Genetic, Pathophysiological and Clinical Aspects of Nephrocalcinosis

Ben Oliveira[1], Robert Kleta[1], Detlef Bockenhauer[1], Stephen B Walsh[1]

1. UCL Centre for Nephrology, London, UK

Correspondence to:

Robert Kleta, MD, PhD
Potter Chair of Nephrology
Head of Centre for Nephrology
University College London
Royal Free Hospital / Medical School
1. Floor, Room 1.7007, Rowland Hill Street
London NW3 2PF, United Kingdom

Phone +44-20-7317 7554
Fax +44-20-7472 6476
Email r.kleta@ucl.ac.uk

Running Head: Genetics of Nephrocalcinosis

Key Words: Nephrocalcinosis, Tubular Physiology, Genetics

Contents

Abstract

Nephrocalcinosis describes the ectopic deposition of calcium salts in the kidney parenchyma. Nephrocalcinosis can result from a number of acquired causes, but also an even greater number of genetic diseases, predominantly renal, but also extra-renal. Here we provide a review of the genetic causes of nephrocalcinosis, along with putative mechanisms, illustrated by human and animal data.

Introduction

The term Nephrocalcinosis was first coined by the then physician's assistant Fuller Albright in 1934 [2]. Albright was referring to calcium deposition into the kidney of patients with primary hyperparathyroidism usually associated with impaired kidney function. Currently the term is often used to describe calcium deposition that is seen in the kidneys radiologically which, as we will discover later, does not necessarily constitute nephrocalcinosis as Albright originally meant it. The calcium is in the form of either calcium phosphate or calcium oxalate; some have termed the latter oxalosis, but here we shall use the term nephrocalcinosis to cover both. In the majority of cases the underlying event leading to nephrocalcinosis is hypercalciuria. This may be the result of extra-renal conditions leading to an increased calcium load with subsequent hypercalciuria; for example enhanced absorption of calcium in the gut. Alternatively, hypercalciuria may be caused by impaired renal handing of calcium. Here, we explore the genetic, pathophysiological and clinical aspects of both renal and extra-renal diseases leading to nephrocalcinosis.

Types of Nephrocalcinosis

Oliver Wrong proposed dividing nephrocalcinosis into three different types [78]. The first was *molecular nephrocalcinosis*, in which there is an increase in renal intracellular calcium but no crystal formation; in essence reflecting the renal dysfunction of hypercalcemia. The second was *microscopic nephrocalcinosis*, in which calcium phosphate or oxalate crystals could be identified on light microscopy but not radiologically (as often happens in biopsies of renal tissue with acute tubular necrosis). The third was *macroscopic nephrocalcinosis*, where the calcium salt is visible

radiologically. It is this third type that is typically referred to by the term nephrocalcinosis, and is also the type that we will mainly consider here.

Sites of Nephrocalcinosis

Cortical nephrocalcinosis is rare (2.4% of the largest published series [116]) and usually results from severe destructive disease of the cortex. This has been described in chronic glomerulonephritis (although this is often in the presence of another factor, e.g. increased calcium ingestion) [4], acute cortical necrosis [22], chronic pyelonephritis (which may be focal and therefore asymmetric) [31] and trauma [16].

Medullary nephrocalcinosis is the usual distribution seen, perhaps unsurprising considering the location of the major homeostatic pathway of Ca^{2+} reabsorption (the thick ascending limb of the loop of Henle) and the segment involved with acid base regulation (the collecting duct).

Genetic Aspects of Nephrocalcinosis

A number of monogenetic conditions lead to nephrocalcinosis. Over the last few decades we have been able to classify the genetic basis of a number of conditions causing nephrocalcinosis and these will form the focus of this review.

Renal genetic disease causing Nephrocalcinosis

Proximal Tubule

Dent Disease and Lowe Syndrome

The term Dent disease was first coined by Wrong in 1994 based on the phenotype first described by Dent in the 1960s [26,117]. It is an X-linked recessive disorder characterized by the renal Fanconi syndrome (low-molecular weight proteinuria, aminoaciduria, phosphaturia, uricosuria, glycosuria) hypercalciuria, nephrocalcinosis and nephrolithiasis. It also causes progressive renal impairment with ESRF out of keeping with the degree of nephrocalcinosis, often in the 4^{th} to 5^{th} decade. Dent disease (Dent-1) is caused by mutations of *CLCN5* gene that encodes the chloride transporter CIC-5. ClC-5 is found in endosomes involved in the endocytic reabsorption of low molecular weight (LMW) proteins. 40% of patients with Dent disease do not have *CLCN5* mutations; of these, about a third have a mutation in *OCRL*, these patients are referred to as Dent-2 [45].

Lowe syndrome, which is caused by *OCRL* mutations, has the same renal phenotype as Dent disease in addition to congenital cataracts and mental impairment [10]. There is some overlap between Dent-2 and Lowe syndrome but Dent-2 sufferers tend to have mild or absent oculocerebral symptoms [11].

Loss of CLC-5 leads to impaired lysosomal trafficking to the lumen. As endocytosis is required for reabsorption of proteins in the proximal tubule this will interfere with normal function. ClC-5 is strongly expressed in proximal tubular cells, where it is collocated with vacuolar H^+ATPase in endosomes, which are involved in reabsorption of filtered albumin and low molecular weight proteins. Inactivation of ClC-5 in rodents leads to a severe endosomal trafficking defect, resulting in a loss of the multiligand cubulin and megalin proteins from the proximal tubular cell brush border, and shedding of these proteins into the urine as well as impaired lysosomal processing .

The pathophysiology of hypercalciuria in Dent/Lowe has not been clearly delineated. It has been suggested that decreased absorption of phosphate and vitamin D binding protein in the proximal tubule leads to secondary increases in Vitamin D3 synthesis resulting in hypercalcemia and then hypercalciuria [27]. Another proposed mechanism is that decreased chloride reabsorption in the proximal tubule leads to reduced calcium absorption in downstream nephron segments (presumably by reducing the trans-tubular electrochemical gradient necessary for cation transport) [103]. Nephrocalcinosis may occur in Dent patients without hypercalciuria [66] and there is some evidence that defective ClC-5 in collecting duct cells may cause decreased clearing of calcium crystals from the apical cell surface [87]. Also, decreased proximal tubular endocytosis of parathyroid hormone (PTH), results in an increased luminal concentration of PTH, which stimulates increased 1-alfa hydroxylation and causes decreased expression of NaPi2 at the apical membrane and phosphaturia, favouring calcification [81].

Idiopathic infantile hypercalciuria (IIH) and hereditary hypophosphatemic rickets with hypercalciuria (HHRH)

Pathogenic NaPi2a variants, caused by mutations in *SLC34A1* can cause autosomal recessive IIH, characterised by hypophosphatemia and nephrocalcinosis [89]. Some variants may have a full renal Fanconi syndrome [67].

Homozygous mutations of *SLC34A3* which encode NaPi2c can cause HHRH, causing hypophosphatemia, secondary elevation of 1,25 OH vitamin D and hypercalciuria and osteomalacia. Heterozygous carriers get a similar but less severe phenotype; both homozygotes and heterozygotes are more likely than wild type to develop medullary nephrocalcinosis [25]. NaPi2a deletion in mice causes interstitial deposition of CaPi, associated with phosphate wasting, a secondary elevation in 1,25 OH Vitamin D and subsequent hypercalcemia and hypercalciuria [19].

Fanconi Bickel syndrome

Fanconi Bickel syndrome is caused by mutations in *SLC2A2* which encodes the glucose transporter GLUT2 [68]. This is responsible for transcellular glucose transport in enterocytes as well as proximal renal tubular cells. Pathogenic mutations in this gene cause a glycogen storage disorder resulting in generalized proximal tubular dysfunction. Hypercalciuria is present, as well as hyperphosphaturia; this is thought to be because pathogenic GLUT2 mutations may cause decreased expression of NaPi2c in proximal tubular cells [68].

Loop of Henle

Bartter Syndromes

The Bartter syndromes comprise a group of autosomal recessive disorders caused by mutations in a number of genes characterized by salt wasting in the thick ascending limb of the loop of Henle, resulting in low-normal blood pressure, hypokalemic metabolic alkalosis, and hypercalciuria. Hypercalciuria is responsible for the nephrocalcinosis often seen in this condition [88].

The genetic basis for the condition is well delineated and is due to mutations in different TAL transporter proteins. Bartter I is due to mutations of *SLC12A1* that encodes the NKCC2 cotransporter itself [93]. Bartter II is due to mutations in *KCNJ1* which encodes the apical ROMK potassium channel [94] and Bartter syndrome type III is due to mutations of *CLCNKB* encoding the chloride channel ClCKb on the basolateral membrane [92]. These channels are required for apical potassium and basolateral chloride efflux and for NKCC2 function. Mutations of *BSND* which encodes Barttin, a chaperone protein that is necessary for the correct insertion of ClCNKA and ClCNKB in the basolateral membrane are responsible for Bartter IV [33]. Barttin also acts in the epithelium of the inner ear and patients with Bartter syndrome type IV have sensorineural deafness. Finally, there are rare gain of function mutations of the calcium sensing receptor (CaSR), that can lead to a Bartter-like phenotype [110]; this has also been termed Bartter V. Activation of the CaSR causes inactivation of NKCC2 probably via inhibition of ROMK [109]. This may also partly explain the diuresis commonly seen in hypercalcaemic states. Nephrocalcinosis has been reported in all five types of Bartter syndrome but is a prominent feature in types I, II, and V.

Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis (FHHNC)

This is a rare autosomal recessive condition characterized by severe nephrocalcinosis, nephrolithiasis and progressive renal failure. Defects in two genes have been found, *CLDN16* [95] and *CLDN19* [58] which encode Claudin-16 and Claudin-19 respectively. These proteins are tight junction components crucially involved in the paracellular absorption of calcium and magnesium in the TAL. The pathogenic mutations cause the tight junctions to lose cation selectivity, which allows leakage of anions into the lumen, diminishing the positive lumen potential required to drive the paracellular absorption of calcium and magnesium [48]. The resulting heavy hypercalciuria causes severe nephrocalcinosis and is associated with subsequent renal failure [73].

Insulin Receptor Mutations

Hypercalciuria and nephrocalcinosis has been reported in cases of insulin receptor mutations [96]. These autosomal recessive mutations of *INSR* lead to severe insulin resistance with subsequent hyperinsulinism. The cause of the hypercalciuria has not been delineated, but mirrors the hypercalciuria that occurs with diabetes, which is reversible by insulin, suggesting a role of INSR activation in the regulation of urinary calcium excretion [85]. The patients were also found to have hypermagnesuria and hypokalemic metabolic alkalosis. INSR is expressed throughout the nephron, and the mechanism of INSR mutations in causing nephrocalcinosis is not clear; the association of hypercalciuria and hypermagnesuria suggests a lesion in the TAL or DCT.

Distal Tubule

Approximately 7-10% of the filtered calcium is reabsorbed in the distal convoluted tubule (DCT). Uniquely, this Ca^{2+} transport is transcellular and active. Surprisingly, there are no known monogenetic conditions arising from this segment causing nephrocalcinosis [101].

Familial Hyperkalemic Hypertension (FHHt)

FHHt causes hypertension, hyperkalemia, metabolic acidosis and hypercalciuria. It is caused by mutations of the kinases, WNK1 and WNK4 [111], as well as the ubiquitin ligase Cullin 3 [12] and its adaptor Kelch-like 3 [65]. These pathogenic mutations increase the phosphorylation and activity of the sodium chloride cotransporter. Hypercalciuria is a particular feature of FHHt due to WNK4 mutations; possibly as WNK4 appears to enhance TRPV5 Ca^{2+} transport, and this effect is attenuated with pathogenic WNK4 mutations [53]. Decreased proximal reabsorption in response to volume expansion is also likely, in contrast to the hypocalciuria of Gitelman syndrome or thiazide administration [75]. Although nephrolithiasis secondary to hypercalciuria has been reported in FHHt [100], to date nephrocalcinosis has not.

Cortical collecting duct

Distal Renal Tubular Acidosis (dRTA)

This condition has a high propensity to cause nephrocalcinosis (80% of individuals) and accounts for 19% of all cases in Wrong's series [116]. The chronic metabolic acidosis leads to phosphate being liberated from the skeleton to buffer acid; the mobilized calcium causes hypercalciuria. As calcium phosphate precipitates at a

higher pH, the alkaline urine will tend to favor the formation of calcium phosphate stones and/or nephrocalcinosis [40]. There may also be a lesser effect from an overt metabolic acidosis, which may contribute to hypercalciuria by decreasing expression of TRPV5 in the DCT [74]. dRTA is caused by a failure of the alpha-intercalated cells to acidify the urine. In these cells, CO_2 is hydrated to form a bicarbonate ion and a proton in a reaction catalysed by carbonic anhydrase 2. The proton is extruded apically by the apical proton pump, vacuolar H^+ATPase, while the bicarbonate is reclaimed to the systemic circulation by the chloride bicarbonate counter transporter anion exchanger 1 (AE1). Mutations of the gene *SLC4A1*, which encodes AE1 were proposed as a genetic cause of hereditary dRTA by Unwin and colleagues [103], and this was later proved correct [13]. Subsequently, mutations of *ATP6V1B1* [54] or *ATP6V0A4* [98] which encode the B1 and A4 subunits (respectively) of vH^+ATPase were also shown to lead to dRTA. Deficiency of carbonic anhydrase 2 (CA2) was reported to cause an autosomal recessive syndrome of RTA, proximal bicarbonate wasting, osteopetrosis and cerebral calcification in 1983 [97]. Loss of function mutations of the *CA2* have been subsequently described [37,91], and nephrocalcinosis is reported in these patients [51].

Miscellaneous

Amelogenesis Imperfecta with Nephrocalcinosis (AINC)

AINC is a rare autosomal recessive disorder characterized by dental defects and nephrocalcinosis due to mutations in FAM20A [52]. AINC patients are hypocalciuric rather than hypercalciuric. FAM20A is a putative kinase in the Golgi apparatus with a

proposed role in biomineralisation, hence it's causality in Amelogenesis and nephrocalcinosis [108].

Medullary Sponge Kidney

Described by Lenaduzzi in 1939 [61], medullary sponge kidney (MSK) is characterized by ectatic precalyceal papillary collecting ducts [38] and causes nephrocalcinosis, nephrolithiasis, which may lead to recurrent urosepsis. The condition may be caused by disruption to the normal ureteric bed-metanephric interface, which can be demonstrated as ectatic precalyceal papillary collecting ducts on intravenous pyelography. It has been shown that up to 50% of those with the condition may have an affected family member and that a number of these kindreds have inherited MSK in an autosomal dominant fashion with variable penetrance [36].

During nephrogenesis the metanephric mesenchyme induces ureteric bud growth via glial cell-lined derived neurotrophic factor (*GDNF*) acting on receptor tyrosine kinase (*RET*). Mutations have been found in both of these genes in patients with MSK [29,102]. Impaired nephrogenesis may also explain the other tubular defects seen in MSK, namely; dRTA, proximal tubular dysfunction, hypercalciuria and hypocitraturia.

Calcification in MSK takes a number of forms. Apart from simple stones, there are ductal stones, ductal and inner medullary collecting duct plugs (which take the shape of the tubular segment in which they form, and destroy the epithelium, settling eventually on the basement membrane), plug overgrowths, stones growing on Randal's plaques and pelvic stones [34].The dilated collecting ducts and resultant low flow contributes to stone formation (especially ductal stones), nephrocalcinosis seems to result from ductal plugging and interstitial calcification (e.g. Randal plaques).

MSK was classically diagnosed via intravenous urography (IVU), in which the diagnosis could be made by the finding of cystic dilations associated with a calyceal 'blush' appearance, caused by pooling of contrast. This would often be in addition to the visualization of nephrocalcinosis and nephrolithiasis [38].

IVUs are no longer available in many centers; and although unenhanced CT scans can only confirm nephrocalcinosis, not MSK, more recently, multidetector CT urography has been shown to demonstrate the characteristic radiologic findings of MSK including medullary cysts, calyceal dilatation, nephrocalcinosis and nephrolithiasis [59,71].

Extra-renal genetic disease causing nephrocalcinosis

Intestinal disease

Williams' syndrome

Williams syndrome (also Williams-Beuren syndrome) is a multisystem disorder resulting from the deletion of 26-28 genes from the long arm of chromosome 7[82]. The clinical phenotype is of intellectual impairment with a friendly 'cocktail party' personality, vascular stenosis, characteristic facies and variable hypercalcaemia, sometimes with nephrocalcinosis[83], but without either a raised PTH or vitamin D level. The specific gene responsible has not been identified; but there is evidence to suggest that the Ca^{2+} channel TRPC3 is up regulated in intestine and kidney and that this may be regulated by TF2i, encoded by the transcription factor 2i (*GTF2i*) gene which is located within the deletion on chromosome 7 [62].

Nephrocalcinosis may occur in a number of other monogenic intestinal diseases associated with hypercalcaemia/hypercalciuria, such as congenital lactase deficiency [86], congenital sucrose/isomaltase deficiency [7], glucose/galactose malabsorption [79] and the blue diaper syndrome [30].

Bone disease

Primary hyperparathyroidism with Multiple Endocrine Neoplasia type 1 (MEN1)

MEN1 is an autosomal dominant condition consisting of the strong predisposition to form tumors of the parathyroid glands (in over 90% of MEN1 patients over 50), the anterior pituitary and the pancreas. The resulting hyperparathyroidism causes hypercalcaemia and thus hypercalciuria and subsequent nephrocalcinosis. The causative gene is *MEN1* [18], which encodes a protein, Menin, which is a putative tumor suppressor gene.

Idiopathic Infantile Hypercalcaemia and Hypercalciuric Nephrolithiasis and Nephrocalcinosis

Loss of function mutations of *CYP24A1,* which encodes the enzyme 1,25-dihydroxyvitamin D_3 24-hydroxylase, cause infantile hypercalcaemia [89] (usually in infants who are given vitamin D supplements) and a syndrome of hypercalciuria, nephrolithiasis and nephrocalcinosis in patients presenting in later childhood or early adulthood [28]. 24-hydroxylase catalyzes the 24 and 23-hydroxylation of 25-$(OH)D_3$ and 1,25-$(OH)_2D_3$, probably in order to regulate and attenuate the action of 1,25-$(OH)_2D_3$ on its target tissues. These autosomal recessive loss of function mutations

therefore appear to amplify the effect of native 1,25-$(OH)_2D_3$, causing a suppressed PTH, hypercalciuria, stones and nephrocalcinosis.

Other monogenic diseases affecting bone may also cause nephrocalcinosis by increasing the urinary concentration of calcium; Neonatal self-limited primary hyperparathyroidism [76], Infantile hypophosphatasia [6] and McCune-Albright syndrome [56].

Oxaluria

Primary Hyperoxaluria types 1, 2 & 3

Primary hyperoxaluria (PH) is caused by autosomal recessive defects in hepatic enzymes involved in the metabolism of glyoxalate, resulting in enhanced oxalate production, and thus hyperoxaluria, with subsequent nephrocalcinosis which often leads to end-stage renal failure [46]. PH1 is caused by defects in *AGXT*, which encodes peroxisomal enzyme alanine:glyoxylate aminotransferase (AGT) which converts glyoxylate to glycine [20].

PH2 is caused by inactivating mutations of *GRHPR* which encodes cytosolic enzyme glyoxylate reductase/hydroxypyruvate reductase (GR/HPR) [90]. The GR/HPR enzyme catalyzes the conversion of glyoxalate to glycolate. The increased load of glyoxalate is converted by lactate dehydrogenase to oxalate. Mammals cannot metabolize oxalate and it must be excreted in the urine. Oxalate is poorly soluble and urinary supersaturation leads to both nephrocalcinosis and nephrolithiasis.

PH3 is caused by genetic inactivation of *HOGA1*, which encodes the liver-specific mitochondrial 4-hydroxy-2-oxoglutarate (HOG) aldolase [8] involved in the final step

of hydroxyproline catabolism in mitochondria, catalyzing the cleavage of HOG to pyruvate and glyoxalate. The mechanisms by which this impaired catabolism leads to oxalate accumulation are, currently, not well understood. PH3 patients typically present at a young age with recurrent stone disease, hyperoxaluria and hypercalciuria. The disease usually becomes silent in later childhood and does not progress to end stage renal failure.

Most mutations in PH1 patients cause either a complete absence of AGT or abolish its function [24,112]. However about a third of patients have presence of functional AGT that is mistargeted to the mitochondria rather than peroxisomes. The mistargeting is caused by a specific mutation (Gly170Arg), if combined with a common polymorphism (Pro11Leu) [84]. Pro11Leu is one of the main allelic variants of the AGXT gene; the so-called minor allele with a frequency of about 15 – 20% in European and North American individuals. Even in the absence of the Gly170Arg mutation the minor allele causes about 5% of AGT to be directed to the mitochondria [84]. It has been proposed that this mutation may have arisen through selection pressures of particular diets. In carnivores the main precursor of glyoxylate is hydroxyproline, found mainly in mitochondria, while in herbivores the precursor is mainly glycolate. Found mainly in peroxisomes [50,77]. It is interesting to note that the population with the highest frequency of the mutation is the European arctic Sami people (27%), who have traditionally had a heavily carnivorous diet. This is in contrast to India, China, and Japan where the occurrence is 2-3% [15].

Acquired

Hypercalciuria

Any condition leading to hypercalciuria can in theory lead to nephrocalcinosis but the most common is primary hyperparathyroidism (32% of Wrong's series [116]). Elevated 25-OH or 1,25-OH vitamin D levels (both iatrogenic and in granulomatous disease e.g. sarcoid) can lead to hypercalcaemia, hypercalciuria and subsequent nephrocalcinosis.

Oxaluria

Excess oxalate production and oxaluria will lead to a propensity to form calcium oxalate crystals. Enteric hyperoxaluria, due to pancreatic insufficiency, small bowel resection or calcium restriction may cause nephrocalcinosis [21] by increasing GI absorption of oxalate and thus causing hyperoxaluria. Ingestion of ethylene glycol, which is metabolized into oxalate, will also cause calcium oxalate precipitation in the kidney, either microscopically or macroscopically [99].

Phosphaturia

Oral sodium phosphate is sometimes given as bowel preparation before colonoscopy or bowel surgery, and may cause massive phosphaturia; renal failure and nephrocalcinosis are described [41].

Acquired tubular defects

In the loop of Henle, furosemide, a pharmacological inhibitor of NKCC2, can cause nephrocalcinosis in adults when taken chronically [55], and when administered to neonates and infants [49].

In the collecting duct dRTA causing nephrocalcinosis, may occur with acquired disease, notably Sjögren syndrome [42], other autoimmune conditions, (such as Lupus) and Wilson disease [47].

Pathophysiological Aspects of Nephrocalcinosis

Calcium deposition in nephrocalcinosis can take the form of intratubular deposits, interstitial deposition (or microliths in the dilated tubules of medullary sponge kidneys).

Intratubular nephrocalcinosis

Nephrocalcinosis may occur via calcium phosphate or calcium oxalate crystals precipitating in the distal nephron when the tubular fluid becomes supersaturated. It is likely that a degree of precipitation occurs in tubules of healthy kidneys, however healthy tubular epithelial cells present a non-crystal binding surface to the lumen [105,106] and a loss of epithelial integrity increases crystal adhesion [105]. Crystal nucleation and growth is also inhibited by the urinary environment which is enriched with inhibitors of aggregation such as magnesium and Tamm Horsfall protein [32,60].

Dedifferentiated or regenerating tubular cells express various molecules including annexin 2, phosphatidyl serine hyaluronan and osteopontin, that have been shown to bind crystals [5,9,17,104].

Thus a failure of these defense mechanisms, often as a consequence of tubular injury, results in epithelial crystal adhesion and possible tubular crystal obstruction if the crystal becomes large enough to block the tubular lumen. Tubular obstruction will cause a functional loss of the obstructed tubule, presumably with the same sequelae that accompany other forms of obstruction.

Therefore it may be that tubular injury and/or failure of epithelial or of solute defenses against crystal nucleation and crystal growth are necessary prerequisites to calcification and nephrocalcinosis; perhaps in some part explaining why

nephrocalcinosis occurs in some conditions with modest hypercalciuria, and not in others in which it is more pronounced.

Interstitial nephrocalcinosis

Interstitial calcium deposition can either be formed de-novo or can come from translocation of intratubular deposits [63].

Evidence on the initial site of deposition in nephrocalcinosis is lacking, but in stone formers with Randall's plaques (which are exclusively formed of calcium phosphate), there is good evidence that the initial calcium phosphate deposition is at the basement membrane in the thin limb of the loop of Henle [35]; the basement membrane in the thin limb is unusually thick comprising collagen and a large amount of mucopolysaccarides [14], which could plausibly offer electrostatically charged sites to attract calcium and phosphate, offering a template for crystal formation. As these deposits expand by crystal growth, they invade the interstitium, the suburothelial space and eventually breach the urothelium [35]. Both inflammation and epithelial cell injury are notably absent from the Randall's plaque type of calcium deposition.

Inflammation and oxalate deposition

There is growing evidence that oxalate crystals may be pro-inflammatory. This may be mediated via their activation of the inflammasome nucleotide-binding domain, leucine-rich repeat (NALP-3). An inflammasome is a complex that consists of a sensor protein that once stimulated is able to activate the protease caspase-1. Caspase-1 is able to then cleave biologically inactive IL-1β and IL-18 to activate them [43,70]. Rats

fed an oxalate rich diet develop an interstitial infiltrate associated with calcium oxalate crystals and up-regulation of NALP-3 and IL-1β gene expression and associated renal failure [57]. Furthermore, NALP-3 null mice had decreased levels on renal inflammation and did not develop renal failure despite the presence of calcium oxalate crystal deposition [57].

Thus, it may be the case that in primary hyperoxaluria, inflammation and subsequent tubular epithelial damage occurs after calcium oxalate deposition; oxalate supersaturation in the proximal tubule as a result of an increased filtered load of oxalate in conjunction with active oxalate secretion by proximal tubular cells may cause calcium oxalate crystal nucleation and deposition in the S3 segment of the proximal tubule. This indeed appeared to be the case in PH1 patients with early disease before the onset of renal impairment [114].

Calcium phosphate crystals do not seem to induce inflammation in the same way. In a biopsy series of patients with calcium oxalate stones associated with Randall's plaques it was shown that even when there was extensive calcium phosphate deposition the cells of the associated collecting ducts appeared normal. In contrast, patients with crystal deposition directly in the collecting ducts in intestinal bypass patients (i.e. calcium oxalate crystals) there was associated fibrosis in the surrounding renal tissue [35]. However the same group demonstrated that there is inflammation surrounding crystal deposition within the tubules or dRTA patients where the crystals are likely to be calcium phosphate [34]. It has also been shown that calcium phosphate crystals can activate NALP-3 in vitro [80].

However, the inflammation associated with oxalate deposition may play an important part in the progressive renal failure of primary hyperoxaluria, and indeed the renal

dysfunction in other hyperoxaluric states.

Clinical Aspects of Nephrocalcinosis

Clinical features

Nephrocalcinosis is an insidious process that is usually detected on incidental radiological investigation. However, if nephrocalcinosis does cause symptoms, the most usual symptom is renal colic, either from associated nephrolithiasis, or possibly from the extrusion of an interstitial calcified nodule into a calyx [78].

Medullary nephrocalcinosis can cause defects in the function of the distal nephron, either *distal renal tubular acidosis* or *diabetes insipidus*. Distal renal tubular acidosis may be either a cause or a consequence of nephrocalcinosis, and was first described by Wrong in a patient who had nephrocalcinosis from vitamin D intoxication [116]. Diabetes insipidus leading to a defective urinary concentrating ability may cause polyuria and nocturia. These defects may occur due to medullary deposition of calcium disrupting the structure and function of the loop of Henle and the medullary collecting duct, and may also occur with hypercalcemia, if present [44,113].

As nephrocalcinosis develops within the medullary interstitium it causes relative hypoxia and inflammation. Polycythemia has been reported in nephrocalcinosis and it has been suggested that is in part driven by the hypoxic medulla [1].

Investigations

By definition, only macroscopic nephrocalcinosis is amenable to imaging diagnosis. Nephrocalcinosis can be diagnosed on plain radiographs, ultrasound and CT; magnetic resonance imaging is a poor modality to detect calcification [64]

Early medullary nephrocalcinosis may be demonstrated as echogenic pyramids sonographically before it is visible on a plain film [3,39]; however there may be alterations in renal parenchymal echogenicity in a number of different diseases [69].

Computed tomography (CT) is the most sensitive modality for imaging calcium, and is less operator dependent than ultrasonography. However, a study comparing ultrasound and CT in the diagnosis of nephrocalcinosis demonstrated a sensitivity of 85-91% with ultrasound, 86-92% with CT and only 66-82% with plain kidney-ureter-bladder (KUB) radiograph [20]. Specificity was best (89%) when ultrasound and CT were used together as there is occasionally a lack of concordance in the two modalities. Where calcium deposition is seen at papillary tips it is impossible to distinguish whether these are due to Randall's plaques, intra-tubular crystal deposition or if these are just calyceal stones [72]. Indeed it has been shown that even when there appears to be extensive degrees of nephrocalcinosis on radiological studies these may turn out to be nephrolithiasis in the collecting system [72].

Once the diagnosis of nephrocalcinosis is made, further investigation is warranted to determine the underlying cause; as this affects both the management and prognosis.

Laboratory investigations in a patient with diagnosed nephrocalcinosis are aimed at identifying an underlying cause.

These should include measurement of the serum and urine electrolytes, calcium, phosphate and creatinine. As two of the 3 leading causes of nephrocalcinosis are hyperparathyroidism and dRTA (the other being MSK) [78], a serum parathyroid hormone should be measured to exclude the first and a serum bicarbonate and an accurate urine pH to exclude the latter if there is a metabolic acidosis, or a urinary acidification test with ammonium chloride [115] or furosemide and fludrocortisone [107],

if the serum bicarbonate is in the normal range. Also, two 24-hour urine collections should be made to measure the 24-hour excretion of oxalate, citrate and creatinine.

Clinical course and treatment

In most cases of nephrocalcinosis the lesion is permanent once present, although in rare cases, lessening of medullary nephrocalcinosis has been reported after a metabolic defect has been corrected. In most patients, the underlying disease is not amenable to treatment and the best that can be achieved is to avoid the nephrocalcinosis getting worse. This might be disease specific therapy, such as oral alkali supplementation in dRTA (to reduce systemic acidosis, reduce hypercalciuria and increase urinary citrate) or liver transplantation in primary hyperoxaluria.

Generalized measures to reduce hypercalciuria should help slow the deposition of parenchymal calcium in most cases, although any evidence for this is conspicuously lacking; this could involve dietary restriction of sodium and the administration of thiazide diuretics.

Progressive renal failure is typical in mainly four of the diseases that cause nephrocalcinosis, primary hyperoxaluria, familial hypomagnesemia with hypercalciuria and nephrocalcinosis, Dent disease and Lowe syndrome. These patients need counseling and appropriate monitoring.

Conclusion

Medullary nephrocalcinosis is a relatively frequent finding in nephrological practice. Although nephrocalcinosis can cause a number of clinical problems, progressive renal failure is an uncommon complication in the absence of obstructing stone disease. Pain and recurrent infection are more likely, if symptoms are present at all.

While nephrocalcinosis occurs within a wide spectrum of pathophysiology, the commonest three causes in adults are primary hyperparathyroidism, distal renal tubular acidosis and medullary sponge kidney, in that order [116]. Knowledge of the systemic and renal specific causes of nephrocalcinosis will help to identify the primary lesion, be it genetic, or acquired. As some may cause ESRF, and others may cause systemic disease, making a diagnosis is clinically important.

Our understanding of the pathophysiology behind many of the genetic conditions associated with nephrocalcinosis has advanced significantly over recent decades. There are however many unanswered questions: Why are some forms of nephrocalcinosis associated with progressive CKD and others not? Why do some patients with hypercalciuria consistently fail to develop nephrocalcinosis while others often with lower urinary calcium concentrations do? What is the mechanism of nephrocalcinosis in insulin receptor mutations? And what are the exact molecular players in proximal calcium reabsorption?

Further investigation into these questions concerning renal calcification may lead to greater understanding not only of the physiology of the kidney, but perhaps also to the phenomena of ectopic calcification elsewhere and, eventually, to better treatments.

Acknowledgments

DB and RK acknowledge support from the European Union, FP7 (grant agreement 2012-305608, “European Consortium for High-Throughput Research in Rare Kidney Diseases [EURenOmics]”).

References

[1] Agroyannis B, Koutsikos D, Exarchou HT, Yatzidis H. Erythrocytosis in type I renal tubular acidosis with nephrocalcinosis. Nephrol. Dial. Transplant. 1992 Jan 1;7(4):365–366.

[2] Albright FMD, Baird PCMD, Cope OMD, Bloomberg E. STUDIES ON THE PHYSIOLOGY OF THE PARATHYROID GLANDS. IV. RENAL COMPLICATIONS OF HYPERPARATHYROIDISM. J. Med. Sci. 1934 Jan;187(1):49–64.

[3] Al-Murrani B, Cosgrove DO, Svensson WE, Blaszczyk M. Echogenic rings — An ultrasound sign of early nephrocalcinosis. Clin. Radiol. 1991 Jul 1;44(1):49–51.

[4] Arons WL, Christensen WR, Sosman MC. Nephrocalcinosis Visible By X-Ray Associated With Chronic Glomerulonephritis. Ann. Intern. Med. 1955 Feb 1;42(2):260–282.

[5] Asselman M, Verhulst A, Broe ME de, Verkoelen CF. Calcium Oxalate Crystal Adherence to Hyaluronan-, Osteopontin-, and CD44-Expressing Injured/Regenerating Tubular Epithelial Cells in Rat Kidneys. J. Am. Soc. Nephrol. 2003 Dec 1;14(12):3155–3166.

[6] Barcia JP, Strife CF, Langman CB. Infantile hypophosphatasia: Treatment options to control hypercalcemia, hypercalciuria, and chronic bone demineralization. J. Pediatr. 1997 May;130(5):825–828.

[7] Belmont JW, Reid B, Taylor W, Baker SS, Moore WH, Morriss MC, et al. Congenital sucrase-isomaltase deficiency presenting with failure to thrive, hypercalcemia, and nephrocalcinosis. BMC Pediatr. 2002 Apr 25;2(1):4.

[8] Belostotsky R, Seboun E, Idelson GH, Milliner DS, Becker-Cohen R, Rinat C, et al. Mutations in DHDPSL Are Responsible For Primary Hyperoxaluria Type III. Am. J. Hum. Genet. 2010 Sep 10;87(3):392–399.

[9] Bigelow MW, Wiessner JH, Kleinman JG, Mandel NS. Surface exposure of phosphatidylserine increases calcium oxalate crystal attachment to IMCD cells. Am. J. Physiol. - Ren. Physiol. 1997 Jan 1;272(1):F55–F62.

[10] Bockenhauer D, Bokenkamp A, Hoff W van't, Levtchenko E, Holthe JEK, Tasic V, et al. Renal Phenotype in Lowe Syndrome: A Selective Proximal Tubular Dysfunction. Clin. J. Am. Soc. Nephrol. 2008 Sep 1;3(5):1430–1436.

[11] Bökenkamp A, Böckenhauer D, Cheong HI, Hoppe B, Tasic V, Unwin R, et al. Dent-2 Disease: A Mild Variant of Lowe Syndrome. J. Pediatr. 2009 Jul;155(1):94–99.

[12] Boyden LM, Choi M, Choate KA, Nelson-Williams CJ, Farhi A, Toka HR, et al. Mutations in kelch-like 3 and cullin 3 cause hypertension and electrolyte abnormalities. Nature. 2012 Feb 2;482(7383):98–102.

[13] Bruce LJ, Cope DL, Jones GK, Schofield AE, Burley M, Povey S, et al. Familial distal renal tubular acidosis is associated with mutations in the red cell anion exchanger (Band 3, AE1) gene. J. Clin. Invest. 1997 Oct 1;100(7):1693–1707.

[14] Bulger RE, Trump BF. Fine structure of the rat renal papilla. Am. J. Anat. 1966 May 1;118(3):685–721.

[15] Caldwell EF, Mayor LR, Thomas MG, Danpure CJ. Diet and the frequency of the alanine:glyoxylate aminotransferase Pro11Leu polymorphism in different human populations. Hum. Genet. 2004 Nov;115(6):504–509.

[16] Calviño J, Romero R, Blanco M, Lens XM, Novoa D, Sanchez-Guisande D. Cortical nephrocalcinosis induced by extracorporeal shock wave lithotripsy. Nephron. 1999 Feb;81(2):242–243.

[17] Carr G, Simmons NL, Sayer JA. Disruption of clc-5 leads to a redistribution of annexin A2 and promotes calcium crystal agglomeration in collecting duct epithelial cells. Cell. Mol. Life Sci. CMLS. 2006 Jan 25;63(3):367–377.

[18] Chandrasekharappa SC, Guru SC, Manickam P, Olufemi S-E, Collins FS, Emmert-Buck MR, et al. Positional Cloning of the Gene for Multiple Endocrine Neoplasia-Type 1. Science. 1997 Apr 18;276(5311):404–407.

[19] Chau H, El-Maadawy S, McKEE MD, Tenenhouse HS. Renal Calcification in Mice Homozygous for the Disrupted Type IIa Na/Pi Cotransporter Gene Npt2. J. Bone Miner. Res. 2003 Apr 1;18(4):644–657.

[20] Cheidde L, Ajzen SA, Tamer Langen CH, Christophalo D, Pfeferman Heilberg I. A Critical Appraisal of the Radiological Evaluation of Nephrocalcinosis. Nephron Clin. Pract. 2007 May 22;106(3):c119–c124.

[21] Chikos PM, McDonald GB. Regional Enteritis Complicated by Nephrocalcinosis and Nephrolithiasis. Radiology. 1976 Oct 1;121(1):75–76.

[22] Cramer GG, Fuglestad JR. Cortical calcification in renal cortical necrosis. Am. J. Roentgenol. 1965 Oct 1;95(2):344–348.

[23] Danpure CJ. Advances in the enzymology and molecular genetics of primary hyperoxaluria type 1. Prospects for gene therapy. Nephrol. Dial. Transplant. 1995 Jan 1;10(supp8):24–29.

[24] Danpure C j., Jennings P. Peroxisomal alanine:glyoxylate aminotransferase deficiency in primary hyperoxaluria type I. FEBS Lett. 1986 May 26;201(1):20–34.

[25] Dasgupta D, Wee MJ, Reyes M, Li Y, Simm PJ, Sharma A, et al. Mutations in SLC34A3/NPT2c Are Associated with Kidney Stones and Nephrocalcinosis. J. Am. Soc. Nephrol. 2014 Apr 3;ASN.2013101085.

[26] Dent CE, Friedman M. Hypercalcuric Rickets Associated with Renal Tubular Damage. Arch. Dis. Child. 1964 Jun;39(205):240–249.

[27] Devuyst O, Thakker RV. Dent's disease. Orphanet J. Rare Dis. 2010 Oct 14;5(1):1–8.

[28] Dinour D, Beckerman P, Ganon L, Tordjman K, Eisenstein Z, Holtzman EJ. Loss-of-Function Mutations of CYP24A1, the Vitamin D 24-Hydroxylase Gene, Cause Long-standing Hypercalciuric Nephrolithiasis and Nephrocalcinosis. J. Urol. 2013 Aug;190(2):552–557.

[29] Diouf B, Ka EHF, Calender A, Giraud S, Diop TM. Association of medullary sponge kidney disease and multiple endocrine neoplasia type IIA due to RET gene mutation: is there a causal relationship? Nephrol. Dial. Transplant. 2000 Dec 1;15(12):2062–2063.

[30] Drummond KN, Michael AF, Ulstrom RA, Good RA. The blue diaper syndrome: Familial hypercalcemia with nephrocalcinosis and indicanuria: A new familial disease, with definition of the metabolic abnormality. Am. J. Med. 1964 Dec;37(6):928–948.

[31] Ekengren K. Renal cortical calcification in pyelonephritis. Ann. Radiol. (Paris). 1973 Jan;16(3-4):223–226.

[32] Em W. Inhibitors of stone formation. Semin. Nephrol. 1996 Sep;16(5):474–486.

[33] Estévez R, Boettger T, Stein V, Birkenhäger R, Otto E, Hildebrandt F, et al. Barttin is a Cl- channel β-subunit crucial for renal Cl- reabsorption and inner ear K+ secretion. Nature. 2001 Nov 29;414(6863):558–561.

[34] Evan AP, Lingeman J, Coe F, Shao Y, Miller N, Matlaga B, et al. Renal histopathology of stone-forming patients with distal renal tubular acidosis. Kidney Int. 2007 Apr;71(8):795–801.

[35] Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SB, Shao Y, et al. Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. J. Clin. Invest. 2003 Mar 1;111(5):607–616.

[36] Fabris A, Lupo A, Ferraro PM, Anglani F, Pei Y, Danza FM, et al. Familial clustering of medullary sponge kidney is autosomal dominant with reduced penetrance and variable expressivity. Kidney Int. 2013 Feb;83(2):272–277.

[37] Fathallah DM, Bejaoui M, Sly WS, Lakhoua R, Dellagi K. A unique mutation underlying carbonic anhydrase II deficiency syndrome in patients of Arab descent. Hum. Genet. 1994 Nov;94(5):581–582.

[38] Gambaro G, Feltrin GP, Lupo A, Bonfante L, D'Angelo A, Antonello A. Medullary sponge kidney (Lenarduzzi–Cacchi–Ricci disease): A Padua Medical School discovery in the 1930s. Kidney Int. 2006 Jan 4;69(4):663–670.

[39] Glazer GM, Callen PW, Filly RA. Medullary nephrocalcinosis: sonographic evaluation. AJR Am. J. Roentgenol. 1982 Jan;138(1):55–57.

[40] Goldfarb DS. A Woman with Recurrent Calcium Phosphate Kidney Stones. Clin. J. Am. Soc. Nephrol. 2012 Jul 1;7(7):1172–1178.

[41] Gonlusen G, Akgun H, Ertan A, Olivero J, Truong LD. Renal failure and nephrocalcinosis associated with oral sodium phosphate bowel cleansing: clinical patterns and renal biopsy findings. Arch. Pathol. Lab. Med. 2006 Jan;130(1):101–106.

[42] Goules AV, Tatouli IP, Moutsopoulos HM, Tzioufas AG. Clinically significant renal involvement in primary Sjögren's syndrome: clinical presentation and outcome. Arthritis Rheum. 2013 Nov;65(11):2945–2953.

[43] Gross O, Thomas CJ, Guarda G, Tschopp J. The inflammasome: an integrated view: The inflammasome: an integrated view. Immunol. Rev. 2011 Sep;243(1):136–151.

[44] Higashihara E, Nutahara K, Tago K, Ueno A, Niijima T. Medullary sponge kidney and renal acidification defect. Kidney Int. 1984 Feb 1;25(2):453–459.

[45] Hoopes Jr. RR, Shrimpton AE, Knohl SJ, Hueber P, Hoppe B, Matyus J, et al. Dent Disease with Mutations in OCRL1. Am. J. Hum. Genet. 2005 Feb;76(2):260–267.

[46] Hoppe B. An update on primary hyperoxaluria. Nat. Rev. Nephrol. 2012 Aug;8(8):467–475.

[47] Hoppe B, Neuhaus T, Superti-Furga A, Forster I, Leumann E. Hypercalciuria and Nephrocalcinosis, a Feature of Wilson’s Disease. Nephron. 1993;65(3):460–462.

[48] Hou J, Renigunta A, Konrad M, Gomes AS, Schneeberger EE, Paul DL, et al. Claudin-16 and claudin-19 interact and form a cation-selective tight junction complex. J. Clin. Invest. 2008 Feb 1;118(2):619–628.

[49] Hufnagle KG, Khan SN, Penn D, Cacciarelli A, Williams P. Renal Calcifications: A Complication of Long-Term Furosemide Therapy in Preterm Infants. Pediatrics. 1982 Sep 1;70(3):360–363.

[50] Ichiyama A, Xue HH, Oda T, Uchida C, Sugiyama T, Maeda-Nakai E, et al. Oxalate synthesis in mammals: properties and subcellular distribution of serine:pyruvate/alanine:glyoxylate aminotransferase in the liver. Mol. Urol. 2000;4(4):333–340.

[51] Ismail E a. R, Saad SA, Sabry MA. Nephrocalcinosis and urolithiasis in carbonic anhydrase II deficiency syndrome. Eur. J. Pediatr. 1997 Nov;156(12):957–962.

[52] Jaureguiberry G, De la Dure-Molla M, Parry D, Quentric M, Himmerkus N, Koike T, et al. Nephrocalcinosis (Enamel Renal Syndrome) Caused by Autosomal Recessive FAM20A Mutations. Nephron Physiol. 2012;122(1-2):1–6.

[53] Jiang Y, Ferguson WB, Peng J-B. WNK4 enhances TRPV5-mediated calcium transport: potential role in hypercalciuria of familial hyperkalemic hypertension caused by gene mutation of WNK4. Am. J. Physiol. - Ren. Physiol. 2007 Feb 1;292(2):F545–F554.

[54] Karet FE, Finberg KE, Nelson RD, Nayir A, Mocan H, Sanjad SA, et al. Mutations in the gene encoding B1 subunit of H+-ATPase cause renal tubular acidosis with sensorineural deafness. Nat. Genet. 1999 Jan;21(1):84–90.

[55] Kim Y-G, Kim B, Kim M-K, Chung S-J, Han H-J, Ryu J-A, et al. Medullary nephrocalcinosis associated with long-term furosemide abuse in adults. Nephrol. Dial. Transplant. 2001 Dec 1;16(12):2303–2309.

[56] Kirk JMW, Brain CE, Carson DJ, Hyde JC, Grant DB. Cushing's syndrome caused by nodular adrenal hyperplasia in children with McCune-Albright syndrome. J. Pediatr. 1999 Jun;134(6):789–792.

[57] Knauf F, Asplin JR, Granja I, Schmidt IM, Moeckel GW, David RJ, et al. NALP3-mediated inflammation is a principal cause of progressive renal failure in oxalate nephropathy. Kidney Int. 2013 Nov 1;84(5):895–901.

[58] Konno A, Takada K, Saegusa J, Takiguchi M. Presence of B7-2+ Dendritic Cells and Expression of Th1 Cytokines in the Early Development of Sialodacryoadenitis in the IQI/Jic Mouse Model of Primary Sjögren's Syndrome. Autoimmunity. 2003 Jan 1;36(4):247–254.

[59] Koraishy FM, Ngo T-TT, Israel GM, Dahl NK. CT Urography for the Diagnosis of Medullary Sponge Kidney. Am. J. Nephrol. 2014;39(2):165–170.

[60] Kumar V, Peña de la Vega L, Farell G, Lieske JC. Urinary macromolecular inhibition of crystal adhesion to renal epithelial cells is impaired in male stone formers. Kidney Int. 2005 Oct;68(4):1784–1792.

[61] Lenarduzzi G. Reperto pielografico poco commune (dilatazione delle vie urinarie intrarenali). Radiol Med Torino. 1939;26:346–347.

[62] Letavernier E, Rodenas A, Guerrot D, Haymann J-P. Williams-Beuren Syndrome Hypercalcemia: Is TRPC3 a Novel Mediator in Calcium Homeostasis? Pediatrics. 2012 Jun 1;129(6):e1626–e1630.

[63] Lieske JC, Spargo BH, Toback FG. Endocytosis of calcium oxalate crystals and proliferation of renal tubular epithelial cells in a patient with type 1 primary hyperoxaluria. J. Urol. 1992 Nov;148(5):1517–1519.

[64] LiPuma J. Magnetic resonance imaging of the kidney. Radiol. Clin. North Am. 1984 Dec;22(4):925–941.

[65] Louis-Dit-Picard H, Barc J, Trujillano D, Miserey-Lenkei S, Bouatia-Naji N, Pylypenko O, et al. KLHL3 mutations cause familial hyperkalemic hypertension by impairing ion transport in the distal nephron. Nat. Genet. 2012 Apr;44(4):456–460.

[66] Ludwig M, Utsch B, Balluch B, Fründ S, Kuwertz-Bröking E, Bökenkamp A. Hypercalciuria in patients with CLCN5 mutations. Pediatr. Nephrol. 2006 Jun 29;21(9):1241–1250.

[67] Magen D, Berger L, Coady MJ, Ilivitzki A, Militianu D, Tieder M, et al. A Loss-of-Function Mutation in NaPi-IIa and Renal Fanconi's Syndrome. N. Engl. J. Med. 2010 Mar 25;362(12):1102–1109.

[68] Mannstadt M, Magen D, Segawa H, Stanley T, Sharma A, Sasaki S, et al. Fanconi-Bickel Syndrome and Autosomal Recessive Proximal Tubulopathy with Hypercalciuria (ARPTH) Are Allelic Variants Caused by GLUT2 Mutations. J. Clin. Endocrinol. Metab. 2012 Aug 3;97(10):E1978–E1986.

[69] Manz F, Jaschke W, Kaick G van, Waldherr R, Willich E. Nephrocalcinosis in radiographs, computed tomography, sonography and histology. Pediatr. Radiol. 1980 Mar;9(1):19–26.

[70] Mariathasan S, Newton K, Monack DM, Vucic D, French DM, Lee WP, et al. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. Nature. 2004 Jul 8;430(6996):213–218.

[71] Maw AM, Megibow AJ, Grasso M, Goldfarb DS. Diagnosis of Medullary Sponge Kidney by Computed Tomographic Urography. Am. J. Kidney Dis. 2007 Jul;50(1):146–150.

[72] Miller NL, Humphreys MR, Coe FL, Evan AP, Bledsoe SB, Handa SE, et al. Nephrocalcinosis: re-defined in the era of endourology. Urol. Res. 2010 Dec;38(6):421–427.

[73] Nadarajah L, Khosravi M, Dumitriu S, Klootwijk E, Kleta R, Yaqoob MM, et al. A novel claudin-16 mutation, severe bone disease, and nephrocalcinosis. The Lancet. 2014 Jan;383(9911):98.

[74] Nijenhuis T, Renkema KY, Hoenderop JGJ, Bindels RJM. Acid-Base Status Determines the Renal Expression of Ca2+ and Mg2+ Transport Proteins. J. Am. Soc. Nephrol. 2006 Mar 1;17(3):617–626.

[75] Nijenhuis T, Vallon V, van der Kemp AWCM, Loffing J, Hoenderop JGJ, Bindels RJM. Enhanced passive Ca2+ reabsorption and reduced Mg2+ channel abundance explains thiazide-induced hypocalciuria and hypomagnesemia. J. Clin. Invest. 2005 Jun;115(6):1651–1658.

[76] Nishiyama S, Tomoeda S, Inoue F, Ohta T, Matsuda I. Self-limited Neonatal Familial Hyperparathyroidism Associated With Hypercalciuria and Renal Tubular Acidosis in Three Siblings. Pediatrics. 1990 Sep 1;86(3):421–427.

[77] Noguchi T. Amino Acid Metabolism in Animal Peroxisomes [Internet]. In: Fahimi PDHD, Sies PDH, editors. Peroxisomes in Biology and Medicine. Springer Berlin Heidelberg; 1987 [cited 2016 Jun 30]. p. 234–243.Available from: http://link.springer.com/chapter/10.1007/978-3-642-71325-5_23

[78] O. M. Wrong. Oxford Textbook of Clinical Nephrology. Oxford University Press; 2005.

[79] Pahari A, Milla PJ, Hoff WG van't. Neonatal nephrocalcinosis in association with glucose-galactose malabsorption. Pediatr. Nephrol. 2003 May 7;18(7):700–702.

[80] Pazár B, Ea H-K, Narayan S, Kolly L, Bagnoud N, Chobaz V, et al. Basic Calcium Phosphate Crystals Induce Monocyte/Macrophage IL-1β Secretion through the NLRP3 Inflammasome In Vitro. J. Immunol. 2011 Feb 15;186(4):2495–2502.

[81] Piwon N, Günther W, Schwake M, Bösl MR, Jentsch TJ. ClC-5 Cl--channel disruption impairs endocytosis in a mouse model for Dent's disease. Nature. 2000 Nov 16;408(6810):369–373.

[82] Pober BR. Williams–Beuren Syndrome. N. Engl. J. Med. 2010 Jan 21;362(3):239–252.

[83] Pober BR, Lacro RV, Rice C, Mandell V, Teele RL. Renal findings in 40 individuals with Williams syndrome. Am. J. Med. Genet. 1993 May 15;46(3):271–274.

[84] Purdue PE, Takada Y, Danpure CJ. Identification of mutations associated with peroxisome-to-mitochondrion mistargeting of alanine/glyoxylate aminotransferase in primary hyperoxaluria type 1. J. Cell Biol. 1990 Dec 1;111(6):2341–2351.

[85] Raskin P, Stevenson MRM, Barilla DE, Pak CYC. The Hypercalciuria of Diabetes Mellitus: Its Amelioration with Insulin. Clin. Endocrinol. (Oxf.). 1978 Oct 1;9(4):329–335.

[86] Saarela T, Similä S, Koivisto M. Hypercalcemia and nephrocalcinosis in patients with congenital lactase deficiency. J. Pediatr. 1995 Dec;127(6):920–923.

[87] Sayer JA, Carr G, Simmons NL. Calcium phosphate and calcium oxalate crystal handling is dependent upon CLC-5 expression in mouse collecting duct cells. Biochim. Biophys. Acta BBA - Mol. Basis Dis. 2004 May 24;1689(1):83–90.

[88] Sayer JA, Pearce SHS. Diagnosis and clinical biochemistry of inherited tubulopathies. Ann. Clin. Biochem. 2001 Sep 1;38(5):459–470.

[89] Schlingmann KP, Kaufmann M, Weber S, Irwin A, Goos C, John U, et al. Mutations in CYP24A1 and Idiopathic Infantile Hypercalcemia. N. Engl. J. Med. 2011 Aug 4;365(5):410–421.

[90] Seargeant LE, deGroot GW, Dilling LA, Mallory CJ, Haworth JC. Primary oxaluria type 2 (l-glyceric aciduria): A rare cause of nephrolithiasis in children. J. Pediatr. 1991 Jun;118(6):912–914.

[91] Shah GN, Bonapace G, Hu PY, Strisciuglio P, Sly WS. Carbonic anhydrase II deficiency syndrome (osteopetrosis with renal tubular acidosis and brain calcification): novel mutations in CA2 identified by direct sequencing expand the opportunity for genotype-phenotype correlation. Hum. Mutat. 2004 Sep;24(3):272.

[92] Simon DB, Bindra RS, Mansfield TA, Nelson-Williams C, Mendonca E, Stone R, et al. Mutations in the chloride channel gene, CLCNKB, cause Bartter's syndrome type III. Nat. Genet. 1997 Oct;17(2):171–178.

[93] Simon DB, Karet FE, Hamdan JM, Pietro AD, Sanjad SA, Lifton RP. Bartter's syndrome, hypokalaemic alkalosis with hypercalciuria, is caused by mutations in the Na–K–2Cl cotransporter NKCC2. Nat. Genet. 1996 Jun;13(2):183–188.

[94] Simon DB, Karet FE, Rodriguez-Soriano J, Hamdan JH, DiPietro A, Trachtman H, et al. Genetic heterogeneity of Bartter's syndrome revealed by mutations in the K+ channel, ROMK. Nat. Genet. 1996 Oct;14(2):152–156.

[95] Simon DB, Lu Y, Choate KA, Velazquez H, Al-Sabban E, Praga M, et al. Paracellin-1, a Renal Tight Junction Protein Required for Paracellular Mg2+ Resorption. Science. 1999 Jul 2;285(5424):103–106.

[96] Simpkin A, Cochran E, Cameron F, Dattani M, de Bock M, Dunger DB, et al. Insulin Receptor and the Kidney: Nephrocalcinosis in Patients with Recessive INSR Mutations. Nephron Physiol. 2014;128(3-4):55–61.

[97] Sly WS, Hewett-Emmett D, Whyte MP, Yu YS, Tashian RE. Carbonic anhydrase II deficiency identified as the primary defect in the autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification. Proc. Natl. Acad. Sci. 1983 May 1;80(9):2752–2756.

[98] Smith AN, Skaug J, Choate KA, Nayir A, Bakkaloglu A, Ozen S, et al. Mutations in ATP6N1B, encoding a new kidney vacuolar proton pump 116-kD subunit, cause recessive distal renal tubular acidosis with preserved hearing. Nat. Genet. 2000 Sep;26(1):71–75.

[99] Stapenhorst L, Hesse A, Hoppe B. Hyperoxaluria after ethylene glycol poisoning. Pediatr. Nephrol. 2008 Aug 12;23(12):2277–2279.

[100] Stratton, Mcnicholas, Farrington. Recurrent calcium stones in Gordon's syndrome. Br. J. Urol. 1998;82(6):925–925.

[101] Subramanya AR, Ellison DH. Distal convoluted tubule. Clin. J. Am. Soc. Nephrol. CJASN. 2014 Dec 5;9(12):2147–2163.

[102] Torregrossa R, Anglani F, Fabris A, Gozzini A, Tanini A, Prete DD, et al. Identification of GDNF Gene Sequence Variations in Patients with Medullary Sponge Kidney Disease. Clin. J. Am. Soc. Nephrol. 2010 Jul 1;5(7):1205–1210.

[103] Unwin R, Fine L, Cohen E, Thakker R, Tanner M. Unravelling of the molecular mechanisms of kidney stones. The Lancet. 1996 Dec;348(9041):1561–1565.

[104] Verhulst A, Asselman M, Persy VP, Schepers MSJ, Helbert MF, Verkoelen CF, et al. Crystal Retention Capacity of Cells in the Human Nephron: Involvement of CD44 and

Its Ligands Hyaluronic Acid and Osteopontin in the Transition of a Crystal Binding- into a Nonadherent Epithelium. J. Am. Soc. Nephrol. 2003 Jan 1;14(1):107–115.

[105] Verkoelen CF, Boom BGVD, Houtsmuller AB, Schröder FH, Romijn JC. Increased calcium oxalate monohydrate crystal binding to injured renal tubular epithelial cells in culture. Am. J. Physiol. - Ren. Physiol. 1998 May 1;274(5):F958–F965.

[106] Verkoelen CF, Boom BGVD, Kok DJ, Houtsmuller AB, Visser P, Schröder FH, et al. Cell type-specific acquired protection from crystal adherence by renal tubule cells in culture. Kidney Int. 1999 Apr 1;55(4):1426–1433.

[107] Walsh SB, Shirley DG, Wrong OM, Unwin RJ. Urinary acidification assessed by simultaneous furosemide and fludrocortisone treatment: an alternative to ammonium chloride. Kidney Int. 2007 Apr 4;71(12):1310–1316.

[108] Wang SK, Reid BM, Dugan SL, Roggenbuck JA, Read L, Aref P, et al. FAM20A Mutations Associated with Enamel Renal Syndrome. J. Dent. Res. 2014 Jan;93(1):42–48.

[109] Wang WH, Lu M, Hebert SC. Cytochrome P-450 metabolites mediate extracellular Ca(2+)-induced inhibition of apical K+ channels in the TAL. Am. J. Physiol. - Cell Physiol. 1996 Jul 1;271(1):C103–C111.

[110] Watanabe S, Fukumoto S, Chang H, Takeuchi Y, Hasegawa Y, Okazaki R, et al. Association between activating mutations of calcium-sensing receptor and Bartter's syndrome. The Lancet. 2002 Aug 31;360(9334):692–694.

[111] Wilson FH, Disse-Nicodème S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, et al. Human Hypertension Caused by Mutations in WNK Kinases. Science. 2001 Aug 10;293(5532):1107–1112.

[112] Wise P j., Danpure C j., Jennings P r. Immunological heterogeneity of hepatic alanine:glyoxylate aminotransferase in primary hyperoxaluria type 1. FEBS Lett. 1987 Sep 28;222(1):17–20.

[113] Worcester EM, Bergsland KJ, Gillen DL, Coe FL. Evidence for increased renal tubule and parathyroid gland sensitivity to serum calcium in human idiopathic hypercalciuria. Am. J. Physiol. - Ren. Physiol. 2013 Sep 15;305(6):F853–F860.

[114] Worcester EM, Evan AP, Coe FL, Lingeman JE, Krambeck A, Sommers A, et al. A test of the hypothesis that oxalate secretion produces proximal tubule crystallization in primary hyperoxaluria type I. Am. J. Physiol. - Ren. Physiol. 2013 Dec 1;305(11):F1574–F1584.

[115] Wrong O, Davies HEF. The Excretion of Acid in Renal Disease. QJM. 1959 Apr 1;28(2):259–313.

[116] Wrong OM, Feest TG. Nephrocalcinosis. In: Advanced Medicine. London: Pitman Medical; 1976. p. 394–406.

[117] Wrong OM, Norden AGW, Feest TG. Dent's disease; a familial proximal renal tubular syndrome with low-molecular-weight proteinuria, hypercalciuria, nephrocalcinosis, metabolic bone disease, progressive renal failure and a marked male predominance. QJM. 1994 Aug 1;87(8):473–493.

Figure Legends

Figure 1.

A plain abdominal radiograph showing severe medullary nephrocalcinosis.

Figure 2.

Abdominal ultrasound examination showing a kidney in longitudinal view in a child with Bartter's. The calcified areas of the medulla are echogenic and clearly visible.

Figure 3.

Diagram showing sites of calcium reabsorption through the nephron.

Table 1

LOCATION	Disease	Gene	Gene Product	Inheritance	Phenotype/ Remarks
SYSTEMIC DISEASES	William's Syndrome	GTF2i (speculated)	TF2i (regulates TRPC3 calcium channel in the intestine)	Sporadic	Hypercalciuria Multisystem condition; likely multiple genes involved
	Multiple Endocrine Neoplasia Type 1	MEN1	Menin	Autosomal Dominant	Hypercalciuria
	Idiopathic Infantile Hypercalcaemia	CYP24A1	1,25-dihydroxyvitamin D_3 24-hydroylase	Autosomal Recessive	Hypercalciuria
	Primary Hyperoxaluria types1,2,&3	AGXT, GRHPR, HOGA1 respectively	Alanine:glyoxylate aminotransferase, glyoylate reductase/hydroxypyruvate reductase, mitochondrial 4-hydoxy-2-oxoglutarate aldolase respectively	Autosomal Recessive	Hyperoxaluria. Associated with ERSF
PROXIMAL TUBULE	Dent's Disease	CLCN5	CIC-5	X-linked recessive	Hypercalciuria. Associated with ESRF Fanconi like phenotype
	Lowe Syndrome	OCRL1	PIP_2 5-phosphatase	X-linked recessive	Hypercalciuria Associated with cataracts and mental impairment
	Idiopathic Infantile Hypercalciuria	SLC34A1	NaPi2a	Autosomal recessive	Hypercalciuria
	Hereditary Hypophosphatemia and Nephrocalcinosis	SLC34A3	NaPi2c	Autosomal recessive	Hypercalciuria
	Fanconi Bickel Syndrome	SLC2A2	GLUT2	Autosomal Recessive	Hypercalciuria
LOOP of HENLE	Bartter Syndromes (Types I-IV)	SLC12A1, KCNJ1, CLCNKB, BSND	NKCC2, ROMK, CLCKb, Barttin	Autosomal Recessive	Hypercalciuria Type IV associated with sensorineural deafness
	Familial Hypomagnesemia with Hypercalciuria and Nephrocalcinosis	CLDN 14 CLDN16 CLDN19	Claudin-16, Claudin-19 Claudin-14	Autosomal Recessive	Hypercalciuria Associated with ESRF Hypercalciuria and kidney stones. Sensorineural deafness
	Insulin Receptor Mutations	Insulin Receptor Gene	Insulin Receptor	Autosomal Recessive	

CORTICAL COLLECTING DUCT	Distal Renal Tubular Acidosis	ATP6V1V1, ATP6V0A4	B-subtype unit and a-subtype unit respectively of H(+)ATPase	Autosomal Recessive	Associated with sensorineural deafness
		SLC4A1	AE1	Autosomal Dominant/Recessive	
		CA2	Carbonic Anhydrase	Autosomal Recessive	Associated with co-existing proximal defect
MISCELANEOUS	Medullary Sponge Kidney	RET and GDNF genes (speculated)	RET & GDNF	Autosomal dominant with variable penetrance	Hypercalciuria hypocitraturia
	Amelogenesis Imperfecta with Nephrocalcinosis	FAM20A	Putative kinase in Golgi apparatus	Autosomal Recessive	Hypocalciuria Associated with dental defects

Table Legend

Table 1.

Familial diseases leading to nephrocalcinosis