Kidney International, Vol. 54 (1998), pp. 698–705

CLCN5 chloride-channel mutations in six new North American families with X-linked nephrolithiasis

Richard R. Hoopes, Jr., Paul A. Hueber, Robert J. Reid, Jr., Gregory L. Braden, Paul R. Goodyer, Andrew R. Melnyk, Julian P. Midgley, Donald I. Moel, Alicia M. Neu, Scott K. VanWhy, and Steven J. Scheinman

Department of Medicine, SUNY Health Science Center, Syracuse, New York; Department of Medicine, Baystate Medical Center, Springfield, Massachusetts, USA; Department of Pediatrics, McGill University, Montreal, Quebec, Canada; Departments of Pediatrics and Genetics, Loyola University, Chicago, Illinois, USA; Department of Paediatrics, Alberta Children's Hospital, Calgary, Alberta, Canada; Department of Pediatrics, Johns Hopkins University, Baltimore, Maryland; and Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut, USA

***CLCN5* chloride-channel mutations in six new North American families with X-linked nephrolithiasis.**

Background. X-linked nephrolithiasis, or Dent's disease, encompasses several clinical syndromes of low molecular weight (LMW) proteinuria, hypercalciuria, nephrocalcinosis, nephrolithiasis, and renal failure, and is associated with mutations in the *CLCN5* gene encoding a kidney-specific voltage-gated chloride channel. Some patients from Europe have rickets, and all symptomatic patients confirmed by mutation analysis have been male.

Methods. We analyzed the *CLCN5* DNA sequence in six new families with this disease.

Results. In three probands, a single-base substitution yielded a nonsense triplet at codons 28, 34, and 343, respectively, and in two families, one of which was Hispanic, we found single-base deletions at codons 40 and 44, leading to premature termination of translation. In the sixth family, a single-base change from C to T predicted substitution of leucine for serine at codon 244, previously reported in two European families with prominent rickets, though this patient of Ashkenazi origin did not have rickets. Each of these mutations was confirmed by restriction endonuclease analysis, or repeat sequencing and CFLP. The R34X mutation occurred in a Canadian infant with severe rickets. The family with the R28X nonsense mutation included one woman with recurrent kidney stones and another woman with glomerular sclerosis. In another family, a woman heterozygous for the W343X mutation also had nephrolithiasis.

Conclusions. These studies expand the range of mutations identified in this disease, and broaden the phenotypic range to include clinically affected women and the first North American case with severe rickets.

Several syndromes of X-linked hypercalciuric nephrolithiasis have been reported that are associated with inactivating mutations in the *CLCN5* gene, which encodes a voltage-gated chloride channel expressed in kidney [1–4]. X-linked recessive nephrolithiasis (XRN) [5, 6] and Dent's disease [7] share in common a number of findings including low-molecular-weight (LMW) proteinuria, hypercalciuria, calcium nephrolithiasis, nephrocalcinosis, and progressive renal insufficiency. Asymptomatic Japanese schoolchildren with LMW proteinuria, hypercalciuria, and nephrocalcinosis, identified through a nationwide screening program, also have mutations in this gene [3, 8, 9]. Rickets was a presenting feature in a third of affected males with Dent's disease [7] and in all nine affected males in two unrelated families with "X-linked recessive hypophosphatemic rickets" (XLRH) from Italy [10] and France [11] who also have a mutation in the *CLCN5* gene [2, 11], but in none of the 18 reported patients with XRN from North America [2, 4, 5, 12, 13]. Until now, all affected individuals confirmed by mutation analysis have been male.

We studied ten affected males in six new families with X-linked nephrolithiasis in North America, and have confirmed the presence of inactivating mutations in *CLCN5* in each of them. Prominent features in these affected individuals include evidence of renal tubular dysfunction, particularly LMW proteinuria, as well as hypercalciuria, hematuria, nephrolithiasis or nephrocalcinosis, and progressive renal insufficiency. These patients include the first reported North American case with rickets. Furthermore, several women in these families are the first affected females documented to be heterozygous for a mutation in the *CLCN5* gene who have clinically significant disease. In addition, one family shares the same mutation (S244L) that was also reported in Italian and French patients with X-linked recessive hypophosphatemic rickets, but this North American patient had clinical renal disease and stones without any evidence of rickets. Together these

Key words: X-linked nephrolithiasis, chloride channel, rickets, Dent's disease, kidney stones.

Received for publication November 17, 1997
and in revised form April 8, 1998
Accepted for publication April 14, 1998

Table 1. Clinical features of affected males and females with X-linked nephrolithiasis (Dent's disease)

Patient	Age	Sex	Hypercalciuria	Stones	Nephrocalcinosis	PTH *pg/ml* (nl 10–65)	1.25 $(OH)_2D$ (nl 10–65 pg/ml)	Other
A-II-2	46	male	NA	yes	yes	NA	NA	Aminoaciduria; ESRD
A-II-3	37	male	NA	yes	no	NA	NA	
A-I-2	65	female	NA	no	no	NA	NA	C_{Cr} 36 ml/min; hypertensive
A-I-1	80	female	NA	yes	no	NA	NA	
A-IV-1	6	male	8.4 mg/kg/24 hr	no	no	22	50[a]	
B	15	male	Ca/Cr 0.32–0.57 mg/mg	no	yes	21[b]	76[c]	Rickets, phosphate-wasting; serum creatinine 1.35 mg/dl
C-II-1	13	male	Ca/Cr 0.38 mg/mg	yes	yes	NA	NA	
C-I-1	35	female	Ca/Cr 0.12 mg/mg	yes	no	NA	NA	
D-II-1	54	male	590 mg/24 hr	no	yes	NA	NA	Intermittent aminoaciduria; hypokalemia; glycosuria; polyuria
D-II-2	49	male	"hypercalciuric"	yes	yes	NA	NA	Aminoaciduria; glycosuria; genu valgum; ESRD; transplant
E-II-1	5	male	8 mg/kg/24 hr	no	yes	9	59	Hypouricemia; phosphaturia
E-II-2	2	male	Ca/Cr 0.93–1.9 mg/mg	yes	yes	19	67.3	
F	5	male	6 mg/kg/24 hr	no	yes	22	NA	Aminoaciduria; C_{Cr} 71 ml/min/1.73 m^2

Abbreviations are: NA, not available; C_{Cr}, creatinine clearance; Ca/Cr, mg calcium/mg creatinine.
[a] nl 20–76 pg/ml
[b] age 15; serum creatinine 1.36 mg/dl
[c] at age 2 years

cases illustrate the variety of clinical phenotypes associated with mutations in *CLCN5*. They also provide additional examples of overlap between the various clinical presentations that serve to reinforce the molecular evidence that these syndromes are not separate and discreet, but rather represent variations within a single disease entity.

METHODS

Patients

Patients were identified by their physicians as exhibiting the clinical features of XRN or Dent's disease, and all were documented to have excessive excretion of LMW proteins, particularly β2-microglobulin. Key clinical features of patients with symptomatic disease are summarized in Table 1. Brief clinical summaries of the relevant cases follow.

Family A. Three males and two females in this family have been documented to have clinically apparent disease associated with mutations in *CLCN5* (Figure 1). Several additional male members of the pedigree are known to have had nephrolithiasis or renal failure. While their relationships to these confirmed patients are consistent with X-linked inheritance, they were unavailable for study.

Patient A-II-2 (Fig. 1; male) was noted at age 7 to have crystalluria. Evaluation at age 25 revealed renal insufficiency (serum creatinine 2.2 mg/dl), extensive medullary nephrocalcinosis with multiple stones, and aminoaciduria. The patient required hemodialysis at age 35, and bilateral nephrectomies were performed. A first renal transplant failed when immunosuppressive therapy was withheld because of a dramatic neurologic reaction to cyclosporine A, for which his case was reported [14]. A second transplant has functioned well for four years without recurrence of nephrolithiasis. His brother, patient A-II-3, has recurrent nephrolithiasis and LMW proteinuria. Their mother, patient A-I-2, at age 65 had chronic renal insufficiency (serum creatinine 2.1 mg/dl), LMW proteinuria, and a renal biopsy indicating glomerular sclerosis, interstitial inflammation and tubular atrophy. She also has hypertension, which is unusual in this disease.

Patient A-IV-1 presented at age four years with proteinuria (34 $mg/m^2/hr$) and hematuria. Serologic studies were negative, and renal biopsy revealed glomerular sclerosis with interstitial fibrosis and calcification. He has marked LMW proteinuria, consistent with the degree in affected males, and his mother (A-III-1), maternal grandmother (A-II-1), and maternal great-grandmother (A-I-1) all have moderate LMW proteinuria, consistent with the carrier state [6]. That maternal great-grandmother (A-I-1) has also had recurrent nephrolithiasis.

Family B. Aminoaciduria was detected by screening in the affected boy at infancy. At age 2 he had hematuria, proteinuria, hypercalciuria, renal phosphate wasting (tubular reabsorption of phosphate 72%), aminoaciduria, β2-microglobulinuria, and active rickets. Serum levels of calcium (2.47 mM) and phosphorus (1.74 mM) were normal, and the level of 1,25-dihydroxyvitamin D was high (76 pg/ml). The rickets improved following supplementation with vitamin D and phosphorus. Renal ultrasonography indicated medullary nephrocalcinosis, and biopsy revealed interstitial fibrosis, tubular atrophy, and some glomerular sclerosis. Renal function deteriorated. Recently, at age 15, serum PTH level was normal at 21 pg/ml (normal range 10 to 60 pg/ml) despite progressive renal insufficiency with serum creatinine 1.35 mg/dl.

Family C. This boy presented at age 4 with microscopic hematuria, proteinuria and nephrolithiasis. He has had

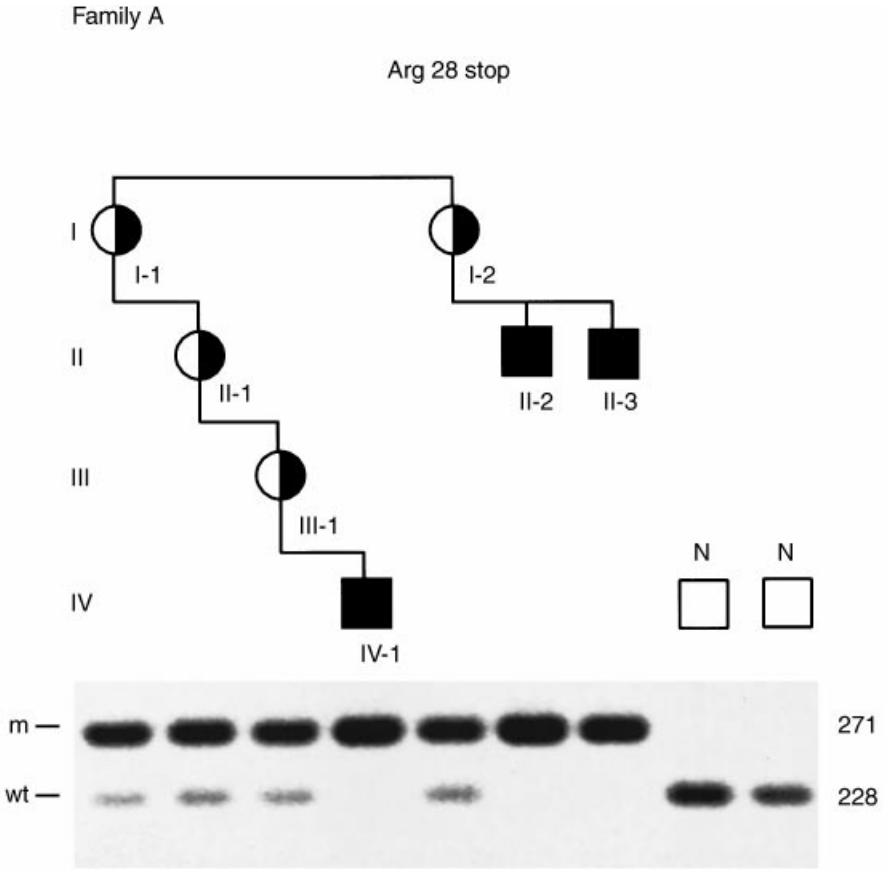

Fig. 1. Segregation of the R28X mutation in Family A. The C-to-T base substitution disrupts an *Xho*I recognition sequence. Thus, when genomic DNA is amplified with exon-specific primers and digested with *Xho*I, this results in a product of 271 bp rather than the normal product size of 228 bp. Heterozyous females display both the mutant (m) and wild-type (wt) alleles, affected males display the mutant allele, and normal males the WT allele. This family includes carrier females I-1, with symptomatic recurrent nephrolithiasis, and I-2, with renal insufficiency and proteinuria.

persistent hypercalciuria and nephrocalcinosis, and markedly excessive LMW proteinuria. With thiazide, urine calcium/creatinine ratio fell from 0.38 to 0.02. Serum calcium and phosphorus levels have been normal. The most recent serum creatinine at age 13 was 1.1 mg/dl. His brother is clinically normal, with normal excretion of LMW proteins. Their mother is adopted and her family history is unavailable. She has had nephrolithiasis.

Family D. This affected male (D-I-1) had polyuria and nocturia from infancy. At age six years he was documented at New York Hospital to have microscopic hematuria, 2.7 g/day of proteinuria, and hypophosphatemia (3.7 mg/dl). At age 17 he was evaluated at the Toronto General Hospital and documented to have nephrocalcinosis, hypercalciuria (442 to 622 mg/day), aminoaciduria, renal glycosuria, mild hypophosphatemia (2.6 mg/dl) and hypokalemia (3.5 mEq/liter), and a serum creatinine of 1.9 mg/dl. Urinary acidifying ability was normal (minimum urine pH 5.14). Hypercalciuria was exacerbated by a Na/K citrate preparation but not by K/H citrate. His mother's sister's son (D-II-2) had renal glycosuria, nonspecific aminoaciduria, phosphate wasting, nephrolithiasis, nephrocalcinosis, and renal failure, and now has a successful kidney transplant. That patient had asymptomatic radiographic evidence of bone demineralization and mild genu valgum deformity, but normal growth. The family history also included the maternal grandfather with polyuria and renal disease, and a maternal uncle with nephrolithiasis.

Family E. Patient E-II-1 presented at 16 months of age with occult hematuria and proteinuria. Evaluation revealed phosphaturia and hypouricemia with an increased fractional urate clearance (23 to 25%). At age five years he had hypercalciuria (8.5 mg/kg/day) and LMW proteinuria. His younger brother (E-II-2) was evaluated at six months of age because of the brother's history, and was found to have normal tubular function and a normal urinalysis, but further studies at age two years indicated microhematuria, severe hypercalciuria (Ca/Cr 1.9), proteinuria (1.8 g/24 hr), marked β2-microglobulinuria, and mild nephrocalcinosis. In both brothers, calcium excretion decreased on treatment with hydrochlorothiazide. The mother excreted normal amounts of β2-microglobulin. The family (of Hispanic origin) included a maternal uncle with nephrolithiasis.

Family F. The 3-year-old male proband was noted incidentally to have microscopic hematuria and proteinuria (720 mg/day). Further evaluation revealed hypercalciuria (5.6 to 6.4 mg/kg/24 hr), aminoaciduria, hyperuricosuria, a reduced GFR, and marked β2-microglobulinuria. The boy is now five years old and lacks any clinical or biochemical evidence for bone disease. The family, of Ashkenazi Jewish

Table 2. Mutations identified in six new families with X-linked nephrolithiasis (Dent's disease)

Family	Codon	Base change	Amino acid change	Restriction enzyme/CFLP
Nonsense				
A	28	**C**GA → **T**GA	Arg → Stop	Xho I
B	34	**C**GA → **T**GA	Arg → Stop	Ban I
C	343	**T**GG → **T**AG	Trp → Stop	Bfa I
Frameshift				
D	40	**A**GC → −GC	7 missense aa and Stop	CFLP
E	44	AC**A** → AC−	3 missense aa and Stop	Bsr I
Missense				
F	244	TCG → T**T**G	Ser → Leu	CFLP

origin, included the boy's mother with LMW proteinuria but otherwise normal renal function, the maternal grandfather who had died at age 39 of renal failure with proteinuria and calcific densities in the kidneys, that grandfather's brother who died at 33 years of age with renal failure, and that granduncle's daughter's son who has had recurrent nephrolithiasis and chronic renal failure without rickets. This latter patient recently received a kidney transplant from his mother (an obligate carrier).

Mutation analysis

Sequencing. Genomic DNA was extracted from peripheral leukocytes using a Puregene DNA Isolation Kit (Gentra Systems, Inc.). The 11 exons comprising the open reading frame were amplified by polymerase chain reaction (PCR) using specific flanking intronic primers as described by Lloyd et al [3]. The DNA sequence of all 746 codons was determined using an automated Perkin Elmer/Applied Biosystems Division 373A Stretch DNA sequencer with 48 cm well-to-read plates. Dye terminator chemistry was used with AmpliTaq-FS DNA polymerase.

Restriction digestion and Cleavase™ fragment length polymorphism (CFLP) assays. All six mutations identified by DNA sequencing were confirmed by an independent method. Four of the mutations either created or destroyed a restriction endonuclease site. These mutations were confirmed by PCR amplification of the relevant exon followed by digestion of the PCR product with the appropriate restriction enzyme and electrophoresis on a 2% agarose gel. Two other mutations neither created nor destroyed any restriction sites and these mutations were confirmed using the Cleavase™ fragment length polymorphism (CFLP) assay (Third Wave Technologies, Inc; http://www.twt.com). CFLP assays were performed according to the manufacturer's recommended protocol. Briefly, subject genomic DNA was amplified by PCR, the PCR product purified by extraction from a denaturing polyacrylamide gel, the CFLP reactions carried out, and the products analyzed by electrophoresis on a DNA sequencing gel. Three hundred nanograms of sample genomic DNA, extracted as described above, were used as template in 100 μl PCR amplification reactions, with 0.1 μM primers, 2.5 units of *Taq* DNA polymerase (Perkin Elmer), 1x PCR buffer (standard, provided with *Taq*), and 50 μM of each dNTP. Amplification reactions were carried out for 42 cycles, with 30-second denaturations at 95°C, one-minute annealing steps at 56°C, and one-minute extensions at 72°C. One of each primer pair for the PCR reactions was end-labeled in a 20 μl reaction, with 40 pmole primer, 34 pmole ^{32}P-ATP, 40 units T4-polynucleotide kinase, using the provided kinase buffer for 30 minutes at 37°C, followed by inactivation at 90°C for five minutes. The 20 μl CFLP assay reactions were carried out in the supplied buffer with 0.2 mM $MnCl_2$, using 5′ end-labeled DNA at 100,000 cpm, and 25 units of Cleavase I enzyme. The DNA template was heated to 95°C for 20 seconds and then cooled to 45°C, before addition to the reaction mix. Reactions were carried out for five minutes at 45°C, and stopped with the addition of stop buffer. Products were heated for two minutes at 80°C just before loading onto a 6% denaturing sequencing gel.

RESULTS

The six mutations identified in these families are summarized in Table 2. In three probands, a single-base substitution yielded a nonsense triplet at codons 28, 34, and 343, respectively. The W343X mutation was previously described in a Japanese kindred with LMW proteinuria, hypercalciuria and nephrocalcinosis [3]. In two families, one of which (E) was Hispanic, we found single-base deletions at codons 40 and 44, leading to frameshifts with 7 or 3 missense amino acids, respectively, followed by premature termination of translation in both cases at codon 47. In the sixth family, a single-base change from C to T led to the substitution of leucine for serine at codon 244. The same mutation has already been reported in an Italian family and a French family in which rickets was prominent [10, 11], though this patient (of Ashkenazi origin) did not have rickets, nor did his affected male relatives.

Four of these mutations either created or disrupted a restriction enzyme site, and all of these were confirmed by restriction enzyme analysis. Figure 1 shows segregation of the R28X mutation with clinical disease in multiple affected individuals in family A, including one woman (A-I-1) with recurrent kidney stones, another woman (A-I-2) with glomerular sclerosis and renal insufficency, and several males with typical clinical features of X-linked nephrolithiasis. Figure 2 illustrates segregation of the W343X nonsense mutation with the disease in Family C, which includes

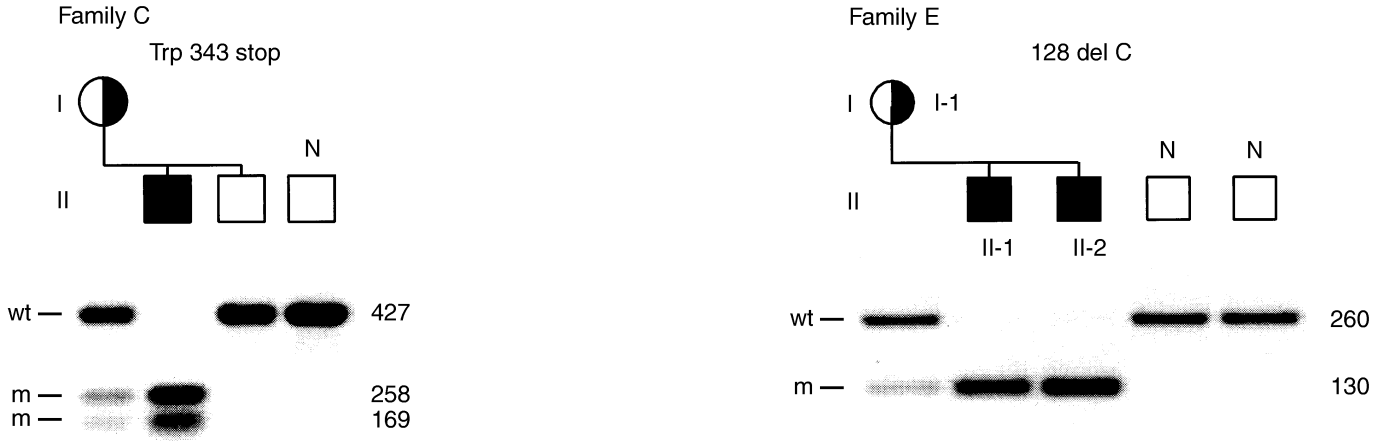

Fig. 2. (*Left panel*) Segregation of the W343X mutation with disease in Family C. The C-to-T transition creates a *Bfa*I recognition site, and the PCR-amplified exon (427 bp) is digested to two fragments (258 and 169 bp) with *Bfa*I. Patient C-I-1 is a woman with nephrolithiasis who is heterozygous for this nonsense mutation. (*Right panel*) Segregation of the single base deletion at codon 44 with disease in Family E. The deletion of a single adenine, which leads to frameshift with premature termination of transcription, creates a *Bsr*I restriction site. The PCR using primers flanking the exon 3 yield a product of 260 bp in normal individuals, and digestion with *Bsr*I produces two products of 126 and 134 bp size which overlap in a single band on this 2% agarose gel.

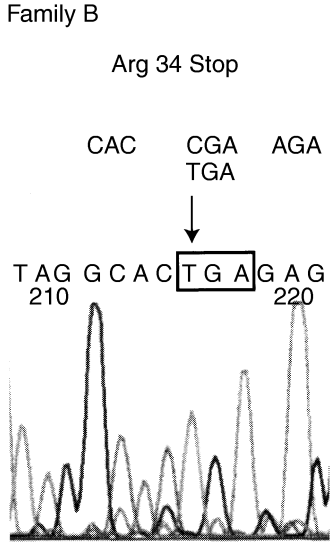

Fig. 3. Transition of C to T at codon 34 in patient B. The resulting TGA nonsense codon leads to termination of transcription.

a heterozygous woman with nephrolithiasis (C-I-1), and also shows segregation of the codon 44 frameshift mutation with disease in Family E. Figure 3 displays the electropherogram documenting the C-to-T transition in patient B, which was confirmed by restriction analysis using *Ban*I (not shown).

Two mutations did not disrupt or create a restriction enzyme site, and these were confirmed using CFLP analysis (Figure 4). The CFLP assay is based on the use of a structure-specific endonuclease that cleaves single-stranded DNA of specific conformations at the base of stem and loop structures. These conformations can be altered by a change in even a single base within 50 bp of the cleavage site. Thus, the location of an abnormal band from the exon being analyzed provides information about mutation location, allowing identification of the mutation by direct sequencing of a small region of the DNA. The CFLP assay is reported to have a sensitivity greater than that of SSCP for the detection of mutations, and comparable to that of DNA sequencing, and it can be performed on much longer regions of DNA sequence than SSCP (more than 2500 bps, compared to the 250 bp limit for SSCP) [15, 16]. In this study we used CFLP to confirm mutations that we had identified by sequencing, and not to screen for mutations, and so we cannot comment on its sensitivity in our hands. We were able easily to confirm the mutations in exons 3 and 7 in families D and F, respectively, for which no restriction site was available for analysis, as well as confirming a mutation in family E, also in exon 3, which had already been confirmed by restriction analysis (Fig. 4).

DISCUSSION

Five of the six mutations reported here are either nonsense mutations creating premature stop codons, or frameshift mutations that also lead to premature termination of translation. Four of these occur at codon 44 or earlier; the fifth occurs at codon 343. Previously reported nonsense mutations at codons 279, 347, 648, and 704 had been expressed in *Xenopus* oocytes, and were found to lead to loss of chloride channel function [2, 17]. Thus, these new mutations cannot be polymorphisms, and can be presumed also to inactivate the chloride channel. The sixth mutation, S244L, has already been reported to occur in three other families [2, 11, 18], and has been demonstrated not to occur in 110 chromosomes from unrelated individuals [2]. Expression of this missense mutation in *Xenopus* yields chloride currents that are markedly reduced, though detectable

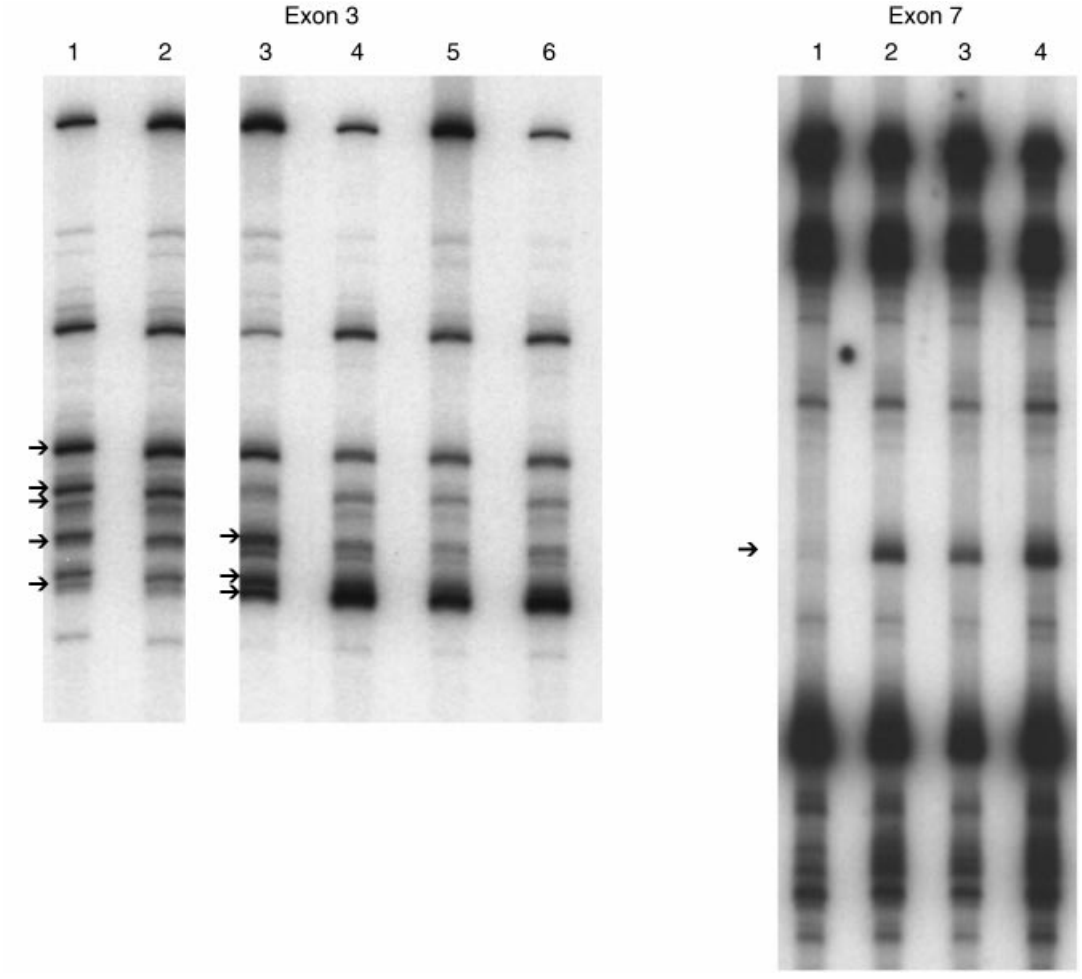

Fig. 4. Cleavase® fragment length polymorphism (CFLP) analyses. (*Left panel*) Deletion of A at codon 40 in exon 3 alters pattern of bands on CFLP analysis in patients D-II-1 (lane 1) and D-II-2 (lane 2) as compared with the normal pattern in lanes 4, 5, and 6. The exon in the patient represented in lane 6 was confirmed to be normal by sequencing. Patient E-II-1, with a different mutation in the same exon (deletion of A at codon 44), is represented in lane 3, where the pattern of bands differs as well. (*Right panel*) C-to-T transition at codon 244 in exon 7 in patient F (lane 1) alters the band indicated by the arrow as compared with normal samples in lanes 2, 3, and 4.

[2]. In addition to the two families in which rickets was prominent [2, 11], the S244L mutation was recently reported in a large kindred with "X-linked renal failure without X-linked recessive hypophosphatemic rickets" [18].

In most respects the patients studied here were typical of X-linked nephrolithiasis or Dent's disease. Presentation in childhood with hematuria, proteinuria, mild polyuria, and often nephrolithiasis was common. Extreme degrees of LMW proteinuria were found in all affected males. Other abnormalities of tubular function included aminoaciduria and hyperuricosuria. Although hyperuricosuria had been described in occasional cases of XRN or Dent's disease [5–7], frank hypouricemia was reported in none of the previously reported cases but was present in patient E-II-1 in the present report. Severe renal insufficiency occurs in adulthood in this disease, although reduction in creatinine clearance rates can begin in childhood. In those patients in whom measurements were available, serum levels of PTH were usually near or below the lower limit of normal, even in the presence of some renal insufficiency, and levels of 1,25-dihydroxyvitamin D were modestly elevated, as is typical of this disease [1].

Female carriers of this recessive disease, who are heterozygous for the presence of a mutation in *CLCN5*, typically have moderate degrees of LMW proteinuria and may have hypercalciuria, but in previous reports lack any clinical evidence of kidney stones, nephrocalcinosis, renal failure, hematuria, or other symptomatic features [1, 6, 7]. Wrong, Norden and Feest reported a history of a 59-year-old woman in one family with Dent's disease who was said to have had renal failure and nephrocalcinosis, but mutation analysis could not be performed in that woman because she had died in 1965 [7]. The families in the present report are therefore remarkable in that three of the heterozygous females have symptomatic disease. Women with symptomatic nephrolithiasis include patient C-I-1, who is heterozygous for the W343X mutation, and patient A-I-1, who is heterozygous for the R28X mutation. In addition,

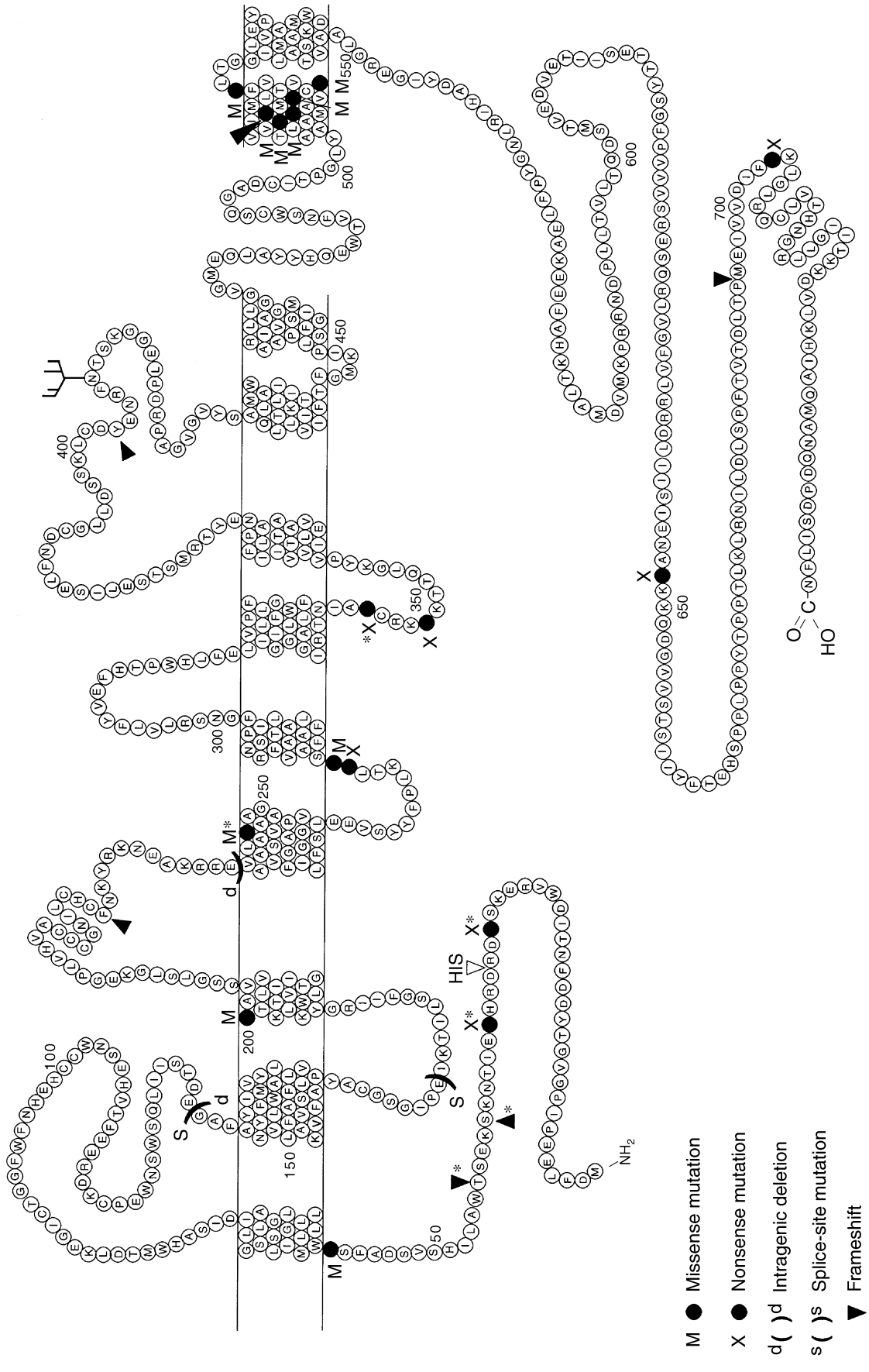
M ● Missense mutation
X ● Nonsense mutation
d( )d Intragenic deletion
s( )s Splice-site mutation
▼ Frameshift
HIS ▽ In-frame insertion of codon

Fig. 5. Schematic summary of the amino acid sequence of the CLC-5 channel protein with predicted topology, revised from Lloyd et al [2] to indicate 27 mutations reported so far ([2–4, 8, 9, 11, 17, 18], and this report). These include 10 missense, 7 nonsense, and 6 frameshift mutations, in addition to 2 splice-site mutations, one intragenic deletion, and one in-frame insertion of a triplet encoding histidine. Not shown are two deletions of 515 kb [19] and 180 kb [9] encompassing the entire gene, so that a total of 29 mutations have been reported to date. The six mutations in the current report are indicated with asterisks (*), including S244L and W343X which had previously been reported in other families [2, 3, 11, 18].

patient A-I-2 has moderate renal insufficiency and proteinuria, although the presence of hypertension and the absence of nephrocalcinosis indicate the possibility that this patient had hypertensive nephropathy. While these women were not tested for the presence of a second mutation on their other X chromosome, patients C-I-1 (Fig. 2) and A-I-2 (not shown) have had normal sons, and thus can be presumed to carry a normal copy of the *CLCN5* gene.

Rickets or osteomalacia is present in a minority of patients with this disease, although two families have been reported in which rickets was the dominant clinical feature in all affected boys [10, 11]. It is notable that the mutation present in both of those families (S244L) was also present in our patient F, who lacked any evidence of bone disease. By history none of the other three affected males in his family had had rickets either. This series also includes patient B, with the R34X mutation, who had symptomatic rickets at age two years. This nonsense mutation leads to termination of transcription very early in the sequence: codon 34 in a protein with 746 amino acids. In previously described families with nonsense mutations, and in the family with a complete deletion of the *CLCN5* gene, only 4 of a total of 16 affected males were reported to have rickets, and the nature of the mutation appears to offer no clue as to the occurrence of rickets. Figure 5 represents a summary of those mutations identified to date.

These studies expand the range of mutations identified in this disease, and broaden the phenotypic range to include clinically affected women and the first North American case with severe rickets.

ACKNOWLEDGMENTS

This study was supported by NIH award DK46838. It was presented in part at the 30th Annual Meeting of the American Society of Nephrology and published in abstract form (*J Am Soc Nephrol* 8:390A, 1997). The authors are grateful to Dr. Anthony Norden for measurement of LMW proteinuria in several patients, and to Dr. Eli Holtzman for helpful discussions.

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

REFERENCES

1. Scheinman SJ: X-linked hypercalciuric nephrolithiasis: Clinical syndromes and chloride channel mutations. *Kidney Int* 53:3–17, 1998
2. Lloyd SE, Pearce SHS, Fisher SE, Steinmeyer K, Schwappach B, Scheinman SJ, Harding B, Bolino A, Devoto M, Goodyer P, Rigden SPA, Wrong O, Jentsch TJ, Craig IW, Thakker RV: A common molecular basis for three inherited kidney stone diseases. *Nature* 379:445–449, 1996
3. Lloyd SE, Pearce SH, Gunther W, Kawaguchi H, Igarashi T, Jentsch TJ, Thakker RV: Idiopathic low molecular weight proteinuria associated with hypercalciuric nephrocalcinosis in Japanese children is due to mutations of the renal chloride channel (CLCN5). *J Clin Invest* 99:967–974, 1997
4. Lloyd SE, Gunther W, Pearce SHS, Thomson A, Bianchi ML, Bosio M, Craig IW, Fisher SE, Scheinman SJ, Wrong O, Jentsch TJ, Thakker RV: Characterisation of renal chloride channel, CLCN5, mutations in hypercalciuric nephrolithiasis (kidney stones) disorders. *Hum Molec Genet* 6:1233–1239, 1997
5. Frymoyer PA, Scheinman SJ, Dunham PB, Jones DB, Hueber P, Schroeder ET: X-linked recessive nephrolithiasis with renal failure. *N Engl J Med* 325:681–686, 1991
6. Reinhart SC, Norden AGW, Lapsley M, Thakker RV, Pang J, Moses AM, Frymoyer PA, Favus MJ, Hoepner JA, Scheinman SJ: Characterization of carrier females and affected males with X-linked recessive nephrolithiasis. *J Am Soc Nephrol* 5:1451–1461, 1995
7. Wrong O, Norden AGW, Feest TG: Dent's disease: A familial proximal renal tubular syndrome with low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, metabolic bone disease, progressive renal failure, and a marked male predominance. *Q J Med* 87:473–493, 1994
8. Nakazato H, Hattori S, Furuse A, Kawano T, Karashima S, Tsuruta M, Yoshimuta J, Endo F, Matsuda I: Mutations in the CLCN5 gene in Japanese patients with familial idiopathic low-molecular-weight proteinuria. *Kidney Int* 52:895–900, 1997
9. Akuta N, Lloyd SE, Igarashi T, Shiraga H, Matsuyama T, Yokoro S, Cox JP, Thakker RV: Mutations of CLCN5 in Japanese children with idiopathic low molecular weight proteinuria, hypercalciuria and nephrocalcinosis. *Kidney Int* 52:911–916, 1997
10. Bolino A, Devoto M, Enia G, Zoccali C, Romeo G: A new form of X-linked hypophosphatemic rickets with hypercalciuria (HPDR II) maps in the Xp11 region. *Eur J Hum Genet* 1:269–279, 1993
11. Oudet C, Martin-Coignard D, Pannetier S, Praud E, Champion G, Hanauer A: A second family with XLRH displays the mutation S244L in the CLCN5 gene. *Hum Genet* 99:781–784, 1997
12. Goodyer P, Kaye M: X-linked recessive nephrolithiasis. (abstract) *Pediatr Res* 33:3676A, 1993
13. Schurman SJ, Norden AGW, Scheinman SJ: X-linked recessive nephrolithiasis: Presentation and diagnosis in children. *J Pediatr* 132:859–862, 1998
14. Scheinman SJ, Reinitz ER, Petro G, Schwartz RA, Szmalc FS: Cyclosporine central neurotoxicity following renal transplantation. Report of a case using magnetic resonance images. *Transplantation* 49:215–216, 1990
15. Brow MA, Oldenburg MC, Lyamichev V, Heisler LM, Lyamicheva N, Hall JG, Eagan NJ, Olive DM, Smith LM, Fors L, Dahlberg JE: Differentiation of bacterial 16S rRNA genes and intergenic regions and Mycobacterium tuberculosis katG genes by structure-specific endonuclease cleavage. *J Clin Microbiol* 34:3129–3137, 1996
16. Rossetti S, Englisch S, Bresin E, Pignatti PF, Turco AE: Detection of mutations in human genes by a new rapid method: Cleavage fragment length polymorphism analysis (CFLPA). *Mol Cell Probes* 11:155–160, 1997
17. Morimoto T, Uchida S, Sakamoto H, Kondo Y, Sasaki S, Marumo F: A mutation of human CLCN5 chloride channel gene in patients with idiopathic low-molecular-weight proteinuria. (abstract) *J Am Soc Nephrol* 7:1617, 1996
18. Kelleher CL, Buckalew VM, Frederickson ED, Rhodes DJ, Conner DA, Seidman JG, Seidman CE: CLCN5 mutation Ser244Leu is associated with X-linked renal failure without X-linked recessive hypophosphatemic rickets. *Kidney Int* 53:31–37, 1998
19. Pook MA, Wrong O, Wooding C, Norden AGW, Feest TG, Thakker RV: Dent's disease, a renal Fanconi syndrome with nephrocalcinosis and kidney stones, is associated with a microdeletion involving DXS255 and maps to Xp11.22. *Hum Molec Genet* 2:2129–2134, 1993