Nephrocalcinosis: molecular insights into calcium precipitation within the kidney

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ABSTRACT

Nephrocalcinosis may be defined as a generalized increase in the calcium content of the kidneys. This renal calcification may occur at a molecular, microscopic or macroscopic level leading to progressive amounts of renal damage. The major causes include those associated with an increase in urinary levels of calcium, oxalate and phosphate. Under these conditions, urine concentration and supersaturation leads to calcium crystal precipitation, which may be an intratubular event or initiate within the renal interstitium. The focus of discussion concerning renal calcification is often limited to factors that lead to renal stones (calculi and nephrolithiasis); however, nephrocalcinosis is a more sinister event, and often implies a serious metabolic defect. This review will discuss the hypotheses concerning initiating lesions of nephrocalcinosis using available laboratory and clinical studies and will examine whether new understanding of the molecular basis of tubulopathies, that lead to nephrocalcinosis, has given further insights.

INTRODUCTION

Nephrocalcinosis may be defined as a generalized increase in the calcium content of the kidney. This increase may reveal itself at a functional level, a histological/microscopic level and eventually at a macroscopic level. Nephrocalcinosis does not necessarily lead to renal calculi, and renal calculi may occur in the apparent absence of (macroscopic) nephrocalcinosis. These two pathologies are therefore distinct, but are intimately related. Medullary nephrocalcinosis is the typical pattern seen in 98% of cases of human nephrocalcinosis [1], where clusters of calcification occur around each renal pyramid. This finding seems to be most commonly associated with overt hypercalcaemia or hypercalciuria, as seen in hyperparathyroidism, for example. The precise cellular events and even the histopathology of such calcification, however, remain in doubt. Thus the exact location of renal calcification may occur at several different sites, such as the renal tubular cells, the interstitium or within the tubular lumen. Each location may be peculiar to the precipitating factors and even the type of crystal formed. The key question is therefore to understand the site of initial calcification and how calcium, phosphate and oxalate ions within the specific anatomical context contribute to the development of nephrocalcinosis. Only recently have studies allowed the precise anatomical location within the kidney to be determined. With this knowledge, there is the even more difficult task of predicting the movement of precipitating ions into each anatomical location.

This review serves to highlight the current understanding of nephrocalcinosis in terms of tubular molecular physiology and renal handling of calcium and anions. Human diseases with a propensity for nephrocalcinosis will then be reviewed within this model to hypothesize mechanisms involved in this complex disorder.

Key words: calcium oxalate, calcium phosphate, hypercalciuria, hyperoxaluria, nephrocalcinosis, renal papilla, tubular transport.

Abbreviations: CaR, calcium-sensing receptor; dRTA, distal renal tubular acidosis; GHS, genetic hypercalciuric stone-forming; IMCD, inner medullary collecting duct; NCX, Na+/Ca2+ exchanger; PMCA, plasma membrane Ca2+-ATPase; TAL, thick ascending limb; TPC1, two-pore channel 1; TRPV, transient receptor potential channel superfamily V.

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RENAL HANDLING OF CALCIUM, PHOSPHATE AND OXALATE

Prior understanding of the tubular transport of calcium, phosphate and oxalate is required in order to formulate mechanistic hypotheses underlying nephrocalcinosis (Figure 1). Firstly, with respect to calcium, the glomerular filtrate contains ionized calcium and calcium complexed to anions such as phosphate and citrate. Protein-bound calcium, representing approx. 50 % of plasma calcium, is unavailable for filtration at the glomerulus. Virtually all (98 %) of the filtered calcium is reabsorbed by the tubule, with the proximal tubule reabsorbing approx. 65 % [2]. This is largely by the paracellular route, driven by the lumen positive potential difference in the S3 (pars recta) segment of the proximal tubule. Urinary and serum calcium concentrations are sensed along the length of the nephron by CaR (calcium-sensing receptor). In the rat proximal tubule, CaR expression is localized to apical membrane [3], where its activation by luminal calcium may limit PTH (parathyroid hormone)-stimulated 1,25-dihydroxyvitamin \( \text{D}_3 \) production and phosphate excretion, and regulate proximal tubule volume reabsorption [4].

Beyond the proximal tubule, there is no evidence for calcium transport (even of limited passive permeability) in the thin descending limb or thin ascending limb of Henle, whereas the TAL (thick ascending limb) of Henle accounts for 20–25 % of calcium reabsorption [2]. This is again paracellular, driven by a lumen positive transtubular voltage and generated by NaCl reabsorption and potassium recycling at the lumen membrane. CaR expression on the basolateral (blood) side of TAL allows regulation of NaCl transport, potential difference and, hence, reabsorbed calcium [3]. Stimulation of the basolateral CaR in the medullary TAL serves to impair NaCl reabsorption and impair the countercurrent mechanism allowing calcium excretion to occur in a less concentrated urine, limiting the risk of calcium salt precipitation [5]. Stimulation of the cortical TAL CaR, where most of the TAL calcium reabsorption takes place, serves to limit potassium recycling via PLA\(_2\) (phospholipase A\(_2\))-mediated arachidonic acid production and its metabolism to 20-HETE (20-hydroxyeicosatetraenoic acid) [6], thus decreasing NaCl uptake and subsequently divalent cation reabsorption.

Finely regulated reabsorption of 8–10 % of filtered calcium occurs in the distal tubule [2]. Here, calcium is reabsorbed actively by transepithelial processes against a transtubular electrochemical gradient and may be regulated independently of sodium reabsorption. Calcium enters the cell via apical TRPV5 (also known as ECaC1) entry channels, a member of the transient receptor potential channel superfamily V [7]. In human kidney, TRPV6 (also known as CAT1 and ECaC2) may also participate as an apical entry pathway, but this is
controversial [8,9]. There is evidence of co-expression of TRPV5 (ECaC1) with the sodium influx channel ENaC in the mouse late distal tubule [10]. Here, a high-sodium load leading to high sodium influx would depolarize the apical membrane and lead to urinary calcium wasting. This may provide a basis for the hypercalciuria sometimes seen in Liddle’s syndrome [11]. Having entered into the cell, calcium is moved across the cell by calcium-binding proteins, such as calbindin, and exits the cell basolaterally, via NCX (Na+/Ca2+ exchanger) and PMCA (plasma membrane Ca2+-ATPase) [7]. In mice lacking TRPV5, there is renal calcium wasting due to a transport defect along the distal convoluted tubule [12]. Regulation of the apical entry calcium transport pathway is multifactorial and includes regulation by CaR [13]. Excessive influx above the efflux capacity of the combined NCX and PMCA pathways would not only increase transtubular transport, but also result in calcium overload and intracellular calcium precipitates. The initial stages of medullary nephrocalcinosis in rats as shown by electron microscopy studies reveal near-basement calcium-crystal deposits (after transtubular transport) and intracellular crystals of calcium phosphate within tubular mitochondria and vacuoles [14,15].

In rat IMCDs (inner medullary collecting ducts), studies on isolated microperfused segments [16] indicate an active calcium reabsorption exists from perfusion bath to lumen. Transport is consistent with an apical calcium entry step, followed by NCX/PMCA-mediated calcium extrusion at the basolateral membrane. Both NCX and PMCA are expressed along the basolateral membrane of collecting duct (as well as the distal convoluted tubule) in human kidney, in contrast with the mouse [17]. Since TRPV5 (ECaC1) is not expressed in the IMCD, an additional calcium-leak pathway must be present. Also in the IMCD, CaR expression is apical and serves to regulate calcium and water reabsorption [18]. At this location, it is hypothesized that hypercalciuria would result in CaR activation and limit vasopressin-stimulated insertion of AQP2 (aquaporin-2) water channels into the apical membrane, thereby limiting urinary concentration in this final nephron segment [18]. As to the identity of the calcium-leak pathway in IMCD, recent experimental data point to a novel apical channel, namely TPC1 (two-pore channel 1) [19,20]. Immunocytochemical analysis has revealed expression of TPC1 at the apical membrane of the rat IMCD [20]. Although of minor quantitative significance to the absorption of the filtered tubular load of calcium, transport of calcium at the IMCD is of special significance for delivery of calcium to the papillary interstitium.

Understanding of renal phosphate transport is focused on transport at the proximal tubule [21]. Approx. 80 % of plasma phosphate is filtered by the glomerulus and, depending upon phosphate balance, 70–100 % of the filtered load is subsequently reabsorbed mainly in the proximal tubule, mediated by apical Na/Pi cotransporters. Luminal expression of Na/Pi is regulated by PTH and dietary phosphate intake, with increases in PTH and serum phosphate causing endosomal retrieval of the renal Na/Pi-2a isoform from the brush-border membrane, thus increasing phosphaturia [22]. Enhanced extracellular fluid volume also increases renal phosphate excretion by decreasing sodium reabsorption in the proximal tubule. Thyroid hormone has the opposite effect causing phosphate reabsorption by increasing Na/Pi expression [23].

As well as the proximal tubule, Na/P expression determined by RT (reverse transcriptase)-PCR was observed in the collecting ducts [24], but was absent from the TAL. Thin segments of Henle’s loop are judged to be essentially impermeable to phosphate in microperfusion studies using isolated rabbit tubules [25]. In contrast, marked phosphate reabsorption in the ‘loop of Henle’ was seen using micropuncture techniques in thyroparathyroidectomized rats [26], although it is likely that S3 proximal tubule segments would have been a confounding factor in these experiments. These micropuncture data are also limited by technical difficulties, interspecies variation and inter-nephron variation and, hence, we know of no data that describes phosphate transport in the human loop of Henle. Phosphate reabsorption continues in the distal tubules and is also influenced by PTH. There is pharmacological evidence that Na/Pi cotransport occurs within this nephron segment [27], but it is still unclear which molecular isoforms are responsible [28].

Collecting duct phosphate reabsorption may continue to be mediated by Na/Pi isoforms [24] and would allow for the final regulation of urinary phosphate. The ability of nephron segments to reabsorb phosphate as required may be an important defence against calcium phosphate precipitation.

In Na/Pi-2 cotransporter gene knock-out in mice (npt2-/-), marked hyperphosphaturia and secondary hypercalciuria generate nephrocalcinosis, evident as early as birth [29]. This finding matches the clinical phenotype (hypophosphataemia, hypercalciuria and nephrolithiasis) of a patient with a mutation of the type 2a Na/Pi cotransporter gene [30]. In adult npt2-/- mice, nephrocalcinosis is evident in distal cortical segments and within the inner medulla. The exact location of these calcium phosphate (apatite) deposits in knock-out animals may have included an intratubular location within collecting ducts, but the considerable tissue distortion made unambiguous assignment difficult [29].

The possible occurrence of Na/Pi transporter expression in IMCD may also be extended by the recent observation of renal expression of the ank gene [31]. ANKH or the 100 % homologous ank mouse protein functions as a transmembrane pyrophosphate export transporter [31,32]. PPi (pyrophosphate), a potent inhibitor of calcification [33], is present in urine and extracellular fluid and is known to limit calcium phosphate crystallization.
ANK expression in the collecting duct would allow the exit of pyrophosphate from cells into the lumen, where it would act as an inhibitor of crystallization. In our own experiments, we have confirmed ANK expression by RT-PCR in mouse IMCD cells (J. A. Sayer, unpublished work). Given the limited biological half-life of PPi, due to pyrophosphatase activity at the cell surface, phosphate produced in this manner would provide substrate for IMCD Na/Pi-2-mediated scavenging.

Oxalate is freely filtered at the glomerulus [34] and its urinary excretion is increased by the renal tubular epithelium, by both passive and active means. Perfused segments of the proximal tubule in rabbit demonstrate net oxalate secretion [35]. Anion exchange proteins in the proximal tubule mediate transcellular oxalate excretion and recycling at the brush-border membrane. Recently identified anion exchangers of the SLC26 family allow for oxalate loss in exchange for chloride, then oxalate uptake in exchange for sulphate loss, energized by Na-sulphate transport in the proximal tubule [36]. Tubular transport of oxalate in other nephron segments has not been defined precisely. SLC26A1 (Sat-1) immunolocalization in rat kidney is consistent with restriction to S1–S3 proximal segments [37]. It should be noted that the SLC26A4 protein (pendrin) highly expressed in the β-intercalated cells of the collecting duct cannot transport oxalate [38]. The renal papilla may be a site for oxalate regulation, as rabbit papillary surfaces exposed to oxalate-containing solutions demonstrated net oxalate absorption [39].

**SITE OF INITIAL RENAL CALCIFICATION**

Alexander Randall [40] described calcium phosphate deposits lying immediately under the papillary epithelium which appeared to be ‘the initiating lesions of renal calculus’. In a summary of his findings, Randall described deposits in 20% of over 1000 necropsies, of which a quarter showed adherent urinary calculi lying in the calyceal system [41]. Are these findings relevant today? Researchers are still trying to solve the ‘riddle of Randall’s plaques’ [42–45]. Randall’s plaques seem to be sites of interstitial crystal deposition at or near the papillary tip and are found in 88% of calcium oxalate stone-formers and 100% of calcium phosphate stone-formers, but are also present in 43% of non-stone-formers [45]. A key insight into our understanding was an examination of biopsy tissue through plaques in the kidneys of patients with calculi, who underwent percutaneous nephrolithotomy [44]. This revealed that Randall’s plaques range from the most minimal scattered calcium phosphate (apatite) deposits located in the basement membranes of the thin loops of Henle, to extensive apatite plaques extending from the basement membrane of the thin loops to the papillary interstitium. In hypercalciuric calcium oxalate stone-formers, the initial crystals were composed of calcium phosphate and located within the basement membrane of the thin limbs of the loop of Henle [44]. These were seen to enlarge into the surrounding interstitium and involve the vasa recta, with larger lesions reaching the basement membrane of the collecting duct and the renal papillae. Remarkably, the cells of the thin loops of Henle and those of the collecting duct appeared unaffected; indeed, no crystals within cells were observed [44]. Is this apparent initial location of renal calcification surprising? Evan et al. [44] argue that the thin limb basement membrane, rich in mucopolysaccharide, offers ionic sites for calcium and phosphate binding, allowing crystal formation, binding and growth. If this plaque subsequently penetrates the urothelium it would act as an anchored site and allow intratubular stone formation.

Previously, animal micropuncture studies have confirmed that fluid in the loops of Henle is able to initiate calcium phosphate crystallization [46]. However, this new understanding of Randall’s plaque argues against intratubular crystallization in cases of hypercalciuria leading to calcium oxalate stones. Bushinsky [47] speculates that, following an increased renal load of calcium, calcium reabsorbed from the TAL would be concentrated in the renal papilla by the medullary countercurrent mechanism. Here, local factors (including pH and basement membrane charge) within the inner medullary interstitium will be crucial. However, it should be noted that a recycling mechanism for calcium equivalent to that observed for urea (urea entry into thin ascending limb, luminal concentration and urea entry into the medullary interstitium from collecting duct [48–50]) has not been identified. Once initiated, the growing plaque of crystal and collagen matrix may rupture into the urinary space, so acting as an anchored site for calcium oxalate adhesion and the formation of a calcium oxalate stone. Continued growth of the crystal collagen matrix, but without rupture of the urothelium, would lead to a patch of medullary nephrocalcinosis, namely an unruptured Randall’s plaque.

Interestingly, in the same study, intestinal bypass patients were noted to display a different pattern of microscopic crystal formation [44]. Importantly, these patients did not exhibit persistent hypercalciuria, rather the underlying metabolic defect was hyperoxaluria. Here, intraluminal calcium phosphate (apatite) crystal formation within the papillary collecting ducts occurred, also described previously by Randall [51] as type 2 deposits. What is remarkable is that the initial crystal type in these patients was also calcium phosphate (apatite) and in this location, contact with urine supersaturated with respect to calcium oxalate would promote heterologous nucleation. Thus intraluminal calcium phosphate crystallization must be occurring within the lumen of the distal nephron at peaks of supersaturation, with perhaps obstruction of ducts of Bellini being the initiating lesion [52], leading to further crystal growth and a renal
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Figure 2  Renal papillary model of nephrocalcinosis
Collecting ducts (CD) together with descending loops of Henle (DLH) and ascending thin loops of Henle (tALH) and vasa recta (VR) are shown through a cross-section of a renal papilla. Possible initial sites of nephrocalcinosis are shown as shaded regions. Mechanisms leading to calcification may include: (1) \( \text{Pi} \) permeability at the papillary thin loops of Henle would allow interstitial loading of phosphate; (2) collecting duct absorption of calcium, possibly via apical calcium channels (e.g. TPC1) and basolateral calcium exit would allow delivery of calcium ions to the interstitium; Na/Pi cotransport at a collecting duct location would also provide additional phosphate ions, which may be derived from PPI delivery into the lumen via ANK; (3) concentration of oxalate from the urinary space into the papillary interstitium allows delivery of oxalate ions; (4) intraluminal crystal formation, both from the loop of Henle (calcium phosphate) and collecting duct (calcium oxalate) may adhere to collecting duct epithelial surfaces, then by endocytosis/transcytosis the crystals are delivered to the papillary interstitium and accumulate. Dissolution by epithelial cells or by interstitial cells (including macrophages) may provide a clearance mechanism.

calculus. This mechanism would only lead to nephrocalcinosis if there was subsequent overgrowth and crystal encapsulation.

CENTRAL ROLE OF THE RENAL PAPILLA?
Thus, knowing these sites of initiating calcification, is it possible to reconcile mechanisms of calcium phosphate and oxalate transport in the thin descending and thin ascending limbs of the loops of Henle and the collecting duct, with both interstitial and tubular lumen loci for initial crystallization?

Interstitial crystallization
Tubular fluid in the papillary loops of Henle is supersaturated with respect to calcium and phosphate ions [46]. Passive fluid reabsorption due to medullary hypertonicity (due to urea) will increase the risk of calcium phosphate precipitation in this nephron segment. The low permeability to NaCl in the thin descending limb of Henle is not seen in the thin ascending limb of Henle, where NaCl permeability is high, allowing its exit to the medullary interstitium [48–50]. The chloride permeability of the thin ascending limb of Henle is mediated via CLC-K1 chloride channels, which, when ablated in mouse knock-out models, results in diabetes insipidus, due to a failure of the concentration mechanisms [53,54]. As already mentioned, the thin loops appear to have low permeabilities to both calcium and phosphate ions [25]. Their tubular fluid/plasma concentration ratios in both thin descending and ascending loop fluid exactly parallel those for inulin, indicating no loss upon transit through the thin loops. The key questions, therefore, are what factors prevent intratubular crystal formation and, for interstitial calcium phosphate deposits, what is the origin of calcium and phosphate ions resulting in their accumulation within the papilla to the extent that precipitation occurs?

The histological location of the initiating plaque is at the boundaries of three separate epithelia, primarily the thin loops of Henle, the papillary urothelium and the collecting duct (Figure 2). A transtubular phosphate permeability at the papillary thin loops would allow removal of this anion and decrease intratubular precipitation, but increase interstitial phosphate thus promoting precipitation. In idiopathic calcium stone-formers, the nature of the presumed phosphate permeability in thin loops is likely to reflect a primary event in the pathophysiological progression. Calcium and phosphate are likely to be
Intraluminal crystallization

It has been noted that crystals in urine are larger and more aggregated in stone-formers than non-stone-formers [61,62]. Non-stone-formers do have crystalluria but, despite this, remain stone free. Therefore intratubular mechanisms must exist to minimize and limit intraluminal and attached crystals. In the intratubular environment, whether calcium phosphate crystals form will be dependent upon the urinary calcium and phosphate load, pH and concentration of inhibitors of crystallization, such as citrate, urinary macromolecules and pyrophosphate. The GHS (genetic hypercalciuric stone-forming) rat is a well-characterized animal model resulting from inbreeding of Sprague–Dawley rats. GHS rats have hypercalciuria resembling human idiopathic hypercalciuria, with excessive intestinal calcium absorption, increased bone resorption and impaired renal calcium reabsorption [63]. GHS rats produce calcium phosphate stones with crystallization occurring only within the urinary space and an absence of nephrocalcinosis [64]. Only after addition of the oxalate precursor hydroxyproline do these animals produce calcium oxalate stones [65] with evidence that this also is an intraluminal process [64]. These calcium oxalate crystals, unlike the calcium phosphate crystals, appeared to induce urothelial proliferation, resulting in crystal entrapment within the interstitium [64]. In the intestinal bypass patients of Evan et al. [44], hyperoxaluria is combined with low values of urinary citrate and, in these conditions, early crystal attachment to the apical surface of collecting duct cells is observed. In some instances, crystallization of the whole tubule lumen occurred. As already noted, analysis of the chemical composition of crystals indicated apatite (phosphate) composition, despite hyperoxaluria.

Where collecting duct fluid becomes supersaturated with respect to calcium oxalate, preformed calcium phosphate crystals from earlier nephron segments or formed locally at the epithelial surface act as nuclei for heterogeneous precipitation. Oxalate transport across the papillary urothelium, crystal inhibitors and a decrease in pH would all serve to protect against local crystallization events. A physical protection may also result from papillary smooth muscle contractions [56]. These transiently occlude the Ducts of Bellini and terminal IMCD; on relaxation of the papilla, a pulse of urine will flush the epithelial surface, thus limiting crystal attachment. Thus, despite overall low fluid flow rates in a concentrating kidney, higher flow and, hence, shear rates are transiently achieved. The potential for mechanical damage to the epithelium at the renal papilla, secondary to contraction against a renal calculus, can only be speculated.

Crystal adhesion, internalization and retention

How does the renal tubular epithelium behave when exposed to microcrystals formed within the lumen and attaching to the apical cell surfaces? Studies using renal epithelial cell lines show that calcium phosphate [66–67] and COM (calcium oxalate monohydrate) crystals [68,69] may bind to the apical cell surface. Specific cell-surface receptors are required for crystal binding and these are strictly regulated. These include CD44 [70], osteopontin [71], hyaluronan [70,72] and annexin II [73]. Up-regulation of these proteins may lead to increased binding and attachment of initial calcium microcrystals, although there is evidence that osteopontin has a protective role against retention [74]. Crystal binding requires anionic sites and may be inhibited by urinary anions, such as uropontin, nephrocalcin, citrate and glycosaminoglycans [75]. Once bound, both calcium phosphate and calcium oxalate crystals are injurious to renal epithelial cells, mediated, in part, by reactive oxygen species [76,77] and modulated by macromolecules (bikunin and osteopontin) [78]. Calcium oxalate crystals induce DNA synthesis and cellular proliferation [64,79–81] and stimulate downstream signalling events, including p38 MAPK (mitogen-activated protein kinase) activation in LLC-PK1 cells [82]. Internalization of calcium phosphate and calcium oxalate crystals via endocytosis is known to occur in...
cultured renal epithelial cells [83,84] and is a postulated physiological clearance mechanism [85]. Internalization of crystals also promotes changes in gene expression, cytoskeletal organization and induces cellular proliferation [86,87]. In a chronic rat model, where a crystal-inducing diet and ethylene glycol lead to intraluminal crystal formation and surface attachment, local proliferation caused overgrowth of a new epithelial layer, resulting in 'interstitial' (sub-epithelial) calcium oxalate crystals [88]. Adherence and uptake of crystals, followed by their 'translocation' to the renal interstitium, may be a minor contributor to interstitial crystal deposits [89,90], but is unlikely to provide the basis for the substantial deposits observed in idiopathic stone-formers by Evan et al. [44].

**TUBULOPATHIES CAUSING NEPHROCALCINOSIS: IS THE PAPILLARY MODEL ROBUST?**

Table 1 lists a number of tubulopathies whose molecular defect and primary tubular localization are known. All result in nephrocalcinosis, with the majority also giving rise to nephrolithiasis. Figure 2 outlines a model in which delivery of precipitating ions to the inner medullary interstitium and the local conditions determine the precise histology of crystal deposition. An additional and striking feature of Table 1 is the almost universal occurrence of hypercalciuria. As already noted, a key tenet of the model is that calcium reabsorption by the
IMCD is responsive to the electrochemical driving force for calcium entry at the apical membrane determined, in part, by hypercalciuria. Clearly, hypercalciuria may not only promote interstitial nephrocalcinosis, but also intratubular crystallization, with both processes ultimately resulting in nephrolithiasis. In detail then, how do selected diseases give rise to nephrocalcinosis?

**Bartter’s syndrome**
The molecular basis of this inherited tubulopathy leading to metabolic alkalosis is now established. Bartter’s syndrome, unlike Gitelman’s syndrome, is associated with nephrocalcinosis and nephrolithiasis [91]. Defects in the apical TAL sodium/potassium/chloride cotransporter NKCC2 [92], the apical ROMK (renal outer medullary K+ channel) [93] and the basolateral chloride channel CLC-Kb [94], together with its subunit barttin [95], give rise to inhibition of TAL NaCl tubular transport and diminution of the transtubular potential difference, thus decreasing paracellular calcium transport. Salt wasting, hypercalciuria and metabolic alkalosis, resulting from aldosterone-stimulated volume contraction, are thus primary, secondary and tertiary aspects of the disease. Renal failure can occur with Barttin mutations, where children develop chronic renal insufficiency secondary to chronic tubulo-interstitial fibrosis and atrophy [96], but is not universal [97].

The biochemical findings in Bartter’s syndrome mimic those of prolonged or high-dose exposure to loop diuretics, such as frusemide, leading to hypokalaemia, metabolic alkalosis, hypercalciuria and NaCl wasting. It is noteworthy that the presence of neonatal nephrocalcinosis has been attributed, at least in part, to use of high-dose frusemide to treat pulmonary oedema in neonates [98]. Severe gain-of-function mutations affecting TAL CaR can also result in a Bartter-like phenotype, because activation of this G-protein-coupled receptor inhibits salt transport in the TAL [99]. The presence of hypercalciuria in Bartter’s syndrome seems to determine the presence of nephrocalcinosis. Gitelman’s syndrome, where mutations affect the thiazide-sensitive transporter, NCCT (NaCl cotransporter), in the distal convoluted tubule, may be distinguished from Bartter’s syndrome by the presence of normocalcaemic hypocalciuria and hypomagnesaemia [91]. The absence of hypercalciuria leads to a corresponding lack of nephrocalcinosis. It has recently become apparent, however, that CLC-Kb mutations may give rise to a mixed Bartter–Gitelman’s syndrome phenotype [100]. Three unrelated patients had childhood salt-wasting episodes suggestive of Bartter’s syndrome, with the subsequent development of hypomagnesaemia and hypocalciuria. These data may be explained on the basis that CLC-Kb is not confined to the TAL, but is also expressed as a basolateral chloride channel in the early distal convoluted tubule. Here, in the presence of the apical thiazide-sensitive NaCl transporter, chloride accumulation above its electrochemical equilibrium holds the cell membrane potential at a depolarized level by chloride exit via CLC-Kb. The driving force for calcium entry via TRPV5 (ECaC1) is thus lowered. When NCCT or CLC-Kb is mutated, the membrane is hyperpolarized and apical calcium entry and, hence, tubular absorption is enhanced [7].

Thus the hypercalciuria seen in Bartter’s syndrome is as a result of defective TAL calcium reabsorption, allowing increased delivery of calcium to a collecting duct location. Unfortunately, the precise histopathological lesion has not been determined. In Bartter’s original report [101], histological sections (of glomeruli and juxtaglomerular apparatus) failed to show any nephrocalcinosis; however, subsequent histological reports have revealed both tubular and interstitial calcifications [102], but give little further insight into the exact location of the initial lesions.

**Hypomagnesaemic hypercalciuric nephrocalcinosis**
Hypomagnesaemic hypercalciuric nephrocalcinosis is a rare autosomal-recessive disorder, which is characterized by marked nephrocalcinosis and nephrolithiasis, associated with hypercalciuria and hypomagnesaemia [103]. Progressive renal failure secondary to nephrocalcinosis occurs by late childhood. The underlying abnormality is a defective TAL tight-junction protein named paracellin (claudin-16) [104] that allows the normal paracellular absorption of calcium and magnesium driven by the transtubular potential difference. Nephrocalcinosis is typically severe in this disorder, so can we understand the underlying molecular mechanisms? Defective paracellin protein, which is expressed in the TAL and the distal convoluted tubule, leads to wasting losses of magnesium and calcium in the urine. Similar to Bartter’s syndrome, delivery of calcium to the medullary interstitium cannot arise from TAL transport and medullary blood flow. The only feasible mechanism is via transcellular transport at the IMCD from the elevated calcium concentrations within the tubular fluid. Additionally, this would lead to intratubular crystallization events downstream from the TAL and distal convoluted tubule. Renal biopsy in affected patients demonstrated a severe medullary nephrocalcinosis and tubular atrophy [105], reflecting established renal failure, and does not allow additional insights into the primary calcification lesions.

**Dent’s disease**
Dent’s disease is a familial X-linked renal stone disease characterized by hypercalciuria, but also with a proximal tubular Fanconi-like defect, leading to low-molecular mass proteinuria and phosphaturia [106,107]. The majority of males develop nephrocalcinosis and renal failure. Women have low-molecular mass proteinuria and sometimes hypercalciuria and are usually spared from
forms of Fanconi syndrome. There are a few cases where female patients have been reported to have nephrolithiasis [106,109,110]. Medullary nephrocalcinosis is unusual in renal Fanconi syndromes and this phenotype in males distinguishes Dent’s disease from other familial forms of Fanconi syndrome.

Of the murine models of Dent’s disease, only one is reported to exhibit nephrocalcinosis [111]. In this model, disruption of exon VI of the CLCN5 gene led to the absence of CLC-5 protein expression. Biochemical features of these animals included low-molecular mass proteinuria, hyperphosphaturia and hypercalciuria [111]. Histological sectioning of kidneys from CLC-5-deficient (−/−) mice using von-Kossa staining demonstrated microscopic nephrocalcinosis at the cortico-medullary junction [111]. Hypercalciuria present in this mouse model almost certainly contributes to the nephrocalcinosis.

A second model created by Piwon et al. [112] used deletion of genomic CLC-5 sequence, which resulted in complete absence of CLC-5 protein. This resulted in low-molecular mass proteinuria, but an absence of hypercalciuria. Despite an up-regulation of α-hydroxylase levels, it has been suggested that urinary loss of vitamin D precursors, secondary to defective proximal tubular endocytosis, prevented a serum rise in active vitamin D levels and, therefore, hypercalciuria [113]. Consequently, in the absence of hypercalciuria, there was no evidence of nephrocalcinosis [112]. A recent review highlighted the difference in the proprietary mouse diets used in these two models. There was absence of hypercalciuria in the Piwon et al. model, where chow contained 1 IU/g of vitamin D3 compared with up to 4.5 IU/g of vitamin D3 in the hypercalciuric model developed by Wang et al. [111]. This may have impacted on the subsequent development of nephrocalcinosis. The third murine model, developed by Luyckx and co-workers [114], only exhibited hypercalciuria under certain feeding conditions and was abolished by dietary calcium removal. Low-molecular mass proteinuria, the marker of proximal tubular endocytic dysfunction, was absent in this model, limiting its applicability to the human disease.

In clinical studies [115], thiazide diuretics have been used successfully to limit the degree of hypercalciuria in Dent’s disease, which is likely to correspond with a decrease in both nephrocalcinosis and renal stone formation. This clinical finding implies that the molecular mechanisms of calcium handling remain intact within the distal convoluted tubule, implicating decreased calcium reabsorption in the proximal tubule and TAL. CLC-5 protein is expressed not only in the proximal tubule, but also in the medullary TAL of Henle and α-intercalated cells of the collecting duct. The contribution of CLC-5 dysfunction at these locations to the overall phenotype is not clear. Our own work [85] has proposed a function in collecting duct cells in which a common cellular role for CLC-5 within recycling endosomes in the collecting duct leads to altered handling of precipitating microcrystals. Renal histology from patients with Dent’s disease is limited to one study [116], where light microscopy revealed intratubular calcified hyaline casts at very early stages of the disease in the outer medulla, possibly in the loop of Henle. Detailed immunocytochemical studies also showed a basolateral, rather than apical, distribution of the H+−ATPase in proximal tubule cells and an absence of apical H+−ATPase in α-intercalated cells of the collecting ducts [116]. These findings allow speculation that an incomplete renal tubular acidosis may also contribute to calcium phosphate precipitation in Dent’s disease.

**dRTA (distal renal tubular acidosis)**

dRTA is associated with an 80% incidence of medullary nephrocalcinosis and accounted for 19% of all cases of nephrocalcinosis in a large human series [1]. Both primary inherited forms of this disease and secondary acquired dRTA may develop nephrocalcinosis. The picture is confused slightly with the fact that nephrocalcinosis itself, by disrupting the tubular epithelium, can lead to dRTA.

The underlying molecular basis of primary dRTA is a defective functioning of α-intercalated cells. There may be failure of acid excretion, secondary to defects in proton pumps, at the apical surface of these cells [117,118], failure of Cl−/HCO3− exchange at the basolateral membrane [119] or defective activity of the enzyme carbonic anhydrase II [120]. Chronic acidosis, resulting in leaching of bone, together with a direct decrease in overall tubular reabsorption of calcium contribute to hypercalciuria. Serum calcium and phosphate levels are normal, but hypokalaemia is usually seen. Mechanisms leading to hypokalaemia are unclear, but increased potassium secretion is postulated to be a result of activation of the renin–aldosterone axis and mechanisms to maintain the electrochemical gradient within the collecting duct epithelium. Urinary citrate is low, due to increased proximal tubular reabsorption to provide bicarbonate ions and combined with an alkaline urinary pH, these factors favour calcium phosphate precipitation [121]; however, the exact mechanisms and the precise sites of calcium phosphate deposition are unknown. Indeed, some patients have marked nephrocalcinosis and no stones, whereas others have the opposite [121].

**Oxalurias**

In primary hyperoxaluria, increased urinary oxalate excretion is a consequence of an increased synthesis of oxalate, causing deposition of calcium oxalate crystals in many organs, including the kidney. The result is extensive nephrocalcinosis, which usually leads to progressive renal failure [122].

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Nephrocalcinosis is usually of a medullary pattern and is seen in both type 1 and type 2 primary hyperoxaluria [1]. Nephrocalcinosis caused by hyperoxaluria is, however, not confined to the familial hyperoxalurias. It can also occur as a result of excess oxalate being absorbed by the enteric route, especially in the context of primary intestinal disease [123], bypass surgery [124] or ingestion of oxalate precursors, such as ethylene glycol.

Mechanisms of nephrocalcinosis must be a result of urine being supersaturated with respect to calcium and oxalate ions. Intratubular precipitation of calcium oxalate crystals seems likely in cases of marked hyperoxaluria, and milder degrees of hyperoxaluria may allow heterologous nucleation of calcium oxalate around calcium phosphate crystals [44]. The degree to which these crystals are removed from the lumen by endocytosis or transcytosis is not known, and such clearance mechanisms remain a hypothesis [75]. Intraluminal calcium oxalate crystals appear to cause an epithelial reaction, leading to inflammation, urothelial overgrowth and movement of crystals into the interstitium [64]. Larger crystals obviously have the potential to cause luminal obstruction and severe tubular damage, resulting in both nephrocalcinosis and nephrolithiasis. Histological sections of a live related donor kidney transplant in a patient with (undiagnosed) primary hyperoxaluria type 1 reveal luminal calcium oxalate crystals, as well as interstitial calcification in the failing graft [125]. In a further case, an acutely failing cadaveric renal transplant, secondary to systemic oxalosis, revealed luminal calcium oxalate crystals, together with calcification of the tubular epithelium and blood vessels [126]. The overall impression is that hyperoxaluria leads to luminal precipitation of calcium oxalate, which, if massive, leads to renal failure secondary to tubular obstruction and inflammation. Thus hyperoxaluria is similar to other chemical causes of intratubular crystal precipitation, such as acyclovir and triamterene [127], and this pathology is separate from nephrocalcinosis secondary to hypercalciumia.

Calcium precipitation is not restricted to the interstitium, but also includes intratubular crystal formation. Whether intratubular crystallization leading to nephrocalcinosis represents only a minority interest requires fine histopathological analysis of further cohorts of idiopathic cases of recurrent stone-formers, and of tubulopathies giving rise to nephrocalcinosis and nephrolithiasis.

CONCLUSION

Almost any condition causing a sustained or intermittent hypercalcaemia or hypercalciuria may cause nephrocalcinosis. Development of interstitial medullary nephrocalcinosis will depend upon tubular delivery of calcium and anions (phosphate and oxalate) to the interstitium, as well as prevailing local conditions, including pH, inhibitory proteins and anions. Key uncertainties include the passive permeability of the thin loops and their behaviour when tubular loop fluid arrives at supersaturated concentrations. In addition, the behaviour and regulation of transtubular transport of calcium at the IMCD needs further investigation.

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Chronic kidney disease (CKD) is a major public health problem. It is estimated that 10–15% of the adult population in industrialized countries have CKD, and the prevalence is even higher in developing countries. The most common causes of CKD are diabetes and hypertension, followed by glomerulonephritis and polycystic kidney disease. Other causes include interstitial nephritis, drug-induced nephropathy, and renovascular disease. The progression of CKD to end-stage renal disease (ESRD) is associated with increased morbidity and mortality. Treatment of CKD includes control of risk factors, such as hypertension and diabetes, and management of symptoms. In severe cases, dialysis and kidney transplantation are the only options for treatment.

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