depletion, children with FHHNC suffer with symptomatic hypomagnesemia but do not have clinically significant salt wasting. Serum levels of renin and aldosterone, which are high in Bartter’s syndrome, are normal in FHHNC. Blanchard et al studied the responses to furosemide in 2 unrelated patients with FHHNC who were homozygous for mutations in PCLN1. Administration of furosemide produces no natriuresis in patients with Bartter’s syndrome, in which transeellular transport in the thick ascending limb of Henle already is impaired. In contrast, furosemide produces a natriuresis in patients with FHHNC that is comparable with the response in control subjects, but unlike the control subjects, furosemide did not increase excretion of calcium or magnesium in patients with FHHNC.

In preliminary studies described so far only in an abstract, mice with targeted disruption of the PCLN1 gene exhibit most of the features of the human FHHNC syndrome. These knockout mice have hypomagnesemia with inappropriate urinary magnesium wasting, hypercalcua, nephrocalcinosis, renal insufficiency, a urinary concentrating defect, and normal salt conservation.

Inadequate urinary acidification was one of the features identified by Michelis, but this was probably not a direct effect of the genetic defect but rather the consequence of medullary interstitial damage. Other findings in FHHNC remain, for the moment, unexplained. Hyperuricemia is present in the majority of patients with this syndrome, but the mechanism is not known. No paracellular secretory process for uric acid has been described or even proposed. Abnormalities of the eye occur in a significant minority of patients. These include nystagmus, severe myopia, macular colobomata, and tapetoretinal degeneration. It is not known whether PCLN1 is expressed in the eye, and what its function there might be.

CONCLUSION

Dent’s disease and FHHNC are both syndromes of hypercalciuria, nephrocalcinosis, and renal failure, for which the pathophysiologic bases certainly would not be so well understood without the success of the genetic studies described earlier. In both cases, identification of families made possible linkage analysis and then positional cloning, which led to the discovery in both diseases of a novel gene. The discoveries of CLC-5 and paracellin-1 have made possible in short order a more complete understanding of disease mechanisms and of normal renal tubular physiology, and also have allowed investigators to frame the right questions for further research. There are other inherited diseases associated with nephrolithiasis for which the molecular basis is known, particularly primary hyperoxaluria and cystinuria. Exciting progress recently has been made in identifying genes for several other inherited syndromes of hypomagnesemia. However, the path to understanding complex traits such as idiopathic hypercalciuria, the most common risk factor for kidney stones, will be much more difficult because in idiopathic hypercalciuria there are multiple mechanisms, probably reflecting polymorphisms in multiple genes. Nevertheless, there is every reason to expect that such obstacles will be overcome and that molecular genetics will continue to teach us about the pathophysiology of monogenic and polygenic diseases.

REFERENCES