Genetic hypercalciuric stone-forming rats

David A. Bushinsky, Kevin K. Frick and Keith Nehrke

Purpose of review

We will describe the pathophysiology of hypercalciuria and the mechanism of the resultant stone formation in a rat model and draw parallels to human hypercalciuria and stone formation.

Recent findings

Through inbreeding we have established a strain of rats that excrete 8–10 times more urinary calcium than control rats. These genetic hypercalciuric rats absorb more dietary calcium at lower 1,25-dihydroxyvitamin D_3 levels. Elevated urinary calcium excretion on a low-calcium diet indicated a defect in renal calcium reabsorption and/or an increase in bone resorption. Bone from hypercalciuric rats released more calcium when exposed to 1,25-dihydroxyvitamin D_3. Bisphosphonate significantly reduced urinary calcium excretion in rats fed a low-calcium diet. Clearance studies showed a primary defect in renal calcium reabsorption. The intestine, bone and kidneys of the hypercalciuric rats had increased numbers of vitamin D receptors. When hydroxyproline is added to their diet they form calcium oxalate stones, the most common stone type in humans. Increased numbers of vitamin D receptors may cause hypercalciuria in these rats and humans.

Summary

Understanding the mechanism of hypercalciuria and stone formation in this animal model will help clinicians devise effective treatment strategies for preventing recurrent stone formation in humans.

Keywords

calcium oxalate, calcium phosphate, idiopathic hypercalciuria, nephrolithiasis, vitamin D receptor

Curr Opin Nephrol Hypertens 15:403–418. © 2006 Lippincott Williams & Wilkins.

Division of Nephrology, Department of Medicine, University of Rochester School of Medicine, Rochester, New York, USA

Correspondence to David A. Bushinsky, MD, Professor of Medicine and of Pharmacology and Physiology, University of Rochester School of Medicine, 601 Elmwood Avenue, Box 675, Rochester, NY 14642, USA
Tel: +585 275 3660; fax: +1 585 442 9201;
e-mail: David_Bushinsky@urmc.rochester.edu

This work was supported in part by grants DK 56788, DK 57716 and AR 46289 from the National Institutes of Health.

Current Opinion in Nephrology and Hypertension 2006, 15:403–418

Abbreviations

CaOx	calcium oxalate
CaR	calcium receptor
1,25(OH)$_2$D$_3$	1,25-dihydroxyvitamin D3
GHS	genetic hypercalciuric stone-forming
IGF-1	insulin-like growth factor 1
PTH	parathyroid hormone
QTL	quantitative trait loci
VDR	vitamin D receptor

1062-4821

Introduction

Nephrolithiasis, with a lifetime incidence of up to 12%, results in significant morbidity as well as substantial economic costs, not only directly from medical treatment but also indirectly through time lost from work [1–10]. Approximately 70% of kidney stones are composed of calcium, generally combined with oxalate and/or phosphate [2–4,7,9,11].

Urinary calcium is distributed over a wide range of values in humans [2,7,8,11–17] and in the rat [13,18–25]. Hypercalciuria is the most consistent metabolic abnormality found in patients with calcium nephrolithiasis [2–9,11,26]. Indeed, idiopathic hypercalciuria, excess calcium excretion with no identifiable metabolic cause, is found in up to 40% of stone-formers [2–4,9,27] but has an incidence of less than 10% in the overall population [28]. The only consistent abnormality in people who excrete large amounts of urinary calcium is an increased risk of renal stone formation [2–4,6–9,11,12,16,29–32]. The elevation in urinary calcium leads to increased supersaturation with respect to a solid phase, generally calcium oxalate (CaOx) or calcium hydrogen phosphate (brushite; $CaHPO_4$), which increases the propensity for kidney stone formation [11,33,34]. Gastrointestinal calcium absorption is generally above normal in these hypercalciuric subjects [2–4,8,9,11,35–40].

Idiopathic hypercalciuria is an inherited metabolic abnormality [25,41–43]. In pediatric patients with nephrolithiasis, 73% had a family history of kidney stones in at least one first or second-order relative, as opposed to a prevalence of 22% in a control population [44]. Of the patients with hypercalciuria, the prevalence of nephrolithiasis in the family history was 69% [44]. Coe *et al.* [45] found a strong inheritance pattern in patients with nephrolithiasis.

In support of a genetic basis for hypercalciuria, we have bred a strain of rats for this disorder. After 70 generations of inbreeding, all of the rats are hypercalciuric: they excrete approximately 8–10 times as much calcium as control animals and uniformly form kidney stones (Fig. 1; each point represents urinary calcium as previously reported [18–20,22,33,46–49,50••,51–60]; reviewed in [25,41,61,62]).

We and others [2–4,9–11,16,26,62] postulate that hypercalciuria may occur through three mechanisms, alone or in combination: (1) increased intestinal calcium absorption may be mediated either by a direct increase in calcium absorption or through excess 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$]-mediated calcium absorption; (2) decreased renal mineral reabsorption of either calcium or phosphorus; (3) enhanced bone resorption. As detailed below we have demonstrated that the increased urinary calcium in the genetic hypercalciuric stone-forming (GHS) rats appears due to independent contributions of each of these three mechanisms [18–20,22,25,33, 41,47–49,51–56,58,61], indicating a systemic dysregulation of calcium transport.

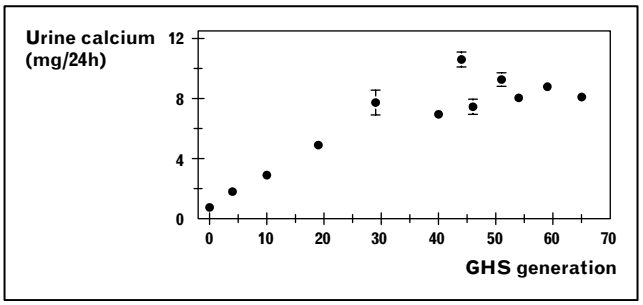
Human hypercalciuria

Analysis of human data reveals that when consuming diets of similar calcium content, net intestinal calcium absorption is significantly elevated in virtually all studies of patients with idiopathic hypercalciuria [3,4,9,10,35, 36,38–40,62–74]. This finding would argue against enhanced bone resorption as the primary etiology of idiopathic hypercalciuria but certainly does not exclude a component of bone resorption in some patients or the possibility that it is the sole cause of idiopathic hypercalciuria in a minority of others. The preponderance of studies indicates that serum 1,25$(OH)_2D_3$ concentrations are normal or elevated in patients with idiopathic hypercalciuria compared to controls [17,39,40,74–83]. This argues against a direct increase in intestinal calcium absorption and also against enhanced bone resorption, but is compatible with other mechanisms of idiopathic hypercalciuria. The majority of studies indicate that parathyroid hormone (PTH) levels are not elevated in most patients with idiopathic hypercalciuria [17,38–40,75–77,79,82–86]. This suggests that decreased renal reabsorption of calcium is not responsible for idiopathic hypercalciuria. Bone density is decreased in several studies of patients with idiopathic hypercalciuria [79,87–90]. This finding is compatible with all mechanisms except for a direct increase in intestinal calcium absorption. Compared to controls, in a number of studies serum phosphorus was significantly decreased [39,40,66,74,76, 78,82] while in others it was unchanged [38–40,63,67,80–82,86,87,91] in patients with idiopathic hypercalciuria. A low serum phosphorus is consistent only with a renal phosphate leak, suggesting that at least some patients with idiopathic hypercalciuria may have excessive renal phosphate excretion [83]. When patients with idiopathic hypercalciuria were placed on a low-calcium diet, there was a continuum from those who were in 'positive calcium balance', suggesting a component of direct increased intestinal calcium absorption, to those in 'negative calcium balance', suggesting other mechanisms of hypercalciuria [17]. The continuum of the response to a low-calcium diet suggests that there are not discrete, independent mechanisms responsible for the hypercalciuria in these patients.

In aggregate, the human data appear most consistent with an excess effect of 1,25$(OH)_2D_3$ or an excess number of receptors for 1,25$(OH)_2D_3$. The serum levels of 1,25$(OH)_2D_3$ are normal to elevated, PTH is not elevated, bone mineral density is decreased, serum phosphate is generally normal and urinary calcium excretion remains elevated after a low-calcium diet. In support of an excess effect of 1,25$(OH)_2D_3$ or an excess number of receptors for 1,25$(OH)_2D_3$ responsible for the hypercalciuria in idiopathic hypercalciuria, Maierhofer and coworkers [92,93] administered 1,25$(OH)_2D_3$ to healthy adults and demonstrated key components of idiopathic hypercalciuria. Administration of 1,25$(OH)_2D_3$ while eating a normal-calcium diet led to an increase in urinary calcium excretion and increased intestinal calcium absorption similar to patients with idiopathic hypercalciuria [92]. When they administered 1,25$(OH)_2D_3$ to control subjects eating a low-calcium diet, there was increased urinary calcium and, in addition, increased bone resorption, again similar to patients with idiopathic hypercalciuria [93].

It is often very difficult to further define the mechanism of hypercalciuria and stone formation in humans. We cannot precisely control diets or environmental factors for long periods of time and often are not able to quantitatively collect all excretions nor collect organs or cells or

Figure 1 Urinary calcium excretion in control rats (generation 0) and in subsequent generations of the inbred genetic hypercalciuric stone-forming (GHS) rats



Values are means ± SEM. Urinary calcium excretion for all generations is greater than that observed in generation 0.

make all of the necessary measurements in humans. In part because of these difficulties with human studies, we have established a GHS rat model [18–20,22,25,33, 41,47–49,51–58,60,61] to aid in our understanding of the mechanisms of hypercalciuria and stone formation in humans.

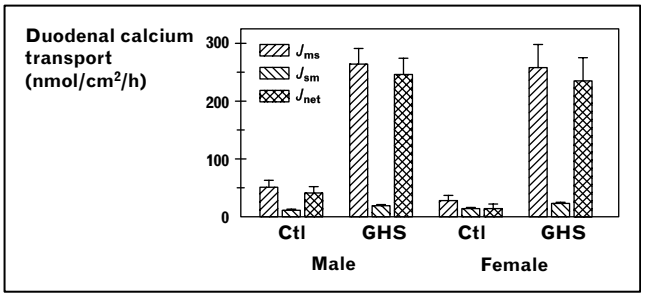
Studies to determine the mechanism of hypercalciuria and stone formation in the genetic hypercalciuric stone-forming rat

Given evidence for a genetic predisposition to hypercalciuria [21,42,45,94], we screened adult male and female Sprague–Dawley rats for hypercalciuria and used the animals with the highest urinary calcium to breed the next generation, followed by subsequent selection and inbreeding of their most hypercalciuric offspring, repeating the selection for 70 generations [18–20,22,25,33, 41,46–49,51–58,60,61]. By the 30th generation, and continuing to the present, the GHS rats (previously termed idiopathic hypercalciuria rats) consistently excrete 8–10 times as much calcium as simultaneously studied control rats (Fig. 1) [18–20,22,25,33,41,46–49,51–58,60,61]. While we plot data from female rats (Fig. 1), urinary calcium in male GHS rats is also approximately 8–10-fold greater than urinary calcium in male Sprague–Dawley rats (data not shown). Compared to similarly fed Sprague–Dawley rats, the GHS rats remain hypercalciuric (8–10-fold increase in urinary calcium) on 1.2, 0.6 and 0.02% calcium diets, on 0.65 and 0.14% phosphorus diets, and in the presence or absence of a thiazide diuretic [18–20,22,33,46–49,51–58]. The rats remain hypercalciuric (8–10-fold increase in urinary calcium) whether they are given continual or intermittent access to food. As detailed below, the GHS rats were found to have defects in calcium transport in the intestine, kidneys and bone, similar to abnormalities found in many patients with idiopathic hypercalciuria [2–11,62]. By the conclusion of an 18-week study, all of the GHS rats formed stones, whereas there was no stone formation in similarly treated Sprague–Dawley controls. The GHS rats, when fed a standard 1.2% calcium diet, form only apatite stones [33,47,48,52,54,58]. However, when 5% hydroxyproline is added to the diet of the GHS rats, they form only CaOx stones [49,56].

Intestinal calcium absorption

Our studies of the GHS rats have shed light on the mechanism of hypercalciuria in this model [33,41,47–49,51–58,60,61]. In the fourth generation of GHS rats we found that urinary calcium and intestinal calcium absorption were greater in GHS rats compared to control Sprague–Dawley rats [22]. In-vitro duodenal net calcium flux was correlated with serum $1,25(OH)_2D_3$; however, with increasing serum $1,25(OH)_2D_3$ there was greater net calcium influx and net calcium absorption in GHS rats than in normocalciuric rats. Thus the hypercalciuria in the GHS rats appears to be due, at least in part, to a primary intestinal overabsorption of dietary calcium. We subsequently studied the tenth-generation GHS rats and found, compared to the fourth generation, that there was a continued increase in urinary calcium and in J_{net} (net calcium flux) due to an increase in J_{ms} (mucosa-to-serosa calcium flux), with no change in J_{sm} (serosa-to-mucosa calcium flux; Fig. 2) [20].

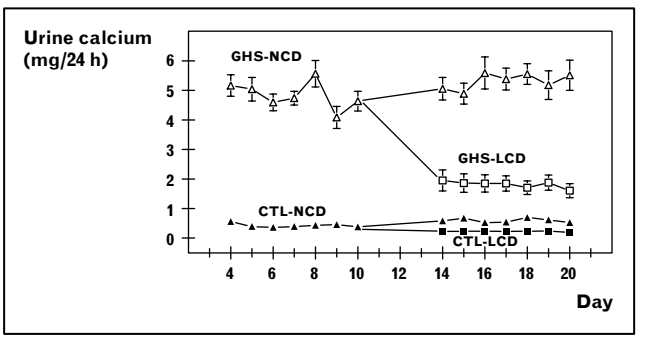
Figure 2 Effects of hypercalciuria on in-vitro duodenal bidirectional calcium fluxes



Ctl, control; GHS, genetic hypercalciuric stone-forming; J_{ms}, mucosa-to-serosa calcium flux; J_{sm}, serosa-to-mucosa calcium flux; $J_{net} = J_{ms} - J_{sm}$. Values are means ± SEM for 5–11 rats per group.

We utilized a low-calcium diet to determine if there were mechanisms in addition to increased intestinal calcium absorption responsible for hypercalciuria in the GHS rats [18]. If increased intestinal calcium absorption was the sole mechanism for the hypercalciuria, then provision of a diet almost devoid of calcium would result in an equalization of urinary calcium between the GHS rats and the controls. However, although low-calcium diet led to a marked decrease in urinary calcium, there was continued hypercalciuria in the GHS rats (Fig. 3), leading to negative calcium balance (urinary calcium excretion was

Figure 3 Daily urinary calcium excretion



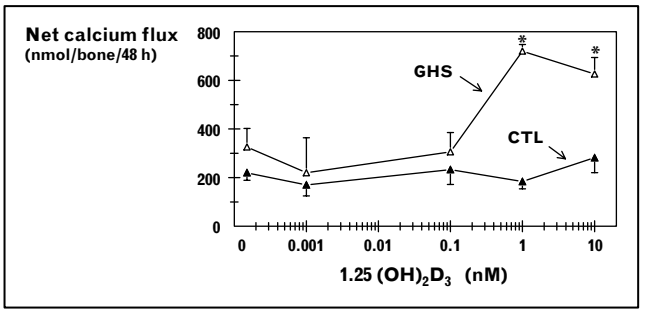
Female genetic hypercalciuric stone-forming (GHS) rats (open symbols) or control (CTL) rats (closed symbols) were fed a normal-calcium diet (NCD; triangles; 0.6% calcium, days 1–10) followed by continuation of the diet (triangles) or switching to a low-calcium diet (LCD; squares; 0.02% calcium) for the next 10 days. Values are means ± SEM.

greater than available dietary calcium), indicating that in addition to enhanced fractional intestinal calcium absorption in GHS rats [22] there must be an additional mechanism of hypercalciuria leading to a loss of bone mineral, the only significant source of calcium in the body [18]. Whether this additional mechanism is a primary bone-resorptive process or due to an inability to conserve urinary calcium was the focus of additional studies. Blood calcium fell in the GHS rats on a low-calcium diet, consistent with a decrease in renal calcium reabsorption; however, serum $1,25(OH)_2D_3$ did not rise, as would be expected with a decrease in renal calcium reabsorption.

Bone calcium resorption

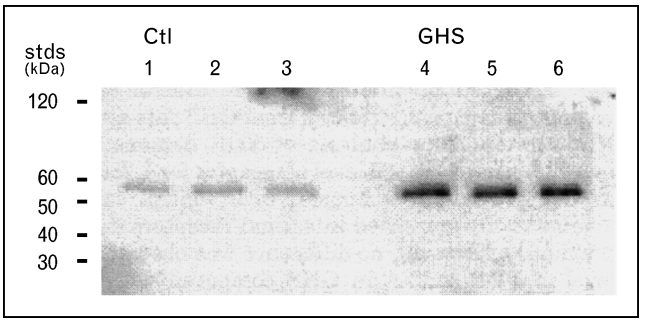
To determine if there is a contribution of bone to the hypercalciuria, we studied cultured neonatal bone (calvariae) from GHS and control Sprague–Dawley rats. We found that cultured calvariae exhibited greater sensitivity to $1,25(OH)_2D_3$ than did bone from control rats [51]. Compared to controls there was significantly greater net calcium efflux from the calvariae of GHS rats at 1 and 10 nM $1,25(OH)_2D_3$ than from control rats; there was no difference in calcium efflux between calvariae from control and GHS rats at lesser concentrations of $1,25(OH)_2D_3$ (Fig. 4). In contrast, PTH induced similar bone resorption in control and GHS calvariae. Immunoblot analysis demonstrated a 4-fold increase in the level of $1,25(OH)_2D_3$ receptors (vitamin D receptors; VDRs) in GHS calvariae (Fig. 5, lanes 4–6) compared to control calvariae (Fig. 5, lanes 1–3), similar to the increased intestinal receptors described previously [20,51,55]. There was no comparable change in VDR RNA levels as measured by slot-blot analysis, suggesting that the altered regulation of the VDR occurs posttranscriptionally. That both bone and intestine display an increased amount of $1,25(OH)_2D_3$ receptors suggests that this may be a systemic disorder of calcium regulation in the GHS rat and that enhanced bone resorption may be responsible, in part, for hypercalciuria in the GHS rat.

Figure 4 Net calcium flux from cultured neonatal rat calvariae



Flux of calcium from calvariae into incubation medium in control (CTL; ▲) or genetic hypercalciuric stone-forming (GHS; △) rats in response to graded concentrations of 1,25-dihydroxyvitamin D_3 [$1,25(OH)_2D_3$]. Values are means ± SEM; *GHS different from CTL, $P < 0.05$.

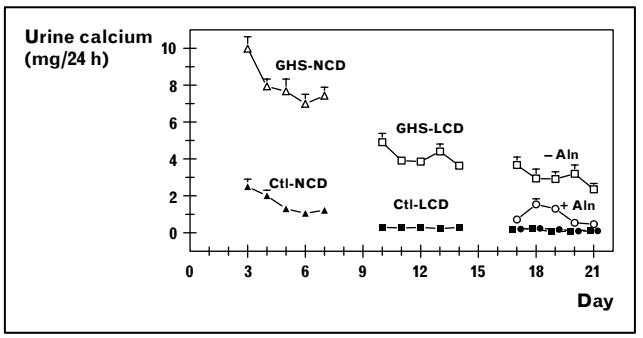
Figure 5 Immunoblot analysis of vitamin D receptor in genetic hypercalciuric stone-forming (GHS) and control (Ctl) rat calvariae



Calvarial protein fractions were obtained from control (lanes 1–3) and GHS (lanes 4–6) rat pups. Molecular-mass markers are indicated (stds).

To help determine the contribution of bone to the increased urinary calcium in the GHS rat *in vivo*, we fed GHS and control rats a high-calcium diet for 7 days, then switched them to a low-calcium diet for 7 days (Fig. 6). Control and GHS rats in each group were then continued on low-calcium diet with or without injection of the bone-resorption inhibitor alendronate (50 μg/kg per 24 h) for an additional 7 days [54]. Urinary calcium was greater in GHS than in control rats on high and low-calcium diets. With the low-calcium diet the GHS rats were in negative calcium balance. Alendronate caused a significant decrease in urinary calcium in GHS, but not in control, rats and brought GHS urinary calcium below

Figure 6 Daily urinary calcium excretion in genetic hypercalciuric stone-forming (GHS) and control (Ctl) rats



During each day of the experiment (duration 3 weeks), urinary calcium excretion of GHS rats (open symbols) exceeded that of control rats (closed symbols). Alendronate (Aln) led to a significant decrease in urinary calcium excretion in GHS but not in control rats. NCD, normal-calcium diet (1.2% calcium; triangles); LCD, low-calcium diet (0.02% calcium; squares); −Aln, continuation of low-calcium diet (squares); +Aln, low-calcium diet with alendronate (50 μg/kg per 24 h; circles). Values are means ± SEM.

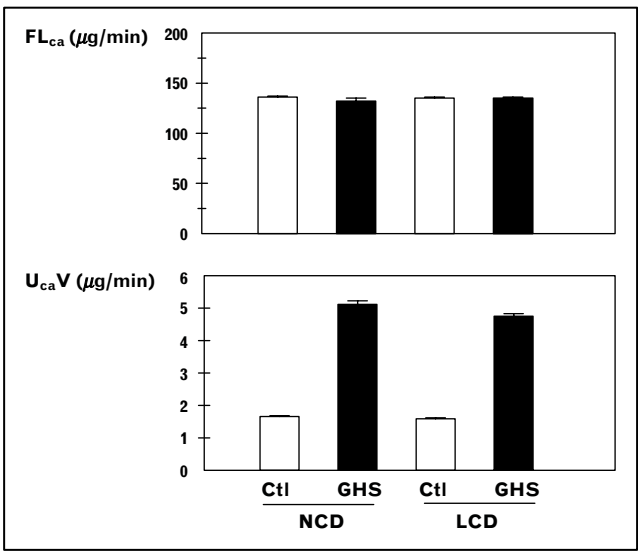
calcium intake. Thus on a low-calcium diet there is a significant contribution of bone calcium to increased urinary calcium in the GHS rat.

There is increased bone formation when bone from VDR-null mice is transplanted into wild-type mice, suggesting that $1,25(OH)_2D_3$ acting through VDR decreases bone formation [95]. Supporting this observation is the finding that primary bone cells from VDR-null mice exhibit enhanced osteoblast differentiation [96]. Thus the presence of VDR appears to enhance bone resorption, leading to increased urinary calcium, consistent with our observations in GHS rats [51].

Renal calcium reabsorption

To determine if GHS rats have a defect in renal calcium reabsorption, we performed ^{14}C-inulin clearance studies [53]. Each rat was parathyroidectomized and infused with $CaCl_2$. Both GHS and control rats had similar glomerular filtration rates and the same ultrafiltrable calcium concentrations, resulting in similar filtered loads of calcium (Fig. 7, top panel) [53]. The GHS rats had approximately three times the fractional calcium excretion and urinary calcium when compared to control rats (Fig. 7, bottom panel). The results were similar whether the rats were fed a normal or a low-calcium diet. Diuretic studies suggested a defect in calcium transport in the thick ascending limb [53].

Figure 7 Renal calcium handling



Control (Ctl) and genetic hypercalciuric stone-forming (GHS) were fed a normal-calcium diet (NCD; 0.6% calcium) or a low-calcium diet (LCD; 0.02% calcium) for at least 7 days and then subjected to selective parathyroidectomy followed by inulin-clearance studies. Filtered load of calcium (FL_{Ca}) was independent of strain and diet (top panel). Compared with controls, GHS rats demonstrated an increased fractional excretion of calcium ($U_{Ca}V$) whether on the normal or low-calcium diet (bottom panel). Values are means ± SEM.

TrpV5 is a principal conduit for renal calcium reabsorption [62,97] and quantitative trait loci (QTL) analysis of the GHS rats suggested that TrpV5 was located in a region of rat chromosome 1 which had a significant LOD score, indicating an association with hypercalciuria [60]. We investigated whether there was an abnormality in the TrpV5 coding region in GHS rats. To sequence the full coding region, primers were identified in both the 5′ and 3′ untranslated regions of the transcript, and cDNA was amplified via PCR from two Sprague–Dawley and two GHS rats. We sequenced clones derived from independent PCR reactions to rule out PCR-induced mutations and used two different sets of primers to rule out selective amplification of a particular splice variant.

We found five sequence variations in the GHS rats compared to the sequence for TrpV5 present in the GenBank nucleotide sequence database. However, these variations were also present in the Sprague–Dawley rats, and the sequence of the variations corresponded to the genomic sequence from rat at the TrpV5 locus. Furthermore, the variations altered the coding sequence such that it more closely resembled TrpV6 at each of these sites. While the sequence variations may represent strain differences, it is quite possible that these variations represent the real coding sequence for rat TrpV5 and that the original sequence reported in the database is in error.

An alternative splice variant was amplified from GHS kidney cDNA that has not been reported in the literature. This variant encoded a deletion RNA that is missing nucleotides 742–888 of the coding sequence, immediately beyond the sequence coding for ankyrin repeats. We designed a set of three primers to amplify simultaneously, yet specifically, a short product from the deletion as well as the Sprague–Dawley sequence, then compared expression levels among the GHS and Sprague–Dawley rats using kidney cDNA. The deletion transcript appeared to be present at similar levels in the Sprague–Dawley rats as the GHS rats, and represents perhaps approximately 25–40% of the total message. We have submitted the coding sequence for the deletion transcript to the GenBank database. The BankIt number is bankit653787 and the GenBank accession code is AY762624.

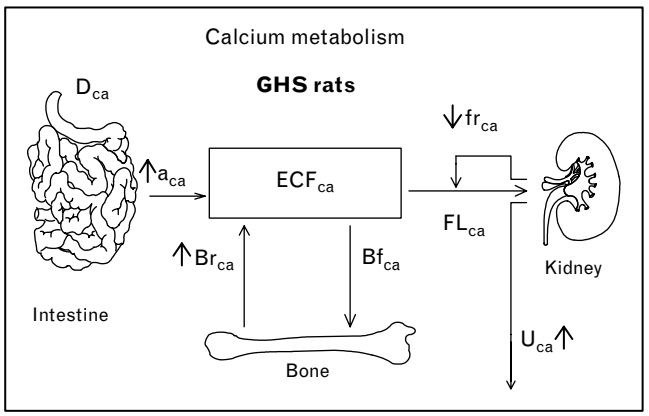
Thus we did not find any differences in either the expression level or primary sequence of TrpV5 between the GHS and Sprague–Dawley rats. It remains possible that there is an alteration in a non-coding sequence of TrpV5 that causes a functional difference in the GHS rats; however that alteration apparently does not change the expression level or size of the transcript and cDNA, respectively. In addition, mutation analysis revealed that the Clcn5 sequence was normal in the GHS rats [57].

Thus, in addition to increased intestinal calcium absorption and increased bone resorption, the GHS rats appear to have a defect in renal calcium reabsorption. These findings indicate that hypercalciuria in the GHS rat [33,41,47–49,51–58,60,61] appears, in large measure, to be analogous to hypercalciuria in humans in that, in both, there is increased intestinal calcium absorption, decreased renal calcium reabsorption and enhanced bone demineralization [2–4,7,9–12,17,25,26,34,62] (Fig. 8).

Vitamin D receptor

The finding of increased intestinal calcium absorption without an elevation in $1,25(OH)_2D_3$ levels led us to hypothesize that alteration of the receptor for vitamin D might be responsible for the abnormal regulation of calcium by enterocytes [20,98]. The magnitude of $1,25(OH)_2D_3$ action correlates with receptor number and saturation [99,100], both in rats *in vivo* [101–103] and in cell culture studies *in vitro* [104,105]. The VDR-rich cytosolic fractions from GHS rat proximal duodenum bound more $[^3H]1,25(OH)_2D_3$ than similar fractions prepared from controls [20]. Using Scatchard analysis, we found that this increase in binding of $1,25(OH)_2D_3$ by the VDR was due to an increase in the number of intestinal binding sites rather than enhanced affinity of the VDR for its ligand. Northern analysis of GHS and control rat mRNA revealed no increased expression of the VDR gene to account for the increase in receptor number. Gene transcription of the VDR was comparable for both groups of rats, as was synthesis of the vitamin D-dependent calcium-binding protein calbindin D_{9K}. Using Western blot analysis, however, more calbindin D_{9K} was detected in intestinal protein from the GHS rats than from controls [20]. Thus there is an increase in $1,25(OH)_2D_3$ action in GHS rats despite normal serum levels of $1,25(OH)_2D_3$. A recent human study demonstrated increased VDR in monocytes in patients with idiopathic hypercalciuria [106].

Figure 8 Dysregulation of calcium transport in the genetic hypercalciuric stone-forming (GHS) rat



As compared to control rats fed comparable dietary calcium (D_{Ca}), GHS rats have increased intestinal absorption of calcium (a_{Ca}), increased bone resorption rate (Br_{Ca}), and decreased renal reabsorption (fr_{Ca}), resulting in increased urinary calcium (U_{Ca}). Bf_{Ca}, bone-formation rate; ECF_{Ca}, extracellular-fluid calcium content; FL_{Ca}, filtered load of calcium.

We then found that gene expression of VDR in the GHS rats hyperresponded to minimal doses of $1,25(OH)_2D_3$ [55], which occurred through an increase in VDR mRNA stability without involving alterations in VDR gene transcription, de-novo protein synthesis or mRNA sequence. $1,25(OH)_2D_3$ administration also led to an increase in duodenal and renal calbindin mRNA levels in GHS rats, whereas levels were either suppressed or unchanged in wild-type animals. Thus hyperresponsiveness appears to be of functional significance in that it affects VDR-responsive genes in $1,25(OH)_2D_3$ target tissues. The normal serum $1,25(OH)_2D_3$ levels, increased VDR protein and normal VDR mRNA suggest that high rates of expression of vitamin D-responsive genes mediate hypercalciuria in the GHS rats. The mechanism of elevated VDR protein and state of receptor function are not known.

To determine if degradation of VDR protein is altered in GHS rats, GHS and non-stone-forming control male rats (normal control rats) fed a normal calcium diet were injected intraperitoneally with $1,25(OH)_2D_3$ (300 ng/kg of body weight) or vehicle 24 h prior to cycloheximide injection (6 mg/kg intraperitoneally), and then killed after 0–8 h [50••]. Duodenal VDR was measured by ELISA and Western blots, and duodenal and kidney calbindins (9 and 28 kDa) were measured by Western blots. Duodenal and kidney VDR were increased in GHS compared with normal control rats, and $1,25(OH)_2D_3$ increased VDR or calbindins (9 and 28 kDa) further in normal control but not GHS rats. Duodenal VDR half-life was prolonged in GHS rats (2.6 ± 0.2 compared with 1.8 ± 0.2 h, $P < 0.001$). $1,25(OH)_2D_3$ prolonged duodenal VDR half-life in normal control rats compared to that in untreated GHS rats. This study supports the hypothesis that prolongation in VDR half-life increases VDR tissue levels and mediates increased VDR-regulated genes that result in hypercalciuria.

Calcium-sensing receptor

The calcium receptor (CaR) is a seven-transmembrane-spanning protein that is a member of the G-protein-coupled receptor family of plasma membrane receptors [107]. The CaR is expressed in a wide variety of tissues including parathyroids, kidney and gastrointestinal tract [62,108,109]. There is marked homology between the parathyroid and kidney CaR in a variety of animals, including humans and rats [107,109]. In the thick ascending limb of the loop of Henle the secretion of potassium into the lumen, through the potassium channel ROMK, increases the lumen positive voltage and drives calcium

reabsorption through the paracellular space [62]. At this tubular site elevation of the blood calcium level is detected by the CaR, located on the plasma (anti-luminal) membrane, which decreases potassium traffic through ROMK, resulting in decreased luminal positivity, decreased calcium reabsorption, increased urinary calcium and a reduction in the concentration of serum calcium.

The GHS rats have been found to have elevated levels of VDR in the intestinal mucosa, bone and renal cortex [20,50••,51,55]. Analogously, human stone formers also have been shown to have elevated VDR in their circulating monocytes [106]. As the CaR contains vitamin D-response elements in its promoter region [110], we asked whether there was increased CaR in kidneys of the GHS rats. We determined renal CaR gene expression and regulation in response to $1,25(OH)_2D_3$ in GHS and control rats [111•]. Male GHS and wild-type normocalciuric non-stone-forming control Sprague–Dawley (normal control) rats were fed a diet adequate in calcium (0.6% calcium). Western blotting revealed a marked increase in CaR protein in the GHS rats compared to normal controls (Fig. 9) [111•]. Northern blot analysis of extracts of renal cortical tissue revealed the major CaR transcript of 7 kb and a less abundant 4-kb transcript, both of which were present in greater abundance in the GHS rats under basal conditions. Administration of $1,25(OH)_2D_3$ increased renal CaR mRNA levels in a dose and time-dependent manner in both GHS and wild-type control rats. However, in GHS rat kidney, $1,25(OH)_2D_3$ induced a markedly greater and a more sustained elevation of CaR mRNA level. The upregulation of CaR expression in response to $1,25(OH)_2D_3$ was through both increased gene transcription and stabilization of the mRNA. Along with the hyperresponsiveness of VDR regulation as described above, the enhanced upregulation of the CaR gene in response to $1,25(OH)_2D_3$ might lead to excess CaR, which then contributes to the hypercalciuria. Thus we found that not only was there increased CaR mRNA and protein in the GHS rat kidney, but also that $1,25(OH)_2D_3$ increased CaR through both elevated CaR gene expression and prolonged tissue half-life [111•].

The calcimimetics, such as cinacalcet, are small organic molecules that act as allosteric activators of the CaR, increasing the sensitivity of the CaR to serum calcium and substantially lowering PTH levels [112–114]. In patients with secondary hyperparathyroidism treated with cinacalcet there is a marked reduction of PTH (approximately 50%) and a modest reduction of serum calcium (approximately 10%) [112,114]. The effect of cinacalcet on human urinary calcium and supersaturations with respect to common solid phases responsible for kidney stones are complex. A reduction in PTH should increase urinary calcium; in addition, increasing the sensitivity of the renal CaR to calcium should lead to an increase in urinary calcium. However, the lowered filtered load of calcium from cinacalcet-induced hypocalcemia should lower urinary calcium. To determine the effect of cinacalcet on urinary calcium, GHS and Sprague–Dawley rats (as controls) were fed a 0.6% calcium diet (normal-calcium diet) for 14 days (Fig. 10) [59]. As expected, urinary calcium was far higher in the GHS rats ($P < 0.001$). The addition of cinacalcet did not significantly alter urinary calcium in the GHS rats but increased urinary calcium in the control rats ($P < 0.01$). Rats were then placed on a 0.02% calcium diet (low-calcium diet). As expected, urinary calcium remained higher in the GHS rats than in the control rats [18]. The addition of cinacalcet led to a significant decrease in urinary calcium in the GHS ($P < 0.01$), but not the control, rats fed a low-calcium diet. At the conclusion of the experiment, serum calcium fell with cinacalcet in both Sprague–Dawley and GHS rats. Interestingly, PTH was lower in the GHS rats than in the Sprague–Dawley rats (121 ± 30 compared with 291 ± 29 pg/ml, $P < 0.05$), suggesting that secondary hyperparathyroidism does not contribute to the hypercalciuria in the GHS rats.

Figure 9 Ca receptor (CaR) protein in normal control (NC) and genetic hypercalciuric stone-forming (GHS) kidney

(a) Lanes 1–3, renal CaR protein from normal control kidney; lanes 4–6, renal CaR protein from GHS kidney. CaR migrates to 120.8 kDa. β-Actin was used to compare sample loading. Blots were scanned, and CaR for each sample was adjusted for loading (b). Values are means ± SEM from three rats per group; $^*P < 0.008$ compared with normal controls.

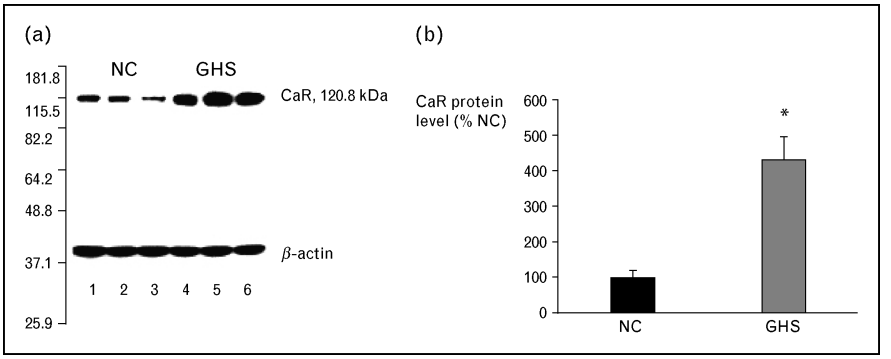
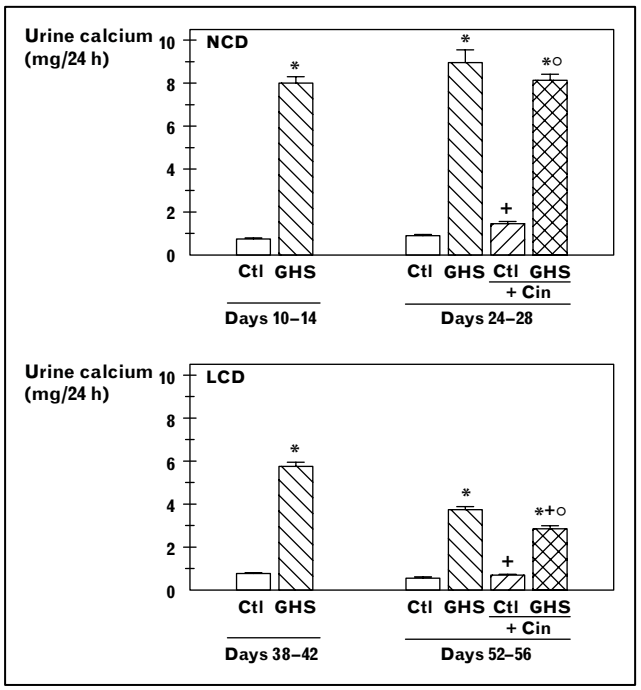


Figure 10 Effects of cinacalcet on urinary calcium excretion



On days 1–14 each rat in each group was fed a normal-calcium diet (NCD; 0.6% Ca and 0.65% P). During the last 5 days of this period (days 10–14) five successive 24-h urine samples were obtained. On days 15–28, half of each group was continued on normal-calcium diet and the other half were fed normal-calcium diet supplemented with cinacalcet (Cin; 30 mg/kg of rat per day). Urine was collected as above on days 24–28. On days 29–42 all genetic hypercalciuric stone-forming (GHS) and control (Ctl) rats were fed a low-calcium diet (LCD; 0.02% Ca and 0.65% P) while no rat received cinacalcet. Urine was collected as above on days 38–42. On days 43–56 half of each group was continued on low-calcium diet without modification and the other half (that had previously received cinacalcet) was fed low-calcium diet supplemented with cinacalcet. Urine was collected as above on days 52–56. $^{*}P < 0.05$ compared with controls in the same time period; $^{+}P < 0.05$ compared with GHS in the same time period; $^{\circ}P < 0.05$ compared with controls+cinacalcet in the same time period. Values are means ± SEM.

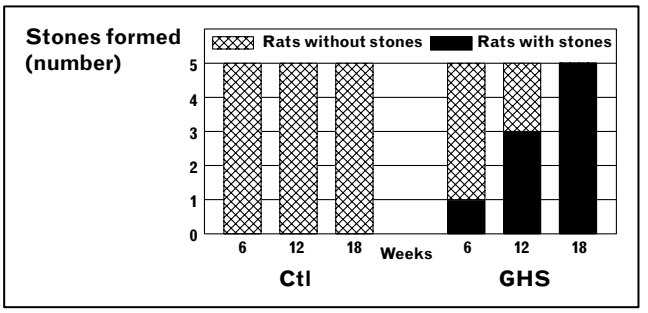
The addition of cinacalcet led to a marked fall in PTH and calcium in both groups of rats, indicating that cinacalcet was effective in lowering PTH and appears to reset the level at which serum calcium is regulated (as it appears to do in humans [112–114]). Urinary calcium is significantly greater than dietary calcium intake in the GHS rats on a low-calcium diet, which means that the source of the additional urinary calcium is bone. That urinary calcium actually fell with cinacalcet in the GHS rats suggests that PTH is necessary to resorb bone mineral and contributes to the hypercalciuria of the GHS rats fed low-calcium diet. The Sprague–Dawley rats fed cinacalcet increased urinary calcium on a normal-calcium diet and did not lower their urinary calcium on low-calcium diet with cinacalcet (in spite of a lower filtered load of Ca, creatinines were similar in all rats), suggesting that cinacalcet may have also affected the renal CaR, resulting in a decreased renal tubular calcium reabsorption.

Effects of alterations in urinary supersaturation on stone formation in the genetic hypercalciuric stone-forming rat

To determine the effect of alterations in dietary calcium on urine supersaturation with respect to $CaHPO_4$ and CaOx, we placed GHS and control rats on 1 week each of a 0.02% calcium diet, then a 0.6% calcium diet and finally a 1.2% calcium diet [19]. With increasing dietary calcium content there was increasing urine supersaturation with respect to $CaHPO_4$ and CaOx, especially in the female GHS rats. In addition these female GHS rats had greater kidney calcium content than either normocalciuric females or males or GHS males [19].

To determine the effect of the increased urinary calcium on urine supersaturation and stone formation, we fed GHS and control rats a 1.2% calcium diet for 18 weeks, measured urine supersaturation every 2 weeks and examined the urinary tract of one-third of the rats for the presence of stones every 6 weeks [48]. Urinary calcium was increased in the GHS compared to controls, resulting in greater supersaturation with respect to $CaHPO_4$ at all times and to CaOx at most times. There was a progressive increase in the incidence of stone formation in GHS rats with one rat having stones at 6 weeks, three of five with stones at 12 weeks and all five with stones at 18 weeks (Fig. 11). There were no stones formed in similarly treated control Sprague–Dawley rats. Analysis revealed the stones to be poorly crystalline apatite, which is a solid phase of calcium and phosphorus. Compared to controls, in the GHS rats the saturation ratio for $CaHPO_4$ increased proportionally more than that for CaOx, perhaps explaining why the rats formed apatite and not CaOx stones.

Figure 11 Stone formation in control (Ctl) and genetic hypercalciuric stone-forming (GHS) rats

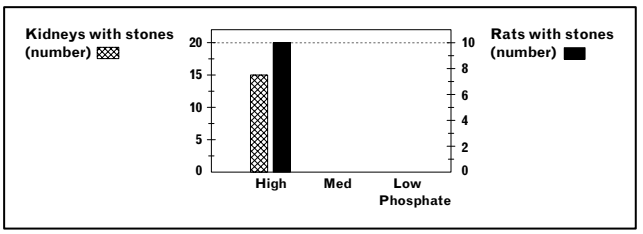


There was a progressive increase in the number of stones formed with time in the GHS rats; by 18 weeks all rats had formed stones. There was no stone formation in the control rats.

We then tested the hypothesis that $CaHPO_4$ supersaturation regulated stone formation in the GHS rats. We tested whether an isolated reduction in urine supersaturation, achieved by decreasing urine phosphorus excretion, would decrease stone formation [33]. Female GHS rats were randomly divided into three groups. Ten rats received a high-phosphorus diet (0.565% P), 10 a medium-phosphorus diet (0.395% P) and 10 a low-phosphorus diet (0.225% P) for a total of 18 weeks. The lower dietary phosphorus would be expected to result in a decrease in urine phosphorus and a decrease in urine supersaturation with respect to the $CaHPO_4$. A decrease in stone formation with reduction in urine supersaturation would support the hypothesis that supersaturation alone can regulate stone formation. Decreasing dietary phosphorus led to a progressive decrease in urine phosphorus and an increase in urinary calcium. There was a progressive decrease in supersaturation with respect to $CaHPO_4$ solid phase. Fifteen of the 20 kidneys from the 10 rats fed high phosphorus had radiographic evidence of stone formation while no kidneys from the rats fed either medium or low phosphorus developed stones (Fig. 12). A decrease in urine phosphorus not only led to a decrease in urine supersaturation with respect to the calcium phosphorus solid phase but to elimination of renal stone formation, supporting the hypothesis that variation in supersaturation alone can regulate renal stone formation.

We next studied the relationship between supersaturation and crystal inhibition [47]. CaOx and apatite crystals do not precipitate in large amounts in normal urine despite considerable supersaturation, partly because urine inhibits crystal nucleation, aggregation, and growth [115]. In control rats, a high-calcium diet (1.2% Ca) raised the supersaturation of CaOx to 8.2 compared to 0.8 when rats were fed a low-calcium diet (0.02% Ca). The high-calcium diet also raised the upper limit of metastability of CaOx (the supersaturation at which CaOx crystals form in urine) from 11.8 to 36. In GHS rats, the diet change altered CaOx supersaturation from 1.5 to 12, and the upper limit of metastability from 17 to 50 (all $P < 0.001$). Because the upper limit of metastability rose with supersaturation, the increased supersaturation had little potential to increase CaOx stone risk. For $CaHPO_4$, however, supersaturation rose from 0.6 to 2.4 and from 1.1 to 8 in normal and GHS rats ($P < 0.001$ for both), respectively, whereas the upper limit of metastability for $CaHPO_4$ did not increase significantly (8 compared with 7 and 7 compared with 11; $P = \text{NS}$ for both changes). Therefore, brushite supersaturation rose close to the upper limit of metastability, posing a high stone risk. In the GHS rat, increasing the CaOx supersaturation by dietary means raises the upper limit of metastability for CaOx, thereby offsetting the risk of CaOx stones in rats.

Figure 12 Urinary supersaturation regulates stone formation



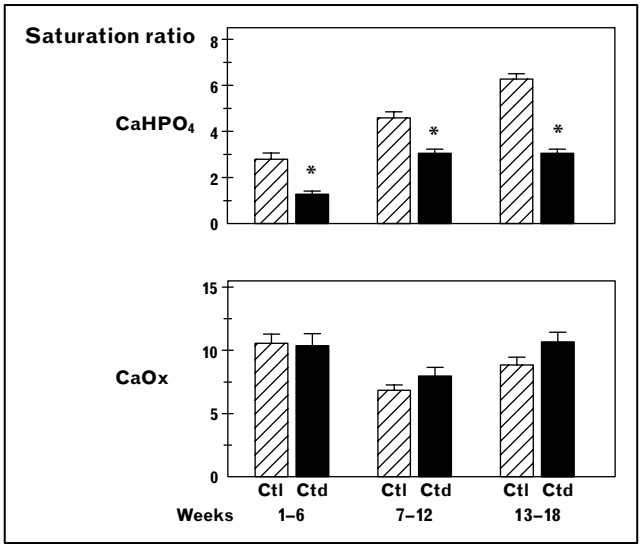
Genetic hypercalciuric stone-forming (GHS) rats were fed 13 g/day of either a high, medium or low-phosphorus diet for a total of 18 weeks and then assessed for stone formation by radiography. High, GHS rats fed 13 g/day of a 0.565% phosphorus diet, $n = 10$; Med, GHS rats fed 13 g/day of a 0.395% phosphorus diet, $n = 10$; Low, GHS rats fed 13 g/day of a 0.225% phosphorus diet, $n = 10$; dashed line, the maximum number of kidneys or rats with stones.

We subsequently tested the hypothesis that the thiazide diuretic chlorthalidone would decrease urinary calcium, supersaturation and stone formation [46]. All GHS rats received a fixed amount of a standard 1.2% calcium diet with 5% hydroxyproline so that the rats would exclusively form CaOx stones (see below). Half of the rats had chlorthalidone (4–5 mg/kg per day) added to their diets while the other half continued on the unsupplemented diet (control). Compared to controls, the addition of chlorthalidone led to a significant reduction of urinary calcium and urine phosphorus while urine oxalate excretion increased significantly. Supersaturation with respect to $CaHPO_4$ fell, while supersaturation with respect to CaOx was unchanged (Fig. 13). There was a significant direct correlation between both urinary calcium and urinary phosphorus and supersaturation with respect to $CaHPO_4$ and a significant inverse correlation between urinary oxalate and supersaturation with respect to $CaHPO_4$. Rats fed chlorthalidone had fewer stones than the control rats. The provision of chlorthalidone reduces urinary $CaHPO_4$, but not CaOx, supersaturation or stone formation in GHS rats. As a calcium phosphate complex appears to be the preferred initial solid phase in patients with CaOx kidney stones [34,116], the reduction in supersaturation with respect to $CaHPO_4$ may be the mechanism by which thiazides reduce CaOx stone formation.

Calcium oxalate stone formation in the genetic hypercalciuric stone-forming rat

The GHS rats form poorly crystalline apatite stones while the majority of human stones are composed of CaOx [2–4,9,26]. We next tested whether alterations in urine pH [58] or oxalate [52] would alter urine supersaturation and/or the type of stone formed in the GHS rat. A reduction in urine pH will increase the solubility of $CaHPO_4$ stones and perhaps predispose to CaOx stones. To determine the effect of acidosis on urine ion excretion and supersaturation, we fed GHS rats a 1.2% calcium diet

Figure 13 Chlorthalidone reduces the saturation relative to $CaHPO_4$ but not calcium oxalate (CaOx) in genetic hypercalciuric stone-forming (GHS) rats



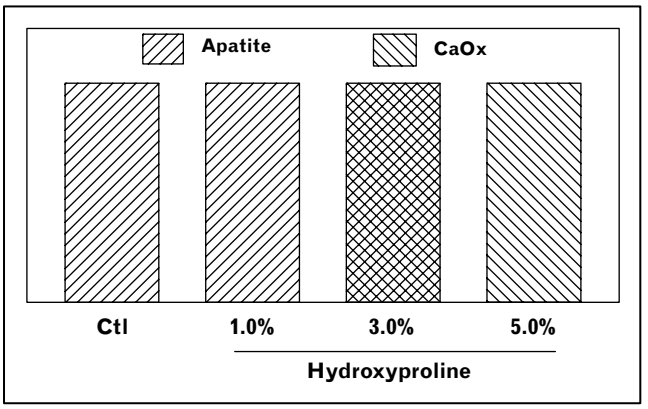
GHS rats were fed a standard calcium diet with 5% hydroxyproline added. Ctl, GHS rats fed a standard-calcium diet with 5% hydroxyproline added; Ctd, rats fed as in Ctl with added chlorthalidone (1 mg/15 g of food to provide approximately 4–5 mg/kg of body weight per 24 h); *, different from the Ctl treatment in the same time period, $P < 0.05$. Values are means ± SEM.

with 0.0, 0.5, 1.0 or 1.5% NH_4Cl in the drinking water for 18 weeks [58]. Increasing NH_4Cl led to a fall in urine pH, citrate and oxalate and an increase in urinary calcium, while supersaturation with respect to both CaOx and $CaHPO_4$ fell. In spite of differences in supersaturation, most rats in each group formed stones, which were a poorly crystalline apatite and not CaOx. Thus, while urine acidification alters ion excretion and supersaturation, it does not change the character of the stones formed in GHS rats.

To determine the effect of increasing dietary oxalate on stone formation we fed GHS rats a standard-calcium diet alone or with added sodium oxalate at 0.5, 1.0 or 2.0% for a total of 18 weeks [52]. Increasing dietary oxalate from 0 to 2.0% increased urine oxalate excretion and decreased urinary calcium excretion. Increasing dietary oxalate decreased CaOx supersaturation, due to the decrease in urinary calcium offsetting the increase in urine oxalate, and decreased $CaHPO_4$ supersaturation. Each rat in each group formed stones. The stones were a poorly crystalline apatite; there were no CaOx stones. Thus increasing dietary oxalate led to a decrease in CaOx and $CaHPO_4$ supersaturation, and did not alter the universal stone formation found in these rats, nor the type of stones formed.

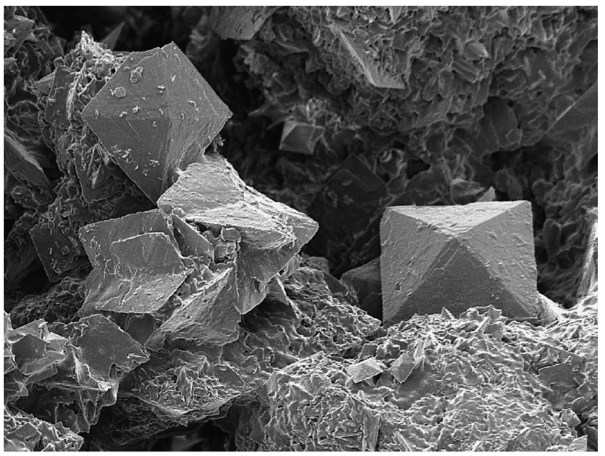
We reasoned that increasing urine oxalate without increasing dietary oxalate (and lowering urinary calcium) would not only increase supersaturation with respect to the CaOx solid phase but would also increase the ratio of CaOx to $CaHPO_4$ supersaturation and result in CaOx stone formation. We increased urine oxalate by adding an oxalate precursor, hydroxyproline, to the diet of male GHS rats [49]. The GHS rats were fed a standard 1.2% calcium diet alone or with 1, 3 or 5% *trans*-4-hydroxy-L-proline (hydroxyproline). The addition of 1% hydroxyproline to the diet of GHS rats led to an increase in urinary oxalate and urinary CaOx supersaturation, neither of which increased further with the provision of additional hydroxyproline. The addition of 1 and 3% hydroxyproline did not alter urine supersaturation with respect to $CaHPO_4$ while the addition of 5% hydroxyproline tended to lower this supersaturation. Compared to rats fed the control and the 3% hydroxyproline diet, the addition of 5% hydroxyproline increased the ratio of CaOx supersaturation to $CaHPO_4$ supersaturation. Virtually all rats formed stones. In the control and 1% hydroxyproline group all of the stones were composed of calcium and phosphate (apatite); in the 3% hydroxyproline group the stones were a mixture of apatite and CaOx while in the 5% hydroxyproline group all of the stones were CaOx (Figs 14 and 15). The provision of additional dietary hydroxyproline to GHS rats increases urinary oxalate, CaOx supersaturation and the ratio of CaOx to $CaHPO_4$ supersaturation, resulting in the formation of CaOx kidney stones. Thus, with the addition of a common amino acid, the GHS rats now not only model the most common metabolic abnormality found in patients with nephrolithiasis, hypercalciuria, but form the most common type of kidney stone, CaOx.

Figure 14 Providing additional dietary hydroxyproline to genetic hypercalciuric stone-forming (GHS) rats results in the formation of calcium oxalate (CaOx) kidney stones



GHS rats were fed a standard 1.2% calcium diet alone or with 1, 3 or 5% hydroxyproline for 18 weeks. At the end of this period, stone formation was assessed radiographically and stones were removed and analyzed by X-ray diffraction. CaOx refers to calcium oxalate monohydrate and dehydrate.

Figure 15 Scanning electron micrograph of kidney stones from genetic hypercalciuric stone-forming (GHS) rats from the 5% hydroxyproline-supplemented group

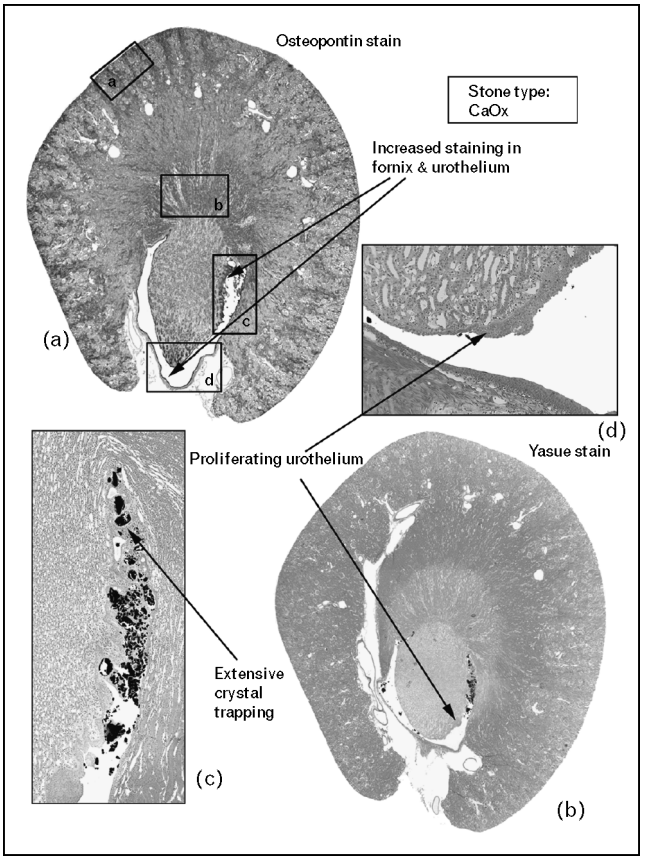


Note the large bipyramidal crystals of calcium oxalate dihydrate resembling stones found in humans.

Pathologic location of the solid phase

The GHS rats develop apatite stones when fed a normal 1.2% calcium diet [33,47,48,52,54,58]. GHS rats that had 5% hydroxyproline added to their diet formed only CaOx stones [49]. We then determined the localization of stone formation and if this solid phase resulted in pathological changes to the kidneys. GHS rats were fed 15 g of standard diet or diet supplemented with 1, 3 or 5% hydroxyproline for 18 weeks [56]. A separate group of Sprague–Dawley rats, fed the standard diet for a similar duration, served as an additional control. At 18 weeks, all kidneys were perfusion-fixed for structural analysis. There were no crystal deposits found in the kidneys of Sprague–Dawley rats. Crystal deposits were found in the kidneys of all GHS rats and this Yasue-stained material was detected only in the urine space (Fig. 16). No crystal deposits were noted within the cortical or medullary segments of the nephron and there was no evidence, by light microscopy, of tubular damage in any group. The only pathological changes occurred in the 3 and 5% hydroxyproline groups, with the 5% group showing the most severe changes. In these rats, which formed only CaOx stones, focal sites along the urothelial lining of the papilla and fornix of the urine space demonstrated a proliferative response characterized by increased density of urothelial cells that surrounded the crystal deposits (Fig. 16). At the fornix some crystals were lodged within the interstitium, deep to the proliferative urothelium. There was increased osteopontin immunostaining in the proliferating urothelium. Carr [117] has described a similar pattern in human stone formers.

Figure 16 Midtransverse paraffin sections through a kidney from a genetic hypercalciuric stone-forming (GHS) rat fed a 5% hydroxyproline-supplemented diet



These rats had stone only in the urinary space, but not in any nephron segments. No tubular injury was detected. (a) Immunohistochemical staining for osteopontin. Extensive urothelial proliferation was noted along the side of the renal papilla extending to the fornix. (b–d) Yasue stain for calcium deposits. Crystal entrapment was common at those sites of proliferation leading to isolation of some crystal from the urinary space. While osteopontin immunostaining of cortical and medullary nephron segments was identical to that described for the Sprague–Dawley controls, sites of urothelial proliferation and crystal entrapment in GHS rats showed intense staining. CaOx, calcium oxalate.

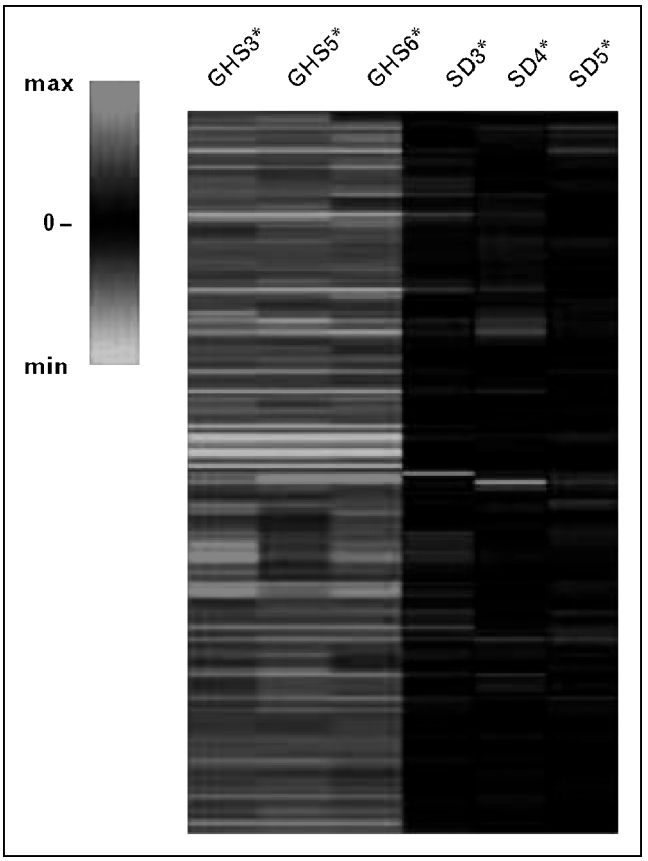
Genomic comparison of kidney gene expression in Sprague–Dawley and genetic hypercalciuric stone-forming rats

The independent dysregulation of calcium handling by the intestine, kidney and bone suggest a systemic defect in calcium handling resulting in hypercalciuria [25,41, 61]. Mathematically the rate of increase in calcium excretion over 30 generations (Fig. 1) suggests that at least seven genes are responsible for the hypercalciuria, and QTL analysis has resulted in five suggestive linkages to particular chromosomal regions [60].

Microarray analysis can complement QTL analysis when identifying potential regulatory systems and genotypic differences responsible for polygenic diseases. The use of

microarray technology in this context allows an efficient, objective, quantitative evaluation of genes in the QTL and has the potential to reduce the overall effort needed in identifying genes causally associated with traits of interest. Often, this is accomplished by comparing individual inbred strains, to reduce the influence of genetic diversity. In our case, however, we chose to compare changes in kidney gene expression profiles between normal, parental strain Sprague–Dawley and hypercalciuric GHS rats. Twelve-week-old rats were pair fed 15 g/day of a 0.6% calcium diet for 2 weeks, and an Affymetrix 230A high-density oligonucleotide microarray was probed with labeled kidney cRNA prepared from three rats of each strain (with three rats reserved for Northern blots and/or further arrays). This study was intended as a candidate gene approach, rather than as a means of identifying so-called eQTLs (changes in gene expression reproducibly associated with a particular trait), and even without the benefit of large sample numbers, the statistical significance of our observations was reasonable and, in some cases, revealing. The resulting data were interpreted through Robust Multi-chip Analysis (RMA) using Iobion GeneTraffic v3.2. To enhance the reliability of the data, only expression hybridization values (signal intensities) in the top one-third were considered for further analysis. Targets where at least one of the three animals displayed a 1.4-fold change or greater relative to the Sprague–Dawley mean expression baseline were clustered and displayed as a color-intensity map. This approach ensured that we did not mask potential candidates due to a single false-negative (or false-positive). Next, the individual hybridization values in the 446 targets thus identified were compared between the Sprague–Dawley and GHS rats using a two-class unpaired *t* test with Benjamini–Hochberg *P* value correction, and the resulting *P* values were plotted against the mean $\log_2$ ratio. This approach led to the identification of 184 genes or expressed-sequence-tag clusters (Fig. 17), where the changes in the GHS rat kidney were significant ($P < 0.05$).

Figure 17 Changes in kidney gene expression profiles between normal, parental strain Sprague–Dawley and genetic hypercalciuric stone-forming (GHS) rats

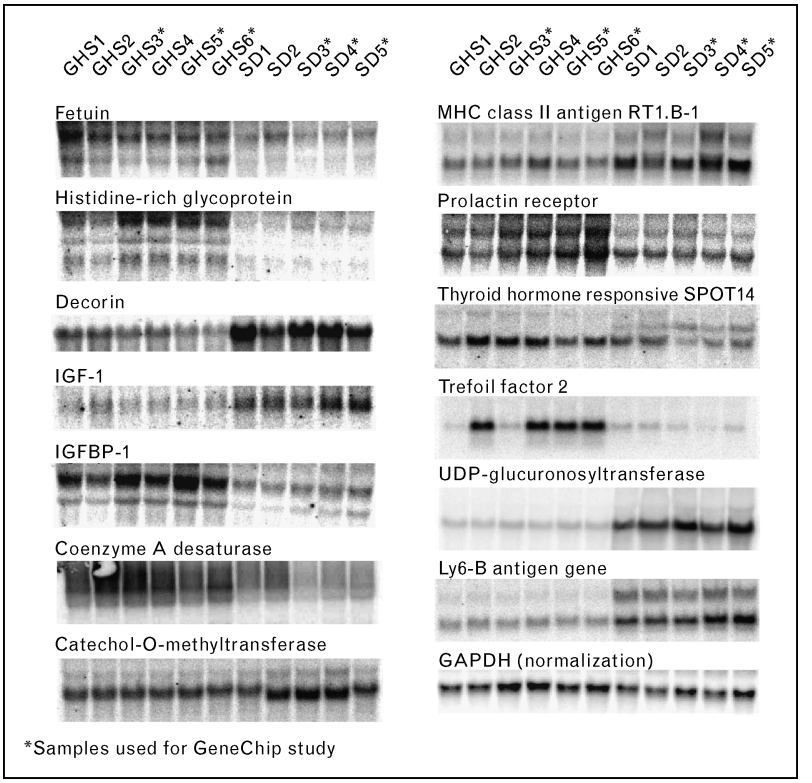


Twelve-week-old rats were pair fed 15 g/day of a 0.6% calcium diet for 2 weeks, and an Affymetrix 230A high-density oligonucleotide microarray was probed with labeled kidney cRNA prepared from three rats of each strain (individual samples designated as GHS3*, GHS5*, GHS6*, or SD3*, SD4*, SD5*). Data were interpreted through Robust Multi-chip Analysis using Iobion GeneTraffic v3.2. We identified 184 genes or expressed-sequence-tag clusters where the changes in the GHS rat kidney compared to Sprague–Dawley were significant ($P < 0.05$).

Several targets that showed changes in the microarray screen were further studied by Northern analysis of kidney RNA from Sprague–Dawley ($n = 5$) and GHS ($n = 6$) rats. Phosphorimager quantitation of the Northern signal intensity, normalized to glyceraldehyde phosphate dehydrogenase, confirmed the microarray results (Fig. 18; the RNA samples used for microarray analysis are denoted by an asterisk). Fetuin, a secreted cystatin-domain protein suggested to inhibit the precipitation of calcium phosphate crystals [118], was upregulated 3.4-fold ($P = 0.025$; compared to a 2.0-fold increase observed using the microarray, $P < 0.0001$), and a related histidine-rich glycoprotein was upregulated 3.0-fold ($P < 0.0001$; compared to 1.9-fold increase observed using the microarray). Decorin, a small leucine-rich proteoglycan of the extracellular matrix that induces calcification in arterial smooth muscle cell cultures and colocalizes to mineral deposition in human atherosclerotic plaque [119], was decreased by 3.41-fold ($P = 0.0007$; compared to a 2.1-fold decrease using the microarray, $P < 0.0001$). Insulin-like growth factor 1 (IGF-1) was decreased by 4.1-fold ($P = 0.0001$; compared to a 1.8-fold decrease using the microarray, $P = 0.01$) and IGF-binding protein 1 was increased by 2.93-fold ($P = 0.0004$; compared to a 2.1-fold increase using the microarray, $P < 0.0001$). Since IGF-binding protein is known to regulate the effective levels of IGF-1, the combined effects of these two changes may be to alter growth hormone regulation in the kidney, and perhaps elsewhere. IGF-1 modulates bone growth [120] and a QTL associated with skeletal acquisition has been localized to an IGF-1 allele [121]. IGF-1 deficiency may lead to $1,25(OH)_2D_3$ deficiency

Figure 18 Northern analysis of kidney RNA for selected microarray targets

Probes were produced by reverse transcriptase PCR, using primers derived from public database sequences. RNA samples also used for microarray analysis are denoted by an asterisk. IGF-1, insulin-like growth factor 1; IGFBP-1, IGF-binding protein 1; MHC, major histocompatibility complex; GAPDH, glyceraldehyde phosphate dehydrogenase.



and elevated PTH levels even under a normal-calcium diet [122]. Ferritin, an abundant transcript that produced a saturated signal on the GeneChip, was expressed at similar levels in both Sprague–Dawley and GHS animals.

These changes potentially represent three classes of genes: those that are fundamentally altered and lead to hypercalciuria, those that are selected for by inbreeding (and may be genetically linked to the first set), and those that are altered in response to hypercalciuria and/or stone formation. Other genes beyond the 184 that we have identified may be dysregulated in the GHS rat as well, but do not statistically meet our criteria with $n = 3$ in each group. This may be due to the low sample number, or it may be due to inherent genetic variability in the Sprague–Dawley rats influencing the baseline signal. This variability is apparent in Fig. 17, when comparing signal values from the Sprague–Dawley to the GHS rats, as might be expected for an outbred compared with an inbred strain.

Furthermore, we have identified several chromosomal 'hotspots' where clusters of genes demonstrate significant changes in kidney expression levels in GHS relative to Sprague–Dawley rats. Some of these changes could be attributable to a reduction in genetic complexity at loci that have variable expression levels in the outbred Sprague–Dawley rat strain. However, other changes may be due to a genetic linkage to loci that are selected for by the process of breeding hypercalciuric rats. Because this is a candidate gene approach, we consider clustering to be a rationale for further exploration of a particular genetic locus, and we found that two of our more dramatic clusters correspond to the cytobands containing interleukin-1 and tumor necrosis factor-α, both of which have profound effects on bone mineralization and density, and could potentiate a systemic defect in calcium handling.

Conclusion

To explain more fully the mechanism of idiopathic hypercalciuria in humans, we have developed an animal model of this disorder. Through more than 70 generations of successive inbreeding of the most hypercalciuric progeny of hypercalciuric Sprague–Dawley rats, we have established a strain of rats that excrete 8–10 times as much urinary calcium as control Sprague–Dawley rats. These hypercalciuric rats have a systemic abnormality in calcium homeostasis; they absorb more intestinal calcium, they resorb more bone, and they do not adequately reabsorb filtered calcium, similar to findings in humans with idiopathic hypercalciuria. Because each one of the hypercalciuric rats forms renal stones, we have described them as GHS rats. These studies suggest that an increased number of VDRs may be the underlying

mechanism for hypercalciuria in these rats and perhaps, by analogy, in humans. In a recent clinical study, circulating monocytes from humans with idiopathic hypercalciuria were shown to have an increased number of VDRs. Understanding the mechanism of hypercalciuria and stone formation in this animal model will help clinicians devise effective treatment strategies for preventing recurrent stone formation in humans.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 464).

1 Pak CY. Nephrolithiasis. Current Ther Endo Metab 1997; 6:572–576.

2 Bushinsky DA. Renal Lithiasis. In: Humes HD, editor. Kelly's Textbook of Medicine. New York: Lippincott Williams & Wilkins; 2000. pp. 1243–1248.

3 Bushinsky DA, Nephrolithiasis. J Am Soc Nephrol 1998; 9:917–924.

4 Monk RD, Bushinsky DA. Nephrolithiasis and nephrocalcinosis. In: Johnson R, Frehally J, editors. Comprehensive clinical nephrology, 2nd ed. London: Mosby; 2003. pp. 731–744.

5 Consensus Conference. Prevention and treatment of kidney stones. JAMA 1988; 260:977–981.

6 Pak CYC. Pathophysiology of calcium nephrolithiasis. In: Seldin DW, Giebisch G, editors. The kidney: physiology and pathophysiology, 2nd ed. New York: Raven Press; 1992. pp. 2461–2480.

7 Coe FL, Parks JH, Asplin JR. The pathogenesis and treatment of kidney stones. N Engl J Med 1992; 327:1141–1152.

8 Asplin JR, Favus MJ, Coe FL. Nephrolithiasis. In: Brenner BM, editor. The kidney, 6th ed. Philadelphia: WB Saunders Company; 2000. pp. 1774–1819.

9 Monk RD, Bushinsky DA. Kidney stones. In: Larsen PR, Kronenberg HM, Melmed S, Polonsky KS, editors. Williams textbook of endocrinology, 10th ed. Philadelphia: WB Saunders; 2003. pp. 1411–1425.

10 Bushinsky DA. Recurrent hypercalciuric nephrolithiasis – does diet help? N Eng J Med 2002; 346:124–125.

11 Coe FL, Bushinsky DA. Pathophysiology of hypercalciuria. Am J Physiol Renal Fluid Electrolyte Physiol 1984; 247:F1–F13.

12 Parks JH, Coe FL. Pathogenesis and treatment of calcium stones. Semin Nephrol 1996; 16:398–411.

13 Bushinsky DA, Krieger NS. Integration of calcium metabolism in the adult. In: Coe FL, Favus MJ, editors. Disorders of bone and mineral metabolism. New York: Raven Press; 1992. pp. 417–432.

14 Bushinsky DA, Krieger NS. Role of the skeleton in calcium homeostasis. In: Seldin DW, Giebisch G, editors. The kidney: physiology and pathophysiology, 2nd ed. New York: Raven Press; 1992. pp. 2395–2430.

15 Hodgkinson A, Pyrak LN. The urinary excretion of calcium and inorganic phosphate in 344 patients w/calcium stone of renal origin. Br J Surg 1958; 46:10–18.

16 Coe FL, Parks JH. Familial (idiopathic) hypercalciuria. In: Coe FL, Parks JH, editors. Nephrolithiasis: pathogenesis and treatment, 2nd ed. Chicago: Year Book Medical Publishers; 1990. pp. 108–138.

17 Coe FL, Favus MJ, Crockett T, *et al.* Effects of low-calcium diet on urine calcium excretion, parathyroid function and serum $1,25(OH)_2D_3$ levels in patients with idiopathic hypercalciuria and in normal subjects. Am J Med 1982; 72:25–32.

18 Kim M, Sessler NE, Tembe V, *et al.* Response of genetic hypercalciuric rats to a low calcium diet. Kidney Int 1993; 43:189–196.

19 Bushinsky DA, Kim M, Sessler NE, *et al.* Increased urinary saturation and kidney calcium content in genetic hypercalciuric rats. Kidney Int 1994; 45:58–65.

20 Li X-Q, Tembe V, Horwitz GM, *et al.* Increased intestinal vitamin D receptor in genetic hypercalciuric rats: a cause of intestinal calcium hyperabsorption. J Clin Invest 1993; 91:661–667.

21 Favus MJ, Coe FL. Evidence for spontaneous hypercalciuria in the rat. Mineral Electrolyte Met 1979; 2:150–154.

22 Bushinsky DA, Favus MJ. Mechanism of hypercalciuria in genetic hypercalciuric rats: inherited defect in intestinal calcium transport. J Clin Invest 1988; 82:1585–1591.

23 Bushinsky DA, Favus MJ, Langman C, Coe FL. Mechanism of chronic hypercalciuria with furosemide: increased calcium absorption. Am J Physiol Renal Fluid Electrolyte Physiol 1986; 251:F17–F24.

24 Bushinsky DA, Favus MJ, Coe FL. Mechanism of chronic hypocalciuria with chlorthalidone: reduced calcium absorption. Am J Physiol Renal Fluid Electrolyte Physiol 1984; 247:F746–F752.

25 Bushinsky DA. Genetic hypercalciuric stone forming rats. Semin Nephrol 1996; 16:448–457.

26 Monk RD, Bushinsky DA. Pathogenesis of idiopathic hypercalciuria. In: Coe F, Favus M, Pak C, *et al.*, editors. Kidney stones: medical and surgical management. Philadelphia: Lippincott-Raven; 1996. pp. 759–772.

27 Levy FL, Adams-Huet B, Pak CY. Ambulatory evaluation of nephrolithiasis: an update of a 1980 protocol. Am J Med 1995; 98:50–59.

28 Robertson WG, Morgan DB. The distribution of urinary calcium excretion in normal persons and stone-formers. Clin Chim Acta 1972; 37:503–508.

29 Coe FL. Uric acid and calcium oxalate nephrolithiasis. Kidney Int 1983; 24:392–403.

30 Maschio G, Tessitore N, D'Angelo A, *et al.* Prevention of calcium nephrolithiasis with low-dose thiazide, amiloride and allopurinol. Am J Med 1981; 71:623–626.

31 Coe FL. Treated and untreated recurrent calcium nephrolithiasis in patients with idiopathic hypercalciuria, hyperuricosuria, or no metabolic disorder. Ann Int Med 1977; 87:404–410.

32 Coe FL, Parks JH, Nakagawa Y. Protein inhibitors of crystallization. Semin Nephrol 1991; 11:98–109.

33 Bushinsky DA, Parker WR, Asplin JR. Calcium phosphate supersaturation regulates stone formation in genetic hypercalciuric stone-forming rats. Kidney Int 2000; 57:550–560.

34 Bushinsky DA. Nephrolithiasis: site of the initial solid phase. J Clin Invest 2003; 111:602–605.

35 Birge SJ, Peck WA, Berman M, Whedon GD. Study of calcium absorption in man: a kinetic analysis and physiologic model. J Clin Invest 1969; 48:1705–1713.

36 Wills MR, Zisman E, Wortsman J, *et al.* The measurement of intestinal calcium absorption by external radioisotope counting: application to study of nephrolithiasis. Clin Sci 1970; 39:95–106.

37 Pak CYC, East DA, Sanzenbacher LJ, *et al.* Gastrointestinal calcium absorption in nephrolithiasis. J Clin Endocrinol Metab 1972; 35:261–270.

38 Pak CYC, Ohata M, Lawrence EC, Snyder W. The hypercalciurias: causes, parathyroid functions, and diagnostic criteria. J Clin Invest 1974; 54:387–400.

39 Kaplan RA, Haussler MR, Deftos LJ, *et al.* The role of 1,25 dihydroxyvitamin D in the mediation of intestinal hyperabsorption of calcium in primary hyperparathyroidism and absorptive hypercalciuria. J Clin Invest 1977; 59:756–760.

40 Shen FH, Baylink DJ, Nielsen RL, *et al.* Increased serum 1,25-dihydroxyvitamin D in idiopathic hypercalciuria. J Lab Clin Med 1977; 90:955–962.

41 Bushinsky DA. Genetic hypercalciuric stone-forming rats. Curr Opin Nephrol Hypertens 1999; 8:479–488.

42 Mehes K, Szelid Z. Autosomal dominant inheritance of hypercalciuria. Eur J Pediatr 1980; 133:239–242.

43 Harangi F, Mehes K. Family investigatons in idiopathic hypercalciuria. Eur J Pediatr 1993; 152:64–68.

44 Polito C, La Manna A, Cioce F, *et al.* Clinical presentation and natural course of idiopathic hypercalciuria in children. Pediatr Nephrol 2000; 15:211–214.

45 Coe FL, Parks JH, Moore ES. Familial idiopathic hypercalciuria. N Engl J Med 1979; 300:337–340.

46 Bushinsky DA, Asplin JR. Thiazides reduce brushite, but not calcium oxalate, supersaturation and stone formation in genetic hypercalciuric stone-forming rats. J Am Soc Nephrol 2005; 16:417–424.

47 Asplin JR, Bushinsky DA, Singharetnam W, *et al.* Relationship between supersaturation and crystal inhibition in hypercalciuric rats. Kidney Int 1997; 51:640–645.

48 Bushinsky DA, Grynpas MD, Nilsson EL, *et al.* Stone formation in genetic hypercalciuric rats. Kidney Int 1995; 48:1705–1713.

49 Bushinsky DA, Asplin JR, Grynpas MD, *et al.* Calcium oxalate stone formation in genetic hypercalciuric stone-forming rats. Kidney Int 2002; 61: 975–987.

50 Karnauskas AJ, van Leeuwen JP, van den Bemd GJ, *et al.* Mechanism and •• function of high vitamin D receptor levels in genetic hypercalciuric stone-forming rats. J Bone Min Res 2005; 20:447–454.
This study supports the hypothesis that prolongation of VDR half-life increases VDR tissue levels and mediates increased VDR-regulated gene expression that result in hypercalciuria through actions on vitamin D-regulated calcium transport in intestine, bone, and kidney.

51 Krieger NS, Stathopoulos VM, Bushinsky DA. Increased sensitivity to $1,25(OH)_2D_3$ in bone from genetic hypercalciuric rats. Am J Physiol Cell Physiol 1996; 271:C130–C135.

52 Bushinsky DA, Bashir MA, Riordon DR, *et al.* Increased dietary oxalate does not increase urinary calcium oxalate saturation in hypercalciuric rats. Kidney Int 1999; 55:602–612.

53 Tsuruoka S, Bushinsky DA, Schwartz GJ. Defective renal calcium reabsorption in genetic hypercalciuric rats. Kidney Int 1997; 51: 1540–1547.

54 Bushinsky DA, Neumann KJ, Asplin J, Krieger NS. Alendronate decreases urine calcium and supersaturation in genetic hypercalciuric rats. Kidney Int 1999; 55:234–243.

55 Yao J, Kathpalia P, Bushinsky DA, Favus MJ. Hyperresponsiveness of vitamin D receptor gene expression to 1,25-dihydroxyvitamin D_3: a new characteristic of genetic hypercalciuric stone-forming rats. J Clin Invest 1998; 101: 2223–2232.

56 Evan AP, Bledsoe SB, Smith SB, Bushinsky DA. Calcium oxalate crystal localization and osteopontin immunostaining in genetic hypercalciuric stone-forming rats. Kidney Int 2004; 65:154–161.

57 Scheinman SJ, Cox JPD, Lloyd SE, *et al.* Isolated hypercalciuria with mutation in CLCN5: relevance to idiopathic hypercalciuria. Kidney Int 2000; 57:232–239.

58 Bushinsky DA, Grynpas MD, Asplin JR. Effect of acidosis on urine supersaturation and stone formation in genetic hypercalciuric stone forming rats. Kidney Int 2001; 59:1415–1423.

59 Bushinsky DA, LaPlante K, Asplin JR. Effect of cinacalcet on urine calcium excretion and supersaturation in genetic hypercalciuric stone-forming rats. Kidney Int 2006 (in press).

60 Hoopes RR, Reid R, Sen S, *et al.* Quantitative trait loci for hypercalciuria in a rat model of kidney stone disease. J Am Soc Nephrol 2003; 14:1844–1850.

61 Bushinsky DA. Bench to bedside: lessons from the genetic hypercalciuric stone forming rat. Am J Kidney Dis 2000; 36:61–64.

62 Frick KK, Bushinsky DA. Molecular mechanisms of primary hypercalciuria. J Am Soc Neph 2003; 14:1082–1095.

63 Lemann F J Jr. Pathogenesis of idiopathic hypercalciuria and nephrolithiasis. In: Coe FL, Favus MJ, editors. Disorders of bone and mineral metabolism. New York: Raven Press; 1992. pp. 685–706.

64 Henneman PH, Benedict PH, Forbes AP, Dudley HR. Idiopathic hypercalciuria. N Engl J Med 1958; 259:802–807.

65 Jackson WPU, Dancaster C. A consideration of the hypercalciuria in sarcoidosis, idiopathic hypercalciuria, and that produced by vitamin D. A new suggestion regarding calcium metabolism. J Clin Endocrinol Metab 1959; 19:658–681.

66 Edwards NA, Hodgkinson A. Metabolic studies in patients with idiopathic hypercalciuria. Clin Sci 1965; 29:143–157.

67 Liberman UA, Sperling O, Atsmon A, *et al.* Metabolic and calcium kinetic studies in idiopathic hypercalciuria. J Clin Invest 1968; 47:2580–2590.

68 Harrison AR. Some results of metabolic investigation in cases of renal stone. Br J Urol 1959; 31:398–403.

69 Dent CE, Harper CM, Parfitt AM. The effect of cellulose phosphate on calcium metabolism in patients with hypercalciuria. Clin Sci 1964; 27:417–425.

70 Nassim JR, Higgins BA. Control of idiopathic hypercalciuria. Br Med J 1965; 1:675–681.

71 Caniggia A, Gennari C, Cesari L. Intestinal absorption of ^{45}Ca in stone-forming patients. Br Med J 1965; 1:427–429.

72 Ehrig U, Harrison JE, Wilson DR. Effect of long-term thiazide therapy on intestinal calcium absorption in patients with recurrent renal calculi. Metabolism 1974; 23:139–149.

73 Barilla DE, Tolentino R, Kaplan RA, Pak CYC. Selective effects of thiazide on intestinal absorption of calcium in absorptive and renal hypercalciurias. Metabolism 1978; 27:125–131.

74 Zerwekh JE, Pak CYC. Selective effect of thiazide therapy on serum 1, 25-dihydroxyvitamin D, and intestinal absorption in renal and absorptive hypercalciuria. Metabolism 1980; 29:13–17.

75 Broadus AE, Dominguez M, Bartter FC. Pathophysiological studies in idiopathic hypercalciuria: use of an oral calcium tolerance test to characterize distinctive hypercalciuric subgroups. J Clin Endocrinol Metab 1978; 47: 751–760.

76 Bataille P, Bouillion R, Fournier A, *et al.* Increased plasma concentrations of total and free 1,25(OH)2D3 in calcium stone formers with idiopathic hypercalciuria. Contr Nephrol 1987; 58:137–142.

77 Duncombe VM, Watts RWE, Peters TJ. Studies on intestinal calcium absorption in patients with idiopathic hypercalciuria. Q J Med 1984; 209: 69–79.

78 Broadus AE, Insogna KL, Lang R, *et al.* Evidence for disordered control of 1,25-dihydroxyvitamin D production in absorptive hypercalciuria. N Engl J Med 1984; 311:73–80.

79 Bataille P, Achard JM, Fournier A, *et al.* Diet, vitamin D and vertebral mineral density in hypercalciuric calcium stone formers. Kidney Int 1991; 39:1193–1205.

80 Shen FH, Baylink DJ, Nielsen RL. Increased serum 1,25-dihydroxy cholecalciferol (1,25 $diOHD_3$) in patients with idiopathic hypercalciuria (IH). Clin Res 1975; 23:423A.

81 Haussler MR, Baylink DJ, Hughes MR. The assay of 1,25-dihydroxy vitamin D_3: physiologic and pathologic modulation of circulating hormone levels. Clin Endocrinol Metab 1976; 5:151S–165S.

82 Gray RW, Wilz DR, Caldas AE, Lemann F J Jr. The importance of phosphate in regulating plasma $1,25(OH)_2$ vitamin D levels in humans: studies in healthy subjects, in calcium stone formers and in patients with primary hyperparathyroidism. J Clin Endocrinol Metab 1977; 45:299–306.

83 Broadus AE, Insoqna KL, Lang R. A consideration of the hormonal basis and phosphate leak hypothesis of absorptive hypercalciuria. J Clin Endocrinol Metab 1984; 58:161–169.

84 Coe FL, Canterbury JM, Firpo JJ, Reiss E. Evidence for secondary hyperparathyroidism in idiopathic hypercalciuria. J Clin Invest 1973; 52:134–142.

85 Bordier P, Ryckewart A, Gueris J, Rasmussen H. On the pathogenesis of so-called idiopathic hypercalciuria. Am J Med 1977; 63:398–409.

86 Insogna KL, Broadus AE, Dryer BE, *et al.* Elevated production rate of 1,25-dihydroxyvitamin D in patients with absorptive hypercalciuria. J Clin Endocrinol Metab 1985; 61:490–495.

87 Barkin J, Wilson DR, Manuel MA, *et al.* Bone mineral content in idiopathic calcium nephrolithiasis. Min Electro Metab 1985; 11:19–24.

88 Pietschmann F, Breslau NA, Pak CYC. Reduced vertebral bone density in hypercalciuric nephrolithiasis. J Bone Miner Res 1992; 7:1383–1388.

89 Lawoyin S, Sismilich S, Browne R, Pak CYC. Bone mineral content in patients with calcium urolithiasis. Metabolism 1979; 28:1250–1254.

90 Alhava EM, Juuti M, Karjalainen P. Bone mineral density in patients with urolithiasis. Scand J Urol Nephrol 1976; 10:154–156.

91 Malluche H, Tschoepe W, Ritz E, *et al.* Abnormal bone histology in idiopathic hypercalciuria. J Clin Endocrinol Metab 1980; 50:654–658.

92 Maierhofer WJ, Lemann F J Jr, Gray RW, Cheung HS. Dietary calcium and serum 1,25(OH)2-vitamin D concentration as determinants of calcium balance in healthy men. Kidney Int 1984; 26:752–759.

93 Maierhofer WJ, Gray RW, Cheung HS, Lemann F J Jr. Bone resorption stimulated by elevated serum $1,25-(OH)_2$-vitamin D_3 concentrations in healthy men. Kidney Int 1983; 24:555–560.

94 Pak CYC, McGuire J, Peterson R, *et al.* Familial absorptive hypercalciuria in a large kindred. J Urol 1981; 126:717–719.

95 Tanaka H, Seino Y. Direct action of 1,25-dihydroxyvitamin D on bone: VDRKO bone shows excessive bone formation in normal mineral condition. J Steroid Biochem Mol Biol 2004; 89–90:343–345.

96 Sooy K, Sabbagh K, Demay MB. Osteoblasts lacking the vitamin D receptor display enhanced osteogenic potential in vitro. J Cell Biochem 2005; 94:81–87.

97 Hoenderop JGJ, Bindels RJM. Epithelial Ca2+ and Mg2+ channels in health and disease. J Am Soc Nephrol 2005; 16:15–26.

98 Favus MJ. Hypercalciuria: lessons from studies of genetic hypercalciuric rats. J Am Soc Nephrol 1994; 5:S54–S58.

99 Pols HAP, Birkenhager JC, Schilte JP, Visser TJ. Evidence that self-induced metabolism of 1,25-dihydroxyvitamin D3 limits the homologous up-regulation of its receptor in rat osteosarcoma cells. Biochim Biophys Acta 1988; 970: 122–129.

100 Reinhardt TA, Horst RL. Self-induction of 1,25-dihydroxyvitamin D3 metabolism limits receptor occupancy and target tissue responsiveness. J Biol Chem 1989; 264:15917–15921.

101 Favus MJ, Mangelsdorf DJ, Tembe V, *et al.* Evidence for in vivo upregulation of the intestinal vitamin D receptor during dietary calcium restriction in the rat. J Clin Invest 1988; 82:218–224.

102 Strom M, Sandgren ME, Brown TA, DeLuca HF. 1,25-Dihydroxyvitamin D3 up-regulated the 1,25 dihydroxyvitamin D3 receptor in vivo. Proc Natl Acad Sci USA 1989; 86:9770–9773.

103 Sandgren M, DeLuca HF. Serum calcium and vitamin D regulate 1,25-dihydroxyvitamin D3 receptor concentration in rat kidney in vivo. Proc Natl Acad Sci USA 1990; 87:4312–4314.

104 Chen TL, Hauschka PV, Cabrales S, Feldman D. The effects of 1,25-dihydroxyvitamin D_3 and dexamethasone on rat osteoblast-like cell cultures: receptor occupancy and functional expression patterns for three different bioresponses. Endocrinology 1986; 118:250–259.

105 Costa EM, Hirst MA, Feldman D. Regulation of 1,25-dihydroxyvitamin D_3 receptors by analogs in cultured mammalian cells. Endocrinology 1985; 117:2203–2210.

106 Favus MJ, Karnauskas AJ, Parks JH, Coe FL. Peripheral blood monocyte vitamin D receptor levels are elevated in patients with idiopathic hypercalciuria. J Clin Endocrinol Metab 2004; 89:4937–4943.

107 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 USA 1995; 92:131–135.

108 Goodman WG. Calcium-sensing receptors. Semin Nephrol 2004; 23:17–24.

109 Brown EM, MacLeod RJ. Extracellular calcium sensing and extracellular calcium signaling. Physiol Rev 2001; 81:239–297.

110 Canaff L, Hendy GN. Human calclium-sensing receptor gene Vitamin D response elements in promoters P1 and P2 confer transcriptional responsiveness to 1,25-dihydroxyvitamin D. J Biol Chem 2002; 277:30337–30350.

111 • Yao J, Karnauskas AJ, Bushinsky DA, Favus MJ. Regulation of renal calcium-sensing receptor gene expression in response to $1,25(OH)_2D_3$ in genetic hypercalciuric stone forming rats. J Am Soc Nephrol 2005; 16:1300–1308.
The GHS rats were found to have high levels of CaR gene expression and CaR protein that may contribute to hypercalciuria and calcium nephrolithiasis.

112 Quarles LD, Sherrard DJ, Adler S, *et al.* The calcimimetic AMG 073 as a potential treatment for secondary hyperparathyroidism of end-stage renal disease. J Am Soc Neph 2003; 14:575–583.

113 Drueke TB. Modulation and action of the calcium-sensing receptor. Nephrol Dial Transplant 2004; 19:20–26.

114 Block GA, Martin KJ, de Francisco AL, *et al.* Cinacalcet for secondary hyperparathyroidism in patients receiving hemodialysis. N Eng J Med 2004; 350:1516–1525.

115 Coe FL, Parks JH. New insights into the pathophysiology and treatment of nephrolithiasis: New research venues. J Bone Miner Res 1997; 12:522–533.

116 Evan AP, Lingeman JE, Coe FL, *et al.* Randall plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. J Clin Invest 2003; 111:607–616.

117 Carr RJ. A new theory on the formation of renal calculi. Br J Urol 1953; 26: 105–117.

118 Price PA, Lim JE. The inhibition of calcium phosphate precipitation by fetuin is accompanied by the formation of a fetuin-mineral complex. J Biol Chem 2003; 278:22144–22152.

119 Fischer JW, Steitz SA, Johnson PY, *et al.* Decorin promotes aortic smooth muscle cell calcification and colocalizes to calcified regions in human atherosclerotic lesions. Arterioscler Thromb Vasc Biol 2004; 24:2391–2396.

120 Rosen CJ. Insulin-like growth factor I and bone mineral density: experience from animal models and human observational studies. Best Pract Res Clin Endocrinol Metab 2004; 18:423–435.

121 Rosen CJ, Ackert-Bicknell C, Beamer WG, *et al.* Allelic differences in a quantitative trait locus affecting insulin-like growth factor-I impact skeletal acquisition and body composition. Pediatr Nephrol 2005; 20: 255–260.

122 Kasukawa Y, Baylink DJ, Wergedall JE, *et al.* Lack of insulin-like growth factor I exaggerates the effect of calcium deficiency on bone accretion in mice. Endocrinology 2003; 144:4682–4689.