Aquaporin 2 and Apical Calcium-Sensing Receptor: New Players in Polyuric Disorders Associated With Hypercalciuria

G. Procino, PhD,* L. Mastrofrancesco,* A. Mira, PhD,* G. Tamma, PhD,* M. Carmosino, PhD,* F. Emma, MD,† M. Svelto, PhD,* and G. Valenti, PhD*

Summary: The kidney plays a critical role in regulating water homeostasis through specific proteins highly expressed in the kidney, called *aquaporins*, allowing water permeation at a high rate. This brief review focuses on some nephropathies associated with impaired urinary concentrating ability and in particular analyzes the role of aquaporin 2 in hypercalciuria, the most common metabolic abnormality in patients with nephrolithiasis. Specifically, this review discusses the relationship between hypercalciuria and impaired aquaporin 2–mediated water handling in both acquired and inherited disorders characterized by hypercalciuria, including those affecting the sensor of extracellular calcium concentration, the calcium-sensing receptor, which represents the principal target for extracellular calcium regulation of several tissues including parathyroid glands and kidney. In the kidney, the calcium-sensing receptor regulates renal calcium excretion and influences the transepithelial movement of water and other electrolytes. Understanding the molecular basis of alteration of kidney concentrating ability found in hypercalciuria will help for devising strategies for reducing the risk of nephrocalcinosis, nephrolithiasis, and renal insufficiency.

Semin Nephrol 28:297-305

Keywords: *Calcium sensing receptor, aquaporin, stones, nephrolithiasis, ADH, calcilytic drugs*

Nephrolithiasis is the major health problem in industrialized countries and it affects about 10% of the population. Several factors including lifestyle and diet habits may be responsible for kidney stone formation.[1] Kidney stones are composed mainly from oxalate or calcium phosphate and hypercalciuria characterizes nearly 40% of stone formers.[2] Hypercalciuria is a biological syndrome defined as excretion in the urine of more than 0.1 mmol/kg/24 hours of calcium in the absence of dietary manipulation. A number of endocrine, renal, and bone diseases can cause hypercalciuria. Urinary calcium excretion is influenced substantially by dietary intakes of calcium, sodium, protein, carbohydrates, alcohol, and potassium: a poorly balanced diet can result in hypercalciuria.[1]

Hypercalciuria is the most common risk factor for kidney stones and has a recognized familial component and the influence of genetic factors appears to be more relevant than diet habit.[3-5] Calcium-containing kidney stones originate by the formation and subsequent growth of calcium oxalate or phosphate crystals in the tubule of kidney medulla. It is a common disorder in developed countries, with incident rates as great as 0.3% to 1%.[6,7] Kidney stones are responsible for about 10% of urologic hospital admissions per year and account for a significant number of visits to hospital emergency departments.[1]

*Department of General and Environmental Physiology and Center of Excellence in Comparative Genomics, University of Bari, Bari, Italy

†Department of Nephrology and Urology, Division of Nephrology and Dialysis, Bambino Gesù Children's Hospital, Rome, Italy

Supported by grants from Telethon (GGP04202 to G.V.), PRIN (Research Program of National Interest to G.V.), Centro di Eccellenza di Genomica in campo Biomedico ed Agrario (CEGBA), and from the Regional Project 2007 (G.V.).

Address reprint requests to G. Valenti, Department of General and Environmental Physiology and Center of Excellence in Comparative Genomics, University of Bari, Bari, Italy. E-mail: g.valenti@biologia.uniba.it

0270-9295/08/$ - see front matter

 doi:10.1016/j.semnephrol.2008.03.007

The mechanism causing the formation and the precipitation of calcium crystals in the urinary tract are multiple and not well defined. Regarding crystal formation, a possible mechanism involved in this process, which seems to depend predominantly on the presence of regenerating/(re)differentiating cells in the renal tubules, can be mediated by a number of luminal membrane molecules, including acidic fragment of nucleolin-related protein, annexin-II, osteopontin, and hyaluronan.[8] On the other hand, as mentioned, hypercalciuria is considered an important risk factor for stone formation.

HYPERCALCIURIA AND WATER REABSORPTION IN THE COLLECTING DUCT: RISK IN STONE FORMATION

The total urinary calcium concentration in healthy human beings with unrestricted access to fluids has been estimated as 2.3 ± 0.3 mmol/L.[9] However, urinary calcium concentration may increase significantly higher this value when water intake is restricted because increases in vasopressin levels stimulate water reabsorption in the collecting duct. Antidiuresis might cause an increased incidence of recurrent stone formation in patients with nephrolithiasis.[10]

Vasopressin acutely regulates the water permeability of the kidney collecting duct by trafficking of the water channel aquaporin 2 (AQP2) from intracellular vesicles to the luminal plasma membrane. As a consequence, water is reabsorbed, leading to urine concentration.[11–14] In several pathologic conditions, characterized by urinary concentrating defects such as nephrogenic diabetes insipidus, postobstructive polyuria, and acute or chronic renal failure, the apical expression of AQP2 is reduced drastically or even absent.[15,16] AQP2 can be detected in the urine and is excreted through the secretion of exosomes from collecting duct epithelial cells.[17] Urinary excretion of AQP2 is a potent marker for the diagnosis of water balance disorders.[18–21] Compared with healthy subjects, the urinary excretion of AQP2 is very low in patients with central diabetes insipidus and conversely is much higher in patients with impaired water excretion.[22,23]

In a previous work, we showed that urinary AQP2 excretion is a good marker for the clinical evaluation of primary nocturnal enuresis, a frequent disease in children, characterized by nocturnal polyuria and appearing to follow an autosomal-dominant mode of inheritance.[24] Specifically, we showed that hypercalciuria was associated with alterations of urinary AQP2 levels indicating clearly that external calcium activity modulates AQP2 excretion in the urine.[24]

However, the factors that link hypercalciuria-associated polyuria and AQP2 are unknown. Indeed, urinary concentrating defects and polyuria are the most important renal manifestations of hypercalcemia and the resulting hypercalciuria. This is of particularly high health relevance because other genetic diseases such as Bartter syndrome are characterized by severe hypercalciuria leading to nephrocalcinosis and reduced water concentrating capacity.[3–5,25]

The close relation between hypercalciuria and AQP2 was first suggested by Sands et al[26,27] and subsequently by Puliyanda et al.[28] The investigators reported that in rats, dihydrotachysterol induces hypercalcemia/hypercalciuria associated with polyuria and AQP2 water channel down-regulation. A calcium-dependent calpain activation is proposed to modulate AQP2 levels through AQP2 proteolysis.[28]

The kidney is a key organ for calcium homeostasis, and its ability to sense extracellular calcium levels in the urinary filtrate and the interstitial fluid is owing to the extracellular calcium-sensing receptor (CaR), which is expressed in multiple sites along the nephron.[29,30] Renal CaR plays a crucial role in the regulation of divalent mineral cation transport.[29–33]

This minireview discusses a link between calcium and water homeostasis with a particular focus on the role of AQP2 and CaR.

HYPERCALCIURIA, AQP2, AND CAR INTERPLAY IN NOCTURNAL ENURESIS

Nocturnal enuresis (NE) is a pathologic state that is more frequent in 5- to 10-year-old children and is characterized by urine loss during the night in children over the age at which bladder control is supposed to be present. Its frequency is 15% to 20% in 5-year-old children, decreasing to 7% by the age of 10, and to 1% to

2% in adults, particularly men.[34,35] In normal subjects, the decreased urine production observed during the night, about half of that produced during the day, is associated with a nocturnal increase in vasopressin secretion. However, data obtained by treating primary nocturnal enuresis with desmopressin acetate (DDAVP; a selective V2-receptor agonist) suggest that DDAVP may be useful in some but not all patients with nocturnal enuresis.

Pace et al[36] suggested that NE can be caused by absorptive hypercalciuria and can be treated with a combination of diet and DDAVP. Valenti et al[24] analyzed whether the vasopressin-sensitive water channel AQP2 might play a role in the osmoregulatory function of the kidney in children who experience primary NE and reported the first detailed analysis of alterations of AQP2 excretion associated with NE in human beings. In that study 80 children who were experiencing primary NE were examined and compared with 9 healthy children as a control group. AQP2 urine excretion was evaluated in all patients.

In consideration of the nocturnal increase of the vasopressin levels in human beings and the consequent effect on AQP2 shuttling on the apical membrane and excretion in the urine, a crucial strategy was to divide the 24-hour urine into 2 portions: collected during the night and collected during the day from each patient. Urinary AQP2 levels, normalized for creatinine, were semiquantified by densitometric scanning and reported as a ratio between the intensity of the signal in the day urine sample versus the night urine sample (D/N AQP2 ratio). Interestingly, in most of the enuretic children tested the D/N AQP2 ratio was significantly higher compared with healthy children. In particular, the D/N AQP2 ratio was 0.59 ± 0.11 ($n = 9$) in healthy children and increased to 1.27 ± 0.24 ($n = 10$) in a subpopulation of enuretic children having low nocturnal vasopressin levels. In enuretic children displaying hypercalciuria, and having normal vasopressin levels, the D/N AQP2 ratio value was 1.05 ± 0.27 ($n = 8$). These data indicate that reduced secretion of vasopressin and absorptive hypercalciuria are associated independently to an increase by about 2-fold of the urinary D/N AQP2 ratio. When low nocturnal vasopressin levels were associated to hypercalciuria a nearly 3-fold increase of the D/N AQP2 ratio was observed (1.67 ± 0.41, $n = 11$). In addition, in all enuretic patients tested, the urinary D/N AQP2 ratio correlated perfectly with the severity of the disorder (nocturnal polyuria). In contrast, in healthy children the D/N AQP2 ratio was associated with a nighttime diuresis approximately half that of the daytime diuresis (N/D diuresis ratio, 0.48 ± 0.01).

Interestingly, more than 40% of the enuretic children analyzed in that study displayed hypercalciuria, suggesting that hypercalciuria might play a crucial role in inducing NE.

It has to be outlined that, in that work, urinary excretion of AQP2 was not evaulated as absolute values but as a ratio between the densitometric signals observed in the D/N urine samples, implying that the alteration in the ratio is constistent with the hypothesis that hypercalciuria causes an impairment in vasopressin response by reducing the amount of AQP2 reaching the apical membrane. This might prevent formation of the precipitation of calcium during antidiuresis. However, no conclusion on the absolute amount of AQP2 present on the apical membrane in the absence and in response to vasopressin can be drawn.

After that observation, in a follow-up study the same group analyzed the effect of a therapeutic treatment in 46 enuretic children, of whom 26 (57%) were hypercalciuric.[37] Before the therapeutic intervention, the hypercalciuric patients were exposed to high calcium levels during the night, but not during the day. The resulting hypercalciuria contributed to the production of a renal concentrating defect manifested as nocturnal polyuria. All patients were treated with DDAVP for 3 to 6 months. Hypercalciuric patients received in addition a low-calcium diet (approximately 500 mg/d) for the same period. At the end of the treatment, the bedwetting episodes stopped in 80% of the 46 patients tested. In those patients with low vasopressin levels before the therapy, circulating vasopressin concentration returned to normal and the hypercalciuria was resolved in hypercalciuric patients. In hypercalciuric patients the D/N AQP2 ratio returned to values close to

those found in healthy children. This study clearly showed that urinary calcium concentration can modulate AQP2 excretion and this finding has been shown to be useful for the treatment of children with enuresis.[37] A key finding of this study was the observation that the bedwetting episodes restarted shortly after suspension of the low-calcium diet in the children despite the treatment with DDAVP. This condition was accompanied by a simultaneously significant increase of the nighttime urinary calcium (UCa) and the D/N AQP2 ratio.

IN VITRO EVIDENCE FOR CAR INVOLVEMENT IN ATTENUATION OF VASOPRESSIN RESPONSE

The signals involved in the strict inverse correlation existing in vivo between hypercalciuria and AQP2 excretion remain to be discovered but may represent a means by which short- and long-term modulation of urinary concentrating ability can be controlled. A signal transduction pathway must link urinary calcium levels to AQP2 expression/trafficking in the collecting duct. As mentioned, Sands et al[26] reported that in rats hypercalcemia induces alterations in vasopressin-regulated water permeability, suggesting that calcium-sensing signal transduction complex (CaR) could possibly integrate calcium and water homeostasis.

CaR, originally cloned from the bovine parathyroid gland,[38] also has been found to be expressed in several nephron segments including the collecting duct.[39,40] CaR are G-protein–coupled receptors that transduce extracellular calcium binding into several intracellular signals including increase of inositol 1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) levels. IP3 facilitates calcium release from intracellular stores, whereas DAG activates protein kinase C (PKC).[41,42] In the collecting duct CaR are expressed in the apical membrane, thus implying that they might be activated by urinary calcium. Based on the observation obtained in hypercalciuric subjects, the inteplay between the CaR signaling and the vasopressin signaling was evalutaed in vitro by Procino et al,[43] using a collecting duct cell line (CD8 cells) stably transfected with AQP2.

CD8 cells[44] were found to expresses endogenous and functional CaR.

Interestingly, short-term treatment with both high calcium levels (5 mmol/L) and gadolinium, a non–membrane-permeable CaR agonist, strongly inhibited forskolin-stimulated increase in AQP2 expression in the apical plasma membrane. At least 3 intracellular pathways activated by extracellular calcium were found to contribute to this effect. First, the increase in cyclic adenosine monophosphate (cAMP) levels in response to forskolin stimulation was reduced drastically in cells pretreated with high calcium. Second, exposure to high calcium levels activated PKC, known to counteract vasopressin response. Third, quantification of F-actin showed that high calcium caused a nearly 2-fold increase in F-actin content compared with basal conditions. All these effects were mimicked by gadolinium.

Together, these data show that extracellular calcium, possibly acting through the endogenous CaR, antagonizes forskolin-induced AQP2 translocation to the apical plasma membrane in CD8 cells in the short term.

In a more recent study, Bustamante et al[45] showed that a high concentration of extracellular calcium attenuates vasopressin-induced AQP2 expression by activating the CaR and reducing the efficiency of coupling between V2 receptor and adenylate cyclase. This effect was prevented by calmodulin, suggesting a calmodulin-dependent reduction of cAMP accumulation in mpkCCD cells. The effect of extracellular calcium on the vasopressin-dependent AQP2 expression was observed after several hours and most likely results from diminished vasopressin-stimulated AQP2 gene transcription.[46]

Together the data obtained in cell culture models suggest that different mechanisms may be involved in short- and long-term modulation of CaR by extracellular calcium with distinct consenquences on AQP2 trafficking and expression, respectively (Fig. 1).

ABNORMALITIES OF RENAL WATER CONSERVATION AND CALCIUM HOMEOSTASIS IN HUMAN BEINGS

CaR Mutations

The intriguing possibility that the apical CaR expressed in the collecting duct epithelial cells

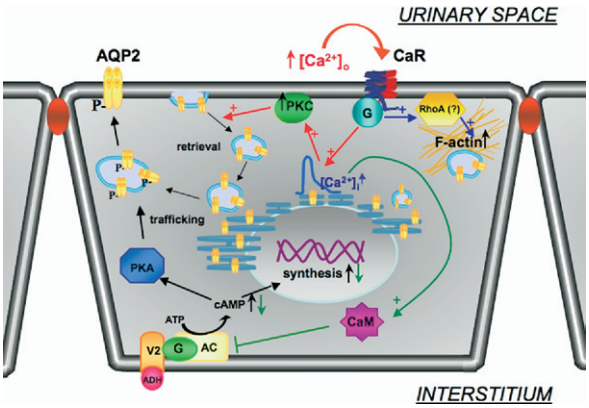

Figure 1. Proposed model of the multiple pathways through which CaR signaling counteracts vasopressin-stimulated AQP2 trafficking as well as AQP2 synthesis in renal collecting duct principal cells. In renal collecting duct CD8 cells extracellular calcium activates the G-protein–coupled CaR, leading to increases in [Ca2+]i via phospholipase C activation, and of IP3 and DAG production. IP3 facilitates calcium release from intracellular stores, whereas DAG activates PKC. The resulting PKC activation/membrane association might result in increasing the rate of AQP2 retrieval from the apical plasma membrane (red pathway). An alternative/parallel pathway activated by short-term CaR stimulation might involve RhoA activation and stabilization of actin cytoskeleton, resulting in a reduced AQP2 exocytosis (blue pathway). A third mechanism through which CaR activation counteracts AQP2 exocytosis is cAMP production.[43] On the other hand, long-term stimulation of the CaR inhibits vasopressin-induced AQP2 expression in mpkCCD cells by activating calmodulin (CaM) (green pathway), thus reducing the efficiency of coupling between V2 receptor and adenylate cyclase (AC).[45]

may act as a sensor for the urinary calcium activity to fine-tune water absorption during antidiuresis can be evaluated in human beings based on the kidney water concentrating ability in the presence of alteration of calcium homeostasis.

It is known that in case of an increase in systemic calcium load, alteration in peritubular calcium concentration reduces both NaCl and calcium reabsorption in the cortical thick ascending limb (TAL).[31] CaR activation inhibits passive calcium reabsorption in the TAL through inhibition of the sodium-chloride-potassium cotransport activity and, thus, the lumen-interstitium electric potential driving passive paracellular calcium reabsorption.[47,48]

As a result, more calcium and NaCl are delivered to the inner medullary collecting duct (IMCD) and a high luminal calcium concentration could activate the CaR in the IMCD apical membrane of collecting duct principal cells, reducing vasopressin-induced water reabsorption, thus preventing maximal water reabsorption and preventing a further increase in urinary calcium concentration.

In this condition both the increase in luminal NaCl and calcium are expected to reduce the transepithelial osmotic gradient in the terminal medulla, reducing in turn the driving force for water reabsorption. Indeed the impaired urinary concentrating ability observed in hypercalcemia results from several factors including urinary and renal interstitial concentrations of calcium, NaCl, and urea.

Inherited human diseases caused by CaR gene mutations show the relevance of CaR in divalent mineral ion as well as water reabsorption. Heterozygous mutations causing loss of function of CaR result in a lower sensitivity of CaR to extracellular calcium as obseved in the familial hypocalciuric hypercalcemia characterized by an inappropriate hypocalciuria.[49] Although the symptoms of familial hypocalciuric hypercalcemia are relatively benign, the homozygous forms of the disease (neonatal severe hyperparathyroidism) are very severe and may be lethal. In this condition the kidney excretes less calcium, causing hypocalciuria.[49–51] In contrast, CaR gain-of-function mutations such as in autosomal-dominant hypocalcemia (ADH) lead to hypercalciuria.[52,53]

Since 1994 more than 90 different mutations (both activating and inactivating) have been described. It appears that the mutations are not distributed evenly throughout the CaR sequence. Those confined to the extracellular domain of the CaR affect its response to extracellular calcium while the mutations expressed in the carboxy-terminus of the receptor affect receptor interaction with its intracellular effectors.[54] For instance, in the C terminal tail of the CaR an Alu repetitive insertion at codon 876 results in a receptor with severely impaired function despite adequate cell surface expression.[55] Also, a deletion between amino acid 895-1075 produces gain-of-function of the receptor owing to enhanced cell surface expression and increased sensitivity to external calcium.[56]

Interestingly, subjects carrying inactivating CaR mutations are not at increased risk for renal stones and do not have impaired urinary concentrating ability despite the hypercalcemia.[49,57] In contrast, subjects carrying activating CaR mutations display hypocalcemia and may present with nephrocalcinosis despite rather normal levels of serum calcium concentrations. Although these observations would fit well with the proposed model of an inhibition of vasopressin-elicited osmotic water permeability by an apical CaR, definitive evidence for such CaR involvement still is lacking. A potent gain-of-function of the CaR (L125P) has been associated to salt-losing tubulopathy, resulting in kaliuresis, hypokalemia associated with hypercalciuria, and nephrolitiasis.[58] A possible explanation for these symptoms is the apical inhibition of the renal outer medullary potassium channel (ROMK) secondary to the constitutive activation of the CaR, leading to a decreased paracellular calcium reabsorption in the TAL. This genetic defect has been described as Bartter-like syndrome type V to distinguish it from the Bartter syndrome type I, involving the mutation in the Na-K-2Cl cotransporter and type II, related to the mutation of the ROMK both associated to hypercalciuria and nephrocalcinosis, whereas the classic Bartter syndrome type III (mutation in the basolateral ClC-Kb) ischaracterized by variable calciuria.

CaR Polymorphisms

In addition to point mutations, 3 single nucleotide polymorphisms of CaR gene have been identified in the portion of exon 7 coding for the CaR intracellular tail and recently the R990G polymorphism of CaR has been reported to produce a gain-of-function predisposing to primary hypercalciuria.[59]

Interestingly, compared with normocalciuric stone formers or healthy subjects the 990G variant allele was more frequent in hypercalciuric subjects forming calcium kidney stones.[60] It is suggested that R990G single nucleotide polymorphism increases the susceptibility to primary hypercalciuria in stone-forming patients. This hypothesis is supported by the observation that the prevalence of primary hypercalciuria is approximately 10% in the general population, whereas it is 40% to 50% among calcium kidney stone formers.[61] A careful analysis of a possible AQP2 dysregulation in those patients would help in elucidating these aspects.

To this respect, a study performed on children with idiopathic hypercalciuria is particulary intersting (Emma, unpublished observations). Forty-one children with idiopathic hypercalciuria have been evaluated to analyze their renal tubular function. Among these patients, 21 had hematuria. Of note, the maximal urinary concentration was significantly lower in hypercalciuric patients without hematuria. These patients also showed increased urinary NaCl (+41%; $P < .002$) and urea excretion (+20%; $P < .02$), which suggest inhibition of NaCl reabsorption in the TAL and underestimate the urinary dilution because of increased urine osmolyte excretion.

This was overcome by analyzing changes in the urine/plasma creatinine ratio, which was found to be 53% lower in hypercalciuric children without hematuria when compared with children with hematuria or with normal controls. The net result of these processes was that despite similar UCa/urinary creatinine (UCreat) ratio, the absolute urine calcium concentration was 43% lower and the absolute calcium X phosphate product was 51% lower in children with decreased urine concentration ability. These preliminary data confirm that children with hypercalciuria can be divided into 2 distinct groups, underlying different renal mechanisms of salt and water handling causing hypercalciuria.

The first group has normal urinary concentration ability and a high prevalence of hematuria. The second group has decreased urinary concentration ability that we may speculate is induced by calcium-mediated AQP2 inhibition and has increased urinary excretion of NaCl and urea that may be related to inhibition of salt reabsorption in the TAL through activation of the CaR.

NEGATIVE ALLOSTERIC CAR MODULATORS AS THERAPEUTIC AGENTS FOR DISORDERS OF MINERAL METABOLISM AND RELATED WATER IMBALANCE

The CaR represents an interesting target for drugs representing novel therapies for selected

disorders of bone, mineral, and water metabolism. Negative allosteric modulators of the CaR decrease the receptor activity and in turn stimulate the parathyroid hormone release. In the kidney, the action of these drugs is expected to reduce hypercalciuria and consequently water loss. The principal negative allosteric modulators are the so-called *calcilytic drugs* such as Calhex-231,[62] NPS2143[63] and NPS89636.[64]

NPS 2143 has been shown to inhibit in vitro the activity of ADH mutations. By decreasing the sensitivity of the CaR to calcium and enhancing parathyroid hormone secretion and renal calcium reabsorption, this drug may represent a good alternative to the current treatment of ADH with vitamin D and calcium supplements, which results in hypercalciuria and water unbalance. Further studies are required to evaluate the ability of these drugs to correct the principal clinical disorders seen in ADH.

CONCLUSIONS

Our understanding of the relationship among hypercalciuria, AQP2 trafficking, and CaR in genetic and acquired diseases characterized by hypercalciuria has improved recently by the identification of rare mutations affecting distinct tubular transporters of the kidney. In this context, observations in human beings and in cell culture models discussed here support the hypothesis of a functional cross-talk between vasopressin signaling and CaR signaling leading to an impaired AQP2 targeting and thus contributing to a reduced kidney concentrating ability in hypercalciuria. However, despite considerable experimental work, the precise pathogenetic mechanisms causing this disorder have not been defined fully, particularly for the specific role of transporters expressed in the vasopressin-responsive epithelial cells of the inner medullary collecting duct. Future work will provide insights to clarify the relative alterations of renal water conservation and calcium homeostasis in human beings that may contribute to the pathogenesis of renal stone formation.

REFERENCES

1. Taylor EN, Curhan GC. Diet and fluid prescription in stone disease. Kidney Int. 2006;70:835-9.
2. Coe FL, Evan A, Worcester E. Kidney stone disease. J Clin Invest. 2005;115:2598-608.
3. Gambaro G, Vezzoli G, Casari G, et al. Genetics of hypercalciuria and calcium nephrolithiasis: from the rare monogenic to the common polygenic forms. Am J Kidney Dis. 2004;44:963-86.
4. Moe OW, Bonny O. Genetic hypercalciuria. >J Am Soc Nephrol. 2005;16:729-45.
5. Devuyst O, Pirson Y. Genetics of hypercalciuric stone forming diseases. Kidney Int. 2007;72:1065-72.
6. Asplin JR, Mandel NS, Coe FL. Evidence of calcium phosphate supersaturation in the loop of Henle. Am J Physiol. 1996;270:F604-13.
7. Johnson CM, Wilson DM, O'Fallon WM, et al. Renal stone epidemiology: a 25-year study in Rochester, Minnesota. Kidney Int. 1979;16:624-31.
8. Verkoelen CF, Verhulst A. Proposed mechanisms in renal tubular crystal retention. Kidney Int. 2007;72: 13-8.
9. Jacobson AL, Singhal PC, Mandin H, et al. Urinary ionic calcium and binding sites in normocalciuric idiopathic calcium urolithiasis. Invest Urol. 1979;17: 218-22.
10. Strauss AL, Coe FL, Deutsch L, et al. Factors that predict relapse of calcium nephrolithiasis during treatment: a prospective study. Am J Med. 1982;72: 17-24.
11. Knepper MA, Inoue T. Regulation of aquaporin-2 water channel trafficking by vasopressin. Curr Opin Cell Biol. 1997;9:560-4.
12. Klussmann E, Maric K, Rosenthal W. The mechanisms of aquaporin control in the renal collecting duct. Rev Physiol Biochem Pharmacol. 2000;141:33-95.
13. Brown D. The ins and outs of aquaporin-2 trafficking. Am J Physiol Renal Physiol. 2003;284:F893-901.
14. Valenti G, Procino G, Tamma G, et al. Minireview: aquaporin 2 trafficking. Endocrinology. 2005;146:5063-70.
15. Knoers NV, Deen PM. Molecular and cellular defects in nephrogenic diabetes insipidus. Pediatr Nephrol. 2001;16:1146-52.
16. Procino G, Carmosino M, Marin O, et al. Ser-256 phosphorylation dynamics of aquaporin 2 during maturation from the ER to the vesicular compartment in renal cells. FASEB J. 2003;17:1886-8.
17. Pisitkun T, Johnstone R, Knepper MA. Discovery of urinary biomarkers. Mol Cell Proteomics. 2006;5: 1760-71.
18. Kanno K, Sasaki S, Hirata Y, et al. Urinary excretion of aquaporin-2 in patients with diabetes insipidus. N Engl J Med. 1995;332:1540-5.
19. Deen PM, van Aubel RA, van Lieburg AF, et al. Urinary content of aquaporin 1 and 2 in nephrogenic diabetes insipidus. J Am Soc Nephrol. 1996;7:836-41.
20. Saito T, Ishikawa SE, Sasaki S, et al. Alteration in water channel AQP-2 by removal of AVP stimulation in collecting duct cells of dehydrated rats. Am J Physiol. 1997;272:F183-91.
21. Murer L, Addabbo F, Carmosino M, et al. Selective decrease in urinary aquaporin 2 and increase in pros-

taglandin E2 excretion is associated with postobstructive polyuria in human congenital hydronephrosis. J Am Soc Nephrol. 2004;15:2705-12.
22. Ishikawa S. Urinary excretion of aquaporin-2 in pathological states of water metabolism. Ann Med. 2000;32:90-3.
23. Schrier RW. Body water homeostasis: clinical disorders of urinary dilution and concentration. J Am Soc Nephrol. 2006;17:1820-32.
24. Valenti G, Laera A, Pace G, et al. Urinary aquaporin 2 and calciuria correlate with the severity of enuresis in children. J Am Soc Nephrol. 2000;11:1873-81.
25. Shaer AJ. Inherited primary renal tubular hypokalemic alkalosis: a review of Gitelman and Bartter syndromes. Am J Med Sci. 2001;322:316-32.
26. Sands JM, Naruse M, Baum M, et al. Apical extracellular calcium/polyvalent cation-sensing receptor regulates vasopressin-elicited water permeability in rat kidney inner medullary collecting duct. J Clin Invest. 1997;99:1399-405.
27. Sands JM, Flores FX, Kato A, et al. Vasopressin-elicited water and urea permeabilities are altered in IMCD in hypercalcemic rats. Am J Physiol. 1998;274:F978-85.
28. Puliyanda DP, Ward DT, Baum MA, et al. Calpain-mediated AQP2 proteolysis in inner medullary collecting duct. Biochem Biophys Res Commun. 2003; 303:52-8.
29. Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. Physiol Rev. 2001;81:239-97.
30. Ward DT, Riccardi D. Renal physiology of the extracellular calcium-sensing receptor. Pflugers Arch. 2002;445:169-76.
31. Hebert SC, Brown EM, Harris HW. Role of the Ca(2+)-sensing receptor in divalent mineral ion homeostasis. J Exp Biol. 1997;200:295-302.
32. Pearce SH, Thakker RV. The calcium-sensing receptor: insights into extracellular calcium homeostasis in health and disease. J Endocrinol. 1997;154:371-8.
33. Hofer AM, Brown EM. Extracellular calcium sensing and signalling. Nat Rev Mol Cell Biol. 2003;4:530-8.
34. Alon US. Nocturnal enuresis. Pediatr Nephrol. 1995; 9:94-103.
35. Mark SD, Frank JD. Nocturnal enuresis. Br J Urol. 1995;75:427-34.
36. Pace G, Aceto G, Cormio L, et al. Nocturnal enuresis can be caused by absorptive hypercalciuria. Scand J Urol Nephrol. 1999;33:111-4.
37. Valenti G, Laera A, Gouraud S, et al. Low-calcium diet in hypercalciuric enuretic children restores AQP2 excretion and improves clinical symptoms. Am J Physiol Renal Physiol. 2002;283:F895-903.
38. Brown EM, Gamba G, Riccardi D, et al. Cloning and characterization of an extracellular Ca(2+)-sensing receptor from bovine parathyroid. Nature. 1993;366: 575-80.
39. Riccardi D, Lee WS, Lee K, et al. Localization of the extracellular Ca(2+)-sensing receptor and PTH/PTHrP receptor in rat kidney. Am J Physiol. 1996; 271:F951-6.
40. Riccardi D, Park J, Lee WS, et al. Cloning and functional expression of a rat kidney extracellular calcium/polyvalent cation-sensing receptor. Proc Natl Acad Sci U S A. 1995;92:131-5.
41. Brown EM. Physiology and pathophysiology of the extracellular calcium-sensing receptor. Am J Med. 1999;106:238-53.
42. Brown EM, Chattopadhyay N, Vassilev PM, et al. The calcium-sensing receptor (CaR) permits Ca2+ to function as a versatile extracellular first messenger. Recent Prog Horm Res. 1998;53:257-81.
43. Procino G, Carmosino M, Tamma G, et al. Extracellular calcium antagonizes forskolin-induced aquaporin 2 trafficking in collecting duct cells. Kidney Int. 2004; 66:2245-55.
44. Valenti G, Frigeri A, Ronco PM, et al. Expression and functional analysis of water channels in a stably AQP2-transfected human collecting duct cell line. J Biol Chem. 1996;271:24365-70.
45. Bustamante M, Hasler U, Leroy V, et al. Calcium-sensing receptor attenuates AVP-induced aquaporin-2 expression via a calmodulin-dependent mechanism. J Am Soc Nephrol. 2008;19:109-16.
46. Hasler U, Mordasini D, Bens M, et al. Long term regulation of aquaporin-2 expression in vasopressin-responsive renal collecting duct principal cells. J Biol Chem. 2002;277:10379-86.
47. Bai M. Structure-function relationship of the extracellular calcium-sensing receptor. Cell Calcium. 2004; 35:197-207.
48. Wang WH, Lu M, Hebert SC. Cytochrome P-450 metabolites mediate extracellular Ca(2+)-induced inhibition of apical K+ channels in the TAL. Am J Physiol. 1996;271:C103-11.
49. Pollak MR, Brown EM, Chou YH, et al. Mutations in the human Ca(2+)-sensing receptor gene cause familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. Cell. 1993;75:1297-303.
50. Ho DS, Jerkins GR, Williams M, et al. Ureteropelvic junction obstruction in upper and lower moiety of duplex renal systems. Urology. 1995;45:503-6.
51. Bai M, Quinn S, Trivedi S, et al. Expression and characterization of inactivating and activating mutations in the human Ca2+o-sensing receptor. J Biol Chem. 1996;271:19537-45.
52. Pollak MR, Brown EM, Estep HL, et al. Autosomal dominant hypocalcaemia caused by a Ca(2+)-sensing receptor gene mutation. Nat Genet. 1994;8:303-7.
53. Pearce SH, Williamson C, Kifor O, et al. A familial syndrome of hypocalcemia with hypercalciuria due to mutations in the calcium-sensing receptor. N Engl J Med. 1996;335:1115-22.
54. Hendy GN, D'Souza-Li L, Yang B, et al. Mutations of the calcium-sensing receptor (CASR) in familial hypocalciuric hypercalcemia, neonatal severe hyperparathyroidism, and autosomal dominant hypocalcemia. Hum Mutat. 2000;16:281-96.

55. Bai M, Janicic N, Trivedi S, et al. Markedly reduced activity of mutant calcium-sensing receptor with an inserted Alu element from a kindred with familial hypocalciuric hypercalcemia and neonatal severe hyperparathyroidism. J Clin Invest. 1997;99:1917-25.
56. Lienhardt A, Garabedian M, Bai M, et al. A large homozygous or heterozygous in-frame deletion within the calcium-sensing receptor's carboxylterminal cytoplasmic tail that causes autosomal dominant hypocalcemia. J Clin Endocrinol Metab. 2000;85: 1695-702.
57. Marx SJ, Attie MF, Stock JL, et al. Maximal urine-concentrating ability: familial hypocalciuric hypercalcemia versus typical primary hyperparathyroidism. J Clin Endocrinol Metab. 1981;52:736-40.
58. Vargas-Poussou R, Huang C, Hulin P, et al. Functional characterization of a calcium-sensing receptor mutation in severe autosomal dominant hypocalcemia with a Bartter-like syndrome. J Am Soc Nephrol. 2002;13:2259-66.
59. Vezzoli G, Terranegra A, Arcidiacono T, et al. R990G polymorphism of calcium-sensing receptor does produce a gain-of-function and predispose to primary hypercalciuria. Kidney Int. 2007;71:1155-62.
60. Vezzoli G, Tanini A, Ferrucci L, et al. Influence of calcium-sensing receptor gene on urinary calcium excretion in stone-forming patients. J Am Soc Nephrol. 2002;13:2517-23.
61. Hodgkinson A, Pyrah LN. The urinary excretion of calcium and inorganic phosphate in 344 patients with calcium stone of renal origin. Br J Surg. 1958;46:10-8.
62. Petrel C, Kessler A, Dauban P, et al. Positive and negative allosteric modulators of the Ca2+-sensing receptor interact within overlapping but not identical binding sites in the transmembrane domain. J Biol Chem. 2004;279:18990-7.
63. Gowen M, Stroup GB, Dodds RA, et al. Antagonizing the parathyroid calcium receptor stimulates parathyroid hormone secretion and bone formation in osteopenic rats. J Clin Invest. 2000;105:1595-604.
64. Dvorak MM, Siddiqua A, Ward DT, et al. Physiological changes in extracellular calcium concentration directly control osteoblast function in the absence of calciotropic hormones. Proc Natl Acad Sci U S A. 2004;101:5140-5.