

## How Bartter's and Gitelman's Syndromes, and Dent's Disease Have Provided Important Insights into the Function of Three Renal Chloride Channels: ClC-Ka/b and ClC-5

Marie Briet Rosa Vargas-Poussou Stephane Lourdel Pascal Houillier  
Anne Blanchard

Département de Physiologie, Hôpital Européen Georges Pompidou, Assistance Publique-Hôpitaux de Paris;  
INSERM U.356, IFR 58, and Rene Descartes University and School of Medicine, Paris, France

### Key Words

Dent's disease · Bartter's syndrome · ClC-5 · ClC-Ka · ClC-Kb · Renal tubule · Ion transport · Gitelman's syndrome, genetic approach · Bartter's syndrome, genetic approach

### Abstract

Chloride channels are expressed in almost all cell membranes and are potentially involved in a wide variety of functions. The kidney expresses 8 of the 9 chloride channels of the ClC family that have been cloned so far to date in mammals. This review focuses on the pathophysiology of two renal disorders that have contributed recently to our understanding of the physiological role of chloride channels belonging to the ClC family. First are the related syndromes of Bartter's and Gitelman's, in which inactivating mutations of the genes encoding either of the ClC-Ks, or their regulatory  $\beta$ -subunit barttin, have shown the important contribution of these chloride channels to renal tubular sodium and chloride (NaCl) transport along the loop of Henle and distal tubule. Second is the renal Fanconi syndrome known as Dent's disease, in which ClC-5 disruption has revealed the key role of this

endosomal chloride channel in the megalin-mediated endocytotic pathway in the proximal tubule. The underlying pathophysiology of this inherited disorder demonstrates how ClC-5 is directly or indirectly required for the reabsorption of filtered low-molecular-weight proteins and bioactive peptides, also expression of membrane transporters, and clearance of calcium-based stone-forming crystals.

Copyright © 2006 S. Karger AG, Basel

### Introduction

In mammals the ClC channel family consists of 9 *CLC* gene products classified on the base of their homologies into three subfamilies. The first subfamily comprises plasma membrane channels, whereas the proteins encoded by the other two subfamilies are thought to be located (mainly) in intracellular membranes [1]. The cloning of the genes encoding these channels has allowed detailed studies of their structure and function, but has failed to establish their physiological role(s). Two members of the *CLC* gene family are expressed predominantly in the human kidney: ClC-Ka and ClC-Kb. These channels are ex-

pressed on the basolateral membrane of the thin ascending limb (CIC-Ka only), thick limb (TAL) and distal convoluted tubule (DCT) cells, as well as intercalated cells (CIC-Ka and -Kb) of the collecting duct (CD). In mice, disruption of the *CLCNK1* gene (the mouse ortholog of CIC-Ka) leads to a form of nephrogenic diabetes insipidus, demonstrating the crucial role of CIC-K1 in NaCl reabsorption in the mouse thin ascending limb and its contribution to the urinary concentrating mechanism [2].

The aim of this review is to highlight how recent clinical data have contributed to our understanding of the physiological function of CIC-Ka and CIC-Kb, as well as the description of a new CIC channel, CIC-5, which is involved in endosomal endocytosis and intracellular trafficking.

### **Evidence for Physiological Involvement of CIC-Ka and -Kb NaCl Reabsorption along the Renal Tubule: Lessons from Bartter's and Gitelman's Syndromes**

Bartter's syndrome is a heterogeneous group of renal tubular disorders that can be classified into three distinct phenotypes: the 'antenatal' and 'classic' Bartter's syndromes, in which there is renal salt wasting, polyuria and hypercalciuria, and Gitelman's syndrome, in which there is renal salt wasting and normal or low urinary calcium excretion and marked hypomagnesemia, but no polyuria [for a review, see 3]. Antenatal Bartter's syndrome is sometimes also referred to as the hyperprostaglandin E syndrome, and is the most severe form of the disease. The salt and water losses lead to antenatal polyhydramnios, premature birth, life-threatening episodes of salt and water depletion in the neonatal period, and failure to thrive. An antenatal variant associated with deafness is particularly severe and most affected patients develop early onset renal failure. Classic Bartter's syndrome occurs in infancy or childhood, when it presents with dehydration, hypokalemia, failure to thrive, and hyper- or normocalciuria; rarely, renal failure and nephrocalcinosis occur.

Gitelman's syndrome is also characterized by hypokalemia, although the symptoms and signs are usually milder, consisting of failure to thrive in childhood and/or transient muscle weakness and spasms, but also acute epileptic seizures in adulthood. The later onset of Gitelman's and the presence of hypomagnesemia and hypocalciuria (especially) are considered hallmarks of the disease, but are not always present. Based on the early observation of

an impaired natriuretic response to the loop diuretic bumetanide in Bartter's syndrome and the thiazide diuretic hydrochlorothiazide in Gitelman's syndrome, the site of the defect in NaCl transport was thought to be the TAL and DCT, respectively. Although cell models of NaCl transport along the TAL and DCT had already been well characterized, and the likely transport processes affected in Bartter's and Gitelman's syndromes known, their actual identity remained elusive. However, based on a molecular genetic approach, Bartter's and Gitelman's syndromes have now been linked to at least seven gene defects, corresponding to five Bartter's and two Gitelman's subtypes; although involvement of as yet unidentified genes is still possible [3].

Antenatal Bartter's syndrome is most often due to inactivating mutations of the *SLC12A1* gene encoding the  $\text{Na}^+, \text{K}^+ - 2\text{Cl}^-$  co-transporter NKCC2 (type 1) or in the *KCJN1* gene encoding the apical K channel ROMK (type 2) (fig. 1). Classic Bartter's is usually due to mutations in the *CLCNKB* gene encoding for CIC-Kb (type 3), providing the first evidence for the contribution of this basolateral chloride channel to NaCl transport along the TAL. More recently, the antenatal Bartter's subtype associated with deafness has been shown to be due to mutations in the *BSND* gene, which encodes a novel protein barttin (type 4) [4]. This accessory protein co-localizes with CIC-Ka/b proteins in kidney epithelial cells, as well as in the inner ear. Subsequent functional studies established that barttin is the regulatory  $\beta$ -subunit required for normal trafficking and function of CIC-Ka and -Kb channels [5]. Finally, a last subtype of classic Bartter's syndrome (type 5) has been attributed to a gain-of-function mutation in the *CASR* gene that encodes the calcium receptor located on the basolateral membrane of TAL cells. In this form of Bartter's syndrome, constitutive activation of the calcium receptor exerts an inhibitory effect on NaCl reabsorption, as well as on divalent cation transport, along the TAL.

The first subtype of Gitelman's syndrome is due to mutations in the gene *SLC12A3* encoding the NaCl (NCCT) co-transporter on the apical membrane of DCT cells. More recently, a second subtype has been described due to mutations in the *CLCNKB* gene, demonstrating that CIC-Kb is also involved in basolateral exit of  $\text{Cl}^-$  from DCT cells [6, 7]. Various phenotypes ranging from severe antenatal Bartter's to Gitelman's syndromes have been reported in related subjects bearing the same homozygous mutation in *CLCNKB* gene [3], but none of these patients were deaf, in contrast to patients with mutations in the *BSND* gene. These data suggest that an alternative

**Fig. 1.** NaCl reabsorption in the thick ascending limb (a) and distal convoluted tubule (b). The transepithelial transport of NaCl in the TAL involves the apical bumetamide-sensitive  $\text{Na}^+, \text{K}^+-2\text{Cl}^-$  co-transporter and the basolateral exit of  $\text{Cl}^-$  via a  $\text{Cl}^-$  channel.  $\text{Na}^+, \text{K}^+-2\text{Cl}^-$  co-transporter (NKCC2) activity is driven by the low intracellular concentration of  $\text{Na}^+$  generated by basolateral  $\text{Na}^+, \text{K}^+-\text{ATPase}$ , and is sustained by  $\text{K}^+$  recycling across an apical luminal  $\text{K}^+$  channel. The lumen-positive electrical potential generated by the luminal recycling of  $\text{K}^+$  and basolateral exit of  $\text{Cl}^-$ , drives the paracellular reabsorption of divalent cations. Evidence of crucial role of  $\text{ClC-Kb}$  and the closely related chloride channel  $\text{ClC-Ka}$  in NaCl reabsorption in human TAL and DCT were first provided by Gitelman's and Bartter's syndromes in patients bearing either homozygous or compound heterozygous mutations in the gene encoding  $\text{ClC-Kb}$  protein [32], mixed heterozygous mutations in the genes  $\text{ClCKa}$  and  $\text{b}$  [8] or in the gene encoding their regulatory  $\beta$  subunit barttin [4].



basolateral pathway for  $\text{Cl}^-$  exit might compensate for  $\text{ClC-Kb}$  inactivation in the inner ear and (more variably) in the kidney. At least three pieces of evidence suggest that  $\text{ClC-Ka}$  may contribute to such compensation: (1)  $\text{ClC-Ka}$  expression overlaps with that of  $\text{ClC-Kb}$  in TAL and DCT, as well as in the inner ear [1]; (2) the severity of the phenotype observed in patients bearing mutations in the *BSND* gene suggests that the compensatory mechanism for  $\text{ClC-Kb}$  loss requires functional barttin, as does  $\text{ClC-Kb}$  itself, and (3) a phenotype resembling a barttin defect has been reported in a child with combined loss of function of  $\text{ClC-Ka}$  and  $\text{ClC-Kb}$  due to a digenic defect in their closely adjacent genes, *CLCNKA* and *CLCNKB* on chromosome 1p36 [8].

Data obtained in patients with Bartter/Gitelman syndromes related to a  $\text{ClC-Kb}$  defect prompted some groups to look for activating mutations or polymorphisms in *CLCNKB* as a genetic factor in hypertension. A possible genetic predisposition due to a T481S *CLCNKB* polymorphism proposed by Jeck et al. has recently been reviewed and criticized [9]. Moreover, the linkage of this polymorphism to hypertension was not confirmed in a similar study in Japan [10].

### Dent's Disease and the Crucial Role of $\text{ClC-5}$ in Endosomal Function

Dent's disease includes a heterogeneous group of X-linked disorders in which hemizygous males develop abnormalities that include low-molecular-weight proteinuria, hypercalciuria with nephrocalcinosis/nephrolithiasis, and in many cases renal failure. Proximal tubule solute wasting is variable, causing an incomplete renal Fanconi syndrome with hypophosphatemic rickets, proximal tubular acidosis, glucosuria, and aminoaciduria. Carrier women usually present with a milder phenotype (due to lyonisation), but rarely develop nephrocalcinosis or chronic renal failure [11]. In the past decade, positional cloning in affected families successfully identified the primary defect in a subgroup of patients: mutation of a novel gene *CLCN5*, encoding a chloride channel with unknown function,  $\text{ClC-5}$ . [12]. More recently, in a subgroup of patients with the Dent's phenotype, but with a normal *CLCN5* genotype, Hoopes et al. [13] found mutations in the gene for Lowe's syndrome, *OCRL1*. Unlike patients with typical Lowe's syndrome, mental retardation was absent or mild, and none of the patients had metabolic acidosis or ocular abnormalities on slit-lamp examination. This milder phenotype was not related to

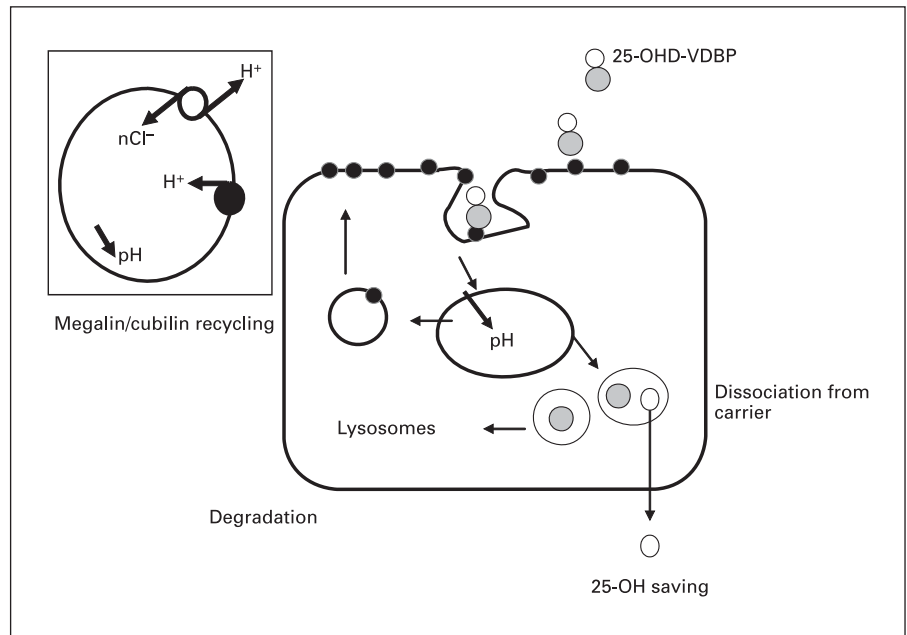
less severe changes in protein expression or enzyme activity, as both were significantly reduced or absent. Moreover, in this study a small group of patients with the Dent's phenotype were negative for mutations in *CLCN5* and *OCRL1*, suggesting that at least one other gene may cause this phenotype.

CIC-5 is expressed mainly in the kidney in endosomes of proximal tubular cells and to a lesser extent medullary thick ascending limb cells and intercalated cells of the collecting duct [14]. To study the pathogenesis of Dent's disease due to CIC-5 inactivation, several models have been reported (knockout or knockdown mouse models and cell lines) that reproduce at least some of the features of the human disease [15–17]. These models revealed that low-molecular-weight proteinuria is due to a defect in early endosomal function, which impairs the internalization and degradation of low-molecular-weight proteins by proximal tubule cells. Piwon et al. [17] first demonstrated that iodinated  $\beta_2$ -microglobulin injected into the bloodstream was lost in the urine of *CLCN5*<sup>-/-</sup> male mice in much larger quantities than in wild-type mice. CIC-5 affects endocytosis in a cell-specific manner, as is evident in heterozygous (*CLCN*<sup>+/-</sup>) females. Due to random X-inactivation, these females are chimeras, in which proximal tubule cells either express the wild-type or mutant gene product. These same authors showed that cells lacking normal CIC-5 take up less protein than their neighboring wild-type cells [17]. Because CIC-5 co-localizes with H<sup>+</sup>-ATPase, the central hypothesis to explain the endocytosis defect is that CIC-5 may provide an electrical shunt for electrogenic H<sup>+</sup> secretion into early endosomes, and that loss of function of CIC-5 would impair endosomal acidification, a crucial step in normal endosomal function [for a review, see 18, 19]. However, this hypothesis is now under scrutiny. Evidence for an alteration in endosomal acidification [20] is still lacking [19, 21] and the acidification defect, if present, may not be the only defect in endocytosis that is related to CIC-5 loss of function. Christensen et al. [21] have shown that CIC-5 inactivation inhibits (primarily) apical protein endocytosis in proximal tubule cells by causing a trafficking defect of megalin and cubilin, though in the absence of any ultrastructural alterations in the apical endocytic apparatus (see later). By contrast, drugs that abolish vacuolar acidification do not affect the rate of endocytic uptake, but do inhibit recycling, or arrest transfer from endosomes to lysosomes [19]. This uncertainty is reinforced by recent data from two groups showing that CIC-5 does not function as a Cl<sup>-</sup> channel, as was originally thought, but as an electrogenic Cl<sup>-</sup>/H<sup>+</sup> exchanger [22, 23]. An exchanger

could still regulate endosomal pH, as the initial endosomal [Cl<sup>-</sup>] should be high (and similar to extracellular fluid) relative to intracellular [Cl<sup>-</sup>], and so promote H<sup>+</sup> entry and endosomal acidification (even in the absence of an H<sup>+</sup>-ATPase).

The work of Hryciw et al. [24] demonstrated that CIC-5 is likely to contribute to the protein-protein interactions required for receptor-mediated endocytosis (i.e., from internalization of the ligand-receptor complex in clathrin-coated pits to degradation of the ligand in lysosomes after dissociation from its receptor in 'late endosomes'). They showed that CIC-5 interacts with cofilin to alter the actin cytoskeleton in the vicinity of the endocytic complex. Thus, CIC-5 expressed at the plasma membrane might be a rate-limiting step in protein uptake by proximal tubules. They also showed that uptake of albumin by proximal tubule cells requires Nedd-4, which by ubiquitinating CIC-5 may shuttle it from plasma membrane to endosomes.

The defect in endocytosis in Dent's disease does not readily explain the hypercalciuria [25]. Studies in patients with this disease are difficult, because it is rare, many patients when diagnosed already have chronic renal failure and often those with normal renal function are too young for study. However, two observations suggest that the primary defect is in the kidney: in humans, CIC-5 is expressed almost exclusively in the kidney and hypercalciuria did not recur in 2 patients who had renal transplants. Two renal defects may explain the hypercalciuria: a decrease in tubular reabsorption of calcium and/or a primary increase in 1,25-dihydroxyvitamin D (calcitriol) production. A decrease in renal tubular calcium reabsorption would increase urinary calcium excretion, lowering serum calcium concentration and stimulating PTH secretion and calcitriol synthesis. Reinhart et al. [26] studied 6 affected males and documented an absorptive (intestinal) hypercalciuria in 5. However, in this study the presence of fasting hypercalciuria in 3 patients also suggested an abnormality in tubular handling of calcium. Our own data in 40 unrelated kindred indicate that the calcitriol concentration is disproportionately elevated relative to the PTH level, which is in the low-normal range, and associated with both fasting and non-fasting hypercalciuria (unpublished observations). The findings in *CICN5* knockout mice are inconsistent. In two models hypercalciuria and nephrocalcinosis occur [15, 16], but not in the model of Jentsch and co-workers [17]. Two studies have demonstrated that urinary calcium excretion depends on dietary calcium intake [15, 27]. However, more detailed physiological studies have demonstrated a renal loss of calcium, but they could not detect an increase in intesti-



**Fig. 2.** Physiological implication of CIC-5 in receptor-mediated endocytosis. Proximal tubule cells through the megalin-cubilin endocytic pathway take up most of filtered proteins, such as the 25-hydroxyvitamin D (25-OHD)-vitamin D-binding protein (VDBP) complex. Proteins (including VDRP, gray circles) are directed toward lysosomal degradation, the dissociation from VDRP allows 25-OHD (open circle) saving, while megalin and cubilin (black circles) are redirected toward the plasma membrane. The mechanisms by which endosomes take up the appropriate proteins and deliver

them to the appropriate compartment (i.e., lysosomes or plasma membranes) remain largely unknown. CIC-5 is involved in these processes in at least two possible ways. First, CIC-5 operates as electrogenic  $\text{Cl}^-/\text{H}^+$  exchanger and likely contributes to the control of intravesicular pH and  $\text{Cl}^-$  content (inset). Second, two specific domains of CIC-5 (CBS and PY motif) are involved in both trafficking and gating of the channel and are required for normal protein uptake.

nal absorption of calcium, despite elevated calcitriol levels [27]. The mechanism of the renal calcium loss is still unclear. A defect in  $\text{Ca}^{2+}$  transport by renal tubule cells seems unlikely, because CIC-5 is not expressed at the major sites of transepithelial calcium reabsorption, i.e., the cortical TAL, DCT and connecting segment. Hypercalciuria might be due to a defect in  $\text{Na}^+$  reabsorption in a nephron segment where  $\text{Ca}^{2+}$  reabsorption is linked to  $\text{Na}^+$  reabsorption, such as in proximal tubule [25]. Further studies of segmental nephron function in vivo and in vitro are necessary to understand the basis of this variability in calciuria in Dent's disease and its relationship to CIC-5.

A hypothesis that may explain the phenotypic variation in Dent's disease has been put proposed by Piwon et al. [17]. PTH and vitamin D binding protein (VDBP) are normally filtered by the glomerulus and taken up from the tubule lumen via the megalin-mediated endocytotic route (fig. 2). Thus, urinary excretion of intact PTH and VDBP is increased in patients with Dent's disease [28],

as well as in *CLCN5* knockout mice [17]. Intact bioactive PTH may then act from the lumen to stimulate internalization of the apical  $\text{NaPi-2}$  co-transporter, so reducing phosphate absorption. Because the apical PTH receptor expressed along the proximal tubule is coupled to phospholipase C, which exerts an inhibitory effect on  $1\alpha$ -hydroxylase activity, phosphate depletion, rather than luminal PTH (as the authors had suggested), may explain the increase in calcitriol synthesis [25]. A combination of these effects could occur in individual patients to produce 25-hydroxyvitamin D deficiency (due renal losses of its carrier protein VDBP) and phosphate depletion, which favors bone disease, and stimulation of  $1\alpha$ -hydroxylase activity, which enhances intestinal calcium absorption and together with a renal leak of calcium cause hypercalciuria.

It is generally thought that nephrocalcinosis contributes to progressive renal impairment in Dent's disease. However, the pathophysiology of nephrocalcinosis is unclear and its presence and severity are not consistently

related to renal failure or degree hypercalciuria. In fact, many patients with idiopathic hypercalciuria have similar rates of urinary calcium excretion, but do not develop nephrocalcinosis. Sayer et al. [29] have reported in vitro findings suggesting that nephrocalcinosis in Dent's disease may be due to a combination of hypercalciuria and a defect in the handling of calcium oxalate and phosphate (which is more usual in Dent's disease) crystals in the medullary collecting duct. They showed that transient expression of antisense *CICN5* mRNA in an inner medullary collecting duct cell line reduced the ability of these cells to bind calcium oxalate or calcium phosphate crystals and favored the formation of crystal agglomerates.

To date, the aim of most treatments given to patients with Dent's disease is to reduce hypercalciuria (e.g., by thiazide diuretics) in the hope of limiting the progression of nephrocalcinosis and possibly renal failure. However, as already mentioned, the link between nephrocalcinosis and renal failure has never been conclusively established; moreover, in other forms of nephrocalcinosis, progressive loss of renal function is unusual. Low-molecular-weight proteinuria might be a cause of the decline in renal function in Dent's disease. Proteinuria is evident early on and

can precede renal failure by 10–20 years [11]. Cutillas et al. [30] studied the proteins excreted in the urine of patients with Dent's disease using mass spectroscopy and found abnormally high levels of proteins linked to progressive renal injury, including cytokines, apolipoproteins and complement components. They suggested that the cubilin-megalin receptor complex may have a greater affinity for carrier proteins and potentially toxic proteins of plasma origin than for other classes of proteins and so normally 'protect' the tubule (especially more distal segments) from such potentially bioactive peptides. However, this concept is at odds with a recent suggestion that toxicity in glomerular proteinuria is related to an increase in proximal tubular endocytosis of filtered proteins, and that a potentially beneficial effect of HMG-CoA reductase inhibitors in proteinuric renal disease is related to their inhibition of endocytosis [31], which may be associated with tubular proteinuria. It is becoming apparent that studying Dent's disease and other forms of renal Fanconi syndrome may provide novel insights into the renal tubular handling of proteins and ultimately the pathophysiology of proteinuric renal disease.

## References

- Jentsch TJ, Poet M, Fuhrmann JC, Zdebek AA: Physiological functions of CLC Cl<sup>-</sup> channels gleaned from human genetic disease and mouse models. *Annu Rev Physiol* 2005;67: 779–807.
- Matsumura Y, Uchida S, Kondo Y, et al: Overt nephrogenic diabetes insipidus in mice lacking the CLC-K1 chloride channel. *Nat Genet* 1999;21:95–98.
- Zelikovic I: Hypokalaemic salt-losing tubulopathies: an evolving story. *Nephrol Dial Transplant* 2003;18:1696–1700.
- Birkenhager R, Otto E, Schurmann MJ, et al: Mutation of *BSND* causes Bartter syndrome with sensorineural deafness and kidney failure. *Nat Genet* 2001;29:310–314.
- Estevez R, Boettger T, Stein V, et al: Barttin is a Cl<sup>-</sup> channel  $\beta$ -subunit crucial for renal Cl<sup>-</sup> reabsorption and inner ear K<sup>+</sup> secretion. *Nature* 2001;414:558–561.
- Jeck N, Konrad M, Peters M, Weber S, Bonzel KE, Seyberth HW: Mutations in the chloride channel gene, *CLCNKB*, leading to a mixed Bartter-Gitelman phenotype. *Pediatr Res* 2000;48:754–758.
- Konrad M, Vollmer M, Lemmink HH, et al: Mutations in the chloride channel gene *CLCNKB* as a cause of classic Bartter syndrome. *J Am Soc Nephrol* 2000;11:1449–1459.
- Schlingmann KP, Konrad M, Jeck N, et al: Salt wasting and deafness resulting from mutations in two chloride channels. *N Engl J Med* 2004; 350:1314–1319.
- Geller DS: A genetic predisposition to hypertension? *Hypertension* 2004;44:27–28.
- Kokubo Y, Iwai N, Tago N, et al: Association analysis between hypertension and *CYBA*, *CLCNKB*, and *KCNMB1* functional polymorphisms in the Japanese population – the Suita Study. *Circ J* 2005;69:138–142.
- Wrong O, Norden A, Feest T: 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. *QJM* 1994;87:473–493.
- Lloyd S, Pearce S, Fisher S, et al: A common molecular basis for three inherited kidney stone diseases. *Nature* 1996;379:445–449.
- Hoopes RR Jr, Shrimpton AE, Knohl SJ, et al: Dent disease with mutations in *OCRL1*. *Am J Hum Genet* 2005;76:260–267.
- Devuyst O, Christie P, Courtoy P, Beauwens R, Thakker R: Intra-renal and subcellular distribution of the human chloride channel, *CLC-5*, reveals a pathophysiological basis for Dent's disease. *Hum Mol Genet* 1999;8:247–257.
- Luyckx VA, Leclercq B, Dowland LK, Yu AS: Diet-dependent hypercalciuria in transgenic mice with reduced *CLC5* chloride channel expression. *Proc Natl Acad Sci USA* 1999;96: 12174–12179.
- Wang S, Devuyst O, Courtoy P, et al: Mice lacking renal chloride channel, *CLC-5*, are a model for Dent's disease, a nephrolithiasis disorder associated with defective receptor-mediated endocytosis. *Hum Mol Genet* 2000;9: 2937–2945.
- Piwon N, Gunther W, Schwake M, Bosl M, Jentsch T: *CLC-5* Cl<sup>-</sup> channel disruption impairs endocytosis in a mouse model for Dent's disease. *Nature* 2000;408:369–373.
- Marshansky V, Ausiello DA, Brown D: Physiological importance of endosomal acidification: potential role in proximal tubulopathies. *Curr Opin Nephrol Hypertens* 2002;11:527–537.
- Devuyst O, Jouret F, Auzanneau C, Courtoy PJ: Chloride channels and endocytosis: new insights from Dent's disease and *CLC-5* knockout mice. *Nephron Physiol* 2005;99:69–73.
- Gunther W, Piwon N, Jentsch TJ: The *CLC-5* chloride channel knock-out mouse – an animal model for Dent's disease. *Pflügers Arch* 2003; 445:456–462.

- 21 Christensen E, Devuyst O, Dom G, et al: Loss of chloride channel CLC-5 impairs endocytosis by defective trafficking of megalin and cubilin in kidney proximal tubules. *Proc Natl Acad Sci USA* 2003;100:8472–8477.
- 22 Scheel O, Zdebek A, Lourdel S, Jentsch T: Voltage-dependent electrogenic chloride-proton exchange by endosomal CLC proteins. *Nature* 2005;436:424–427.
- 23 Picollo A, Pusch M: Chloride/proton antiporter activity of mammalian CLC proteins CLC-4 and CLC-5. *Nature* 2005;436:420–423.
- 24 Hryciw DH, Ekberg J, Lee A, et al: Nedd4-2 functionally interacts with CLC-5: involvement in constitutive albumin endocytosis in proximal tubule cells. *J Biol Chem* 2004;279:54996–55007.
- 25 Yu AS: Role of CLC-5 in the pathogenesis of hypercalciuria: recent insights from transgenic mouse models. *Curr Opin Nephrol Hypertens* 2001;10:415–420.
- 26 Reinhart SC, Norden AG, Lapsley M, et al: Characterization of carrier females and affected males with X-linked recessive nephrolithiasis. *J Am Soc Nephrol* 1995;5:1451–1461.
- 27 Silva IV, Cebotaru V, Wang H, et al: The CLC-5 knockout mouse model of Dent's disease has renal hypercalciuria and increased bone turnover. *J Bone Miner Res* 2003;18:615–623.
- 28 Norden A, Scheinman S, Deschodt-Lanckman M, et al: Tubular proteinuria defined by a study of Dent's (CLCN5 mutation) and other tubular diseases. *Kidney Int* 2000;57:240–249.
- 29 Sayer JA, Carr G, Pearce SH, Goodship TH, Simmons NL: Disordered calcium crystal handling in antisense CLC-5-treated collecting duct cells. *Biochem Biophys Res Commun* 2003;300:305–310.
- 30 Cutillas PR, Chalkley RJ, Hansen KC, et al: The urinary proteome in Fanconi syndrome implies specificity in the reabsorption of proteins by renal proximal tubule cells. *Am J Physiol Renal Physiol* 2004;287:F353–F364.
- 31 Verhulst A, D'Haese PC, De Broe ME: Inhibitors of HMG-CoA reductase reduce receptor-mediated endocytosis in human kidney proximal tubular cells. *J Am Soc Nephrol* 2004;15:2249–2257.
- 32 Simon DB, Bindra RS, Mansfield TA, et al: Mutations in the chloride channel gene, CLCNKB, cause Bartter's syndrome type III. *Nat Genet* 1997;17:171–178.