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PERSPECTIVES IN RENAL MEDICINE

X-linked hypercalciuric nephrolithiasis: Clinical syndromes and chloride channel mutations

STEVEN J. SCHEINMAN

Department of Medicine, SUNY Health Science Center, Syracuse, New York, USA

The recent discovery of a gene responsible for a particular form of hypercalciuric nephrolithiasis has created excitement because it promises to shed light on mechanisms involved in both calcium stone formation and renal epithelial function. The disease that I will refer to here as X-linked hypercalciuric nephrolithiasis (XLHN) has been the subject of some confusion owing to the fact that it has been described over the past five years by different groups using different names, all but one of them polysyllabic: "X-linked recessive nephrolithiasis with renal failure" in North America, "Dent's disease" in the United Kingdom, "X-linked recessive hypophosphatemic rickets" in Italy, and "low-molecular-weight proteinuria with hypercalciuria and nephrocalcinosis" in Japan. These syndromes differ in degree from each other, but common themes include proximal tubular reabsorptive failure, nephrolithiasis, nephrocalcinosis, progressive renal insufficiency, and in some cases rickets. The fact of mutations in the same gene in each of these syndromes has now established that they are phenotypic variants of a single disease and not separate entities.

Clinical studies of these patients produced few clues as to the pathophysiology of the condition, and the gene responsible for this disease was identified through a positional cloning approach rather than through the study of logical candidate genes. The gene identified, *CLCN5*, was novel. Sequence homology suggested, and expression studies confirmed, that the gene product was a member of the CLC family of voltage-gated chloride channels. This was a surprise, since it is not immediately clear how mutations in such a chloride channel could explain the clinical findings in this disease. The physiology of the gene product, designated CLC-5, is at present only partly understood, and is the subject of active research.

SYNDROMES OF X-LINKED HYPERCALCIURIC NEPHROLITHIASIS

Four syndromes of X-linked hypercalciuric nephrolithiasis (XLHN) were reported independently, but mutations in the chloride channel gene *CLCN5* have been identified in all four syndromes, justifying the view that these together represent a single disease. Table 1 summarizes the features of these syndromes as originally described. An understanding of these reports is valuable in that it emphasizes the range of phenotypes in this disease, and as an effort to clarify the relationships among what some might otherwise be tempted to consider as separate entities.

X-linked recessive nephrolithiasis

In 1991, we reported a disease among members of a single large family from Watertown in northern New York State that affected only males, and gave it the descriptive name "X-linked recessive nephrolithiasis with renal failure" (XRN) (McKusick catalog no. 310468) [1]. The essential clinical features of this syndrome were urinary solute wasting suggestive of proximal tubular dysfunction, calcium nephrolithiasis, nephrocalcinosis, and progressive renal failure. The disease presented with proteinuria and microscopic hematuria, in some cases within the first year of life. Kidney stones occurred as early as three to six years of age in some patients, in others first occurred in adulthood, and were composed of calcium oxalate, often with calcium phosphate as well. Progression to renal failure occurred in many but not all patients at some time in adult life [1]. All affected individuals were male; carrier females were asymptomatic with only mild biochemical abnormalities detectable in the urine [2].

While we were unable to establish an underlying pathophysiologic mechanism, the pedigree established clearly that inheritance was X-linked recessive (Fig. 1). There were no instances of male-to-male transmission (among 11 sons of affected males). Daughters of affected males were themselves clinically unaffected, with no stones or renal insufficiency in any carrier female, but 50% of their male offspring were affected [1]. Virtually all of the carrier females had some degree of low-molecular-weight proteinuria and about one-third of carrier females were hypercalciuric [2].

One patient has maintained a functioning kidney transplant, which is radiographically free of nephrocalcinosis after 12 years, suggesting that the defect is intrinsic to the renal tubule and is not systemic.

The original report of this family included several patients with moderate renal insufficiency in whom calcium excretion was normal [1], but with further study of a larger number of patients it became clear that hypercalciuria was present in all affected individuals if studied at a time when renal function was still preserved [2]. The most consistent urinary abnormality of all, however, was excessive excretion of low-molecular-weight (LMW) proteins, in both affected males and carrier females. Moderate

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Table 1. Four syndromes of X-linked hypercalciuric nephrolithiasis

Syndrome	XRN	Dent's disease	X-linked rickets	LMW proteinuria/ nephrocalcinosis
References	[1–5]	[8]	[10,12]	[16,17]
Location of reports	U.S.A., Canada	U.K.	Italy, France	Japan
LMW proteinuria	+	+	+	+
Hypercalciuria	+	+	+	+
Nephrocalcinosis	+	+	+	+
Calcium stones	+	+		
Renal failure	+	+	+	
Male predominance	+	+	+	+
Rickets		+	+	
CLCN5 mutations	+	+	+	+

polyuria occurred in affected males only, from early in childhood. Other abnormalities of renal tubular function, such as glycosuria, aminoaciduria, phosphaturia, kaliuresis, or uricosuria, were found variably [1, 2]. These are discussed in detail below.

Patients with XRN have now been reported in families from Montreal [3], Calgary [4], and St. Petersburg, Florida [5]. In all cases boys presented with hematuria, proteinuria (primarily LMW), hypercalciuria, nephrocalcinosis or nephrolithiasis, and a family history including, in each case, the maternal grandfather with a similar history including stones and terminating in end-stage renal failure [3–5]. Renal biopsy findings in these cases, as in the original family with XRN, showed a nonspecific pattern of tubular atrophy, interstitial fibrosis, and glomerular sclerosis. All together, we have data on eleven families in North America with this syndrome. These include one family in which a woman has had recurrent nephrolithiasis and another woman has proteinuria and glomerular sclerosis (unpublished observation). Of the 30 affected males represented in these eleven families, there is one patient with rickets (P. Goodyer, personal communication).

Dent's disease

In 1964, Dent and Friedman reported the cases of two unrelated English boys who on evaluation for childhood rickets were found to have renal tubular dysfunction including tubular proteinuria, aminoaciduria, phosphate wasting, and hypercalciuria, with a normal ability to acidify the urine. They reported no occurrence of kidney stones or renal failure, and no kidney problems or rickets in any family members [6]. However, Oliver Wrong and colleagues have followed these and other patients over the succeeding 30 years, and have established that stones, renal failure, and mendelian inheritance are clearly features of what they have named "Dent's disease," which they described briefly in an abstract in 1990 [7], and subsequently reported fully [8].

The value of LMW proteinuria as a consistently detectable abnormality in one of these syndromes was first reported by Wrong, Norden, and Feest in Dent's disease [7, 8]. Other renal functional defects, and the progression to renal failure, are also essentially indistinguishable from those found in XRN, and will be discussed in detail below. In Dent's disease there is also a marked male predominance, although Wrong et al did describe historic information about one woman who was known to have died in renal failure at age 56 with nephrocalcinosis. In general, female carriers with Dent's disease are asymptomatic, although all have low molecular-weight proteinuria, and half are hypercalciuric [8].

Urinary acidification was defective in some of their patients, but they did not consider this to be a primary feature of the disease, since in all cases it could be attributed to renal insufficiency or nephrocalcinosis. The one feature of Dent's disease that appeared to distinguish it definitively from XRN was rickets or osteomalacia, which in five children was the presenting feature. Overall, however, rickets was present in only a third of affected males with Dent's disease [8].

X-linked recessive hypophosphatemic rickets

The third related syndrome, X-linked recessive hypophosphatemic rickets, was initially described in a family in Genoa, Italy. Five males in this family presented with rickets or osteomalacia, hypophosphatemia, and a reduced renal threshold for phosphate reabsorption, but a number of features were atypical of X-linked dominant hypophosphatemic rickets (HYP). For example, these patients had hypercalciuria, and high levels of 1,25-dihydroxyvitamin D. In addition, they had proteinuria of up to 3 grams per day. Patients developed nephrocalcinosis, with progressive renal failure in early adulthood [9, 10].

In additional contrast with findings expected in X-linked dominant hypophosphatemic rickets, female carriers in this family were not hypophosphatemic, and lacked any biochemical abnormalities other than hypercalciuria [10]. It is of interest that two of the four hypercalciuric females had "mild symptoms of bone disease," though no further details were given [9]. No member of this family, male or female, had hypokalemia or aminoaciduria.

The locus of the HYP gene at Xp22, on the telomeric end of the short arm of the X chromosome, is known, and the gene has been cloned [11]. Using genetic markers that flank the HYP locus, a group headed by Prof. Giovanni Romeo excluded the HYP gene from linkage with the disease in this family. Further studies established definite linkage to the pericentromeric region at Xp11.22 that is also linked to XRN and Dent's disease [10]. A second such family was recently identified in France in which the four affected males all had symptomatic rickets, and in whom the disease linked to the same chromosomal region [12]. Urinary excretion of LMW proteins, not measured in the Italian report, was excessive in the affected French males.

This clinical syndrome differs from XRN and Dent's disease only in degree, but is potentially instructive, particularly regarding the occurrence and severity of rickets. It is interesting that both families share the same mutation (Table 2).

Low-molecular-weight proteinuria with hypercalciuria and nephrocalcinosis

Because of the annual urine-screening program performed on school children in Japan since 1974, and more recently in preschool children as well, the entity of asymptomatic low molecular-weight proteinuria had been recognized in the 1980s. Other abnormalities found in these children included microscopic hematuria, glycosuria, aminoaciduria, and hypophosphatemia [13, 14]. Biopsy findings were most often normal or nearly so, though a third of patients had evidence of tubular atrophy and glomerular sclerosis. Murakami and Kawakami noted a tendency to short stature in the older children, and some patients had a decreased creatinine clearance [14]. All but one of the 58 patients in these

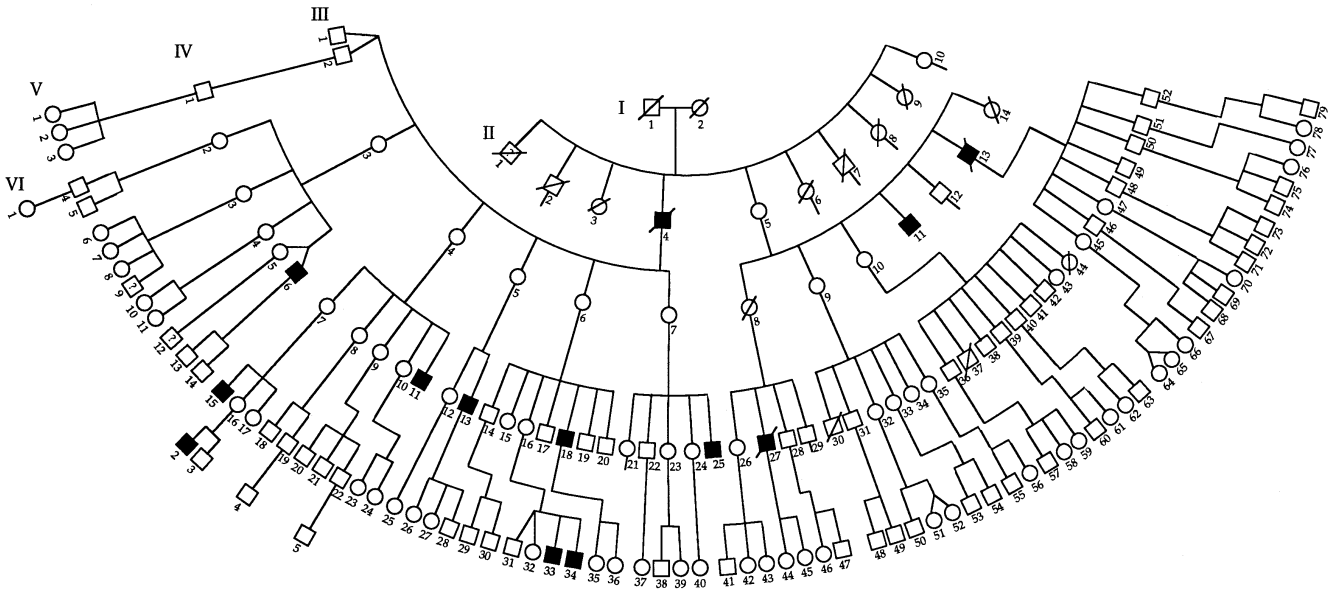


Fig. 1. Pedigree of extended family with X-linked recessive nephrolithiasis. Males are indicated by squares, females by circles, and affected patients by solid symbols. Four patients not initially identified in the original report as affected are now indicated by solid symbols (subjects III-11, V-15, V-34, and VI-2). (Adapted from Frymoyer et al [1], with permission. Copyright © 1991, Massachusetts Medical Society. All rights reserved.)

two reports were male. Isolated tubular proteinuria with hypercalciuria has also been reported in one boy in England [15], who has now been found to have a mutation in *CLCN5* (#14 on Table 2) [19].

Igarashi and colleagues from Tokyo were the first to report hypercalciuria in association with this syndrome. They described seven such children from six different families, all of whom had nephrocalcinosis. Six of the seven were boys, and had hypercalciuria; the one girl did not. No mention was made of any evidence for bone disease. Two boys had aminoaciduria, two had glycosuria, and all had a mild impairment in urinary concentration [16].

A subsequent report included clinical data on additional boys, as well as molecular data to be described below [17]. Of these eight Japanese boys with idiopathic LMW proteinuria, hypercalciuria, and nephrocalcinosis, none was more than 14 years old, and so it is not surprising that serum creatinine measurements were normal in all [16, 17], or that the six measured creatinine clearance rates were normal as well [17]. These children were identified by screening rather than by symptomatic disease, and thus the absence of symptomatic renal colic or rickets should not be viewed as distinguishing these patients from patients with XRN or Dent's disease. Nevertheless, the presence of nephrocalcinosis in boys as young as three or five years of age [16, 17] indicates significant disease, in which the prognosis may be no milder than that of patients in North America or Britain.

Other reports

The literature contains several other descriptions consistent with XLHN. Only one of these reported families has been studied for mutation in the *CLCN5* gene. This is an Italian family in which males developed hematuria, LMW proteinuria, hypercalciuria, phosphaturia, high 1,25-dihydroxyvitamin D levels, impaired urinary concentration, and progressive renal insufficiency [18]. This family also had a mutation in *CLCN5* [19]. Bone mineral density was reduced, but growth was normal and clinical rickets was absent [18].

Buckalew et al reported a large (64-member) kindred in which clinical features included hypercalciuria, nephrocalcinosis, nephrolithiasis, proteinuria with prominent LMW excretion, aminoaciduria, polyuria, and varying degrees of renal failure. Biopsy findings included tubular atrophy and glomerular hyalinosis. Although the report was entitled "Hereditary renal tubular acidosis" complete RTA was present in only one patient and impaired urinary acidification without acidosis in another three, while six had nephrocalcinosis and 19 had hypercalciuria, and the authors themselves made the point that the acidification defect was probably a consequence of the hypercalciuria [20]. Thus, the clinical features of this inherited renal tubular disorder would be completely consistent with the diagnosis of XLHN.

In 1962, Gentil and colleagues in Paris described two boys with hypophosphatemic rickets, hypercalciuria, urinary loss of both albumin and LMW proteins, polyuria, and normal ability to acidify the urine. Renal biopsies documented an "interstitial nephropathy," consistent with those described in documented cases of XLHN [21]. In 1979, Salti and Hamadi reported a Lebanese boy with hypercalciuric rickets, "tubular" proteinuria, glycosuria, aminoaciduria, hypophosphatemia, impaired urinary concentration, and normal urinary adicification. The authors were struck by the similarities between their patient and those of Gentil et al and of Dent and Friedman [22].

Carey and Hopfer described a 12-year-old Puerto Rican boy with hypophosphatemic rickets, hypercalciuria, β2-microglobulinuria, aminoaciduria, and microscopic hematuria. His maternal grandfather had nephrolithiasis and his sister had hypercalciuria. Hypercalciuria was diet-dependent, and also improved on therapy with thiazide [23].

Furuse et al reported two Japanese families in which an inherited Fanconi syndrome occurred in six male individuals [24].

Urinary abnormalities included low-molecular weight proteinuria and hypercalciuria, and appeared to be progressively more severe with advancing age. This condition resembled the syndromes of XLHN in the occurrence of renal failure, the findings on renal biopsy, and the excessive urinary excretion of β2-microglobulin in all of the patients and unaffected carriers. The pedigree was consistent with an X-linked recessive mode of inheritance [24]. To my knowledge no molecular studies have been pursued in these patients.

One condition that is tempting to compare with XLHN is that described in a Bedouin tribe by Tieder et al as hereditary hypophosphatemic rickets with hypercalciuria (HHRH) [25, 26]. Patients with HHRH had rickets, hypophosphatemia with appropriate elevation in serum levels of 1,25-dihydroxyvitamin D, hypercalciuria, and relatively low PTH levels. Thus, in several ways they resembled the Italian and French families with X-linked rickets discussed above. Hypercalciuria appeared to reflect intestinal hyperabsorption of calcium. One of these six children had cystolithiasis, but none had proteinuria, aminoaciduria, glycosuria, or uricosuria, and all had normal glomerular filtration rates [25]. It is of great interest that about 40% of members of this tribe had (apparently asymptomatic) hypercalciuria with a similar metabolic pattern, though milder and without rickets [26]. Interpretation of the inheritance pattern was difficult in view of the extensive intermarriage among families in this tribe, but several instances of male-to-male inheritance of hypercalciuria argued against X-linked inheritance [26], and the absence of proteinuria in particular also makes it unlikely that this represents a form of XLHN. The metabolic features of this syndrome make it tempting to propose that it is explained by dysfunction of the Na-dependent phosphate transporter NaPi2, and this transporter is encoded by a gene on chromosome 5 (5q35) [27].

COMMON CLINICAL FEATURES OF THE X-LINKED HYPERCALCIURIC NEPHROLITHIASIS SYNDROMES

If X-linked hypercalciuric nephrolithiasis is to be classified as a form of the Fanconi syndrome it must be kept in mind that while LMW proteinuria is a consistent finding, other defects of proximal tubular function are variable and intermittent, that proximal renal tubular acidosis does not occur, and that it differs from most other forms of the Fanconi syndrome in the nature of the abnormalities of calcium metabolism.

Proteinuria

The degree of proteinuria is relatively consistent across the spectrum of reports of this disease. Total excretion of proteins in most cases amounts to 0.5 to 2 grams per day in adults and up to 1 g per day in children [1, 2, 8, 10, 13, 16, 24], with 50 to 70% of the total consisting of LMW proteins with molecular mass of less than 40,000 daltons [13, 14, 16, 24] (and Anthony Norden, personal communication). Albumin represents less than half of the protein excreted by these patients, and the nephrotic syndrome does not occur in any of the X-linked hypercalciuric nephrolithiasis syndromes. The defects in renal tubular function, the degree of proteinuria, and the histopathology (described below) together most strongly suggest that the albuminuria reflects glomerular damage secondary to tubulointerstitial disease.

Figure 2 illustrates the excretion of LMW proteins in the large pedigree that formed the basis for the description of XRN. Affected males excreted β2-microglobulin in amounts that exceeded the upper limit of normal by 100- to 300-fold, and retinol-binding protein in amounts that exceeded the upper limit of normal by 1000- to 3000-fold. Obligate carrier females excreted a moderate excess of β2-microglobulin and retinol-binding protein. Excretion of α1-microglobulin followed a similar pattern [2]. This is consistent with X-linked recessive inheritance, with random inactivation of the X chromosome yielding a variable phenotype in females that is almost always less severe than in affected males. Essentially identical findings have been reported in the British families [8] one of the Italian families [18], and the recently-described French family [12], and a number of Japanese reports described similar findings for β2-microglobulin, α1-microglobulin, and lysozyme [13, 14, 16, 24].

Although low molecular-weight proteinuria is a nonspecific finding in many tubulointerstitial diseases, it is uncommon in stone disease in the absence of infection or obstruction from recurrent stones [28]. In contrast, low-molecular-weight proteinuria is one of the earliest and certainly the most consistent abnormality found in affected males in all families with XLHN, and for this reason excretion of these low molecular-weight proteins is useful as a screening test for this disease.

Urinary protein markers of tubular injury were normal in British patients with Dent's disease [8]. Urinary levels of n-acetyl glucosaminidase were abnormal in a number of patients in two Japanese reports [14, 24], but one of these was a report of LMW proteinuria with no mention of hypercalciuria or nephrocalcinosis [14], and neither has been confirmed by anlysis of *CLCN5* for mutations.

Hypercalciuria

Hypercalciuria in adults with XLHN tends to be modest, usually in the range of 4 to 6 mg/kg body wt even on a high calcium intake and even when renal function is still well-preserved. In some children, however, we have seen calcium excretion rates as high as 10 mg/kg body wt. In cases in which thiazides have been given, hypercalciuria often has improved, though usually not to normal. These anecdotal observations on thiazides need to be pursued in properly controlled studies.

In studies involving dietary deprivation and loading, we have found fasting hypercalciuria in about half of patients with XRN, but all patients have an exaggerated calciuretic response to oral calcium loading [2]. In two Japanese children with LMW proteinuria and nephrocalcinosis, Igarashi observed fasting hypercalciuria with a post-absorptive rise in urinary calcium. They observed a mildly increased urinary cAMP excretion on fasting that suppressed with oral calcium loading, but PTH levels were in the low-normal range in both patients [16], as was found in 15 of the 16 published cases on three continents (Fig. 3). Serum calcium levels in XRN, Dent's disease, and the Japanese children, have all been normal, with no tendency to hypocalcemia [1, 2, 8, 16].

Figure 3 depicts the serum levels for PTH and 1,25 dihydroxyvitamin D only in those patients with normal rates of creatinine clearance, in each of the four published XLHN syndromes, and in additional patients whom we are currently studying. Patients from North America, England, Italy, and Japan, with only one exception, all have PTH levels below the mean of the normal range and often frankly low, and all have 1,25 dihydroxyvitamin D levels that are above the normal mean or frankly elevated. Serum levels of phosphorus were normal in most of these patients, though often

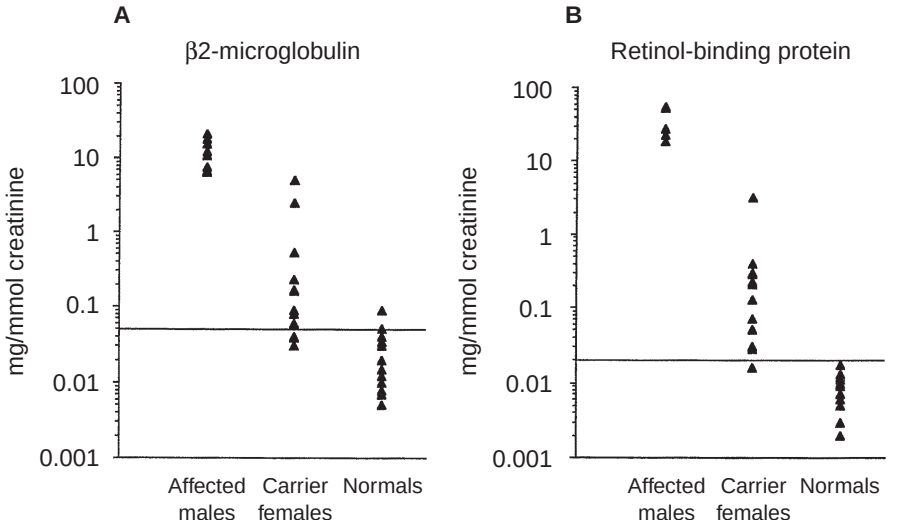


Fig. 2. Urinary excretion of the low-molecular-weight proteins β2-microglobulin and retinol-binding protein in affected males, obligate carrier females, and normal members of the large family reported with XRN. Horizontal lines indicate the established upper limit of the normal range. (Adapted from Reinhart et al [2], with permission.)

near the low end of the normal range. In these respects, calcium metabolism in XRN is similar to one pattern commonly seen in patients with idiopathic hypercalciuria. In addition, the combination of hypercalciuria with high levels of 1,25 dihydroxyvitamin D contrasts with other renal diseases in which hypocalciuria and normal to low levels of 1,25 dihydroxyvitamin D is a more common pattern.

If the molecular defect in this disease led directly to a renal tubular leak of calcium, one might expect PTH levels to be high, or at least not low. This, and the exaggerated calciuretic response to dietary calcium loading, make it reasonable to speculate that the hypercalciuria may be the consequence of abnormal regulation of 1-hydroxylation of vitamin D, possibly as a part of more generalized dysfunction of the proximal tubular cell.

Factors contributing to stone risk

The most significant factor contributing to calcinosis and stone formation appears to be hypercalciuria. Urinary supersaturation for calcium oxalate is high, particularly when corrected for the patients' large urine volumes [29]. Urinary excretion of oxalate was slightly elevated in one of six male patients with Dent's disease [8] and was normal in all of the published cases of XRN [1, 2] but was slightly high in one patient with XRN we have subsequently seen (unpublished observation). Urinary excretion of citrate was normal in all patients with XRN [1, 2] and in all 5 patients with Dent's disease in whom it was measured, except for one patient who had renal insufficiency [8]. Wrong and colleagues pointed out that this is unlike the findings in typical Fanconi's syndrome, in which citrate excretion is often high [8].

Other defects of proximal tubular function

While LMW proteinuria is universal in X-linked hypercalciuric nephrolithiasis, other defects of proximal tubular function occur less consistently. Aminoaciduria occurred in 14 of 15 of Wrong's patients with Dent's disease, and in five included most prominently glycine. However, we observed a nonselective pattern of aminoaciduria in patients with advanced renal failure in XRN [1], while a specific pattern of dibasic aminoaciduria happened to occur in a number of normal family members and in two carrier females but in none of the other affected males, and could be demonstrated by linkage analysis to segregate independently of X-linked nephrolithiasis [2]. Aminoaciduria was reported in 1 of 5 patients in Suzuki's report of LMW proteinuria in Japan [13], 2 of 6 males in Igarashi's report [16], and 5 of 37 in Murakami and Kawakami's report [14]. Dibasic aminoaciduria occurred in 6 of 6 male members of Furuse's families with "familial progressive renal tubulopathy" [24].

Renal glycosuria occurred in fewer than half of patients in each of a number of reports [2, 8, 14, 16, 24] and was often intermittent [8]. Hypokalemia was certainly uncommon, but when it occurred could require substantial quantities of potassium replacement [1, 2, 8, 24]. Hypophosphatemia with a reduced threshold for phosphate reabsorption also occurred in a minority of patients [1, 2, 8, 24]. Hyperuricosuria was reported in 3 patients with XRN [1] and in 2 of 15 patients with Dent's disease [8], but all had normal serum levels of uric acid so this should not be interpreted to reflect an alteration in tubular reabsorption [1, 2, 8]. We have seen one young boy with mild hypouricemia (1.4 mg/dl) and hyperuricosuria (unpublished observation).

Defects in renal sodium reabsorption have not been demonstrated and patients have not been studied after severe restriction of dietary sodium intake. The absence of hypertension in all reported patients (except one with advanced renal failure [8]) argues against an inappropriate retention of sodium. Our patients were able to tolerate a moderate (2 g/day) restriction of sodium intake without alteration in blood pressure [2]. Wrong reported one patient with an exaggerated natriuretic response to a large dose of thiazide diuretic [8].

Defective urinary concentration and acidification

Moderate polyuria and relative resistance to vasopressin occurred in many patients including young children [2, 8]. Osmolar clearance was normal indicating that the polyuria did not result from a solute diuresis from proximal tubular failure, nor did it correlate with the presence or absence of hypokalemia [2]. In all cases the impaired concentrating ability was consistent with the degree of renal insufficiency or nephrocalcinosis [2, 8]. Metabolic acidosis was absent in all but those patients with advanced renal

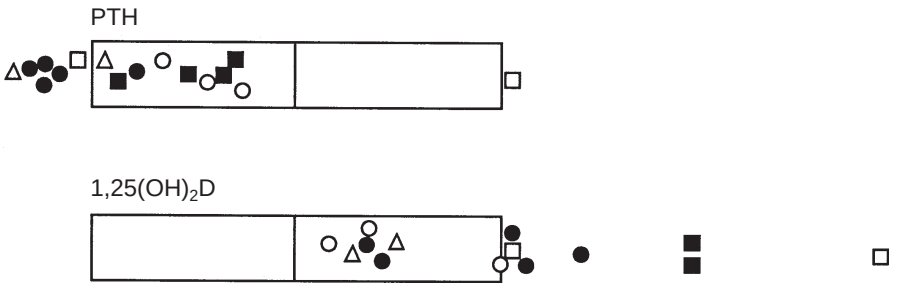


Fig. 3. Serum levels of intact PTH and 1,25-dihydroxyvitamin D in patients with XLHN syndromes in whom creatinine clearance rates were documented to be normal. Except for two of the Japanese cases, all patients shown have been confirmed to have *CLCN5* mutations. The boxes indicate the limits and midpoints of the normal ranges for PTH and 1,25 dihydroxyvitamin D, respectively. Symbols indicate sources of data: North American patients with XRN (original report, ● [2]; other North American cases, ■ [3–5, and unpublished observations]); British patients with Dent's disease (□, [8]); Italian cases with rickets (△, [10]); and Japanese cases with LMW proteinuria and nephrocalcinosis (○, [14]).

insufficiency. Urinary acidification defects detectable with acid-loading were identified in a number of patients and again could be explained in each case by renal insufficiency or nephrocalcinosis [2, 8].

Renal failure

The occurrence of renal failure in this disease is variable. Some patients have significant depression of GFR by late childhood and may reach end-stage renal disease in their 30s, while others may have only modest impairment of renal function into old age. The cause of renal failure is not clear. Nephrocalcinosis is a common finding in virtually all patients from teenage years onwards, and is occasionally detectable in young children. Histologically and radiographically, the nephrocalcinosis is medullary. However, the severity of nephrocalcinosis does not correlate consistently with the presence or degree of renal insufficiency.

Findings on renal histopathology are nonspecific. Most prominent is tubular atrophy and interstitial fibrosis, with glomeruli exhibiting varying degrees of sclerosis, and some glomeruli hypertrophic. The biopsy appearance is that of a focal tubulopathy with atrophy and scarring but little if any inflammation, and secondary glomerular damage. Ultrastructural examination by electron microscopy reveals normal basement membranes, and immunofluorescence studies have been negative [1, 8, 16].

I am aware of at least nine cases of XRN and Dent's disease, both published [1, 8] and unpublished, in which renal transplantation has been performed, and which have been followed for as long as 12 years. In no case has nephrolithiasis or nephrocalcinosis developed in the transplant. The persistence of LMW proteinuria or aminoaciduria [8] after transplantation is of uncertain significance, as they could reflect nonspecific allograft injury or some residual function from the native kidneys.

Rickets/osteomalacia

Rickets was a prominent feature in about a third of patients reported with Dent's disease, and in all nine affected males in the Italian and French families with X-linked recessive hypophosphatemic rickets. The cause of the rickets was and remains unclear. No reported patient with any of these syndromes was hypocalcemic, and bone disease could not be explained by acidosis. Hypophosphatemia was present in six of fifteen affected males with Dent's disease, but the degree of hypophosphatemia did not correlate with the presence of rickets, and in most cases did not distinguish patients with rickets from those with either Dent's disease or XRN who did not have rickets. For example, of the three patients in the report by Wrong, Norden and Feest with the most severe hypophosphatemia, only one had rickets or osteomalacia [8]. Two of the four rachitic French patients were normophosphatemic [12].

Serum levels of alkaline phosphatase were elevated in patients with clinical bone disease [8] but were normal in patients without bone disease [1, 2, 8]. In addition to normal levels of alkaline phosphatase, patients with XRN had normal bone mineral densities measured by dual-energy x-ray absorptiometry [2]. Healing of the bone disease occurred with large doses of vitamin D in all of the rachitic children with Dent's disease [8].

POSITIONAL CLONING OF THE GENE *CLCN5*

The clinical findings in patients with X-linked hypercalciuric nephrolithiasis did not provide clear clues to the nature of the primary pathophysiologic defect. Early attempts to address this included measurements in erythrocytes of the activities of the Na-K-Cl cotransporter, K-Cl cotransporter, Na/K-transporting-ATPase, and carbonic anhydrases B and C, all of which were were normal in patients with XRN [1]. The disease was difficult to explain *a priori* on the basis of a defect in any single transport protein or pathway.

However, the availability of an extended family with XRN with a pedigree providing compelling evidence of X-linkage made possible a genetic approach to localize the gene and identify it by positional cloning. Working with Dr. Raj Thakker of the Hammersmith Hospital in London, we focused our localization efforts on the X chromosome, using restriction fragment length polymorphisms (RFLPs) and microsatellite polymorphisms on DNA extracted from peripheral blood leukocytes in 102 members of the extended pedigree. We were extremely fortunate in that the first marker we tried that was informative in this family exhibited linkage with the disease. This marker was a highly polymorphic RFLP at locus DXS255 identified by the probe M27β after digestion with the restriction enzyme *Eco*RI. Figure 4 illustrates in one small branch of this pedigree the results with this RFLP. In this branch and across the entire family, affected males all displayed the 7.5 kb allele, carrier females were heterozygous for this allele, and unaffected males displayed other alleles. With only one unaffected recombinant (3.6% recombination), this locus was highly linked with the disease, with a LOD score of 5.91, indicating odds favoring linkage of nearly one million to one for this locus at Xp11.22, in the pericentromeric region of the short arm of the X chromosome [30]. This region harbors genes

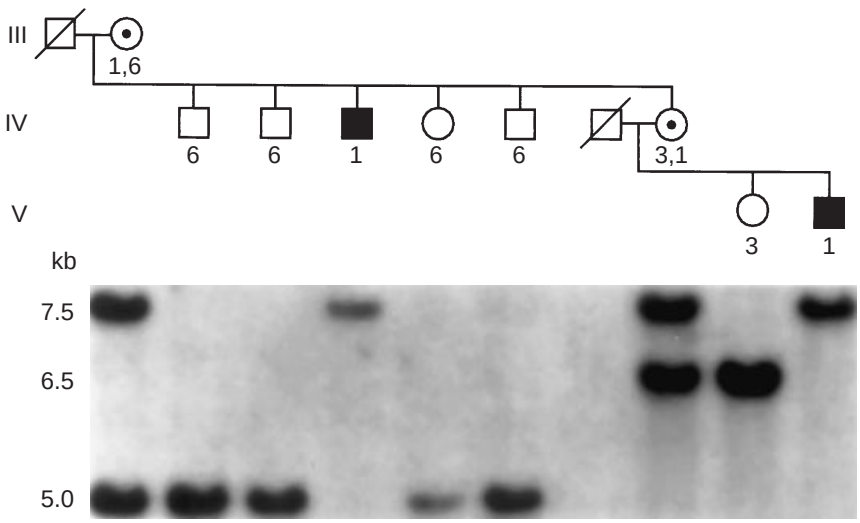


Fig. 4. Restriction fragment length polymorphism revealed by probe M27β identifying locus DXS255 in one branch of the extended family with XRN reported by Frymoyer et al [1]. Five micrograms of leukocyte DNA was digested to completion with a fourfold excess of enzyme (*Eco*RI), electrophoresed and transferred to a nylon membrane (Hybond N) by Southern blotting. The DNA probe was labeled using α-^{32}P dCTP, hybridized to the Southern blot and detected by autoradiography. Seven alleles were identified in the entire family for this highly polymorphic marker, of which three are seen on this Figure. The affected males (■) had the 7.5 kb allele, and none of the unaffected males (□) had this allele. Two obligate heterozygote females are illustrated, indicated by a dot in the circle; both are heterozygous for this marker, and both have the 7.5 kb allele. Altogether in the family there were 24 meioses informative for this marker, of which 23 were non-recombinant. (Data from Scheinman et al [28].)

responsible for a number of eye diseases, but detailed ophthalmologic examination of patients with XRN failed to reveal any structural or functional abnormalities [2].

This localization, as well as the clinical and histologic features, allowed a definitive answer to the question of the relationship between XRN and two other important diseases. One is the oculocerebrorenal syndrome of Lowe, the only other X-linked Fanconi syndrome. Patients with Lowe's syndrome occasionally develop renal failure. However, the neurologic and eye findings of Lowe's syndrome are absent in XLHN. The other disease, Alport's syndrome, is the most common X-linked cause of renal failure, but Alport's syndrome is not associated with nephrolithiasis, no patient with XRN had a hearing deficit, and most importantly, renal biopsies in patients with XRN or other XLHN syndromes all failed to demonstrate the abnormalities of the glomerular basement membrane that are characteristic of Alport's syndrome. In addition to these important phenotypic differences, the chromosomal location of the gene for XRN, in the pericentromeric region of the short arm of the X-chromosome (Xp11.22), clearly distinguished it from both of these other conditions which have been mapped to the long arm (Lowe's at Xq25 to 26 [31] and Alport's at Xq22 [32]). Figure 5 illustrates the relative positions on the X chromosome of the diseases affecting renal function that have been mapped on that chromosome.

Knowledge of the localization of XRN also made it possible to address the issue of the relationship between XRN and Dent's disease. In the British patients with Dent's disease, the apparent occurrence of clinical disease in one female, and the low molecular-weight proteinuria in all female carriers, originally led Wrong and colleagues to propose that this syndrome was autosomal [7]. However, using markers in the region to which XRN had been localized, the Thakker group discovered in the largest of the British families with Dent's disease not just linkage to the same locus, but exciting evidence for a deletion of that locus [33]. In collaboration with Dr. Ian Craig's group at Oxford University they determined the size of this deletion in this family to be about 515 kb [34]. Yeast artificial chromosomes that spanned the deletion were used to screen a human renal cDNA library, and a candidate gene was identified [34].

This gene had a novel sequence, and was homologous to the family of voltage-gated chloride channels. Current nomenclature is unfortunately confusing, with the gene designated *CLCN5*, and the channel protein it encodes designated CLC-5. The gene had 13 exons, and the transcript was 9.5 kb in size, with an open reading frame of 2238 bases encoding a product of 746 amino acids, with 12 predicted transmembrane domains [35].

The first of the genes in this family had been identified by Thomas Jentsch by expression cloning from the marine ray *Torpedo marmorata*. The mammalian members of this family of channels are represented in Figure 6. These include CLC-1, the major chloride channel in human muscle, which is the gene mutated in both the dominant and recessive forms of congenital myotonia, the only other disease currently associated with mutations in this family of genes [36]. CLC-Ka and CLC-Kb are expressed exclusively in kidney, and their axial distribution along the nephron has been studied [37]; one of them appears to be the basolateral chloride channel in medullary thick ascending limb [38]. In the rat, expression of the homologues of CLC-Ka and CLC-Kb can be induced by dehydration [39, 40]. These two channels, however, share only about 30% amino acid identity with CLC-5. Sequences for CLC-3 and CLC-4, both of which are expressed broadly including in kidney, are about 77 to 78% identical to CLC-5 [35].

On Northern analysis CLC-5 appears to be expressed in humans primarily in the kidney [34]. In the rat, on the other hand, CLC-5 expression can be demonstrated in brain, liver, and lung, though as in human the predominant expression is in kidney [41]. Bone cells have not yet been examined for expression of CLC-5. In the rat, CLC-5 is expressed throughout the nephron with the exception of the glomerulus [41], but particularly since the distribution of CLC-5 expression in extrarenal tissues differs significantly between rat and humans [41, 42], we must be cautious about extrapolating observations on intrarenal localization from rat kidney to human.

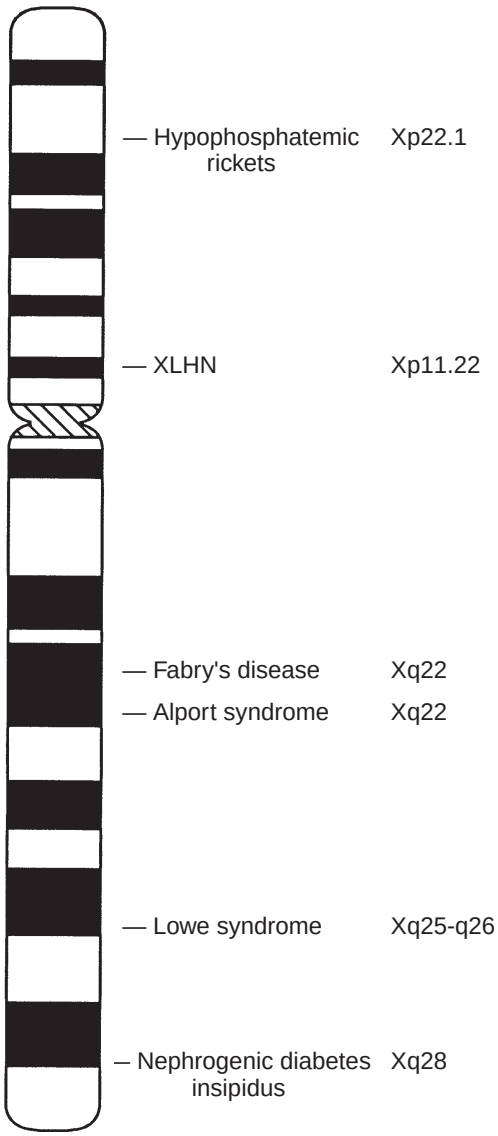


Fig. 5. Genes responsible for X-linked disorders affecting the kidney are indicated schematically at their relative positions on this cartoon of the X chromosome. Localization data taken from references [11, 31, 32, 77, 78].

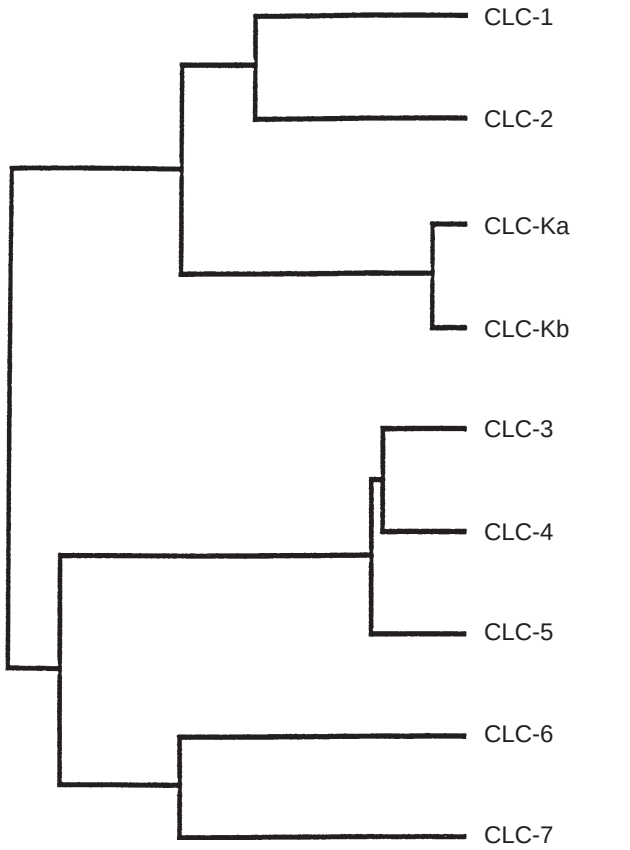


Fig. 6. The nine known members of the CLC family of voltage-gated chloride channels in mammals. The dendrogram represents the relative similarities among these channel proteins based on amino acid sequence (From Brandt and Jentsch [79], used with permission).

CLCN5 MUTATIONS IN X-LINKED HYPERCALCIURIC NEPHROLITHIASIS

Upon identification of a candidate gene, mutations were sought and identified in patients with XRN, Dent's disease, and X-linked recessive hypophosphatemic rickets, demonstrating that these three syndromes with overlapping phenotypes all shared a common molecular basis [43]. Soon thereafter, mutations were found in Japanese families with LMW proteinuria and nephrocalcinosis. Thus, the question posed by Igarashi and colleagues in the title to their report, "Is the disease identical to Dent's disease in the United Kingdom?" [16], has been answered in the affirmative. Mutations have been found in four families with XRN [19, 43], ten families with Dent's disease including one of Dent's original patients [19, 43], and the Italian and French families with X-linked recessive hypophosphatemic rickets [12, 43] as well as in the Italian family without rickets [19]. Five mutations have been documented in six Japanese families [17, 44].

To date 19 different mutations have been reported in 22 unrelated families (Table 2 and Fig. 7). These include 8 missense mutations leading to substitutions of single amino acids; 5 nonsense mutations leading to stop codons that yield a truncated protein; one in-frame insertion of an triplet encoding histidine; 2 donor splice-site mutations and one in-frame deletion that lead to loss of one or more transmembrane domains; one insertion of a single base resulting in a frameshift that truncates the protein; and the deletion of the entire gene found in the family from Kent.

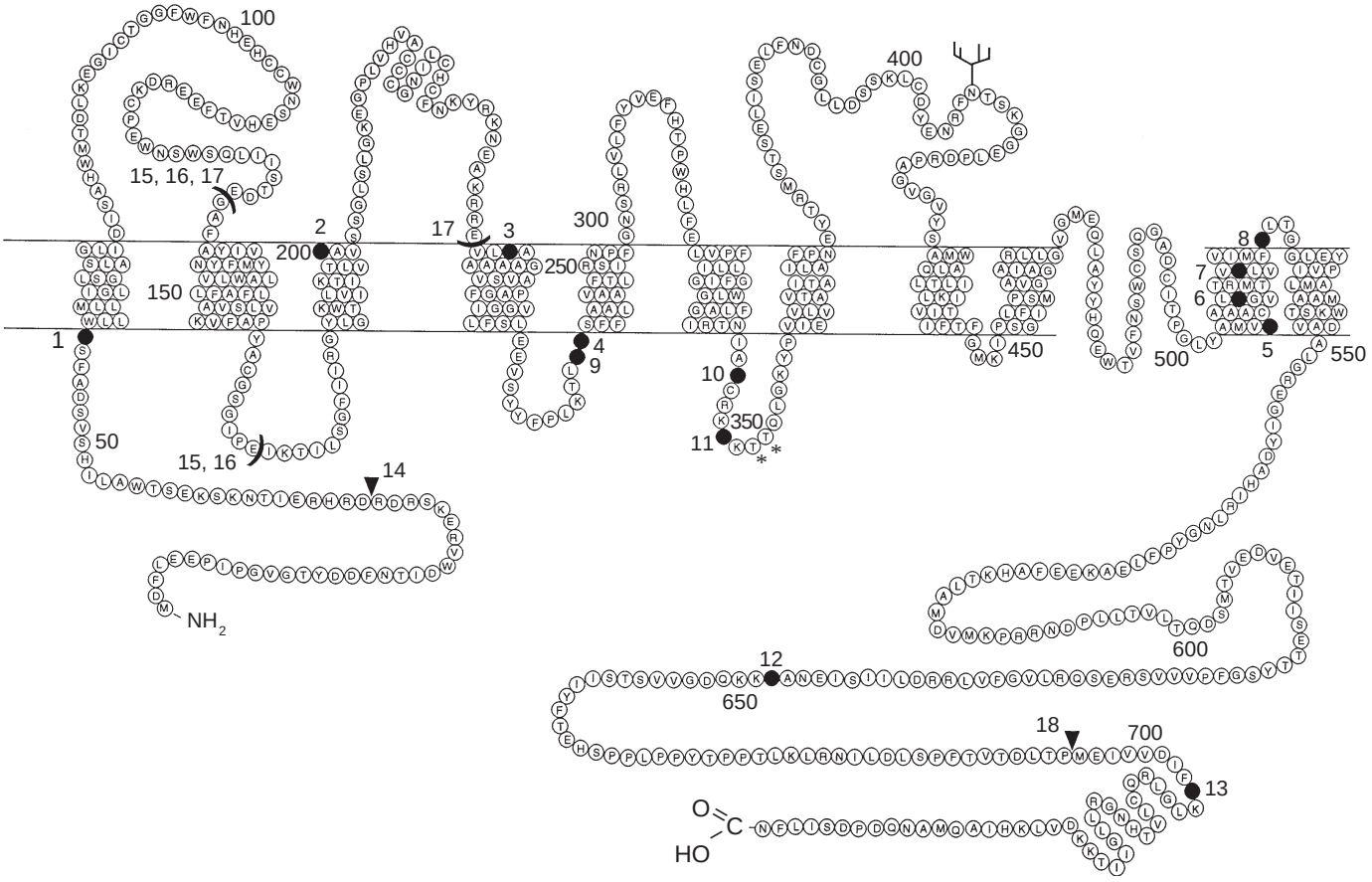
The identification of point mutations in *CLCN5* not only

Fig. 7. Schematic representation of the amino acid sequence of CLC-5 with predicted topology, revised from Lloyd et al [43] to indicate the 18 mutations published to date. Mutations are identified by numbers as listed on Table 2. Solid circles represent missense (1 to 8) and nonsense (9 to 13) mutations; triangles indicate insertion of a triplet codon [14] and a single base causing a frameshift [18]; and pairs of curved brackets represent the boundaries of the sequence of amino acids deleted as a result of two donor splice-site mutations (15 and 16) and an intragenic deletion [17]. (A chromosomal microdeletion encompassing the entire gene represents the 19th known mutation.) (Figure is used with permission of *Nature*.)

Table 2. Published mutations in CLCN5 in patients with X-linked hypercalciuric nephrolithiasis

	Codon	Base change	Amino acid change	Predicted effect	Expression	Source	Phenotype[a]	Ref
Missense								
1	57	G**G**C→G**T**C	Gly→Val	D1 helix	Reduced	U.S.A.	1,2,3,4	[19]
2	200	C**T**G→C**G**G	Leu→Arg	D3 charge distribution	Abolished	U.K.	1,2,3,4,5	[43]
3	244	T**C**G→T**T**G	Ser→Leu	D5 helix	Reduced	Italy	2,3,4,5	[43]
	244	T**C**G→T**T**G	Ser→Leu	D5 helix		France	1,2,5	[12]
4	280	C**G**T→C**C**T	Arg→Pro	at border of D6	Reduced	Japan	1,2,3	[17]
5	506	G**G**G→G**A**G	Gly→Glu	D11 charge distrib	Abolished	U.S.A.	1,2,3,4	[43]
6	512	**G**GT→**C**GT	Gly→Arg	D11 charge distrib	Abolished	U.S.A.	1,2,3,4	[19]
7	520	**T**CT→**C**CT	Ser→Pro	D11 helix	Reduced	U.K.	1,2,3,4,5	[43]
8	527	GA**A**→GA**T**	Glu→Asp	?[highly conserved]	Abolished	India	1,2,3,4,5	[19][b]
Nonsense								
9	279	TG**G**→TG**A**	Trp→Stop	Lose 467 aa	Abolished	U.K.	1,2,3,4	[43]
	279	TG**G**→TG**A**	Trp→Stop	Lose 467 aa		Japan	1,2,3	[17]
10	343	T**G**G→T**A**G	Trp→Stop	Lose 404 aa		Japan	1,2,3	[17]
11	347	**C**GA→**T**GA	Arg→Stop	Lose 399 aa		Japan	1	[44]
12	648	**C**GA→**T**GA	Arg→Stop	Lose 98 aa	Abolished	U.K.	1,2,3,4,5	[43]
	648	**C**GA→**T**GA	Arg→Stop	Lose 98 aa		Italy	1,2,3,4	[19]
13	704	**C**GA→**T**GA	Arg→Stop	Lose 42 aa	Abolished	Canada	1,3,4	[43]
Insertion								
14	30	ACC in-frame insertion	30:H insertion	?charge distrib	Reduced	U.K.	1,2	[19][b]
Splice-site								
15	132-172 del	g**t**→g**g**	Lose 41 amino acids	Lose D2	Abolished	U.K.	1,2,3,4,5	[43]
16	132-172 del	g**t**→g**a**	Lose 41 amino acids	Lose D2	Abolished	U.K.	1,2,3,4	[43]
Deletions								
17	132-241 (2 kb)		Lose 110 amino acids	Lose D2-D4	Abolished	U.K.	1,2,3,4,5	[43]
(ns.) entire gene (515 kb)			Protein absent	No protein		U.K.	1,2,3,4,5	[33]
18	695	ΔC	Δ695fs→699 Stop	Lose 47 aa		Japan	1,2,3	[17]

[a] Phenotypic features: 1 = LMW proteinuria; 2 = hypercalciuria; 3 = nephrocalcinosis/stones; 4 = renal insufficiency; 5 = rickets
[b] Clinical data from Prof. Oliver Wrong, personal communication



established that this is the gene responsible for the disease phenotype, but also provides information regarding on structure/function relationships in the CLC-5 channel. Nearly all of these mutations have been studied by expression in *Xenopus* oocytes in the laboratory of Dr. Thomas Jentsch. Each mutation when expressed yielded a channel protein that either abolished or markedly reduced the chloride currents measured at positive voltages. Thus, the disease X-linked hypercalciuric nephrolithiasis results from inactivation of this chloride channel. This is consistent with the recessive pattern of inheritance.

Missense mutations, designated 1 to 8 on Figure 7, result in substitution of a single amino acid in a channel that is otherwise unaltered. All of these 8 occur in or near transmembrane domains, and all affect sequences that are highly conserved among members of the CLC channel family. Four of these (Leu200Arg, Gly506Glu, Gly512Arg, and Glu527Asp) yielded chloride currents in *Xenopus* that were indistinguishible from water-injected controls, and thus appear to abolish channel function. Three others (Ser244Leu, Ser520Pro, and R280P) led to chloride currents that were markedly reduced. A fourth missense mutation (Gly57Val) and an in-frame insertion of a triplet encoding histidine (designated #14 on Fig. 7) both led to currents that were moderately (50 to 70%) reduced, compared with currents produced with the wild-type sequence. These latter two mutations occur near the amino-terminal end of the protein, in sequences that are less well-conserved among members of the CLC chloride-channel family [19].

One of the missense mutations (Glu527Asp) and the triplet insertion (30:His) are *de novo* mutations, as evidenced by the absence of the mutation in the parents in each case. The Glu527Asp mutation was passed along by the affected male patient to each of his two daughters.

The five nonsense mutations leading to truncation of the protein occur throughout the sequence. All four of these that have been expressed have resulted in abolition of chloride currents, including the least disruptive one, R704X, which leads to loss of 42 amino acids from the cytoplasmic C-terminal end of the protein. A frameshift at codon 695 has not been expressed, but as this mutation results in a stop codon at 699 it should similarly abolish function. In addition, the two donor-splice-site mutations (mutations 15 and 16 on Fig. 7) lead to loss of 41 amino acids encompassing the second transmembrane domain D2. Expression of this deletion in *Xenopus* yielded chloride currents indistinguishable from water-injected controls, indicating abolition of channel function. The functional importance of D2 had been questioned based on a report that expression of a variant of rCLC-K2 deleted for D2 still led to a functional channel [45]. However, this observation has not been widely accepted [39, 46, 47], and Jentsch's expression of the D2 deletion in CLC-5 supports a significant role for this segment of the protein, most likely as a transmembrane domain [43].

Two of the nonsense mutations have each been found in separately identified and unrelated families. The G-to-A transversion at codon 279 that results in a stop codon has been found in a British family and in a Japanese family [17, 43], and the C-to-T transversion yielding a premature stop signal at codon 648 occurred in an Italian family and in a British family [19, 43]. In each case haplotype analysis using microsatellite polymorphisms confirmed that the families were unrelated.

There appears to be no correlation between the nature of mutation and the phenotype. Individuals with missense mutations may be as severely affected as others with stop codons or deletion of the entire gene. The mutation with the mildest effect on chloride currents when expressed in *Xenopus* (50% reduction) is the arginine-for-glycine substitution at the start of the first transmembrane domain (mutation #1), and yet several members of this family (from Florida) have had recurrent nephrolithiasis and progressive renal failure requiring dialysis and transplantation, though not rickets [5]. In addition, within families, individuals sharing the same mutation can be mildly or severely affected. In the American family in which we first described XRN, the mutation (#5) resulting in substitution of glutamate for glycine in the 11th transmembrane domain is associated with severe renal failure before age 40 in some patients, recurrent nephrolithiasis with preserved renal function in other patients [1, 2], and a more benign course in at least two patients (unpublished observations). Some patients in the British family with complete deletion of the entire gene are less severely affected than some individuals in other families with missense mutations.

Symptomatic rickets, presenting with deforming bone disease in childhood, occurred in some of the patients with Dent's disease originally reported by Wrong et al [8], who turn out to have a variety of mutations including missense (mutations #2 and #7), nonsense (mutation #12), and a splice-site mutation leading to loss of a transmembrane domain (#15). In each of these four families, only one member has rickets, and in each of these families, other affected individuals share the same mutation but manifest disease with differing severities and without rickets. In only two families has rickets appeared to be the predominant clinical feature; these are the Italian and French families reported as X-linked recessive hypophosphatemic rickets [10, 12]. Affected members of both of these families have a missense mutation leading to substitution of leucine for serine at the fifth transmembrane domain, which yields a markedly reduced though detectable chloride current when expressed in *Xenopus* [43], and is not clearly different from a number of the other mutations.

Such phenotypic variation is not uncommon in genetic diseases. In XLHN, it is not yet known to what extent environmental factors or other modifying genes may alter severity of the disease phenotype, although it is clear that the nature of the *CLCN5* mutation alone is not sufficient to determine the expression of the clinical disease.

STRUCTURE OF CLC CHLORIDE CHANNELS AND FUNCTION OF CLC-5

Hydropathy analysis of the amino acid sequences predicts a similar basic transmembrane structure for all members of the CLC family of voltage-gated chloride channels [35]. A model of a possible structure is illustrated on Figure 7, which displays 12 transmembrane domains, as proposed by Jentsch et al [46]. A weakly hydrophobic sequence once considered a transmembrane domain (D4) is now felt to be extracellular. The sequence linking the 8th and 9th transmembrane domains had been thought to be intracellular, but contains a potential glycosylation site that has now been confirmed in several other members of this channel family, using *in vitro* translation and site-directed mutagenesis, in fact to be glycosylated, establishing its extracellular position [46]. The segment from the 9th through the 12th transmembrane

domains as pictured represents a broad hydrophobic region, essentially the same in all members of the CLC family, in which the number of transmembrane crosses is proposed to be uneven [35, 46].

Chloride channels of this family are thought to function as multimeric complexes, which would explain the dominant negative effect of mutations in CLC-1 in the dominant form of human congenital myotonia (Thomsen's disease) [46]. Our current understanding of the electrophysiologic properties of these channels is dependent largely upon study of them as expressed in *Xenopus* oocytes, and some members of this gene family do not yield chloride currents when expressed, or have led to controversial results [36, 46], perhaps because physiologic activity requires the presence of other subunits. Preliminary studies so far have not revealed functional interactions when CLC-5 was coexpressed with other members of this family [48]. If CLC-5 does function physiologically as a heteromultimer, the electrophysiologic features of the functional channel in humans may well differ from that observed when it is expressed alone in *Xenopus*. Both the human [17, 19, 43] and rat [41] CLC-5 sequences have been expressed by Jentsch, and resemble the other CLC channels that have been expressed successfully (CLC-0, CLC-1 and CLC-2) in having a high selectivity for chloride. However, CLC-5 differs from those members of the CLC family in that it yields currents that are strongly outwardly rectifying and are activated at inside-positive plasma membrane voltages exceeding + 10 to 20 mV [36, 41–43].

CLC-5 bears little if any relation to the cystic fibrosis transmembrane conductance regulator (CFTR), which also conducts chloride currents and appears to be expressed in all segments of human renal epithelium [36]. Microscopic nephrocalcinosis has been reported in cystic fibrosis, occurring even at infancy, and with hypercalciuria in a third of such patients. However, clinically significant renal dysfunction is absent and histology is otherwise unremarkable even at sites of calcinosis [49]. While the sequence for CLC-5 predicts a potential cytoplasmic cAMP-dependent phosphorylation site, the rat CLC-5 expressed in *Xenopus* failed to respond to raising intracellular cAMP concentrations [41].

PHYSIOLOGY OF CLC-5 AND POSSIBLE PATHOPHYSIOLOGY OF X-LINKED HYPERCALCIURIC NEPHROLITHIASIS

We do not yet know enough about the physiology of the CLC-5 chloride channel to say how these mutations result in a syndrome of proximal tubulopathy, hypercalciuria, and renal failure. Since the earliest and most consistent clinical features are low-molecular-weight proteinuria and hypercalciuria, it will be important to discover the role this chloride channel plays in proximal tubular function and calcium metabolism. When the expression of CLC-5 along the human nephron becomes known, we might reasonably expect that it will be found at least in proximal tubule. The vasopressin-resistant polyuria might suggest a role for CLC-5 in distal nephron as well, since polyuria occurs in patients whose hypercalciuria appears too mild to account for it.

Acidification of endocytic vesicles in proximal tubule has been shown to involve a protein kinase A-regulated chloride conductance [50]. A number of authors have pointed out the potential importance of chloride channels in the lysozomal vacuoles that degrade proteins taken up from the proximal tubular lumen. Such channels would provide an inward chloride flux to dissipate the positive charge resulting from active proton secretion into these vacuoles. Thus, it has been speculated that inactivation of CLC-5 might lead to impaired vacuolar acidification and thus impaired reabsorption of LMW proteins [35, 41, 43, 51]. This hypothesis has been challenged, however, based on the voltage-gating characteristics of CLC-5 [52]. Chloride channels have been studied in endosomal vesicles from proximal tubule, and the conductance, ion selectivity, and inhibitor sensitivities [53] all differ from those reported for CLC-5 [41].

Several authors have speculated on the possible role of CLC-5 in calcium reabsorption [47, 51, 52]. It is difficult to propose a specific effect of CLC-5 on calcium reabsorption in the proximal tubule, where transepithelial movement of calcium is mainly passive and paracellular. Chloride channels may play a role in calcium reabsorption in the medullary thick ascending limb (MTAL), where a basolateral chloride channel participates in transepithelial chloride transport. However, the ion selectivity, inhibition by NPPB, and activation by cAMP reported for the basolateral chloride current in MTAL all differ from the properties of CLC-5, at least in the rat [41]. Furthermore, recent studies in the rabbit indicate that CLC-Ka is the basolateral chloride channel in the thick limb [38]. In the distal convoluted tubule, both thiazides and PTH have been reported to bring about their stimulatory effect on calcium transport through activation of chloride channels with consequent cell hyperpolarization [54, 55]. Thus, inactivation of such chloride channels could lead to hypercalciuria. However, renal hypercalciuria might be expected to lead to a secondary increase in PTH levels, which was clearly absent in virtually all cases in which PTH levels were measured (Fig. 3).

The intestinal hyperabsorption of calcium in these patients points to the potential physiologic importance of the high 1,25-dihydroxyvitamin D levels. This, in the setting of LMW proteinuria, may be most consistent with dysfunction of the proximal tubular cell with resulting inappropriate activation of 1-hydroxylation of 25-hydroxyvitamin D. Such activation could be a consequence of renal phosphate loss (an inconsistent finding) or a primary abnormality in the regulation of 1-hydroxylation of 25-hydroxyvitamin D; either would place the blame on the proximal tubule. Confirmation of this speculation will require knowledge of the localization of the CLC-5 message within the nephron and the CLC-5 protein within the tubular epithelial cell, and of the role of CLC-5 in renal epithelial cell function.

The cause of renal failure is also not clear, but it may not be the result of hypercalciuria alone. Patients with XLHN are no more hypercalciuric than many patients with idiopathic hypercalciuria in whom renal failure does not occur in the absence of obstruction and infection from recurrent stones, although some young children have been very hypercalciuric. Histopathology indicates tubulointerstitial disease, but the extent to which hypercalciuria is the cause, a contributing factor, or merely an epiphenomenon, is not at all known.

GENETICS OF IDIOPATHIC HYPERCALCIURIA

X-linked hypercalciuric nephrolithiasis is uncommon, and its clinical features, particularly low-molecular-weight proteinuria, renal failure, and rickets, clearly distinguish it from the common forms of hypercalciuric stone disease. Idiopathic hypercalciuria (IH), in contrast, is common, and although it is widely thought to

have an important genetic component, the genetics of IH are poorly understood. IH is present in 40% of patients with calcium nephrolithiasis [56]. A family history of kidney stones can be obtained in as many as 45% of patients with IH [57, 58]. Reports of such families have been interpreted to indicate autosomal inheritance of hypercalciuria [59–61]. However, there are several reasons why it may not be correct to assume that IH is inherited as a simple mendelian trait. One is that the distribution of calcium excretion in the population is not bimodal, but a continuum [62], and definitions of hypercalciuria depend upon statistical criteria for the upper limit of the normal range that are by nature arbitrary. Another is that there are a number of patterns of hypercalciuria, and each may potentially represent a different metabolic abnormality that is inherited separately or in combination in individual patients.

Idiopathic hypercalciuria, defined as hypercalciuria without identifiable systemic cause, can be divided into as many as five categories, including three separate patterns of absorptive hypercalciuria [63]. There is evidence indicating that these may represent separate pathophysiological mechanisms in which inheritance patterns may differ [59, 64]. In addition, familial hypercalciuria can occur in the setting of other tubular dysfunction, including hypophosphatemia [25, 26] or hyperuricosuria [65]. Nephrocalcinosis in very low-birth-weight infants is strongly associated with a history of nephrolithiasis in first- or second-degree relatives of the parents [66].

The variety of forms of IH, and the continuous distribution of calcium excretion in the normal population, suggest that multiple factors, possibly multiple genes, contribute to produce the phenotype of high calcium excretion [57, 67]. Other common conditions, including hypertension and diabetes, also appear to result from polygenic inheritance, in contrast to the less common and often more distinctive diseases that reflect monogenic mendelian inheritance. This raises the question of whether *CLCN5* may be one gene among others contributing to hypercalciuria in some patients with IH.

A large group of patients with idiopathic hypercalciuria have exaggerated intestinal absorption of calcium, elevated serum levels of 1,25-dihydroxyvitamin D, suppressed PTH levels, and serum phosphorus levels that run at the lower end of the normal range [63]. Thus, it is reasonable to speculate that some patients with IH may have mutations in *CLCN5* that may lead to less dramatic loss of chloride-channel function than in XLHN, leading to hypercalciuria but perhaps not the fully expressed syndrome. To address this, we have been studying a large group of patients with IH for clinical or molecular evidence of XLHN. Using the most sensitive phenotypic marker of XLHN, that is, LMW proteinuria, we have screened nearly 100 patients, and so far have found substantial degrees of LMW proteinuria in none of them [68]. We have also sequenced the *CLCN5* gene in 8 patients with IH, and all were normal [69]. This work continues, but at present it appears that XLHN does not represent a large proportion of patients with IH.

TREATMENT OPTIONS IN X-LINKED HYPERCALCIURIC NEPHROLITHIASIS

At this stage in our understanding of XLHN, a proposed therapeutic approach cannot be informed either by data from clinical trials, of which there have been none, or by an understanding of the pathophysiology, which we currently lack despite identification of the disease gene.

Although it is not clear how large a role is played by hypercalciuria in contributing to progressive renal insufficiency in XLHN, it does appear to be the major factor responsible for the nephrolithiasis. It is therefore reasonable to attempt to reduce calcium excretion by following prudent principles that guide most therapy in idiopathic hypercalciuria. First of all, I believe that restriction of dietary calcium intake has no place in the therapy of this disease. Many patients with XLHN have hypercalciuria on fasting [16] or following calcium restriction [2], so that dietary restriction may exacerbate the risk of bone disease. Dietary calcium restriction is no longer recommended in idiopathic hypercalciuria, in which patients have a tendency to lower bone density [70], and particularly in view of the data in men [71] and women [72] that lower dietary calcium intakes are associated with a higher risk of nephrolithiasis.

Thiazide diuretics will stimulate distal tubular calcium reabsorption and thereby promote a positive balance of calcium, with beneficial effects on bone mineralization [73]. A few patients with XLHN have significant potassium wasting, and one patient developed acute volume-depletion and hypokalemia when given a large dose of hydrochlorothiazide [8], so patients should be observed closely at least initially when starting therapy with this class of diuretic. I am aware of a number of patients, particularly children with XRN, in whom therapy with a thiazide has been well-tolerated and has reduced urinary calcium excretion, though not always to normal. Understandable reluctance to use diuretics in children is based on concerns over long-term side effects, and it is sensible to identify the minimal dose that achieves the desired effect on calcium excretion. Amiloride should also stimulate calcium reabsorption [74], and because of its effect to inhibit potassium secretion may be useful in XLHN, either alone or in combination with thiazide. Restriction of dietary intake of sodium and meat will also reduce urinary calcium loss [75], and are consistent with good nutritional advice anyway; in addition, the protein restriction may retard the progression of renal failure once established [76].

Wrong and colleagues have reported successful healing of rickets in Dent's disease using "pharmacologic amounts" of vitamin D, and they recommend "the use of small amounts of vitamin D or its derivatives" in patients with rickets or osteomalacia [8]. Since therapy with vitamin D will increase urinary calcium excretion, it would be important to titrate the dose carefully, following both serum levels of alkaline phosphatase and urinary calcium excretion.

Those patients who develop end-stage renal failure are often excellent candidates for transplantation, and also do well on dialysis. Patients without preexisting rickets do not appear to have any particular predisposition to renal osteodystrophy, if they are compliant with conventional therapy.

CONCLUSION

The pace of progress in our knowledge of this disease has been remarkable. Barely five years from the definition of the syndrome as X-linked and associated with nephrolithiasis, and before it had reached wide recognition as a clinical entity, the responsible gene had been cloned and mutations identified. Much remains to be done before we can claim to understand the pathophysiology of

X-linked hypercalciuric nephrolithiasis, but we should look forward to a fuller knowledge of the physiology of this new chloride channel leading us to a more complete understanding of renal tubular physiology.

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Reprint requests to Steven J. Scheinman, M.D., Department of Medicine, SUNY Health Science Center, 750 East Adams Street, Syracuse, New York 13210, USA.
E-mail: scheinms@vax.cs.hscsyr.edu

NOTE ADDED IN PROOF

Two recent reports describe a total of eight mutations in Japanese patients with LMW proteinuria [80, 81]. Two of these mutations had been reported previously, and one was another deletion of the entire gene, so that at present a total of 24 distinct mutations have been reported in 30 families. The new mutations include two missense and three frameshift mutations. In addition, the report by Akuta, et al. included the first description of a polymorphism in the *CLCN5* gene, with a substitution of C for T at the third base of codon 484 that leads to no amino acid change. These patients were young and the phenotype was in general mild, with several even lacking hypercalciuria. One girl (age 14) had nephrocalcinosis, but a *CLCN5* mutation was not detected in this patient (Akuta, et al.). The report by Nakazato, et al. included documentation of a *CLCN5* mutation in the families described by Furuse, et al. [24] and discussed above (in "Other reports").

A third human disease has now been associated with mutations in a member of the CLC gene family. Simon and colleagues [82] recently reported mutations in *CLCNKB* in a significant subset of patients with Bartter's syndrome. This gene encodes the human CLC-Kb chloride channel, the homolog to the rabbit CLC-Ka channel that is the basolateral chloride channel in the thick ascending limb. It is very interesting that the 17 kindreds in whom mutations were found included no patients with nephrocalcinosis, in contrast with patients who have mutations in the apical bumetanide-sensitive Na-K-2Cl cotransporter or the apical ATP-sensitive K channel ROMK, in whom nephrocalcinosis does occur. It is possible that the inactivation of this CLC-Kb channel might protect the cell from accumulating calcium by depolarizing the membrane, but this is only speculation.

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