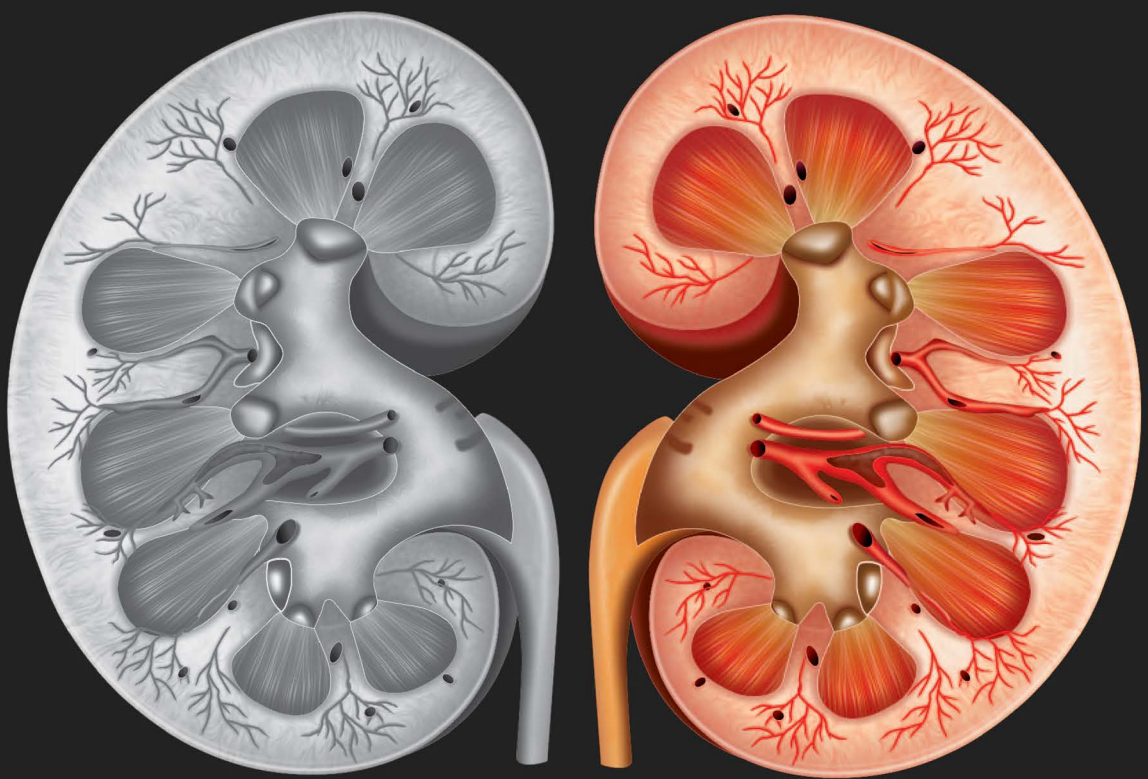
KIDNEY STONES

MEDICAL AND SURGICAL MANAGEMENT



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SECTION 1: Characteristics and Formation of Kidney Stones

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3 Biology and Clinical Relevance of Urine Crystallization Inhibitors

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INTRODUCTION

Supersaturation (SS), the presence of a substance in solution at a concentration above its solubility, is the driving force for crystallization.[1] In urine, SS for calcium oxalate (CaOx), calcium phosphate (CaP) and uric acid (UA) are the most relevant contributors to the formation of the majority of human kidney stones.[2] Stone-forming patients generally have elevated SS values compared to healthy people; these SS values are effective in identifying causes of stones and are used to guide treatment.[3] However, even normal human urine is frequently supersaturated for stone-forming salts, yet most people do not form stones. This is due to the well-known ability of urine to inhibit the nucleation, growth and aggregation of crystals.[4] This ability is widely considered an essential defense against stones that is indeed abnormal among male and female stone formers.[5,6] Crystallization inhibition is mediated by molecules in urine that slow the formation of crystals. It is possible that some molecules may exhibit abnormalities, which result in reduced inhibition effectiveness in a stone-forming population. Many molecules, large and small, in urine are involved in controlling crystal nucleation, growth and aggregation, as well as crystal interaction with the renal tubular epithelial cells and crystal retention within the kidney. Aggarwal et al. have provided a comprehensive review of molecules found in urine and stone matrix, and how they modulate stone formation.[7] To date, no individual defects have been shown to be a sole cause of stones. In this chapter, urinary molecules that are thought to contribute the most to the overall crystallization-inhibitory effect of urine on calcium stone-forming salts will be discussed. Since calcium-containing stones represent the vast majority of human stones (>80%) and urinary inhibitors are not so important in preventing the formation of other types such as UA, only inhibitors of calcium crystallization will be considered.[8]

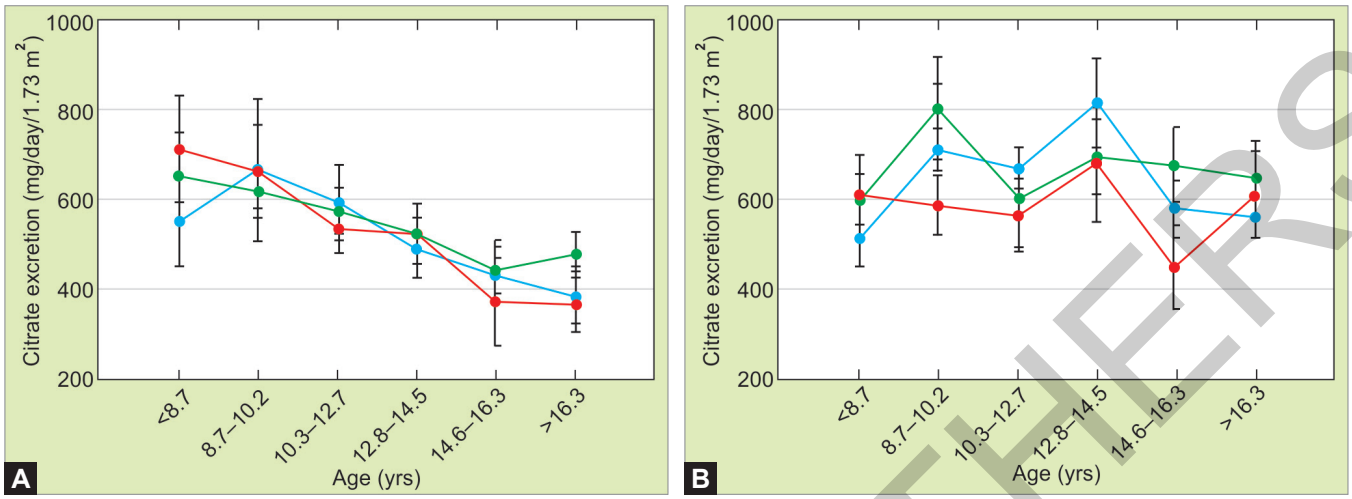
SMALL MOLECULES

Part of the capacity of urine to inhibit calcium crystallization is found in low-molecular-weight (LMW), dialyzable substances that are known to reduce SS of calcium salts.[1]

Citrate

Citrate is an established kinetic retardant of calcium crystallization.[9] Citrate creates soluble complexes with calcium in urine, thereby inhibiting the nucleation, growth and aggregation of CaOx and CaP crystals.[10–14] Citrate has generally been found to be present at reduced levels in the urine of adult stone formers, a deficiency that is thought to facilitate stone formation.[15,16] Two randomized controlled trials have shown citrate supplementation to be effective in reducing recurrence of calcium stones.[17,18] Consequently, citrate is often prescribed as a treatment to decrease risk of urine crystallization in calcium stone-forming patients.[19,20]

However, the effective clinical use of citrate is complicated since urinary citrate levels are influenced by many factors, including age, sex and metabolism. Miyake et al. found that the urinary excretion rate of citrate in healthy children is two- to threefold higher than in adults, when adjusted for body size,[21] supporting a role for citrate in the reduced incidence of nephrolithiasis in children compared to adults. We and others have found sex-dependent differences in citrate excretion starting around puberty, with citrate excretion in boys declining with age and girls having significantly higher urinary citrate than boys after about the age of 12 years (Figs. 3.1A and B).[22,23] Likewise, among healthy adults, citrate excretion is higher in females than males.[24] Thus female sex hormones may play an important role in maintaining elevated urinary citrate levels. However, citrate excretion has been found to be increased among overweight and obese stone formers of both sexes compared to nonobese stone formers.[25,26] Changes in urinary citrate level can also be affected by the acid–base



Figs. 3.1A and B: Urine citrate by sex and age. Daily excretions of citrate are expressed per 1.73 m^2 body surface area in boys (A) and girls (B) by age sextile. Values are mean ± standard error of the mean. Citrate declines with age in boys, $p < 0.001$ for the regression.[22] (Blue: Stone formers; Red: Non-stone forming siblings of stone formers; Green: Unrelated non-stone forming children).

status, with citrate excretion being decreased under conditions of systemic or intracellular acidosis.[23,27] The physiological mechanisms governing urine citrate excretion are not completely understood, but may involve differential regulation of the Na^+-dependent dicarboxylate co-transporter, NaDC-1, which facilitates citrate reabsorption in the proximal tubule.[28] Administration of citrate can raise urine pH, and therefore CaP SS, in addition to urine citrate concentration, which may increase risk of crystallization, although this effect may be offset by its ability to chelate and lower urine calcium.[29–31] Altogether, more research is needed to determine which patients may benefit most from treatment with citrate, with age, sex, weight and urine pH potentially affecting the risk-to-benefit ratio for stone prevention. The reader is referred to a series of excellent articles by Fredric Coe on the subject of the relationship of citrate to the formation and prevention of kidney stones.[32]

Magnesium

Magnesium (Mg) is present at millimolar concentrations in urine where it competes with calcium for binding of oxalate.[33] Magnesium ions destabilize calcium oxalate ion pairs and reduce the size of their aggregates.[34] Magnesium oxalate is two orders of magnitude more soluble than calcium oxalate; therefore, urine Mg reduces the risk of crystallization of insoluble calcium salts and subsequent stone formation.[35] The incidence of hypomagnesiuria, defined as <3 mmol per day for men and <2.2 mmol per day for women, has been reported to be 19–60% in calcium stone formers.[36,37] As such, there has been much interest in the use of Mg supplements as therapy for CaOx nephrolithiasis. However, evidence from clinical trials has not justified the use of Mg as a sole therapy for stones, possibly due to poor gastrointestinal (GI) absorption.[33] In a double-blind, randomized, placebo-controlled trial, Ettinger et al. were not able to show a difference between the recurrence rates of the Mg and placebo groups in 51 patients with a history of at least two stones.[38] However, the addition of Mg to potassium citrate therapy seems to improve outcomes; providing Mg as a mixed salt (Mg potassium citrate) reduced calcium stone recurrence by 90%, similar to potassium citrate, but with better GI tolerance.[17]

Pyrophosphate

Pyrophosphate inhibits crystallization of CaOx and CaP in vitro.[39,40] At normal urinary concentrations, pyrophosphate is a more potent inhibitor of hydroxyapatite crystal growth than either citrate or Mg.[41] Studies comparing pyrophosphate levels in the urine of stone formers versus healthy controls have produced mixed results, with some studies showing reduced pyrophosphate excretion in stone formers[42,43] and others showing no difference.[44,45] Pyrophosphate excretion varies with age and sex, with a tendency toward higher excretion with increasing age and higher excretion in men than in women.[43] Urinary pyrophosphate is presumably of endogenous origin and is likely a

reflection of bone metabolism.[44] Interestingly, in a study of the effect of chronic stress on urine composition of CaOx stone patients, measures of stress were positively correlated with blood cortisol and urinary pyrophosphate levels.[46] Cortisol triggers bone mineral resorption and increases urine calcium excretion,[47] so pyrophosphate excretion may be correspondingly elevated to protect against urine crystallization.[46] Men have been found to have a higher cortisol response to stress compared with women,[48] which is in accord with the sex difference in pyrophosphate excretion. All in all, urine pyrophosphate levels may have more to do with demographic and lifestyle factors than with stone-forming status.

Phytate

Phytate, or inositol hexaphosphate, is a natural compound that is found in abundance in plants, especially in whole grains, seeds and nuts.[49] Phytate contains six phosphate groups, each with two hydroxyl moieties, which allow chelation of divalent metal ions including Fe^{2+}, Zn^{2+}, Mg^{2+} and Ca^{2+}.[50] In vitro studies have shown phytate to be a powerful inhibitor of CaOx and CaP crystallization due to the multiple negatively charged phosphate groups that bind calcium in solution.[35,49,51] Phytate is normally found in urine at levels that are directly related to dietary intake,[52] and urinary phytate has been found to be reduced in calcium stone formers relative to healthy controls.[53] A large epidemiologic study of >96,000 women in the United States showed that phytate intake was associated with a reduced risk of stone formation.[54] The major potential drawback for phytate as a treatment to reduce stone risk is that it forms insoluble metal ion complexes that are poorly absorbed by the GI tract.[55] Excretion of insoluble complexes of phytate with essential minerals such as iron and zinc can lead to reduced bioavailability and mineral deficiency.[56] In fact, micronutrient malnutrition due to phytate consumption from plants is a recognized problem worldwide, and breeding low-phytate crop varieties is an active area of agricultural research.[57] There is also evidence that CaOx stone formers who consume vegetarian diets should avoid foods that are high in phytate, as chelation of calcium by phytate in the GI tract results in an increase in intestinal absorption and urinary excretion of oxalate.[58] Thus, the potent ion-chelating ability of phytate represents a potential benefit to stone patients in inhibiting urine crystallization that is countered by the disadvantage of depleting essential minerals and increasing oxalate excretion.

MACROMOLECULES

Most of the capacity of urine to inhibit crystallization appears to reside not in the ionic milieu of urine but in the larger molecules that cannot be removed by dialysis.[59,60] One such class of molecules is glycosaminoglycans (GAGs) such as chondroitin sulfate, heparan sulfate and hyaluronic acid.[61] Another class is urine proteins with crystallization inhibitory properties, including prothrombin fragment 1 (PF1),[62] osteopontin (OPN; uropontin),[63] inter-alpha-trypsin inhibitor (ITI),[64] bikunin (BK, a portion of the ITI),[65] Tamm–Horsfall protein (THP),[66] albumin[67] and calgranulin.[68] These macromolecules are all expressed in the kidney, and may contribute to the overall crystallization inhibitory effect of urine. Because of their ability to bind calcium, many so-called inhibitors can also act as promoters of crystal formation and deposition depending upon experimental conditions and whether the molecules are fixed to a substrate.[69] Organic macromolecules, including GAGs and proteins, form the matrix of calcium stones and comprise 2–3% of the weight of the stone.[50] In addition to their effects on crystallization, all of these molecules have other normal biological functions including, but not limited to, modulation of tubule and transporter functions, mediation of inflammation and the innate immune response, formation and stabilization of extracellular matrix, facilitation of wound healing and tissue repair, and antimicrobial protection.[70–75] Thus, the clinical relevance of these molecules to kidney stone disease may not be solely due to their effects on calcium crystallization inhibition but also to their other functions in the kidney and urinary tract. Here, the clinical relevance of these proteins to the pathophysiology of calcium nephrolithiasis will be examined.

Glycosaminoglycans

Glycosaminoglycans are long unbranched polysaccharides consisting of a repeating disaccharide unit.[76] They are synthesized by epithelial cells lining the urinary tract where they serve to form a water-tight barrier to shield the cell surface from urinary solutes, proteins, bacteria and crystals.[77] The most abundant GAGs in urine are chondroitin sulfates A and C and heparan sulfate; also present at lower levels are hyaluronic acid, dermatan sulfate and keratan sulfate.[78] Glycosaminoglycans are highly negatively charged and have been shown to be potent inhibitors of growth and aggregation of CaOx and CaP crystals in vitro.[40,79-81] These inhibitory properties are largely due to the effects of chondroitin sulfate and, to a lesser extent,

heparan sulfate.[50] Altered abundance of urinary GAGs has been shown to be associated with interstitial cystitis,[82,83] but a plethora of studies examining the relationship between urine GAG concentration or excretion and stone formation has failed to demonstrate any conclusive association.[50] Urinary GAGs are increased in interstitial cystitis as a result of chronic bladder epithelial damage and neurogenic inflammation.[84] Urinary GAG levels are more likely to be a reflection of inflammatory processes in the kidney and urinary tract than to be related to a marker of stone formation.

Urine Proteins

Prothrombin Fragment 1

Prothrombin fragment 1 is a potent inhibitor of CaOx crystal growth and aggregation[62] and also the most prominent protein in the organic matrix of CaOx crystals precipitated from fresh human urine.[85] Urinary PF1 also inhibits CaOx crystal attachment to cultured cells.[86] Prothrombin fragment 1 comprises the first 155 amino acids of prothrombin, including a 10-residue γ-carboxyglutamic acid (Gla) domain that confers on PF1 a strong ability to bind calcium.[87] The potent inhibitory effect of PF1 on CaOx crystallization has been ascribed to this Gla domain, which is absent from other prothrombin activation fragments including thrombin and the F2 fragment.[88] Liu et al. showed that the Gla composition of urinary PF1 as well as its ability to inhibit CaOx crystal growth was significantly decreased in patients with CaOx stones.[89] However, Buchholz et al. found no difference in the ability of urine from patients taking warfarin to inhibit CaOx crystal growth compared to urine from healthy individuals.[90] Gamma carboxylation is a vitamin-K-dependent process that is inhibited by the drug warfarin, so the absence of increased crystal growth in the presence of warfarin does not support a substantial role for vitamin-K-dependent proteins such as PF1 in inhibiting urine crystallization.

Prothrombin fragment 1 is also highly glycosylated[91] and the level of sialylation may contribute to its effectiveness in inhibiting CaOx crystallization. Webber et al. found a correlation between the genetically determined levels of PF1 sialylation and the incidence of CaOx stone disease in racial groups among the South African population.[92] Glycosylation of PF1 was also found to govern CaOx crystal nucleation and aggregation, but it does not play a role in inhibiting crystal growth.[93]

Prothrombin fragment 1 is produced by thrombin cleavage of a precursor protein, prothrombin, and is associated with activation of blood coagulation.[87] It is primarily produced by the liver, but the prothrombin gene is also expressed in the kidneys of humans and animals.[94,95] Prothrombin conversion to thrombin and prothrombin fragments is the last step in the coagulation cascade, and there is evidence that this can occur at sites of renal injury.[96] Acting via protease-activated receptors in the kidney, thrombin mediates several intracellular-signaling pathways and plays a role in the inflammatory response to tissue damage, stimulation of epithelial cell proliferation and extracellular matrix deposition.[97,98] Immunohistochemical analysis of human kidneys has shown that PF1 is synthesized in tubular epithelial cells of the thick ascending limb of the loop of Henle (TALH) and the distal convoluted tubule, and is more abundant in the kidneys of stone formers than in healthy individuals.[99] It is unclear whether urinary PF1 derives from serum prothrombin or is the product of renal prothrombin gene expression. In a study of first-degree family members of calcium stone formers, we found that prothrombin fragments in urine (mainly larger PF1-containing activation peptides of prothrombin such as PF1+2) were strong predictors of stone formation in men.[100] However, others have found no differences in the relative abundance of PF1 in the urine of CaOx stone formers compared to healthy people.[101]

A genetic link between a prothrombin gene variant and increased kidney stone risk has been identified in female calcium stone-forming patients in Thailand.[102] A nonsynonymous change leading to a threonine (T)-to-methionine (M) substitution at amino acid 165 (T165M) was located in the kringle 1 domain of PF1.[103] Structural modeling of PF1 suggests that the T165M substitution may affect the function of PF1 by blocking hydrogen bond formation with glutamic acid at position 180. The PF1 kringle 1 domain is thought to be important in mediating interactions with other proteins within the blood coagulation cascade such as cofactor Va,[104] and it is believed to play a role in inducing migration and activation of human neutrophils.[105] How this mutation affects the risk of stone formation is yet to be understood. The prevalence of stone disease in the Thai population shows a typical male predominance, with a male-to-female ratio of about 2:1; the reason why this variant plays a role only in females is also unclear.[103]

Osteopontin

Osteopontin is a member of the SIBLING family of proteins (*S*mall *I*ntegrin-*B*inding *Li*gand, *N*-linked *G*lycoprotein),

which plays a role in matrix mineralization, most commonly in bone and dentin.[106] OPN has been found to be expressed in several tissues and cell types, including the mineralized bone matrix, macrophages, dendritic cells, endothelial cells, smooth muscle cells and epithelial cells.[107] It is also expressed in the kidney in glomerular mesangial cells, podocytes, collecting duct, thin loop of Henle and urothelial cells.[108–110] OPN is a chemokine that can modulate cell adhesion as well as autocrine and paracrine factors by interacting with cell surface receptors such as integrins.[111] OPN is rich in aspartic acid residues and is highly negatively charged, making it an avid calcium-binding protein.[112] OPN is abundantly found in the matrix of calcium stones[113,114] and is present in hydroxyapatite Randall's plaques in the renal medullary interstitium.[110,115] OPN is a potent inhibitor of CaOx crystal nucleation, growth and aggregation in vitro.[63,116] However, experiments using atomic force microscopy have shown that OPN may also promote aggregation of crystals and binding to cell surfaces by increasing the adhesiveness of crystals to which it is bound.[117] Similarly, Rodriguez et al. found that OPN can promote intrafibrillar mineralization of collagen with hydroxyapatite in an in vitro polymer-induced liquid-precursor biomineralization system.[118]

Osteopontin expression is induced by angiotensin II in different cell types, such as vascular smooth muscle cells, macrophages and epithelial cells of renal tubules.[107,132,133] Patients with nephrolithiasis have been found to have elevated urinary angiotensinogen,[134] which is a marker of intrarenal angiotensin II activity.[135] High dietary salt intake, which can increase the production of intrarenal angiotensin II, also increases renal OPN expression in rats.[136] Osteopontin expression is increased as well by hormones that regulate bone and mineral metabolism such as parathyroid hormone and vitamin D, which are often elevated in stone formers.[137,138] These results suggest that OPN expression would be expected to be increased in kidneys of stone formers compared to normal controls.

Osteopontin is difficult to measure in urine, likely because it is highly susceptible to cleavage by proteases in urine. There is evidence that urine from kidney stone patients has elevated serine protease activity that generates aberrant OPN species.[119] This may cause technical difficulties in OPN detection and quantification depending on the type of assay used. Most efforts to examine OPN abundance by enzyme-linked immunosorbent assay (ELISA) in urine from stone patients and normal controls have found decreased urinary OPN in stone formers.[120–122] However, other studies have found no difference, either by semiquantitative western blotting[101] or ELISA.[119] These disparities may be due to differences in the antibodies and methods used for detection of OPN; loss of an epitope in OPN due to proteolytic cleavage in urine could lead to failure of an antibody to detect certain fragments and overall underdetermination of OPN. In histopathology studies, Evan et al. found that OPN in renal medullary tissue was similarly expressed in normal and stone-forming subjects except for its localization in Randall's plaques of the stone formers.[110] Overall, whether there are differences in renal or urinary total OPN abundance between stone formers and healthy people is still an open question.

Proteolytic cleavage of OPN by thrombin and matrix metalloproteinases is a normal regulatory process that modulates the activity of OPN and its binding to integrin receptors in some tissues.[123] Thrombin cleavage of OPN produces three fragments that have different crystallization inhibitory properties in vitro, and it is the central fragment that has been found to effectively inhibit hydroxyapatite crystal formation and growth.[124] In urine, OPN may be cleaved by thrombin and/or other proteases that are present. Kolbach et al. have identified four isoforms of OPN in human urine and found that there is a relative deficiency of the two most acidic forms in urine from stone formers compared to that of normal individuals.[125] A decline in the most anionic of the OPN isoforms, that is, those that would interact most strongly with crystals to inhibit growth and aggregation, could be a factor promoting stone formation. Thus, proteolytic cleavage of OPN may affect the crystallization properties of urine and contribute to decreased crystal inhibition in stone formers.

Cleavage by thrombin exposes new epitopes in OPN that increase its capacity to bind and signal through integrin receptors to mediate functions such as cell cycle regulation and actin cytoskeleton dynamics.[126] In human kidneys, $\alpha_v\beta_3$ integrin is expressed in glomerular epithelial cells, Bowman's capsule, vascular endothelium and weakly in tubular epithelial cells; $\alpha_v\beta_5$ is similar, but also on the vascular endothelium, β1 expression is seen in glomerular epithelial cells, Bowman's capsule, the vascular epithelium and tubular epithelial cells.[109,127] In chronic inflammatory conditions with increased thrombin activity such as obesity, atherosclerosis and rheumatoid arthritis, production of thrombin-cleaved OPN fragments in urine is increased.[128] Some of these conditions, including obesity, diabetes and rheumatoid arthritis, are linked to a higher risk of kidney stones.[129,130] Antibodies have been developed

to selectively target protease-cleaved OPN forms, which may have applications in treatment and prevention of various inflammatory diseases and cancer.[126] One such antibody against thrombin-cleaved OPN has been found to reduce renal crystal formation, tubular cell injury and crystal–cell attachment in mice with renal crystals induced by glyoxylate.[131] It is possible that differential protease cleavage of OPN in stone formers may alter its activities both in mediating crystallization and functioning as a chemokine in the kidney.

Mouse strains lacking the *Spp1* (secreted phosphoprotein 1) gene that encodes OPN have been generated.[139,140] Mo et al. found that 10% of the mice lacking OPN spontaneously form renal papillary interstitial deposits of CaP reminiscent of Randall's plaque, which are never seen in wild-type mice.[140] Paloian et al. found that OPN knockout (KO) mice on a high-phosphate diet developed nephrocalcinosis with substantial renal tubular and interstitial calcium deposition, and marked vascular calcification when compared with control mice.[141] In humans, the single nucleotide polymorphism (SNP) rs1126616 affecting Ala 250 in exon 7 of the *SPP1* gene has been linked with reduced OPN levels in serum and urine and increased susceptibility to nephrolithiasis.[142] Safarinejad et al. examined nine SNPs in the *SPP1* gene in CaOx stone formers and healthy individuals in Iran and found one SNP that was protective and three SNPs that were predisposing to CaOx stone formation.[143] Subjects with the protective polymorphism had increased levels of serum OPN and urinary calcium to OPN ratios, while those with the predisposing SNPs had lower serum and urine OPN. Others have found an association between SNPs in the promoter of the *SPP1* gene and risk of stone formation in Japanese and Turkish populations.[144,145] Together these suggest a role for OPN in preventing CaP crystallization and protection against nephrolithiasis that is as yet not clearly defined.

Osteopontin is a multifunctional protein that is upregulated in wound healing, fibrosis, autoimmune disease and atherosclerosis where it is highly expressed at sites of atherosclerotic plaques.[107] It is a potent activator of transcription factor NF-κB (nuclear factor-κB) that is involved in cellular responses to injury and stress; OPN is implicated in promoting epithelial–mesenchymal transition, which is a significant contributor to fibrotic phenotypes and cancer metastasis.[146] OPN expression is upregulated in renal epithelial cells in animal models of renal injury as well as in human renal allografts and diseases such as idiopathic membranous nephropathy, immunoglobulin A nephritis, lupus nephritis and essential hypertension with decompensated arteriolosclerosis.[109] OPN upregulation may mediate an early response to injury and promote regeneration and repair in the kidney. Mice with a disrupted *Spp1* gene exhibit significantly greater ischemia-induced renal dysfunction and structural damage than wild-type mice.[147] With regard to the role of OPN in stone formation, it is not clear whether OPN in the kidney serves to protect against stones by inhibiting crystallization or perhaps promotes interstitial crystal formation (Randall's plaque) and fibrosis seen in CaP stone formers.[148]

Inter-Alpha-Trypsin Inhibitor/Bikunin

Inter-alpha-trypsin inhibitor proteins are a group of serine protease inhibitors that are mainly synthesized in the liver but have also been detected in many other tissues, including intestine, kidney, stomach, placenta and brain.[149] At least four genes are involved in the synthesis of various members of the inter-alpha-trypsin family of proteins, three encoding heavy chains (H1, H2 and H3) of 75–85 kD, and one encoding the light chain, also known as bikunin, BK, with a molecular weight of 35–40 kD.[150,151] Bikunin can be linked covalently through its chondroitin-4-sulfate side chain to one or two heavy chains, forming the pre-alpha-inhibitor (H3 + BK, 125 kD) or ITI (H1 + H2 + BK, 180–220 kD), respectively.[152] All of these genes have been shown to be expressed in kidney cells.[153,154] ITI, pre-alpha-inhibitor, and all individual subunits have also been detected in urine[155,156] as well as in the matrix of calcium stones.[50,114] While the entire ITI molecule has been shown to inhibit CaOx crystallization,[157] the BK portion is itself a potent inhibitor.[65] The heavy chain components do not have inhibitory activity, and BK is thought to be responsible for the ability of ITI to inhibit CaOx crystallization.[158] To date, no polymorphisms in the genes encoding these proteins have been shown to be associated with nephrolithiasis. One study investigated the possible association between BK gene (*AMBP*) polymorphisms and urinary stone formation in a Turkish population, but found no significant differences in allele distribution between patients and controls.[159]

Inter-alpha-trypsin inhibitor expression is associated with response to inflammation or injury.[97] ITI proteins are thought to be part of the systemic innate immune system; they attenuate complement activation and play a role in limiting complement-dependent tissue injury.[149,160,161] Administration of exogenous BK and ITI proteins has been shown to reduce systemic

inflammation in sepsis and systemic inflammatory disorders in mice and humans.[162,163] ITI proteins also play a role in extracellular matrix stabilization in a variety of tissues via binding to hyaluronan.[164] In rats, renal injury induced by hyperoxaluria and crystalluria has been shown to result in significant increases in the level of BK mRNA (messenger ribonucleic acid) expression in the kidney, and concomitant urinary excretion of high-molecular-weight (HMW) BK-containing proteins as well as heavy chains H1 and H3.[155,165,166] In humans, ITI and dimer of BK combined with H1 or H2 were found more often in urine in stone-forming males than in normal males.[156] We also found an increase of HMW (>50 kDa) immunoreactive ITI components in the urine of those who formed stones versus those who did not,[100] and another study confirmed increased urine ITI in stone formers versus normals of both sexes.[101]

Using immunohistochemistry in sections of human kidney tissue, Evan et al. explored the relationship between ITI, BK and calcium stone disease.[110] Heavy chain 3 (HC3) was found to be associated with hyaluronan in the interstitial matrix of the kidney. Widespread HC3 was only present in stone formers, and was found in collecting duct, thin loop and interstitial cells. HC3 colocalized with Randall's plaque (interstitial hydroxyapatite) and urothelial cells, and was also found in the matrix layer of individual plaque spherules. BK was present only in the collecting duct apical membranes and the loop cell cytoplasm of stone formers, colocalizing with HC3. The mechanism linking HC3 to stone disease is unclear but may involve a role for HC3 in stabilizing hyaluronan in the renal interstitial matrix. In the context of pulmonary epithelial cell injury, ITI interacts with hyaluronan and other extracellular matrix components and is an important regulator of cellular repair after injury.[167] Proteomic analysis of renal calculi indicates an important role for inflammatory processes in calcium stone formation.[113] Merchant et al. found that proteomic analysis of stones supports the hypothesis that stone formation induces a cellular inflammatory response and the protein components of this response contribute to the abundant stone matrix proteome. Perhaps ITI in kidney plays a similar role in a cellular inflammatory response in the kidney and in promoting repair of injured renal epithelial and interstitial cells.

Tamm–Horsfall Protein

Tamm–Horsfall protein, also known as uromodulin (UMOD), is a glycoprotein that is the most abundant protein excreted in urine under normal physiological conditions.[168] Made exclusively by epithelial cells in the TALH, THP enters the secretory pathway where it is glycosylphosphatidylinositol anchored, glycosylated, and sorted to the apical plasma membrane. Ultimately, THP is secreted into the urine via proteolytic cleavage by the plasma-membrane-bound serine protease hepsin.[169] In vitro crystallization experiments have shown that THP does not inhibit CaOx crystal nucleation or growth[170,171] but is a potent inhibitor of crystal aggregation.[172] Tamm–Horsfall protein from stone-forming patients inhibits CaOx crystal aggregation to a lesser degree than normal.[173] The high glycan content is important for the physicochemical properties and function of THP. Several studies have reported that stone formers produce THP with reduced levels of glycosylation, particularly sialic acid levels, which leads to reduced negative charge.[66,174] This reduced charge likely decreases the effectiveness of THP binding to Ca^{2+} or CaOx crystals in urine, thus limiting its aggregation-inhibitory activity.[175,176] Some studies have shown that desialylated and low sialic acid forms of THP actually promote crystal aggregation, a property that could ultimately contribute to stone formation.[177,178]

Whether kidney stone formers have defects in THP excretion remains unresolved. Some studies of recurrent calcium stone formers have observed decreased THP in the urine[179] while others have shown increased THP excretion.[180] People with extremely reduced urinary THP levels due to *UMOD* mutations do not show increased rates of renal stone formation.[168] These patients are more likely to have other uromodulin-associated kidney diseases such as medullary cystic kidney disease type 2 and familial juvenile hyperuricemic nephropathy, which, as opposed to calcium nephrolithiasis, are characterized by mild urine concentrating defects, reduced fractional excretion of UA, diffuse tubulointerstitial fibrosis with inflammatory cell infiltrates, cysts at the corticomedullary junction and chronic renal failure.[181,182] Thus, the relative importance of urine THP abundance in protection against stones is still an open question.

In a study of urinary excretion of THP in first-degree family members of calcium stone formers, we found that THP excretion differed by sex and by number of stones formed (K. Bergsland, unpublished). By western blotting, the major THP band (Fig. 3.2) migrated at about 85 kD, in agreement with the 80–90 kD molecular weight observed by others on sodium dodecyl sulfate polyacrylamide gel electrophoresis of

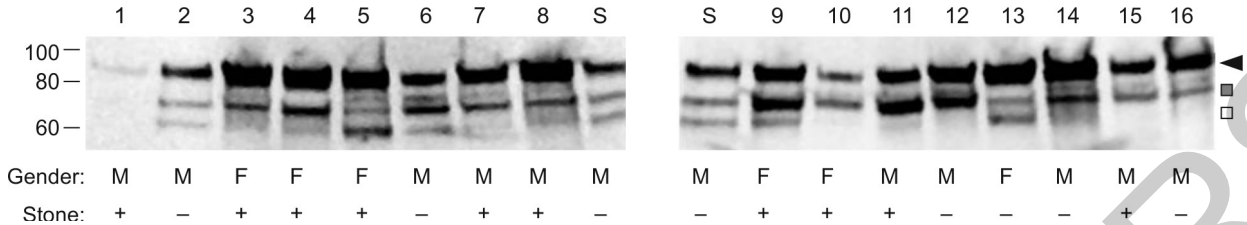


Fig. 3.2: Representative western blots of urinary Tamm–Horsfall protein (THP). Dialyzed urine protein samples were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis on 10% acrylamide gels under reducing conditions, blotted and immunodetected using an antibody to THP. Lanes 1–16 contain samples from different individuals. Lanes S contain a standard urine sample from a nonstone-forming male. Sizes of protein molecular weight standards (in kD) are indicated at left. The major THP band (arrowhead) migrated at about 85 kD; lower-molecular-weight forms likely represent THP precursors lacking glycosylation (57–60 kD, open square) or sialic acid modifications of glycan chains (69–71 kD, closed square). Semiquantitative abundances of various THP forms were calculated by dividing the optical density (OD) of each band by the total OD of lane S on the same blot. (F: Female; M: Male).

properly glycosylated, glycosylphosphatidylinositol-modified and membrane-anchored THP protein excreted in urine.[183] Lower-molecular-weight forms likely represent THP precursors lacking glycosylation (57–60 kD) or sialic acid modifications of glycan chains (69–71 kD).[184–186] When family members of stone formers (50 men, 50 women; half stone formers) were grouped within sex by the presence or absence of kidney stones, THP excretion in all men was the same (Fig. 3.3). Women stone formers tended to have less THP than those who had never had a stone but the difference was not significant. However, when subjects were stratified by the number of stones formed, THP excretion was greater in men with multiple stones compared to men who had formed one stone, a difference that was not observed in women (Fig. 3.3). These sex- and stone number-related differences may help explain the discrepant measurements of urine THP abundance seen in previously published studies, with one study finding less THP in stone formers and another finding more.[179,180] THP excretion in nonstone-forming men was intermediate between male single stone formers and multiple stone formers. Increased excretion of LMW THP was highly significant in men with multiple stones compared to male single stone formers and controls ($p < 0.0001$, both comparisons), but not in women (Fig. 3.4). Thus, increased excretion of both mature THP and LMW forms lacking post-translational modifications is strongly associated with having >1 stone in men. Low-molecular-weight forms of THP that lack post-translational modifications (glycosylation or sialic acid residues) are likely less effective in inhibiting crystal aggregation in urine; the increased proportion of LMW THP in the urine of male multiple stone formers may contribute to their higher rate of stone formation and perhaps to the increased prevalence of stones in men versus women.

Although THP is predominantly targeted to the apical membrane of TALH cells, there also is a lesser but significant basolateral release of THP by a mechanism that is not well understood.[187,188] Tamm–Horsfall protein can be detected in the blood of healthy individuals, suggesting that some degree of basolateral release of THP occurs normally.[187,189] Basolateral release is significantly increased during kidney injury; El-Achkar et al. found a major redirection of THP from the apical membrane toward the basolateral domain and interstitium during recovery from acute kidney injury.[190,191] This redirection corresponds with increased THP in the serum but not in the urine. Evidence suggests that basolateral THP plays an important role in halting intrarenal inflammatory signaling after ischemia reperfusion injury (IRI) by facilitating tubular cross-talk with the neighboring injured proximal tubule S3 segments.[187,191] Tamm–Horsfall protein is known to function as a "cytokine trap," forming in vivo complexes with renal and urinary cytokines, thus limiting their activation of inflammatory signaling pathways.[192] Mice deficient in THP show significantly more functional and histological renal damage after IRI, possibly due to reduced suppression by THP of proinflammatory cytokines and chemokines produced by S3.[193] These mice also have higher expression of toll-like receptor 4 (TLR4) in the basement membranes of injured tubular segments, indicating that THP may also normally act to limit renal injury by decreasing activation of innate immunity mediated by TLR4.

Studies in *Umod* gene KO mice have suggested a role for THP in protection against stone formation in the kidney. Lack of THP in KO mice leads to the formation of CaP (hydroxyapatite) crystals in the kidneys and progressive renal calcification.[140,194] In young mice, both intratubular and interstitial crystals are seen, but in older mice

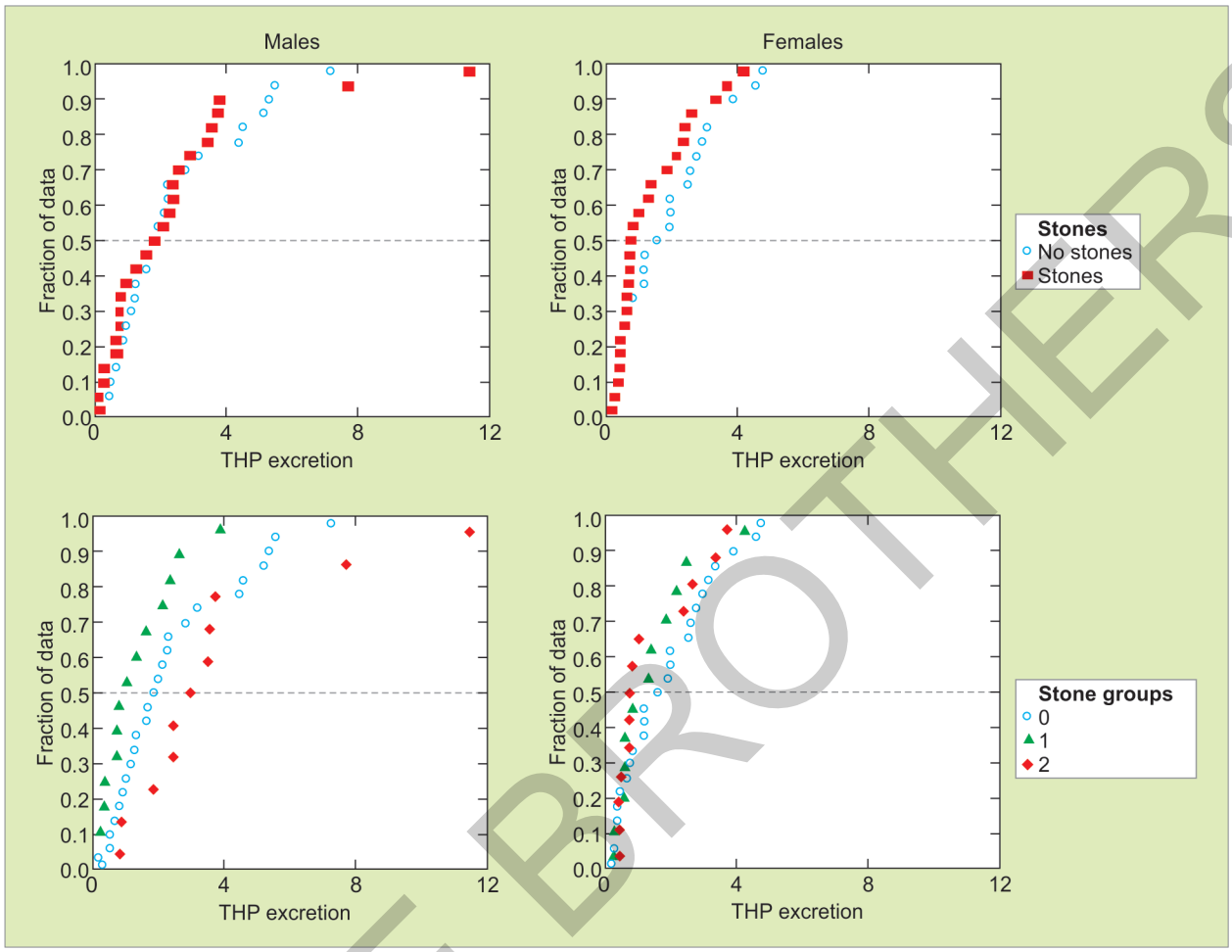


Fig. 3.3: Quantile plots of urine Tamm–Horsfall protein (THP) excretion. Estimations of excretion rates of mature THP were made by multiplying the semiquantitative measurements of each protein (Fig. 3.1) by the 24-hour urine volume. Males are at left and females are at right. In the top panels, subjects are grouped by stone status: nonstone formers (○) and stone formers (■). In the bottom panels, subjects are grouped by stone number: Nonstone formers (○), men with 1 stone, and women with 1–2 stones (▲), and men with ≥2 stones and women with ≥3 stones (♦). In subjects grouped by the presence or absence of kidney stones (top), THP excretion in all men was the same; female stone formers tended to have less THP than nonstone formers but the difference was not significant. In subjects grouped by the number of stones formed (bottom), THP excretion was greater in men with multiple stones compared to men who had formed one stone, a difference that was not observed in women.

interstitial crystals predominate. Tamm–Horsfall protein exerts its protective effect synergistically with OPN as seen in double KO mice.[140,168] The incidence of renal interstitial hydroxyapatite deposits is more than twice as high in the OPN + THP double KO than in either of the single-gene KOs alone. Crystals are mainly located in the renal papilla with the rest of the kidney devoid of any crystals. The pattern of interstitial crystallization in THP KO mice resembles Randall's plaques of human idiopathic CaOx stone formers with respect to histological location, chemical composition, ultrastructural features and lack of accompanying inflammation.[195,196] In humans, however, Randall's plaques also involve the basement membranes of the thin loops of Henle, and there is an overgrowth of CaOx stones atop regions of papillary crystallization, neither of which have been observed in THP KO mice.[197]

In humans, genome-wide association studies have linked a common variant in the *UMOD* gene on chromosome 16p12 to decreased risk of kidney stones.[198] The variant rs4293393-T, which is located in the promoter region of the *UMOD* gene about 550 bp upstream of the translational start, is present at high frequency in most modern populations (70–80% in Africans and Europeans and 92–95% in East Asians) and confers protection against calcium

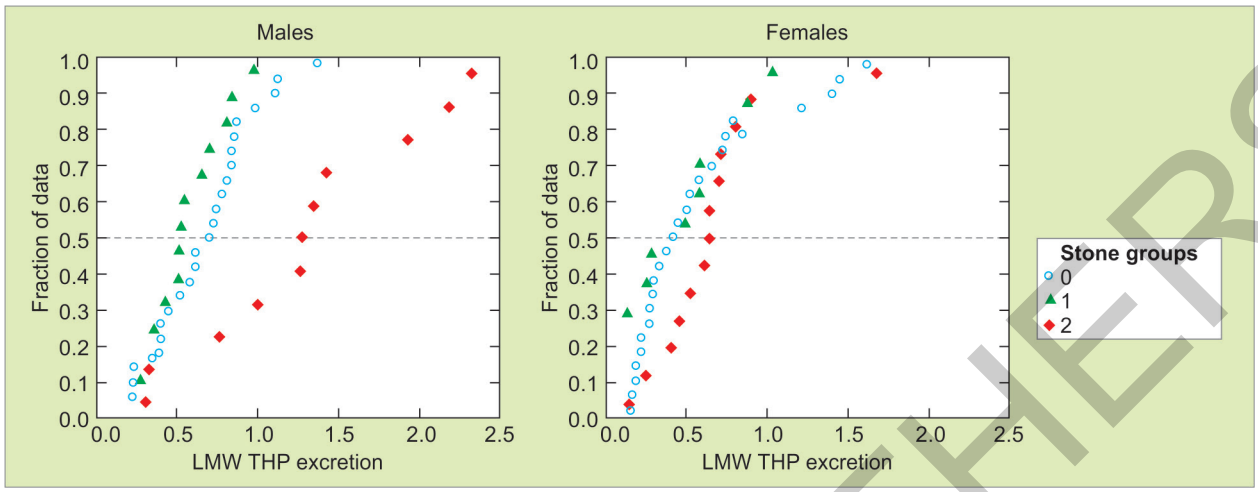


Fig. 3.4: Quantile plots of low molecular weight (LMW) Tamm–Horsfall protein excretion. Estimations of excretion rates of LMW THP forms lacking glycosylation or sialic acid modifications were made by multiplying the semiquantitative measurement of each protein band (Fig. 3.1) by the 24-hour urine volume. Males are at left, and females are at right. Subjects are grouped by stone number: Nonstone formers (○), men with 1 stone and women with 1–2 stones (▲), and men with ≥2 stones and women with ≥3 stones (♦). Increased excretion of LMW THP was highly significant in men with multiple stones compared to male single stone formers and controls ($p < 0.0001$, both comparisons), but not in women. (F: Female; M: Male).

kidney stones.[199] This variant has been shown to increase THP expression approximately twofold both in vivo in kidney tissue by quantitative reverse transcription polymerase chain reaction and in vitro in luciferase reporter assays.[200] Likewise, there is a strong association of this variant with an up to twofold increase in urinary excretion of THP; levels of THP rise in a dose-dependent manner with an increasing number of T alleles.[200,201]

A study of the frequency of the rs4293393-T allele in various modern and ancient human and nonhuman primate populations strongly suggests that this allele has been kept at high frequency through evolution because of its protective effect against urinary tract infections (UTIs).[199] The KO mice lacking THP have an increased susceptibility to UTI.[73,202] Tamm–Horsfall protein functions in defending the urinary tract against infections due to its ability to bind to pathogens and prevent their adherence to the urothelium.[203] There is evidence that, in a similar manner, THP coats CaOx crystals and helps prevent adhesion to cultured epithelial cells.[86,176] Perhaps the selective pressure to maintain the rs4293393-T allele because of its protective effect against UTI has had the fortuitous benefit over time of also protecting against stones. The minor rs4293393-C variant that is the risk allele for kidney stones also potentially increases susceptibility to UTIs.[199] It is interesting that, although a definitive study has not been done, there is evidence to suggest a correlation between the presence of UTI and stones. In several small single-center studies, a significantly higher incidence of UTI was observed in patients with stones, including not only struvite but metabolic stones composed of CaOx and CaP, than in healthy people.[204–206]

The same *UMOD* rs4293393-T variant that is protective against stones is also associated with an increased risk of salt-sensitive hypertension and chronic kidney disease.[198,200,207] Tamm–Horsfall protein is thought to play a role in water and electrolyte balance in the TALH. Evidence from THP KO mice suggests that THP facilitates the activation of the sodium–potassium-chloride transporter (NKCC2) and the renal outer medullary potassium channel (ROMK), the two main ion transporters involved in sodium chloride reabsorption by the TALH.[74,75] In mice lacking THP, both NKCC2 and ROMK accumulate in subapical intracellular vesicles resulting in decreased apical surface expression and reduced transporter activity. On the other hand, overexpression of wild-type THP in transgenic mice enhances NKCC2 activity and increases sodium reabsorption in the TALH, leading to salt-sensitive hypertension.[200] The *UMOD* rs4293393-C variant that is the risk allele for kidney stones would be expected to have the opposite effect, which is to decrease THP expression and NKCC2 activity and to reduce blood pressure. Trudu et al. investigated the role of *UMOD* variants in modulating blood pressure in humans and found that mean diastolic blood pressure was significantly lower in individuals that were heterozygous or homozygous for the rs4293393-

C allele relative to individuals that were homozygous for the T allele.[200] Interestingly, Ko et al. found that male patients with idiopathic hypercalciuria, a common trait among calcium stone formers, have lower systolic blood pressures than normal male patients.[208] Tamm–Horsfall protein also plays a role in modulating urine calcium excretion by stimulating calcium reabsorption in the distal nephron by decreasing endocytosis of the calcium channel TRPV5.[209] Tamm–Horsfall protein KO mice and mice carrying *UMOD* mutations found in human uromodulin-associated kidney diseases that severely reduce urinary THP levels exhibit hypercalciuria. Thus, *UMOD* genotypes that reduce expression of THP may link susceptibility to kidney stones and protection from hypertension via the effect of THP on calcium and salt reabsorption in the kidney.

Albumin

Human serum albumin is the second most abundant protein in urine, after THP. Albumin is primarily synthesized in the liver, functions as a carrier protein for fatty acids and hormones in the blood and plays a major role in stabilizing extracellular fluid volume by contributing to oncotic pressure of plasma. Albumin has an immunomodulatory and extracellular antioxidant defense functions mediated by its high capacity to bind lipopolysaccharide and other bacterial products (lipoteichoic acid and peptidoglycan), reactive oxygen species (ROS), nitric oxide and other nitrogen reactive species, and prostaglandins.[210,211] Albumin has also been found to have antimicrobial properties in exerting fungistatic activity.[212] Albumin is a large component of the matrix of calcium stones.[114,213,214] It does not inhibit CaOx crystal growth in vitro[170,171] and seems to promote nucleation of CaOx crystals, specifically the dihydrate form.[215] By contrast, albumin is an inhibitor of CaOx crystal aggregation in inorganic solutions[171,216] and urine.[170] Baumann and Affolter found that, somewhat paradoxically, when albumin was adsorbed onto CaP crystals it became a promoter of CaOx crystal aggregation rather than an inhibitor.[69] This was attributed to self-aggregation bridging zones of electrostatic repulsion between coated crystals with identical electrical surface charge. This effect was abolished when urine was diluted, arguing for the importance of stone formers maintaining adequate urine volume to diminish urinary SS and risk of crystal aggregation.

The gene encoding albumin may be expressed in kidney,[217] but it is more likely that albumin in urine is filtered from the blood. Pourmand et al. found increased albumin in the urine of recurrent calcium stone formers compared to normal controls.[218] Of 10 urine proteins measured, only albumin and transferrin (another blood protein) were different between the groups, suggesting renal bleeding or perhaps glomerular impairment as the source of these proteins in stone formers. All in all, the presence of albumin in stones is more likely due to its ubiquitous nature in blood, urine and tissue than to any specific role in regulation of crystallization or stone formation.

Calgranulins (S100A8 and S100A9)

S100A8 and S100A9, also known as calgranulin A and B or MRP8 and MRP14, are small, acidic, potent calcium-binding proteins that are abundantly present in the matrix of calcium stones.[114,219–222] S100A8 and S100A9 exist as monomers of about 10 and 14 kD, respectively, but preferentially form a S100A8/A9 heterodimer (also known as "calprotectin") in the presence of Zn^{2+} or Ca^{2+}.[223] S100A8/A9 exhibits antimicrobial activity by inhibiting bacterial adhesion to mucosal epithelia and sequestering essential trace elements such as Zn^{2+}.[224] Both S100A9 and S100A8/A9 can also form higher-order oligomers such as tetramers or even larger oligomers, and this multimer formation may regulate their function.[225–227] Calgranulin (likely S100A8) has been found to be a potent inhibitor of CaOx crystal growth and aggregation in the nanomolar range in vitro.[68] S100A8 and S100A9 are constitutively expressed in myeloid cells (monocytes, neutrophils and dendritic cells), but expression can also be induced in other cell types such as macrophages, vascular endothelial cells and fibroblasts.[219] In the kidney, S100A8 and S100A9 have also been found to be expressed in glomerulus, tubulointerstitial regions and collecting duct.[68,228,229] Polymorphisms in the genes for these proteins have been shown to be associated with periodontitis (S100A8)[230] and insulin resistance and type 2 diabetes (S100A9),[231] but not with nephrolithiasis thus far.[102]

Secreted S100A8 and S100A9 have been shown to be endogenous proinflammatory ligands that can activate cytokine signaling pathways and leukocyte recruitment via the receptor for advanced glycation endproducts and TLR4.[232,233] S100A8 and S100A9 are involved in acute response to injury and early inflammatory program initiation.[234] Circulating levels of S100A8 and S100A9 are increased in many disease conditions including cancer, neurodegenerative disorders, autoimmune and inflammatory diseases, including type 2 diabetes (T2DM) and

obesity.[223,235–238] They are considered to be damage-associated molecular pattern molecules that are overexpressed at sites of local inflammation.[239] The genes for S100A8 and S100A9 are direct targets of NF-κB, which is stimulated downstream of TLR4 activation. Therefore, binding of these damage-associated molecular patterns to TLR4 can set up a positive feedback loop that contributes to perpetuation of the innate immune response.[240] TLR4 is expressed all along the nephron from Bowman's capsule to proximal convoluted tubule, TALH, distal tubule and collecting duct.[241] Continued stimulation of TLR4 in any of these locations could set off a "damage chain reaction" leading to chronic innate immune activation and enhanced tissue injury.[242]

However, these proteins also play anti-inflammatory roles in wound healing[243] and protection against oxidative tissue damage as a result of their extreme ability to scavenge oxidants.[227] Under conditions of oxidative stress, S100A8 and S100A9 may serve as sinks for reactive oxygen species (ROS) including HOCl, H_2O_2, and NO. Binding of ROS causes conformational changes and formation of covalent bonds in S100A8 and S100A9 homodimers and higher order homocomplexes.[227] S100A8 and S100A9 can self-assemble into a variety of amyloid complexes both in vitro and in vivo, and covalently-linked complexes of S100A9 have been identified in brain extracts of patients with familial Alzheimer's disease and in association with senile plaques.[244]

S100A8 and S100A9 may play roles in both normal and dystrophic calcification processes. Both are expressed in human bone and cartilage cells and may play a role in calcification of cartilage matrix and its replacement with trabecular bone.[245] S100A8 and S100A9 are abundant in osteoclasts and may contribute to regulation of the oxidative balance required for bone remodeling. S100A9 has been shown to be highly expressed in atherosclerotic plaque where it is associated with calcified areas.[246] Calcifying microvesicles from carotid artery isolates contain high levels of S100A9, with a lesser amount of S100A8. In addition, age-related protein amyloid structures in prostate, also called prostatic calculi or corpora amylacea, are primarily composed of amyloid forms of S100A8 and S100A9 in combination with hydroxyapatite.[247] To date, expression of these proteins in the kidney has not been investigated in the context of stone or Randall's plaque formation.

Whether the functional outcome of S100A8 and S100A9 expression is protective or injurious may depend on the types of cells expressing these proteins in particular microenvironments, their relative concentrations and post-translational modifications.[248] High circulating levels of S100A8/A9 are suggested to contribute to atherogenesis in patients suffering from acute and chronic inflammatory disorders such as periodontitis, rheumatoid arthritis, obesity and systemic lupus erythematosus.[235,249–251] However, it is possible that the high concentrations of S100A8 released following neutrophil and monocyte/macrophage accumulation in plaque may moderate oxidative damage and reduce inflammation by suppressing mast cell activation, thereby limiting atherogenesis.[248] In the kidney, glomerular and tubulointerstitial S100A8 expression was found to be increased in renal biopsy samples from obese or type 2 diabetic patients compared to controls.[228] Elevated S100A8 in injured tubular epithelial cells and surrounding macrophages was associated with tubulointerstitial fibrosis and progression of proteinuria in obese and type 2 diabetic patients. On the other hand, there is evidence that S100A8/A9 is protective against ischemia/reperfusion (IRI) injury. In a mouse model of IRI, renal abundances of S100A8 and S100A9, likely to be from infiltrating granulocytes, were normally increased in the corticomedullary region in wild-type mice.[252] However, S100A9 KO mice displayed defective tissue repair after I/R, increased renal damage, sustained inflammation, induction of fibrosis and increased expression of collagens, suggesting loss of a protective function of S100A9.

In a study of urinary excretion of S100A9 in first-degree family members of calcium kidney stone formers (same study as for THP, above), we found that S100A9 excretion differed by sex and by the number of stones formed (K. Bergsland, unpublished). Western blots showed a prominent immunoreactive band at 14 kD that is the major monomeric form of this protein (Fig. 3.5).[253] Monomeric S100A9 exists in two isoforms: full length (14 kD) and truncated S100A9* (~13.5 kD), which is translated from an alternate start site in the S100A9 gene and lacking four amino acids at the N-terminus.[225,227] In addition, HMW forms around 28, 42 and 56 kD were also apparent, which most likely represent dimers, trimers and tetramers of S100A9 induced by oxidative modification.[227] These complexes contain covalent bonds that are resistant to the reducing conditions of gel electrophoresis. Among men, but not women, excretion of HMW S100A9 (a sum of all forms ≥15 kD, i.e. larger than the monomer) was significantly increased in recurrent stone formers relative to single stone formers and nonstone-forming controls (Fig. 3.6). There were no differences between subject groups in the

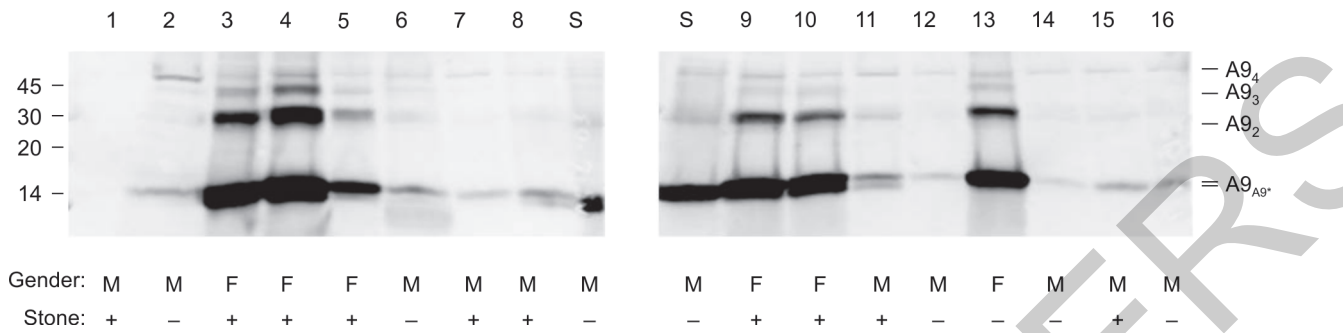


Fig. 3.5: Representative western blots of urinary S100A9. Lanes 1–16 contain samples from different individuals. Lanes S contain a standard urine sample from a nonstone-forming man. Sizes of protein molecular weight standards (in kD) are indicated at left. Positions of monomeric and multimeric forms are indicated at right. S100A9* (~13.5 kD) is a monomeric form which is translated from an alternate start site in the S100A9 gene and lacks four amino acids at the N-terminus.

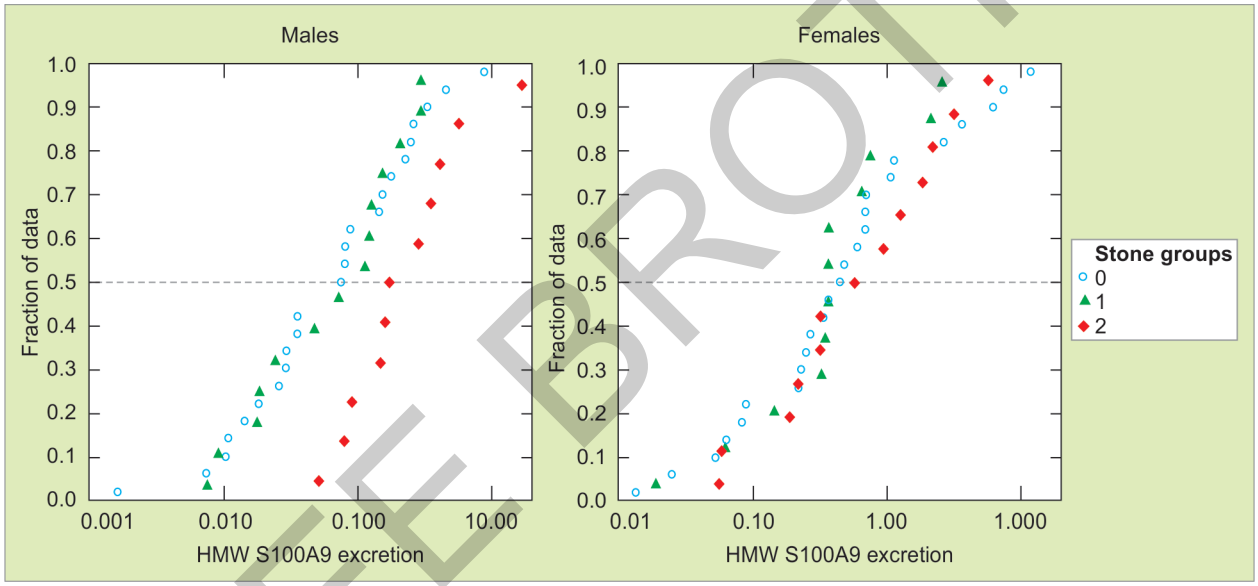


Fig. 3.6: Quantile plots of high-molecular-weight (HMW) S100A9 excretion. Estimations of excretion rates of HMW S100A9 forms (a sum of all forms ≥ 15 kD, i.e. larger than the monomer) were made by multiplying the semiquantitative measurement of each protein band (*see* Fig. 3.5) by the 24-hour urine volume. Males are at left and females are at right. Subjects are grouped by stone number: non-stone formers (○), men with 1 stone and women with 1–2 stones (▲), and men with ≥ 2 stones and women with ≥ 3 stones (♦). Increased excretion of HMW S100A9 was highly significant in men with multiple stones compared to male single stone formers and controls ($p < 0.05$, both comparisons), but not in women.

amount of monomer excreted, except that it was higher in females than males in general. Thus, elevated excretion of multimeric (HMW) forms of S100A9 is a particular feature of men who have had more than one stone.

It is not clear whether the S100A9 in urine from stone formers is coming from an intrarenal source or is filtered into the urine from the blood. Increased total urinary S100A8/A9 has been identified as a diagnostic marker of bladder cancer[254] and intrinsic acute kidney injury,[255] suggesting the urinary tract or kidney as the source. It has been found to be transiently elevated over a period of 48 hours in patients who have undergone renal ischemia during surgery.[256] Higher urinary S100A8/A9 is also a predictor of renal allograft injury and impaired function after kidney transplantation.[257] Other studies have suggested a systemic source for this protein. Ortega et al. found elevated levels of total urinary S100A8/A9 to be associated with chronic low-grade inflammation and insulin resistance in men.[258] By ELISA, urine S100A8/A9 was significantly increased in male patients with impaired glucose tolerance or T2DM

Table 3.1: Functions of crystallization inhibitor proteins in modulating innate immune response.

	Antimicrobial	*Cytokines*	*Antioxidant*	*Tissue repair*
PF1		Thrombin promotes cytokine and chemokine synthesis via PARs[97,98]; PF1 is a marker of thrombin generation		Thrombin interacts with PAR-1 to stimulate a regenerative response to tissue damage[98]
OPN		Potent chemoattractant for macrophages[146]	Repressor of inducible nitric oxide synthase [260]	Role in tissue remodeling and matrix reorganization after injury; promotes wound healing[260]
ITI/Bikunin		BK decreases binding of LPS to TLR4 and inhibits LPS-induced TNF α production[261]		Protease inhibitors, attenuate complement activation[160,161]; interact with hyaluronan to stabilize extracellular matrix after injury[167]
THP	Binds to microbes and prevents adhesion to epithelia[203]	"Cytokine trap" forms in vivo complexes with renal and urinary cytokines; limits activation of TLR4 and inflammatory signaling pathways[192]		
Albumin	Binds lipoteichoic acid and peptidoglycan[210]; antifungal[212]		Binds ROS, NO and other reactive nitrogen species[211]	
S100A8/A9	Inhibit adhesion to mucosal epithelia; potent Zn^{2+} binding limits micronutrient availability for microbes[224]	Endogenous ligand of RAGE and TLR4; promote expression of proinflammatory cytokines[233]	Avid oxidant scavengers; sink for ROS including HOCl, H_2O_2, and NO[227]	

(PF1: Prothrombin fragment 1; OPN: Osteopontin; ITI: Inter-alpha-trypsin inhibitor; THP: Tamm–Horsfall protein; PAR: Protease-activated receptor; TLR4: Toll-like receptor 4; RAGE: Receptor for advanced glycation endproducts; ROS: Reactive oxygen species; LPS: Lipopolysaccharide; TNFα: Tumor necrosis factor alpha).

independently of body mass index and positively associated with homeostasis model assessment–insulin resistance (HOMA-IR), inflammatory markers, and parameters of glucose and lipid metabolism. Diabetes and obesity are both associated with a risk of nephrolithiasis.[129] It is possible that S100A9 in urine from stone formers may be coming from both systemic and renal sources. Interestingly, we found that only higher-order forms of S100A9 were elevated in male recurrent stone formers, suggesting that an oxidative process is at play in these patients. Renal oxidative stress is higher in males compared to females,[259] which may explain this sex difference if urinary S100A9 is coming from the kidney. More research is needed to understand the association of S100A8 and S100A9 with nephrolithiasis and whether these urinary proteins are marking a cause or consequence of stone formation.

CONCLUSION

The overall capacity of urine to inhibit crystallization is determined by the actions and abundance of many molecules in this biological solution. There is much redundancy in the system, such that no one molecule is pre-eminent in this function. Making the situation more complicated is the fact that some molecules considered to be inhibitors of crystallization can, in fact, be promoters given the proper conditions. The sources of these proteins in urine are often unclear, being potentially derived from renal and/or systemic production. The proteins mentioned here all have other additional normal roles in mediating inflammation and the innate immune response, facilitating wound healing and tissue repair, managing oxidative stress or antimicrobial protection (Table 3.1). Observed differences in the context of stone formation may actually be related to

alterations in their function in another system or process. Much more research will be required to untangle the complex interplay of pathways and determine the conditions that constitute optimal crystallization inhibition and factors that tip the balance toward stone formation.

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KIDNEY STONES
MEDICAL AND SURGICAL MANAGEMENT

Salient Features

- For the most common type of stone former, the Idiopathic calcium oxalate stone former (ICSF), stones are found attached to the renal papilla at sites of interstitial plaque
- Interstitial plaque or Randall's plaque is first seen in the basement membranes of thin loops of Henle
- Intraluminal plugs are rarely seen ICSF patients but are commonly seen in all other stone phenotypes we have studied to date
- A stone can grow from the protruding end of intraluminal plugs of a duct of Bellini
- Stone formers with intraluminal plugs have varying degrees if papillary tissue changes while those with interstitial plaque have normal appearing papilla
- Ductal stones are characteristic of medullar sponge and cystine stone disease
- Plaque amount on the papilla surface has a strong correlation with urine calcium excretion and a strong negative correlation with urine pH and volume
- The "vas wash-down" theory best explains plaque formation in a stone patient with idiopathic hypercalciuria.

Fredric L Coe MD is professor of Medicine, Nephrology, University of Chicago, USA. In 1969, he opened what would become the University of Chicago Kidney Stone Center. For 45 years his research was funded by NIH NIDDK, for which he is grateful. In 1995, he founded Litholink Corp to provide kidney stone testing and improved treatment for North America. The company continues in its original purposes as part of LabCorp since 2006. In 2014, he created a website–https://kidneystones.uchicago.edu/–for patients, physicians, and scientists to provide accurate information about kidney stones to a world audience.

Elaine M Worcester MD is Professor in the Department of Medicine, Nephrology, University of Chicago, USA. After completing her Nephrology training at the University of Chicago in 1986, she joined the faculty of the Medical College of Wisconsin, where she continued to pursue research into the causes of kidney stones, as well as continue to provide preventive care to patients with recurrent stones. In 2000, she returned to the University of Chicago where it has been her privilege to participate in the active research and clinical programs started by Dr Fredric L Coe, now encompassing a diverse group of clinicians and scientists. Hope this book will provide encouragement to those caring for patients with stone disease, and also to researchers seeking answers about their cause and prevention.

Andrew P Evan PhD is Professor Emeritus, awarded the Chancellor's Professorship in 2003, Department of Anatomy and Cell Biology, Indiana University School of Medicine, Indianapolis, Indiana, USA. He established the International Kidney Stone Institute with Dr James E Lingeman in 2004 to guide research in stone disease and to channel resources toward projects that deliver positive results in stone disease treatment and prevention. He received 30 years of continuous funding from the National Institute of Health to study kidney disease.

James E Lingeman MD FACS is well known nationally and internationally for his interest in the area of kidney stone disease and minimally invasive urologic techniques. Dr Lingeman performed the first percutaneous stone removal procedure in the State of Indiana in 1983 and performed the first ESWL treatment in the United States at Indiana University Health/Methodist Hospital in Indianapolis in 1984. Currently, Dr Lingeman and Indiana University Health/Methodist Hospital are widely recognized for their expertise in both state-of-the-art techniques for kidney stone removal and also in understanding and preventing kidney stone formation. Dr Lingeman holds several grants from the National Institutes of Health studying extracorporeal shock wave lithotripsy and kidney stone formation. Dr Lingeman is currently Co-Director of the International Kidney Stone Institute. He is Chairman of the Board of the Methodist Research Institute and is a past member of the Board of Directors of Indiana University Health, a healthcare system comprised of 19 hospitals in the State of Indiana. He is a Professor of Urology at Indiana University School of Medicine. He is a past president of the Indiana State Urologic Society and past president of the North Central Section of the American Urological Association. He is a past president of the Endourology Society. He is a member of the American Association of Genitourinary Surgeons (AAGUS). He is the author of over 300 peer review scientific publications.

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