Review

Idiopathic urinary stone disease: possible polygenic aetiological factors

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Introduction

About 12% of men and 5% of women of Western European descent develop at least one urinary stone during their lifetime. The incidence is somewhat less in individuals of sub-Saharan African or Asian descent, although there is a high incidence in the Middle Eastern countries. Calcium oxalate is a major constituent of about 70% of these stones. They are of multifactorial origin, with both genetic and environmental factors involved. The genetic factors influence different components of a highly complex system whereby calcium oxalate, which has a very low solubility in water, remains in solution. When the system is perturbed, the calcium oxalate crystallizes, the crystals aggregate and stones form. The genetic factors operate through mechanisms that are less obvious than the environmental ones such as the patient’s state of hydration and dietary effects on the concentrations of the relevant ionic species.

It is now generally accepted that the crystallization process begins in the distal ducts of the kidney by adhesion of calcium and oxalate to the epithelial surfaces of the tubule cells and their internalization. The calcium oxalate crystal becomes detached, with some incorporated organic materials forming a microlith. This process of microlith formation could arise either because of genetically determined alterations in the surface properties of the cells, or in the composition and/or concentration of substances in the urine that inhibit crystal nucleation and/or aggregation. The microliths pass through the collecting tubules more slowly than calcium oxalate in simple physical solution, aggregating until they reach the pelvicalyceal system, where they grow into stones and pass down the ureters. It has been suggested that a shift in the crystal form from the mono-hydrate to the di-hydrate form reduces the likelihood of such adhesion, and that molecular inhibitors of crystallization operate by favouring the dihydrate form.

Calcium oxalate urolithiasis had, until relatively recently, been thought of primarily in relation to the calcium content of the urine; patients with unexplained hypercalciuria have been labelled as having ‘idiopathic hypercalciuria’, which accounts for 30–40% of calcium oxalate stone formers, and those without hypercalciuria as having ‘idiopathic stone disease’. However, stone formation is also influenced by oxalate, citrate, phosphate, magnesium and the glycoproteins (Tamm-Horsfal protein, osteopontin and nephrocalcin) in the urine.

This paper reviews biochemical sites at which some individually undetectable genetically determined phenotypic changes could act additively to produce calcium oxalate stone disease, which would then appear to be either idiopathic or due to unexplained hypercalciuria.

These postulated mutations with individually undetectable effects would also operate against the background of the whole genome, with its potential for the multiple polymorphisms which are the basis of an individual’s uniqueness and therefore predisposition to disease. Single nucleotide polymorphisms...
SNPs are the most common type of variation in the human genome, and have been calculated to occur in approximately one in every 1200 base pairs. Thus, considering the total size of the human genome, the scope for differences is considerable, even though any two individuals are likely to be 99.92% identical in terms of nucleotide sequence.2

Calcium

The availability of calcium for stone formation depends ultimately on the dietary intake, intestinal absorption, excretion in the faeces, transport across cell membranes from the extracellular to the intracellular components of the body fluids and the renal tubular reabsorption of calcium. Glomerular filtration removes about 60% of the total plasma calcium or about 250 mmol/day. Ninety-seven percent of this filtered load is re-absorbed; 50–60% in the proximal convoluted tubule, mainly passively along electrochemical gradients although a sodium-dependent active process also operates. Active absorption also occurs in the ascending segment of Henle’s loop, in the distal convoluted tubules and in the collecting ducts. Parathyroid hormone (PTH) receptors are widely distributed in the nephron, and one major aspect of PTH function in the maintenance of calcium homeostasis is to increase calcium reabsorption in the distal part of the nephron. A gene which appears to be associated with absorptive hypercalciuria has recently been identified.3,4

The calcium-sensing receptor

The calcium-sensing receptor (CaR) is a 1078 amino-acid G-protein-coupled receptor embedded in the membrane of parathyroid and renal tubule cells. An increase in the concentration of circulating calcium and magnesium ions causes a conformational change in the extracellular domain of the transmembrane protein, which produces an intracellular signal inhibiting PTH secretion and promoting renal calcium secretion, thus producing minute to minute regulation of the plasma calcium concentration.5,6 Mutations with a large effect on the function of the CaR produce overt diseases. Increase in the sensitivity to calcium produces familial benign hypocalciuric hypercalcaemia (FBHH), autosomal dominant hypocalcaemic hypercalciuria (ADHH), in which there is a high incidence of renal complications.

There is phenotypic as well as genetic variations in all of these disorders, and minor changes insufficient to produce an effect on urinary calcium excretion in their own right, but acting in concert with other factors, could contribute to calcium oxalate stone disease.

X-linked calcium oxalate urolithiasis syndromes

The preponderance of males over females in idiopathic calcium oxalate urolithiasis and idiopathic hypercalciuria points towards the existence of an X-linked gene or genes being involved in the regulation of calcium homeostasis. This is seen in its most extreme form in three diseases that are due to mutations in the GLCN-5 gene, which maps to Xp.22. These are: the five-generation 162-member kindred in which calcium oxalate urolithiasis segregated in a sex linked recessive manner;7 X-linked recessive hypophosphataemic rickets;8 and Dent’s disease,9 in which the affected male hemizygotes have hypercalciuria, nephrolithiasis, nephrocalcinosis, hypophosphataemia, generalized amino-aciduria, renal glycosuria, renal calciuriis, defective urinary calcification and concentrating ability, together with juvenile rickets. Dent and Friedman10 originally identified some aspects of the syndrome and called it hypercalciuric rickets. The defective gene product is a chloride-channel protein specific to the renal tubule; it is not directly responsible for calcium transport, and it is suggested that impaired chloride reabsorption reduces water reabsorption in the proximal convoluted tubule, and hence the fluid drag upon which the passive reabsorption of calcium ions in this part of the tubule depends. The low-molecular-weight proteins are taken up into the vacuolar-lysosomal system of the epithelial cells, presumably by receptor-mediated endocytosis. The low pH within the vacuolar-lysosomal system is maintained by an electrogenic H+-ATPase pump, and chloride channels provide a mechanism for dissipating a charge that results from this proton pumping. Dysfunction of this mechanism, with a change in the intra-vacuolar-lysosomal pH, might impair low-molecular-weight protein endocytosis and possibly other tubular reabsorptive functions. Gram11 reported a large kindred in which calcium oxalate stone formation was inherited and expressed.
principally in the male members. This may have been another example of X-linked urolithiasis with partial expression in the female carriers.

**Vitamin D synthesis as a possible factor in urinary stone formation**

The biosynthetic pathway of Vitamin D\textsuperscript{12} or 1α,25-dihydroxycholecalciferol (1α25(OH)\textsubscript{2}D) involves the non-enzymatic conversion of 7-dehydrocholesterol to cholecalciferol in the skin, subsequent 25-hydroxylation of cholecalciferol to 25-dydroxycholecalciferol in the liver by a mitochondrial cytochrome 450 mono-oxygenase and subsequent 1-hydroxylation by the cytochrome 450 oxygenase in the kidney. This second hydroxylation is highly regulated. Vitamin D promotes intestinal calcium absorption and resorption from bones, thus influencing blood and urine calcium levels. Urinary stones are not a feature of the known specific inherited disorders of vitamin D metabolism (hereditary selective and simple deficiency of 1α25(OH)\textsubscript{2}D and hereditary generalized resistance to 1α25(OH)\textsubscript{2}D, in which either the enzymic synthesis of vitamin D or the synthesis of the vitamin D receptor is abnormal). However, small specifically placed mutations in the relevant genes, insufficient to produce the rachitic features seen in the well recognized inborn errors of vitamin D metabolism, could change either the vitamin D level or the affinity of the vitamin D receptor, leading to hypercalciuria in the upper part of the reference range. This would modify the overall effect of mutations in other genes that tend to promote hypercalcaemia and hypercalciuria, and thereby, stone formation. Activation of lymphocytes in sarcoidosis produces detectably high level of 1α25(OH)\textsubscript{2}D and can cause hypercalciuria.

**Oxalate metabolism**

Robertson and his colleagues\textsuperscript{13} developed a statistical approach to the problem of assessing the relative importance of six different factors in the genesis of urolithiasis, and found that urine volume, oxalate concentration, glycosaminoglycan concentration, urine pH and calcium concentration were important, in this order. Oxalate has no known intermediary metabolic role in animals in general and mammals in particular. It is an important carbon source for some bacteria such as *Oxalobacter formigenes* which is a normal commensal in the human colon.\textsuperscript{14,15}

About 5–10% of urinary oxalate is of dietary origin; the remainder is of endogenous origin. Its main immediate metabolic precursor is glyoxylate, which is itself a product of a minor pathway of glycine metabolism (about 1% of the whole.\textsuperscript{16} and catalysed by D-amino acid/glycine oxidase, EC 1.4.3.3). About 40% of the urinary oxalate is derived from glycine. The C\textsubscript{1}–C\textsubscript{3} moiety of ascorbate\textsuperscript{17} also contributes to the urinary oxalate. The remainder comes from other amino acid and carbohydrate sources by metabolic pathways, the exact nature of which remains obscure.

There are two inborn errors of glyoxylate metabolism which cause excessive oxalate synthesis leading to calcium oxalate urolithiasis, and nephrocalcinosis. They are: primary hyperoxaluria type I (PH1), which is due to reduced activity (or absence) of liver peroxisomal alanine:glyoxylate aminotransferase (AAG, EC 2.6.1.44); and primary hyperoxaluria type II (PHII), in which the activity of the widely distributed enzyme glyoxylate reductase (GR, EC 1.1.1.26/79), also known as hydroxypyruvate reductase or D-glycerate dehydrogenase (EC 1.1.1.29), is absent or grossly deficient. Glyoxylate accumulates behind the respective metabolic blocks, and is oxidized to oxalate by lactate dehydrogenase (LDH, EC 1.1.1.22), which is widely distributed. Both of these diseases are genetically and phenotypically heterogeneous. Mutations producing only minor changes in catalytic activity, insufficient to produce overt hyperoxaluria in their own right, could lead to oxalate excretion levels at the upper end of the reference range, and might act in concert with other predisposing risk factors to cause stone formation in patients with idiopathic calcium oxalate stone formation, or make the extent of the stone formation in patients with idiopathic hypercalciuria more severe. Patients with PHI and PHII have hyperglycolic aciduria and D-glyceric aciduria respectively as well as hyperoxaluria, but about 25% of cases of PHI do not show this phenomenon. Absence of D-glyceric acid has been reported in the less common and less extensively studied PHII.

Cases of hyperoxaluria without deficiency of either AAG or GR have also been reported. They have been ascribed to either a primary disorder of hyperabsorption (PHIII) or due to impaired oxidation of oxalate in the colon due to deficiency of *Oxalobacter formigenes*. Attempts are currently being made to exploit orally administered *O. formigenes* as a probiotic way of reducing hyperoxaluria, on the assumption that oxalate within the colon is not only derived from the diet but from body fluids outside the colon also.\textsuperscript{18,19} It is well known that excessive oxalate absorption and secondary hyperoxaluria occurs in diffuse small
intestine disease and after extensive small intestinal resections, but the extent to which oxalate is either secreted into, or diffuses passively, into the colonic lumen where it could be metabolized by *O. formigenes* is at present unknown. Progress in this field would be greatly advanced by the development of animal models such as, gene ‘knock-out’ mice for the genes directing the synthesis of the enzymes involved in the synthesis of oxalate.

**Nephrocalcin**

Nephrocalcin is a strongly acidic glycoprotein (molecular weight 14 kDa) present in normal urine, which inhibits calcium oxalate crystal growth and has been isolated from urinary stone matrix. It contains both hydrophobic and hydrophilic regions and polymerizes readily.

Nakagawa and his colleagues reported that nephrocalcin isolated from stone-formers’ urine and from the organic matrix of calcium oxalate stones lacks the γ-carboxyglutamic acid that is present in nephrocalcin from normal urine. The extent to which nephrocalcin contributes to the overall anticrystallization potency of normal urine is unclear, but mutation in the gene directing its synthesis should be considered as a site at which polymorphisms affecting the γ-carboxyglutamic acid content of the nephrocalcin could influence an individual’s risk of stone formation. Some workers have cast doubt on the concept of nephrocalcin as a discreet entity.

**Osteopontin**

Osteopontin is a 40 kDa glycoprotein whose function has been reviewed by Mazzali and colleagues, and which may have a role as a defence against stone formation. It is involved in the regulation of both normal and abnormal calcification, including bone remodelling and dystrophic calcification (including that associated with atherosclerosis, where it represents an attempt to prevent or limit vascular calcification). In the case of the urinary tract, it is a normal constituent of urine, and has been isolated from urinary stones. Osteopontin is synthesized within the kidney and secreted into the urine by renal epithelial cells of the loop of Henle, the distal tubules and the renal papillae. It inhibits all stages of the stone-forming process: the binding of calcium oxalate to renal epithelial cells, which has been proposed as a first step in stone formation, as well as the nucleation, growth and aggregation of calcium oxalate crystals. In addition, it has been reported to direct the crystallization process towards calcium oxalate dihydrate which is less adherent to renal tubule epithelium than the monohydrate form.

Studies by Rittling and colleagues using osteopontin knock-out mice have demonstrated this directly. They also found that osteopontin expression in the kidney is up-regulated in wild-type mice with ethylene-glycol-induced hyperoxaluria. Osteopontin knock-out mice do not spontaneously develop urolithiasis and/or nephrocalcinosis, indicating the role of other protective factors in urine, at least in the mouse. Wild-type mice with ethylene-glycol-induced hyperoxaluria show up-regulation of osteopontin, lending further support to its putative role in preventing renal tract calcification.

Thus, mutations in the gene directing the synthesis of osteopontin could be another genetic factor predisposing to urinary stone formation.

**The Tamm-Horsfall glycoprotein (uromodulin)**

The Tamm-Horsfall glycoprotein (molecular weight 84 kDa) is the most abundant protein in normal urine (50–100 mg/day). It was originally called uromucoid, and was isolated from normal urine by salt precipitation, and subsequently from pregnancy urine by lectin column chromatography. These two proteins have the same primary amino acid sequence, but have differences in their glycosylation side chains. For the purposes of this discussion they are both referred to as Tamm-Horsfall glycoprotein. It is a potent activator of granulocytes and its physiological function to relate to immunoregulation. Tamm-Horsfall glycoprotein is synthesized in the cells of the thick ascending segment of Henle’s loop, and coats the luminal side of the tubule epithelium. It is a major constituent of urinary casts, and its involvement has been proposed in cast nephropathy, urolithiasis and tubulointerstitial nephritis. Mutations in the Tamm-Horsfall glycoprotein are associated with autosomal dominant medullary cystic disease and juvenile hyperuricaemic nephropathy. It aggregates at high ionic strengths, under which circumstances it forms part of the stone matrix. Conversely, at low ionic strengths, in its unaggregated form, it binds calcium oxalate monohydrate (COM) and is an effective inhibitor of COM aggregation and hence of microlith formation. It also inhibits the binding of COM to the renal tubular epithelium, a mechanism (the fixed particle theory of stone formation) which is currently viewed as the most likely first step in stone formation.
Thus, polymorphisms in the gene encoding the Tamm-Horsfall glycoprotein, leading to structural changes that affected either its ability to bind COM, its degree of aggregation or its ability to influence the binding of COM to the luminal surface of the renal tubule epithelial cells, could modify the propensity of COM to crystallize and form stones. Furthermore, minor alterations in its ability to promote uric acid handling in the kidney, as in juvenile hyperuricaemic nephropathy, could also be significant.

Prothrombin fragment 1 (UPTF1)

Prothrombin fragment 1 (UPTF1) inhibits crystallization in urine, and abnormalities in the excretion of this protein have been proposed as a factor in stone formation for about 20 years. Prothrombin synthesis occurs in the kidney. Differences in the underlying conformation of UPTF1 have been proposed as the sole cause for differences in the incidence of urolithiasis in different ethnic groups. Although the kidney is only a minor site of the total body synthesis of prothrombin, the excretion of one of its degradation products (UPTF1) makes it a site at which genetic polymorphism could act additively with small genetic changes in other potentially protective substances in the urine to alter the overall predisposition to urinary stones. If the observation on protein conformation as the determinant of the inhibitory capacity of a highly purified preparation of UPTF1 is confirmed, it could open up a completely new research area.

The glycosaminoglycans

The glycosaminoglycans are large unbranched acidic polysaccharides which, with the probable exception of hyaluronic acid, occur attached to a protein core, forming the proteoglycans which form part of the extracellular matrix. Two glycosaminoglycans, chondroitin sulphate and heparan sulphate are excreted free in the urine and can inhibit the nucleation, aggregation and growth of calcium oxalate and uric acid crystals (on which calcium oxalate crystals grow epitaxially). Thus polymorphisms in the genes directing their production from tissue proteoglycans could be another genetic factor contributing to calcium oxalate stone formation.

Bikunin

Bikunin is the light chain of inter-z-inhibitor (lzI), which is composed of two heavy chains (H1 and H2) and bikunin. Their synthesis is directed by two separate genes, and they are covalently bonded to form lzI. The bikunin chain originates from a common -microglobulin/bikunin precursor which is separated into the two polypeptides by post-translational cleavage. lzI and related proteins are thought to be synthesized in the liver, and have been detected in various tissues, including the kidneys. Free bikunin is found in plasma and urine. Urinary bikunin inhibits calcium oxalate crystal growth and bikunin isolated from stone-former urine shows less inhibitory activity towards calcium oxalate crystal growth than that isolated from non-stone-former urine. Bikunin also inhibits the nucleation and aggregation of calcium oxalate crystal growth. Thus, bikunin and/or lzI may be physiological protective substances against urinary stone formation, and genetically determined variations in their structure could contribute to the propensities of different individuals to form stones.

References
