

# Novel insights into the role of the renal interstitial microenvironment in Randall's plaque formation (Review)

JIALIANG WANG<sup>1,2</sup>, ZHANGCHENG LIAO<sup>1,2</sup>, MINGHUI LIU<sup>1,2</sup>, YIZHOU WANG<sup>1,2</sup>,  
YU CUI<sup>1,2</sup>, HEQUN CHEN<sup>1,2</sup>, FENG ZENG<sup>1,2</sup> and ZEWU ZHU<sup>1-3</sup>

<sup>1</sup>Department of Urology, Xiangya Hospital, Central South University, Changsha, Hunan 410008, P.R. China;

<sup>2</sup>National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan 410008, P.R. China; <sup>3</sup>Department of Internal Medicine, Section Endocrinology, Yale University School of Medicine, New Haven, CT 06520-8020, USA

Received May 2, 2026; Accepted September 4, 2026

DOI: 10.3892/ijmm.2026.6004

**Abstract.** Randall's plaque (RP) consists of subepithelial hydroxyapatite (HAP) deposits within renal papillae and provides a well-established attachment substrate for a subset of idiopathic calcium oxalate (CaOx) kidney stones. However, RP has traditionally been viewed as a passive mineral surface onto which crystals accrete, with comparatively less focus on the cellular and molecular events that build the plaque itself. The renal papillary interstitium has been indicated as a dynamic microenvironment that undergoes molecular remodeling during plaque-associated mineralization. The present review aimed to organize the current evidence regarding interconnected processes that may overlap *in vivo* rather than occur in a fixed sequence. Physicochemical conditions in the inner medullary interstitium favor HAP nucleation and provide the foundation for plaque formation. Epithelial injury responses, regulated cell death, osteogenic-like fibroblasts and immune remodeling may contribute to the development of a pro-calcific microenvironment; however, the spatial relationship, temporal order and plaque specificity of these processes remain incompletely defined. Macrophage polarization may influence whether local crystal-associated inflammation and mineralization are amplified or reduced. As interstitial HAP deposits enlarge and become exposed through focal disruption of the renal papillary epithelium, these deposits can interact with pelvic urine and support CaOx nucleation and

overgrowth. Systemic metabolic abnormalities and urinary or gut microbiome alterations are associated with stone disease and may modify the papillary microenvironment; however, direct evidence that they alter human RP burden is lacking to date. Of note, available animal and cell models demonstrate selected aspects of mineralization or crystal injury but do not reproduce the chronic, progressive interstitial HAP plaque observed in humans. In conclusion, considering RP as an interstitial microenvironmental disorder may help identify potential biomarkers of early papillary remodeling and generate potential therapeutic strategies for stone prevention in the future.

## Contents

1. Introduction
2. Historical and clinical foundations
3. Molecular architecture of the pro-calcific microenvironment
4. Systemic drivers of the pro-calcific niche
5. Microbiome-metabolism axis
6. Animal models: Strengths, limitations and emerging platforms
7. Unresolved questions and future directions
8. Conclusion

**Correspondence to:** Dr Zewu Zhu or Professor Feng Zeng, Department of Urology, Xiangya Hospital, Central South University, 87 Xiangya Road, Kaifu, Changsha, Hunan 410008, P.R. China  
E-mail: zhuzevxy@csu.edu.cn  
E-mail: urologyxyy@126.com

**Key words:** Randall's plaque, renal interstitial microenvironment, calcium oxalate nephrolithiasis, renal papilla, osteogenic reprogramming

## 1. Introduction

Nephrolithiasis affects ~12% of the global population over a lifetime (1). Population-based studies demonstrate substantial kidney-stone incidence across different economic settings: In Olmsted County, Minnesota, USA, the incidence of confirmed symptomatic kidney stones increased between 1984 and 2012 from 145 to 299 per 100,000 person-years in men and from 51 to 217 per 100,000 person-years in women, whereas prospective urban cohorts in Shanghai, China, reported incidence rates of 3.80 and 2.10 per 1,000 person-years in men and

women, respectively (2,3). Calcium oxalate (CaOx) accounts for 70-80% of all cases with urinary stones, and recurrence rates reach up to 50% within 5 years of the index episode despite conventional interventions (1,4). This high recurrence rate indicates gaps in the current understanding of how stones develop, which have marked clinical costs.

Randall's plaque (RP), comprising subepithelial hydroxyapatite (HAP) deposits within renal papillae, has been recognized as a key attachment substrate for a subset of idiopathic CaOx stone formation, originally identified in the autopsy study by Alexander Randall in 1937 on 429 renal pairs (5). Endoscopic mapping and direct stone-attachment studies indicate that exposed interstitial RP can serve as an attachment and heterogeneous nucleation substrate for CaOx stone overgrowth (6-12). Multimodal imaging further demonstrates spatial and mineral continuity from interstitial HAP-rich plaque, through the exposed papillary surface, to HAP-containing regions at the base of attached CaOx stones (13-16). Quantitative papillary mapping has exhibited higher plaque coverage in individuals with idiopathic CaOx stones (CaOx stone formers) compared with that in non-stone controls and has associated plaque burden with higher urinary calcium and lower urine volume (17). By contrast, the identification of idiopathic CaOx stone formers with little plaque indicates that plaque overgrowth is not the only route to CaOx stone formation (18). Therefore, these observations support RP as a notable origin for a subset of idiopathic CaOx stones, particularly in cases where stones form on exposed interstitial plaque.

Recent ultrastructural and molecular observations have suggested that, in addition to physicochemical supersaturation, local microenvironmental remodeling contributes to RP formation (19-22). The renal papillary interstitium, comprising immune cells, fibroblasts and extracellular matrix (ECM), is a dynamic microenvironment where homeostasis is disrupted by metabolic, inflammatory and osteogenic signals. Therefore, RP may be further understood as a local consequence of widespread interstitial dysfunction, in contrast to solely an anchoring surface for crystal growth.

Previous reviews have addressed specific facets of this process: Khan *et al* (23) identified the roles of immunity and inflammation in RP pathogenesis, while Sivaguru *et al* (21) proposed a GeoBioMed framework in understanding renal biomineralization. However, an attempt to link these factors, from physicochemical priming and cell injury to immune shifts and metabolic signals, within one integrated model remains to be explored. The present review details the clinical foundations (section 2), dissects the molecular architecture of the pro-calcific microenvironment (section 3), examines systemic drivers and microbiome contributions (sections 4 and 5), appraises animal models (section 6) and assesses translational opportunities (section 7).

As research focused specifically on RP remains limited (19-22,24), the hypotheses proposed in the present review focus on RP pathological morphology, RP-associated tissue sequencing, cell culture and animal experiments and CaOx-associated epidemiological studies. To clarify which evidence is considered strong or tenuous, the present review classifies the evidence as follows: i) Level A, pathological and mineral-morphological evidence obtained from RP;

ii) level B, sequencing-based or other molecular observations from RP-associated tissue; iii) level C, *in vitro* cell experiments and animal models; and iv) level D, epidemiological association analyses associated with CaOx stone disease. These labels are provided throughout the discussion of the renal interstitial microenvironment in RP formation for reference. Furthermore, the labels indicate the source of evidence and do not establish causality.

## 2. Historical and clinical foundations

*Randall hypothesis: Original observations and the HAP-CaOx compositional duality.* The concept of RP was derived from the systematic examination of 429 paired kidneys at autopsy by Alexander Randall, published in 1937 (5). Randall documented subepithelial calcified lesions on the renal papillae in ~17% of specimens, of which 28 specimens harbored CaOx stones directly adherent to the plaque surface. A key mineralogical observation was that the plaque core consisted predominantly of HAP, largely carbonate-substituted apatite (carbapatite), whereas the overlying stones were CaOx (5). Modern mineralogical and imaging studies have since confirmed this two-phase mineral pattern, with carbapatite accounting for ~97.6% of the plaque mineral (15,16,25,26). Randall's original hypothesis posited a sequential process in which progressive HAP accumulation within the papillary interstitium breaches the overlying renal papillary epithelium, exposing a mineralized HAP surface to urinary constituents and thereby providing a heterogeneous nucleation site for CaOx crystal attachment and growth (5).

*Endoscopic and imaging validation.* The advent of digital ureteroscopy and intraoperative papillary mapping transformed RP from a postmortem observation into a clinically assessable lesion. In an early endoscopic mapping study, Low and Stoller (6) identified papillary RP in 42 of 57 patients (74%) undergoing stone removal and in 3 of 7 patients (43%) undergoing endoscopic procedures unrelated to stone disease. Due to the small non-stone comparison group, these statistics should be interpreted as descriptive endoscopic observations rather than a definitive population-prevalence estimate. Direct endoscopic evidence of calculus attachment to RP was subsequently provided in a study by Matlaga *et al* (7), which examined 24 kidneys and 172 renal papillae in 23 idiopathic CaOx stone formers; all examined kidneys contained papillary plaque and 11 patients (48%) had attached calculi. Quantitative mapping provided more informative evidence of plaque burden. Kuo *et al* (17) mapped the papillae of 14 idiopathic CaOx stone formers and four non-stone controls and identified significantly higher mean polar fractional plaque coverage in the stone formers (7.4 vs. 0.5%;  $P=0.012$ ). However, within idiopathic CaOx stone formers, plaque burden was heterogeneous. Wang *et al* (18) divided 42 patients into a low-plaque group with <5% plaque coverage per papilla (mean, 1.5%;  $n=32$ ; 76.2%) and a high-plaque group with  $\geq 5\%$  coverage (mean, 10.5%;  $n=10$ ; 23.8%). Compared with high-plaque stone formers, low-plaque stone formers were more frequently obese (50 vs. 10%;  $P=0.03$ ) and had a more frequent history of urinary tract infection (34 vs. 0%;  $P=0.04$ ), but were less likely to have experienced at least four previous stone events (22 vs.

80%;  $P=0.002$ ) and had lower mean 24-h urinary calcium excretion ( $187\pm86$  vs.  $291\pm99$  mg/day;  $P<0.01$ ). The stones in the low-plaque stone formers also lacked a calcium phosphate (CaP) core on micro-CT, and their papillary biopsies demonstrated less interstitial and basement-membrane punctate crystallization. Therefore, these observations supported a major role for plaque-associated stone attachment in a subset of idiopathic CaOx stone formers, while also demonstrating clinical and morphological heterogeneity consistent with alternative or plaque-limited pathways.

Among idiopathic CaOx stone formers with measurable plaque, plaque surface coverage associates positively with stone burden: The RP-to-papilla area ratio scales with stone number after adjusting for disease duration (27) and a 'papillary calcification index' demonstrates a linear relationship with cumulative stone mass (28). This association is stone-type specific; struvite stone formers exhibit RP prevalences statistically indistinguishable from those of non-stone controls (29), supporting a closer plaque-stone relationship in idiopathic CaOx disease.

Micro-CT and multimodal imaging studies have extended these observations to the microstructural level by enabling non-destructive three-dimensional visualization of mineral continuity within intact papillae and stones (11-16). Williams *et al* (12) identified annular HAP-rich deposits at the apex of small CaOx calculi; Miller *et al* (11) demonstrated that free-floating, unattached CaOx stones also harbor internal HAP-rich regions whose mineral signature and spatial orientation are consistent with remnant RP. Multimodal imaging combining micro-CT, confocal autofluorescence microscopy and electron energy-loss spectroscopy has delineated a multi-stage progression model: Nascent HAP crystallites arise within the interstitial matrix, coalesce into plaque, extend through the renal papillary epithelium and are then colonized by CaOx from pelvic urine (13,14,16,30). This continuum of mineralization from the inner medullary interstitium to the stone surface provides structural evidence (level A) that a subset of idiopathic CaOx stones forms on exposed RP.

*Anatomical origin and the physicochemical basis of plaque initiation.* A notable finding by Evan *et al* (19) localized the earliest RP mineral deposits to the basement membranes of the thin limbs of the loop of Henle in the inner medulla. This anatomical specificity carries physicochemical significance: The inner medullary interstitium is a uniquely pro-calcific compartment. The countercurrent multiplication system generates interstitial calcium concentrations several-fold higher compared with that in plasma, while acid loading, through bicarbonate reabsorption and proton secretion, paradoxically alkalinizes the interstitial fluid compared with that in the tubular lumen (19,31). Thus, the combination of elevated calcium activity and high local pH drives CaP supersaturation to levels that favor spontaneous HAP nucleation (32,33). Low medullary blood flow and high tissue osmolality further concentrate solutes, compounding supersaturation. Therefore, these conditions make the inner medullary interstitium the sole site in the kidney where spontaneous apatite nucleation is thermodynamically favorable. The inner medullary interstitium is also where RP consistently originates.

A key observation that any microenvironment-centric model of RP should address is the apparent histological normalcy of plaque-bearing papillary tissue. In a 2003 biopsy study, Evan *et al* (19) noted no overt evidence of cell injury, inflammation or fibrosis in the papillary interstitium of idiopathic CaOx stone formers at the light-microscopic level. This observation may contrast the molecular injury signatures (for example, oxidative stress, immune activation and ECM remodeling) described below.

Canela *et al* (22) provide a notable molecular atlas of the stone-forming papilla. However, these data should not be interpreted as a plaque-exclusive transcriptome. As noted in the original study, an intratubular plug present in one analyzed specimen was not separately segmented from the plaque-focused region (22). The single-nucleus and spatial signatures may therefore include cells adjacent to ductal plugging as well as cells associated with interstitial plaque. In the present review, these findings are classified as level B evidence for molecular remodeling in the stone-forming papilla, rather than as direct proof that each identified pathway is intrinsic to interstitial plaque. This limitation reinforces the need to distinguish interstitial from intratubular pathology in future spatial studies, as discussed later in section 7.

This apparent discordance may reflect molecular remodeling that precedes changes detectable by routine light microscopy; however, the cross-sectional biopsy data cannot distinguish an early transient response from a stable subclinical state. Longitudinal papillary biopsies with matched multi-omics profiling (technically challenging; however, increasingly feasible) will be warranted to distinguish these possibilities in the future.

### 3. Molecular architecture of the pro-calcific microenvironment

The following discussion is organized as a five-phase sequence: Initiation, amplification, transformation, immune regulation and renal papillary epithelial breach (Fig. 1). The present review used this order as it tracks a direct mechanistic logic in the available data; however, it is a conceptual framework, not a validated timeline. Available evidence largely derives from cross-sectional human studies, endpoint histology and experimental cell/animal models (19,22,24,34,35); no longitudinal dataset provides the order in which these processes begin in a patient. These five processes probably run concurrently within the same tissue compartment and may be associated by candidate mediators, including reactive oxygen species (ROS) and the NOD-like receptor family pyrin domain-containing 3 inflammasome (NLRP3), rather than by temporal hand-offs. The processes have been numbered for simplicity, and not to suggest that one process finishes before the next begins. In any patient, one or more processes may predominate depending on the metabolic background; the relative contributions of the processes are discussed in section 7.

Of note, the present review examines the interstitial-plaque pathway, in which CaOx stones can grow on a subepithelial-exposed interstitial HAP plaque (5,19). However, this is not the sole route to CaOx stone formation. Intratubular CaP plugging within the inner medullary collecting ducts and other plaque-independent processes may represent distinct pathways that occur in different anatomical and metabolic

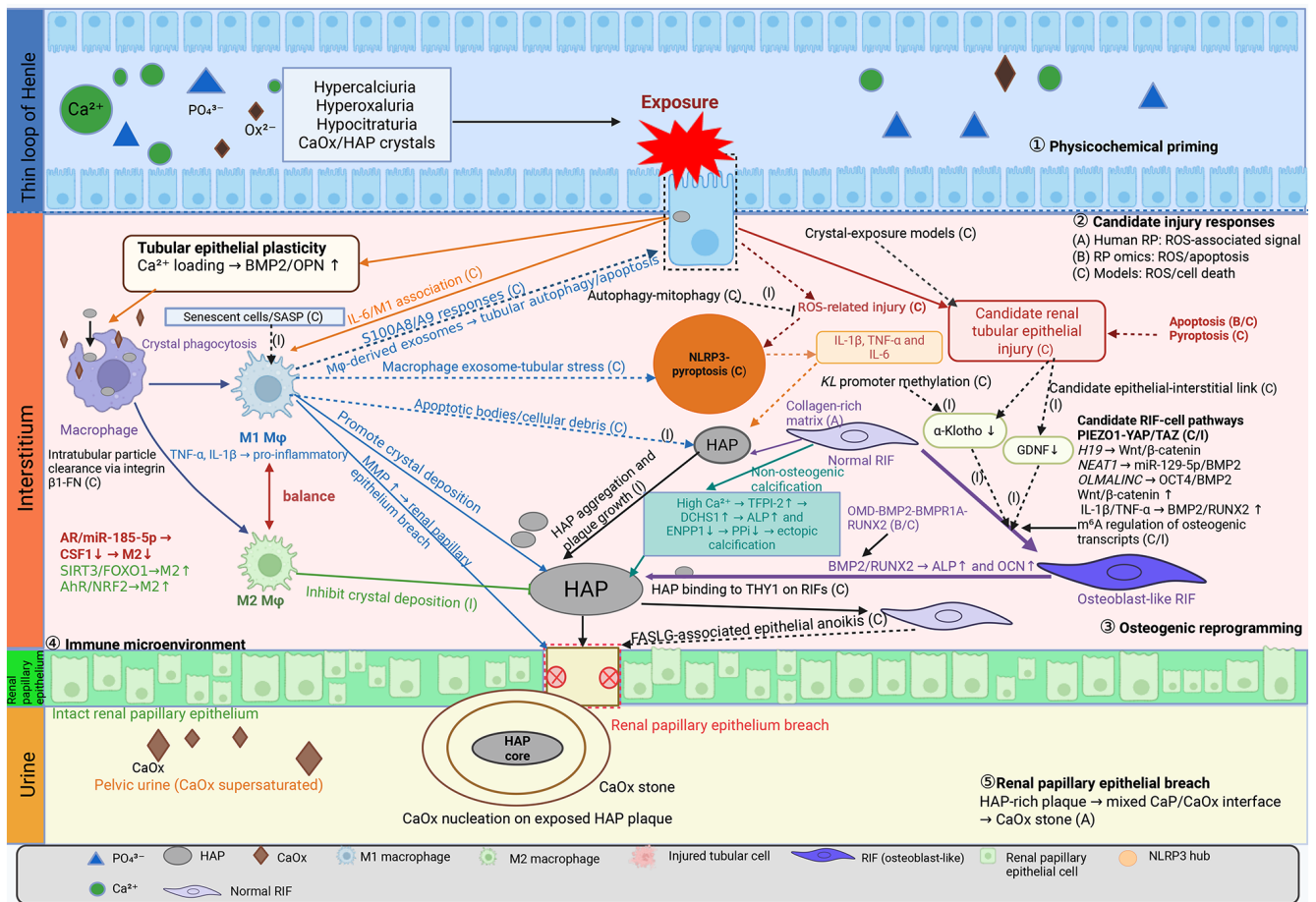


Figure 1. Proposed renal interstitial microenvironment framework for RP formation. The schematic illustrates five interconnected processes involved in RP development and CaOx stone overgrowth: ① Physicochemical priming; ② candidate injury responses; ③ calcification routes of RIFs; ④ immune microenvironment remodeling; and ⑤ renal papillary epithelial breach. Evidence levels indicate the source of supporting evidence: A, morphologically verified human RP pathology or ultrastructural evidence; B, RP-associated human tissue omics or molecular profiling; C, cell or animal model evidence; and I, inferential or hypothesis-generating relationships. Arrow colors represent inflammatory/crystal-handling, injury, anti-calcific, mineralization and osteogenic pathways. Upward ( $\uparrow$ ), downward ( $\downarrow$ ) and rightward ( $\rightarrow$ ) arrows indicate increased expression, decreased expression and directional transition, respectively. Dashed arrows indicate proposed relationships without direct validation in human RP, whereas solid arrows indicate experimentally supported associations. RP, Randall's plaque; HAP, hydroxyapatite; CaOx, calcium oxalate; CaP, calcium phosphate; RIF, renal interstitial fibroblast; SASP, senescence-associated secretory phenotype; ROS, reactive oxygen species; miR, microRNA.

settings (26,36,37). In particular, collecting duct plugging has been documented in a subset of idiopathic CaOx stone formers, whereas other idiopathic CaOx stone formers have low plaque burden (18,37). Therefore, the framework below does not treat all CaOx stone studies as evidence for interstitial plaque formation. Evidence from non-RP models is labeled according to the level A-D framework and is used to generate verifiable hypotheses rather than to establish plaque-specific mechanisms.

**Supersaturation microenvironment.** Prior to discussing specific molecular events, the present review briefly revisits the physicochemical conditions of the inner medullary interstitium, which provide the thermodynamic foundation for RP formation. As described in section 2, the countercurrent multiplication system elevates interstitial calcium concentrations to levels several-fold higher compared with that in plasma, while bicarbonate reabsorption and proton secretion paradoxically alkalize the interstitial fluid compared with that in the tubular lumen (19,31). The combination of high calcium activity and elevated local pH induces CaP supersaturation beyond

the threshold for spontaneous HAP nucleation (32,33). Low medullary blood flow and high tissue osmolality compound the problem by further concentrating solutes. These conditions are sufficient to promote spontaneous HAP nucleation in the absence of any biological catalyst. Idiopathic hypercalciuria, which increases calcium delivery to the medulla, amplifies this baseline supersaturation and is the metabolic phenotype most closely associated with interstitial plaque formation (19,26). These are the baseline conditions within which the molecular events described below unfold.

**Candidate injury responses: Oxidative stress and cell death**  
**Oxidative stress: Evidence from RP tissue and experimental models.** Evidence directly obtained from RP tissue for oxidative stress remains limited. In human RP tissue, dihydroethidium staining combined with E-cadherin and CD34 immunofluorescence demonstrated increased oxidative stress signal in renal tubules adjacent to calcium deposits (level A) (38). In parallel, microdissected RP transcriptomic data and subsequent analyses of RP datasets have identified oxidative stress-associated expression signatures (level B) (24,39). These observations

support an association between RP-related tissue and oxidative stress responses; however, they do not identify the responsible nephron segment, establish temporal order or demonstrate that oxidative stress initiates interstitial HAP deposition.

By contrast, CaOx- or oxalate-exposed proximal tubular human kidney-2 cells, porcine proximal tubular Lilly Laboratories culture-porcine kidney 1 cells and Madin-Darby canine kidney cells, as well as high-calcium normal rat kidney-52E cells and vitamin D-associated rodent models, have reported ROS-associated epithelial injury responses (level C) (40-42). These systems do not reproduce the thin loop basement membrane and papillary interstitial environment in which human RP initially develops (19). Although CaOx crystals may contact tubular luminal surfaces in certain stone formers, these exposure paradigms do not model the initial interstitial HAP mineralization of RP. Thus, these studies have been retained only to generate the verifiable hypothesis that epithelial cells anatomically adjacent to plaque may exhibit associated injury responses; these studies are not interpreted as direct evidence of ROS-mediated plaque initiation.

Whether epithelial or interstitial cells adjacent to early HAP deposits in the human papilla display oxidative stress responses *in vivo* remains to be elucidated. Addressing this question warrants spatially resolved analyses of human papillary tissue and experimental systems that reproduce the local interstitial mineral environment in the future.

**Apoptosis and NLRP3-associated pyroptotic injury.** Apoptosis-related transcriptional programs were enriched in microdissected human RP tissue compared with adjacent non-calcified papillary tissue (level B) (24). In a *Brd4*<sup>+</sup>/M149T mouse model of papillary interstitial calcification, terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling-positive nuclei were observed in interstitial papillary areas corresponding to calcified lesions (level C) (34). These observations identify apoptosis as a candidate injury response in plaque-associated microenvironments; however, these findings do not establish that apoptosis occurs in thin loop cells adjacent to early human RP or that it precedes plaque mineralization.

NLRP3-associated pyroptotic injury has been demonstrated mainly in hyperoxaluria and CaOx crystal-nephropathy models (level C) (43-45). In these systems, CaOx exposure is associated with NLRP3 activation, inflammatory injury and crystal deposition, and interruption of pyroptotic signaling reduces experimental injury readouts. Related nephrocalcinosis models have also implicated NLRP3-associated macrophage responses in mineral-related renal injury (46). However, no study has directly demonstrated NLRP3-mediated pyroptosis in cells adjacent to morphologically verified human interstitial RP, to the best of our knowledge. The relevance of NLRP3-mediated pyroptosis to plaque formation therefore remains a hypothesis warranting plaque-specific validation in future research.

**Autophagy and mitophagy as counter-regulatory responses.** Autophagy is a lysosome-dependent quality control process that can counterbalance regulated cell death by removing damaged proteins and organelles before they become persistent sources of cellular stress (47). In high-glucose-treated HK-2 cells, a mitochondrial fission phenotype was associated with reduced autophagic activity

and increased apoptosis, whereas pharmacological inhibition of autophagy further decreased cell viability, supporting a protective role for basal autophagy in this tubular-cell setting (level C) (48).

Mitophagy provides a more selective form of this response by removing damaged mitochondria that would otherwise sustain mitochondrial ROS (mtROS) production. In iohexol-exposed HK-2 cells and mouse models of contrast-induced acute kidney injury, PTEN-induced kinase 1 (PINK1)/Parkin (PARK)-dependent mitophagy limited mitochondrial damage, mtROS accumulation, NLRP3 inflammasome activation and tubular-cell apoptosis. Silencing or genetic deficiency of *PINK1* or *PARK2* impaired mitophagy and aggravated each of these injury responses (level C) (49). Consistently, in high-glucose-treated murine renal tubular epithelial cells, optineurin-enhanced mitophagy reduced mtROS and suppressed NLRP3 expression, caspase-1 cleavage, and interleukin (IL)-1 $\beta$  and IL-18 release (level C) (50).

However, the effects of autophagy appear to be context-dependent rather than uniformly protective. In renal tissue from patients with CaOx nephrolithiasis, LC3-II and Beclin 1 (*BECN1*) expression and the number of autophagic vacuoles were increased. Mechanistic experiments in CaOx-exposed HK-2 cells further demonstrated that ROS-associated autophagy was enhanced, whereas pharmacological inhibition of autophagy or *BECN1* knock-down attenuated tubular-cell injury; rapamycin produced the opposite effect (level C) (51). This model reflects tubular CaOx crystal injury rather than the initiation of interstitial HAP plaque. Thus, autophagy and mitophagy provide plausible associations among mitochondrial quality control, ROS, NLRP3 activation and regulated cell death; however, no study has directly demonstrated altered autophagic flux or mitophagy in cells adjacent to morphologically verified human RP, to the best of our knowledge. The contributions of autophagy and mitophagy to plaque formation remain hypothetical and warrant plaque-specific spatial validation in future research.

Therefore, the available data do not establish a unified ROS-cell-death-autophagy network in human RP. Oxidative stress-associated signals in RP tissue, apoptosis-related expression changes, and autophagic, mitophagic and pyroptotic responses observed in experimental models identify candidate processes for future investigations. ROS-dependent regulation of multiple cell-death programs provides broader biological context for these experimental observations but is not specific to human RP (52). Their spatial relationship to thin loop basement membrane mineralization, temporal order and contribution to plaque growth remain to be elucidated.

**Transformation: Osteogenic reprogramming of the interstitium.** Cell death is a key process; however, surviving cells undergo changes as well, and what these cells develop into may markedly impact long-term plaque growth. Injury-associated inflammatory signals may favor osteoblast-like reprogramming of resident fibroblasts and tubular cells in experimental systems; whether this transition initiates or drives human RP remains to be investigated in the future.

**Histological and molecular evidence of an osteogenic phenotype.** The present section integrates level A mineral-morphological observations from human RP, level B

molecular observations from stone-bearing papillae and level C evidence from renal cell systems. Previous human RP studies reported calcifying vesicles and HAP-rich plaque architecture, including concentric mineral layers with needle-like morphology, combined with osteopontin (OPN) localization around mineral deposits (16,53,54). Classic biomineralization studies also support the capacity of calcium-phospholipid-phosphate complexes to participate in HAP formation (55). Altered matrix  $\gamma$ -carboxyglutamic acid protein (MGP) and bone morphogenetic protein 2 (BMP2) expression has also been reported in papillary tissue from CaOx stone formers (56). These observations support a mineralization-competent papillary microenvironment, but do not establish that a complete osteogenic transcriptional program is active within morphologically verified human RP.

Ultrastructural analyses such as Evan *et al.* (53,54) used immune-transmission electron microscopy to investigate the calcified core of early RP, identifying calcifying vesicles and HAP crystals intercalated with organic matter and circumscribed by OPN. This molecular architecture defines the nucleation microenvironment at the leading edge of the plaque. Khan *et al.* (57), using scanning and transmission electron microscopy, demonstrated that HAP crystals deposit preferentially within collagen-rich interstitial zones, confirming that the ECM serves as both a structural scaffold and a template for oriented mineral growth. In medullary sponge kidney (MSK) primary renal-cell cultures, spontaneous calcification has been accompanied by osteocalcin (OCN) and osteonectin expression, supporting osteogenic activity in this non-RP system (58).

**Renal interstitial fibroblasts (RIFs): Principal effectors.** Among the cell types in the papilla, RIFs are candidate cellular effectors of mineralization-associated remodeling. However, their contribution to the initiation or progression of morphologically verified human RP remains to be elucidated. Traditionally regarded as matrix-producing support cells, RIFs possess an intrinsic osteogenic differentiation capacity that exceeds that of renal tubular epithelial cells when assessed under identical induction conditions (35). Exposure of human RIFs (hRIFs) to osteogenic medium or elevated extracellular calcium induces their trans-differentiation into osteoblast-like cells, accompanied by robust ECM calcification (35). In primary renal cells derived from papillary tissue of a patient with MSK, spontaneous  $\text{Ca}_2\text{PO}_4$  deposition was associated with reduced glial cell line-derived neurotrophic factor (GDNF) expression and an osteoblast-like phenotype (level C) (58). These MSK-derived primary cell observations and RIF culture studies support the ability of renal interstitial cells to adopt pro-mineralizing phenotypes in non-RP systems; however, these studies do not demonstrate that runt-related transcription factor 2 (RUNX2)- or OCN-dependent reprogramming is established within morphologically verified human RP.

The BMP2/RUNX2 axis is a candidate regulatory circuit implicated in osteogenic differentiation. BMP2 activates SMAD-dependent transcription that converges on RUNX2, which in turn activates mineralization effectors including OCN, alkaline phosphatase (ALP) and OPN. Several long non-coding RNAs, including *H19* (59), *NEAT1* (60) and *OLMALINC* (61), have been reported to modulate BMP2/RUNX2-associated osteogenic responses in RIF

systems; however, their relevance to morphologically verified human RP remains to be established in future research. Furthermore, an osteomodulin (OMD)-mediated osteogenic pathway has been proposed in a previous study investigating renal papillary specimens (62). OMD was increased in vimentin-positive interstitial cells and colocalized with CaP deposits and calcifying vesicles; tissue OMD abundance was also positively associated with BMP2, RUNX2 and OCN expression (level B) (62). In cultured hRIFs, OMD promoted osteogenic-like differentiation through an OMD/BMP2/BMP receptor 1A/RUNX2 positive-feedback loop. Of note, mineral deposits were observed in the renal papillary tissues, including those around the renal tubules, within the interstitium, around the peritubular vessels and in the subepithelial layer of the renal papilla. This distribution pattern was consistent with findings from several studies on calcified renal papillae (19,63,64). However, considering that the aforementioned observations did not reproduce the earliest canonical pattern of thin loop basement membrane deposition described in RP (65), and that the eroded calcific deposits in the subepithelial region could not be definitively excluded as Randall's plugs, the features of this study as a characteristic of RP should be interpreted with caution.

Counterbalancing this pro-osteogenic machinery,  $\alpha$ -Klotho secreted by renal tubular epithelial cells suppresses the Wnt/ $\beta$ -catenin pathway in neighboring fibroblasts, thereby blocking osteoblast-like transformation (35). These findings suggest that reduced tubular  $\alpha$ -Klotho signaling may weaken an anti-osteogenic paracrine brake on neighboring RIFs in experimental systems; whether this mechanism contributes to human RP formation remains unknown (35). Whether  $\alpha$ -Klotho reduction precedes plaque initiation in human RP remains to be elucidated.

Liu *et al.* (66) recently demonstrated that high extracellular calcium activates the tissue factor pathway inhibitor 2 (TFPI-2)/dachsous cadherin-related 1 (DCHS1) axis in these cells, upregulating ALP while suppressing ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), which depletes the endogenous mineralization inhibitor pyrophosphate (PPi). OPN, RUNX2 and BMP2 expression were unchanged during this process, indicating that the TFPI-2/DCHS1 pathway operates independently of the aforementioned osteogenic program. In RIF culture systems, these findings suggested that mineralization can proceed through at least two experimentally distinguishable routes: BMP2/RUNX2-associated and TFPI-2/DCHS1-associated pathways. However, which pathway, if any, predominates during human plaque maturation remains to be elucidated.

RIFs also modulate the mineralization microenvironment through collagen secretion. Alterations in collagen composition have been associated with ectopic calcification (67,68); however, whether collagen-type-specific mineralization occurs in RP remains to be investigated in the future.

**Emerging mechanosensitive, epigenetic and epitranscriptional modifiers.** Mechanosensitive PIEZO1-Yes-associated protein (YAP) signaling, along with the context-dependent effects of YAP/transcriptional coactivator with PDZ-binding motif (TAZ) on osteogenic plasticity, represents a candidate regulatory mechanism relevant to osteogenic reprogramming. The renal inner medulla is continuously exposed to steep

osmotic gradients, providing a biologically plausible setting for mechanosensitive signaling. In mouse inner medullary collecting duct cell models, hyperosmotic NaCl/urea exposure activated the  $\text{Ca}^{2+}$ -permeable channel PIEZO1, increased  $\text{Ca}^{2+}$  influx and mtROS, and reduced cell viability (level C) (69). This finding established the responsiveness of renal medullary collecting-duct epithelial cells to PIEZO1-dependent osmotic signaling; however, the model does not reproduce the thin loop basement membrane or papillary interstitial compartment in which early human RP develops. Furthermore, the study did not examine YAP/TAZ activation or osteogenic differentiation.

Additional evidence directly associating PIEZO1 with osteogenic transcription has been reported in calcific aortic valve disease. In human aortic valve interstitial cells and two mouse models of valve calcification, oscillatory mechanical stress or pharmacological PIEZO1 activation promoted  $\text{Ca}^{2+}$ -dependent nuclear activation of YAP. YAP subsequently regulated glutaminase 1 (GLS1)-mediated glutaminolysis, which enhanced histone acetylation at RUNX2 promoter regions and promoted osteogenic differentiation and mineralization. Inhibition or knockdown of PIEZO1, YAP or GLS1 attenuated these responses (level C) (70). Notably, this study established a PIEZO1-YAP pathway but did not independently demonstrate involvement of TAZ.

The effects of YAP/TAZ are also cell-context dependent. In vascular smooth muscle cells, loss of YAP/TAZ promoted dishevelled segment polarity protein 3 nuclear translocation, upregulated a Wnt-responsive osteogenic program independently of canonical Wnt/ $\beta$ -catenin activation and resulted in vascular calcification (level C) (71). Thus, osmotic or mechanical cues may theoretically influence RIF phenotypes through PIEZO1-YAP/TAZ-related mechanotransduction; however, PIEZO1 activity, YAP/TAZ localization and downstream osteogenic signaling have not been demonstrated in RP-derived RIFs or morphologically verified human RP. This proposed association remains inferential and warrants plaque-specific validation in the future.

Epigenetic silencing of the *KL* (*KLOTHO*) gene represents another candidate modifier of the anti-calcific microenvironment. In cell-based and mouse experiments, cytosine-phosphate-guanine (CpG) methylation of the *KL* promoter restricted its transcriptional activity, whereas DNA-demethylating treatment restored promoter activity and increased *KL* expression (level C) (72). Cross-disease human evidence further demonstrated that renal *KL* promoter methylation was increased in patients with chronic kidney disease (CKD) and was inversely associated with renal  $\alpha$ -Klotho protein immunostaining and estimated glomerular filtration rate, while associating positively with tubulointerstitial fibrosis (73). Regarding pathological mineralization, indoxyl sulfate increased DNA methyltransferase (DNMT)1 and DNMT3A expression, promoted *KL* CpG hypermethylation, reduced Klotho expression and enhanced calcification in human aortic smooth muscle cells and 5/6-nephrectomized rats; these effects were attenuated by 5-aza-2'-deoxycytidine (level C) (74). Tubular cell-derived  $\alpha$ -Klotho has been reported to suppress Wnt/ $\beta$ -catenin-dependent osteogenic differentiation of neighboring RIFs in an experimental renal cell system (35), therefore *KL* promoter hypermethylation could theoretically weaken this paracrine anti-osteogenic brake. However, the

available methylation evidence derives from CKD and vascular calcification rather than idiopathic CaOx stone papillae (73,74), and neither *KL* promoter methylation nor DNMT activation has been demonstrated in morphologically verified human RP, to the best of our knowledge. The proposed association between *KL* methylation and loss of the  $\alpha$ -Klotho-RIF paracrine brake therefore represents a cross-model inference rather than an established mechanism of human RP.

At the epitranscriptomic level,  $\text{N}^6$ -methyladenosine ( $\text{m}^6\text{A}$ ) has emerged as a regulator of vascular aging and related disease processes (75) and may also influence osteogenic plasticity. In human calcified arteries, methyltransferase-like 14 (*METTL14*) expression and total RNA  $\text{m}^6\text{A}$  levels were increased. In indoxyl sulfate-treated primary human artery smooth muscle cells and rat models of uremic vascular calcification, experimental reduction of *METTL14* attenuated  $\text{m}^6\text{A}$  accumulation and calcification and improved vascular repair-associated responses (level C) (76). *METTL14*-dependent  $\text{m}^6\text{A}$  modification also altered the stability and protein output of vascular-protective transcripts, indicating that RNA methylation can influence the balance between anti- and pro-calcific cellular programs (76). Complementary evidence demonstrated that the  $\text{m}^6\text{A}$  reader YTHDF2, an  $\text{m}^6\text{A}$ -binding protein that recognizes  $\text{m}^6\text{A}$ -modified transcripts and can regulate their stability and translation, was downregulated in *in vitro* and *in vivo* vascular calcification models. YTHDF2 overexpression recognized an  $\text{m}^6\text{A}$ -modified site in the transcript encoding *RUNX2*, shortened its half-life and suppressed vascular smooth muscle cell osteogenic transition and calcium deposition (level C) (77). These findings indicated that the effects of  $\text{m}^6\text{A}$  are regulator- and transcript-dependent rather than uniformly pro-calcific. As *RUNX2*-related programs contribute to osteogenic differentiation of RIFs in experimental systems, altered  $\text{m}^6\text{A}$  writing or reading could theoretically modify osteogenic transcript stability in the papillary interstitium. However, global  $\text{m}^6\text{A}$  abundance, *METTL14* or YTHDF2 expression and transcript-specific  $\text{m}^6\text{A}$  marks have not been examined in RP-derived RIFs or morphologically verified human RP. Therefore, this proposed association remains a cross-disease, hypothesis-generating inference.

*Tubular epithelial plasticity and paracrine contribution.* Renal tubular epithelial cells are anatomically adjacent to papillary calcification and, in experimental renal-cell systems, these cells can exhibit injury-associated or osteogenic-like responses under high-calcium or osteogenic-culture conditions (78-80).

Primary tubular cells isolated from patients with idiopathic CaOx stone exhibit dose-dependent upregulation of BMP2 and OPN upon calcium loading ( $\text{Ca}^{2+}$ , 0.5-2.5 mM), consistent with calcium-associated phenotypic remodeling through BMP-related signaling in this primary-cell system (level C) (78). In osteogenic HK-2 cultures, apoptosis-related changes were detected prior to overt CaP deposition (level C) (79). In primary renal cells derived from papillary tissue of a patient with MSK, reduced GDNF expression was associated with  $\text{Ca}_2\text{PO}_4$  deposition and an osteoblast-like phenotype (level C) (58). A subsequent study using this model reported caspase-independent programmed cell death during the calcification process (level C) (80).

These findings are derived from non-RP MSK- or HK-2-based systems and should be interpreted as level C hypothesis-generating evidence rather than proof of a mechanism in human RP. Further research using spatially resolved multi-omics analyses of human papillary biopsies is warranted to investigate how tubular cells and fibroblasts coordinate osteogenic commitment *in vivo*.

**Regulation: Immune microenvironment.** The renal papillary interstitium harbors tissue-resident macrophages that actively patrol the medullary space (81,82). Macrophages are present in the papillary interstitium and may participate in local inflammatory responses. Proposed interactions between macrophages, oxidative-stress signals and fibroblast remodeling are derived mainly from crystal injury models and warrant plaque-specific validation in future research. Whether these interactions favor mineralization or resolution may depend partly on the balance between classically activated, pro-inflammatory M1 macrophages and alternatively activated, repair-associated M2 macrophages.

**Immune mediators in crystal handling.** A network of soluble immune mediators participates in crystal handling and interstitial calcification, with individual factors exerting context-dependent pro- or anti-calcific effects (Table SI) (23,83). Three mechanistic themes are highlighted in the present review. First, fetuin-A, a hepatocyte-derived glycoprotein, inhibits calcification systemically by forming colloidal calciprotein particles that sequester calcium and phosphate (84); its local depletion in the interstitium may lower the threshold for spontaneous CaP nucleation, although this process remains to be investigated in morphologically verified human RP (23). Second, the S100 calcium-binding proteins A8/A9 (S100A8/A9), which are upregulated in M1 macrophages upon crystal exposure, directly promote CaOx crystal aggregation and amplify oxidative injury through a self-amplifying IL-6-dependent circuit (85). Third, fibronectin serves a dual function: Fibronectin mediates crystal adhesion to damaged tubular surfaces but also participates in macrophage-mediated crystal clearance via integrin  $\beta 1$  engagement (81). The net calcific outcome of these factors depends on their local concentration, post-translational modification state and the local balance of pro- and anti-inflammatory mediators and immune-cell phenotypes.

OPN warrants separate discussion because its effects on inflammation and mineralization are highly context-dependent and can be either pro- or anti-calcific depending on its post-translational state. As an immune mediator, OPN interacts with  $\alpha v \beta 3$  and CD44 receptors to regulate macrophage migration, adhesion and survival, and is also a T helper 1-polarizing cytokine (86). OPN has a paradoxical role as a mineralization modulator. Genetic evidence demonstrated that OPN has a protective function: *Opn*<sup>-/-</sup> mice develop exacerbated ectopic calcification when crossed with MGP-deficient backgrounds (87), and OPN deficiency increases CaOx crystal retention in renal tubules (88). However, in bone biology, OPN promotes osteogenesis by binding CaP microspheres and modulating osteoclast activity (89).

The functional effects of OPN depend partly on its post-translational state (88-91). Phosphorylation of OPN by

casein kinases generates a polyanion that adsorbs to crystal surfaces and sterically inhibits further growth (90). By contrast, proteolytic cleavage by thrombin and matrix metalloproteinases generates N- and C-terminal fragments with altered integrin-binding specificity: The thrombin-cleaved N-terminal fragment exposes a cryptic SVVYGLR motif that potentially recruits macrophages and promotes M1 polarization (91). Temporal dynamics provide an additional mechanism: Acute OPN elevation at the crystal-tissue interface may predominantly exert inhibitory effects through intact phosphorylated forms, whereas chronic exposure in an inflammatory milieu favors proteolytic processing and the generation of pro-inflammatory, pro-calcific fragments (89).

Ultrastructural evidence from human RP tissue further clarifies the role of OPN. Immunoelectron microscopy demonstrated that OPN protein tightly circumscribes calcium salt deposits within the plaque interior (53). However, the phosphorylated vs. cleaved fraction of OPN at each stage of plaque maturation remains to be elucidated; spatially resolved phospho-proteomics applied to RP tissue sections could provide further understanding. In persistent inflammatory settings, proteolytic processing of OPN could alter macrophage recruitment and mineral interactions (91). Whether a self-sustaining feedback loop involving OPN, M1-polarized macrophages and osteogenic remodeling operates in morphologically verified human RP remains unknown. Therefore, the net effect of sustained OPN expression in established plaque should not be defined as uniformly protective or pro-calcific.

**Macrophage polarization as a candidate modifier.** Macrophage polarization may influence whether the local microenvironment tends toward mineralization or resolution (Fig. 1). M1/M2-associated expression changes in RP-related tissue are classified as level B observations, whereas mechanistic macrophage-polarization studies are predominantly classified as level C.

CaOx crystals drive the balance toward M1 macrophage polarization: Human monocytes exposed to CaOx *in vitro* differentiate into an inflammatory phenotype secreting tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), IL-1 $\beta$  and IL-8 (92). By contrast, He *et al* (81) reported that tissue-resident renal macrophages remove intratubular particles through integrin  $\beta 1$ -fibronectin-dependent trans-epithelial migration. The study did not assign this clearance program to a canonical M1 or M2 phenotype. In crystal-handling models, macrophage polarization influences the magnitude and character of local inflammatory signaling and the persistence of crystal retention. In microdissected RP-related tissue, M1-associated transcripts were increased whereas M2-associated signatures were reduced (24). Whether macrophage polarization serves a similar regulatory role within human interstitial RP remains to be elucidated.

Colony stimulating factor 1 (*Csf1*)<sup>-/-</sup> mice, which lack M2 macrophages, develop markedly increased renal CaOx crystal deposition under hyperoxaluric conditions; reconstitution with recombinant CSF1 or adoptive transfer of M2 macrophages rescues the phenotype (93). Taguchi *et al* (94) demonstrated that systemic M1 induction (lipopolysaccharide + IFN- $\gamma$ ) promotes crystal formation, whereas M2 induction (IL-4) reduces CaOx crystal deposition. Several signaling axes feed into this balance, including SIRT3/FOXO1-mediated M2

promotion (95), AR-mediated suppression of CSF1-dependent macrophage recruitment and M2-like polarization through microRNA (miR)-185-5p (96) and aryl hydrocarbon receptor- and nuclear factor erythroid 2-related factor 2-dependent M2 commitment (97,98). Bioinformatic analyses suggested that immune polarization and ECM remodeling are transcriptionally linked in RP tissue (39,99).

*Cellular senescence and the senescence-associated secretory phenotype (SASP).* Cellular senescence is a durable state of cell-cycle arrest in which metabolically active cells can acquire an SASP. The composition of the SASP varies according to cell type and stimulus, but commonly includes inflammatory cytokines, chemokines, growth factors and matrix-remodeling mediators that can modify neighboring cells (100). In human nephropathy samples and experimental kidney-injury models, *Wnt9a* expression was associated with tubular p16<sup>INK4A</sup> expression and renal fibrosis. Mechanistic experiments further demonstrated that *Wnt9a*- $\beta$ -catenin signaling accelerated tubular epithelial-cell senescence, while TGF- $\beta$ 1 released by senescent tubular cells promoted renal fibroblast proliferation and activation (level C) (101,102).

A related study identified Brahma-related gene 1 (BRG1) as an upstream regulator of tubular senescence. In mouse and primary renal tubular-cell systems, BRG1 activated *Wnt*/ $\beta$ -catenin signaling, impaired autophagic activity and increased senescence-associated markers. The altered secretome of these senescent tubular cells promoted fibroblast proliferation and activation, whereas BRG1 suppression, *Wnt*/ $\beta$ -catenin inhibition or restoration of autophagy attenuated these effects (level C) (102).

In one vascular-calcification model, the SASP was associated with pathological mineralization. In senescent human aortic smooth muscle cells, miR-34a increased IL-6 secretion and induced a secretome enriched in inflammatory cytokines, chemokines, pro-senescent growth factors and matrix-degrading molecules. Conditioned medium from miR-34a-overexpressing cells accelerated senescence and calcification in neighboring cells, while *Mir34a* deficiency reduced vitamin D-induced medial calcification and IL-6 expression in mice (level C) (103).

Therefore, these cross-disease findings suggested that senescent papillary epithelial or interstitial cells could sustain an inflammatory and matrix-remodeling milieu that influences macrophage activity, fibroblast activation and osteogenic plasticity. However, these mechanisms are derived from renal fibrosis and vascular calcification systems. Senescent-cell burden, SASP composition and their spatial association with early interstitial HAP deposits have not been characterized in morphologically verified human RP. Cellular senescence and the SASP should therefore be regarded as hypothesis-generating modifiers rather than established drivers of plaque formation.

*Exposure of RP to urine: Renal papillary epithelial breach and the interstitium-to-lumen transition.* Interstitial calcification does not solely produce a kidney stone. For stones forming via the interstitial-plaque route, focal disruption of the overlying renal papillary epithelium exposes the interstitial HAP deposit to pelvic urine and permits heterogeneous CaOx crystal nucleation and overgrowth (5,19,20). The interstitium-to-lumen

transition phase in the RP-to-stone sequence remains to be elucidated. The following section examines the available evidence.

*Renal papillary epithelium and its vulnerability.* The epithelial surface of the human renal papilla is a specialized papillary epithelium that is distinct from the transitional urothelium of the renal pelvis (104). To avoid terminological ambiguity, the present review uses 'renal papillary epithelium' when referring to the epithelial surface overlying renal papillae and reserves 'transitional urothelium' for the renal pelvis. In intraoperative biopsy and related structural studies, Evan *et al* (19,20) and Coe *et al* (36) reported that the renal papillary epithelium is intact over papillary areas where RP has not reached the surface, whereas at plaque-associated stone-attachment sites, it is focally lost, exposing the interstitial HAP deposit to pelvic urine. Plaque exposure is therefore a spatially restricted event associated with focal loss of the renal papillary epithelium.

The process by which an enlarging interstitial HAP deposit becomes exposed through the renal papillary epithelium remains to be investigated. Mechanical displacement by an expanding mineral deposit has been proposed as a possible structural explanation; however, human structural studies do not identify the initiating cellular event at plaque-exposure sites (5,12-14,16,19,30). A recent experimental study suggested that a HAP-rich microenvironment may alter RIF signaling and promote epithelial anoikis (level C) (105). Whether this candidate HAP-fibroblast-epithelial pathway operates at human plaque-exposure sites remains to be determined in future research.

*Events at the exposed plaque surface.* Once the renal papillary epithelium is breached, the exposed mineral surface encounters a different chemical environment. Pelvic urine contains CaOx at or beyond supersaturation, along with macromolecular modulators such as OPN and Tamm-Horsfall protein (THP; uromodulin). Detailed ultrastructural analyses by Coe *et al* (36) and Evan *et al* (53,54) of the stone-tissue interface in idiopathic CaOx stone formers revealed a characteristic layered architecture at the attachment site: The interstitial HAP plaque is coated by a ribbon-like structure composed of alternating mineral and organic-matrix layers (termed the 'ribbon'). OPN localizes along the crystal-matrix interface, whereas inter- $\alpha$ -trypsin inhibitor heavy chain 3 is present within the plaque matrix (53,106). Microstructural analysis of RP-associated calcium oxalate monohydrate (COM) stones by Sethmann *et al* (107) provided complementary mineralogical evidence at the plaque-stone interface. The earliest COM crystals on exposed plaque exhibited morphologies consistent with different local levels of COM supersaturation and were frequently coated with CaP. The study proposed that CaP-supersaturated interstitial fluid may diffuse through porous plaque into pelvic urine, locally increasing COM supersaturation and thereby promoting initial COM nucleation. Replenishment of pore fluid within early COM layers may subsequently permit additional CaP precipitation within the interface. Thus, this study associated exposed interstitial HAP plaque, CaOx overgrowth and the mixed CaP/COM interface with local precipitation conditions; however, the proposed ion-diffusion mechanism remains a structural interpretation rather than a directly observed dynamic process (107).

Structural and molecular observations from available studies are consistent with a proposed sequence at this interface. The exposed HAP plaque surface can interact with urinary macromolecules. OPN localizes at the plaque crystal-matrix boundary in human papillae (53), whereas THP is a major urinary crystal-modifying protein; its direct localization or passivating function at the exposed human plaque surface has not been established (108). Urinary prothrombin fragment 1 has also been reported to modulate CaOx crystallization, with its sialylation state potentially influencing CaOx stone formation (109,110); however, its role at the exposed human RP surface has not been established. OPN in its phosphorylated form is a well-established inhibitor of CaOx crystal growth and retention in renal tubules (88), and its immunolocalization to the crystal-matrix boundary in the ribbon zone is consistent with this inhibitory role (53). However, under conditions of sustained urinary CaOx supersaturation may eventually overcome protein-mediated inhibition: CaP and CaOx co-precipitate in the transition zone, and the stone composition progressively shifts to predominantly CaOx (36). This compositional gradient, from an HAP-rich plaque interior through a mixed CaP/CaOx transition zone to predominantly CaOx in the bulk stone, has been confirmed by micro-Fourier-transform infrared spectroscopy across the stone-plaque interface (25,26,36) and provides notable chemical and spatial evidence consistent with direct CaOx overgrowth on exposed RP. However, these observations are cross-sectional rather than dynamic.

Endoscopic observations provide clinical support to the breach model. Borofsky *et al* (10) reported that papillary surface pits (focal depressions in the renal papillary epithelium) are markedly associated with RP stone anchors (RPAs) and proposed that these pits mark the sites where stones were once attached to the papillary plaque before dislodging from the papillary surface. Micro-CT of surgically extracted calculi confirmed that RPAs are discrete HAP-rich regions at the stone base whose mineral signature matches interstitial plaque (10,12); spontaneously passed CaOx stones also contain internal HAP-rich remnants consistent with prior papillary attachment (11). These residual papillary defects may represent potential sites for recurrent crystal nucleation; however, their direct contribution to clinical recurrence risk has not been established.

#### 4. Systemic drivers of the pro-calcific niche

*Conceptual framework: How systemic signals converge on the interstitium.* The systemic and microbiome factors discussed in sections 4 and 5 are hypothesized modifiers of the papillary microenvironment rather than established determinants of RP presence or burden. No systemic or microbiome factor has been reported to alter endoscopic or histological RP burden in humans to date. Current tissue-level evidence directly derived from RP-related human papillary specimens is limited to level B molecular observations, including altered fatty acid-binding protein 4 (FABP4) and  $\alpha$ -Klotho expression (35,111); other proposed associations are derived from level C cell or animal studies and level D stone epidemiology or clinical associations. Fig. 2 distinguishes these evidence sources and presents only proposed routes of influence.

*Dyslipidemia: Remnant cholesterol (RC), FABP4 and inflammatory reprogramming.* Beyond the epidemiological association between dyslipidemia and nephrolithiasis, an important question is how abnormal lipid metabolism may modify the papillary interstitial milieu. Epidemiological data consistently associate dyslipidemia with nephrolithiasis risk. Cross-sectional analyses identify abdominal adiposity (112), elevated RC (113) and reduced high-density lipoprotein (114) as independent risk factors, with RC exhibiting a dose-response relationship when low-density lipoprotein cholesterol is controlled (113). These associations hold across Japanese (115) and American populations (112,114), identifying dyslipidemia as a robust systemic risk factor for nephrolithiasis.

*In vitro* co-culture studies have begun to explore how a metabolic-syndrome-like environment may enhance tubular inflammatory responses and CaOx crystal adhesion. Ichikawa *et al* (116) demonstrated that adipocyte-tubular cell co-culture enhances CaOx crystal adhesion and upregulates IL-6 expression in tubular cells. Zuo *et al* (117) extended this system to include macrophages, demonstrating that the tri-cellular metabolic milieu markedly upregulates monocyte chemoattractant protein-1, OPN and TNF- $\alpha$ , consistent with an M1-promoting inflammatory milieu associated with crystal retention. These findings position adipose tissue-derived paracrine signals as potential contributors to the interstitial inflammatory cascade, although the specific lipid mediators responsible have not been fully identified.

Notably, Taguchi *et al* (111) performed transcriptomic profiling of microdissected human RP tissue and identified marked enrichment of differentially expressed genes in lipid metabolism pathways. Among these, *FABP4* was markedly downregulated in plaque tissue. Functional validation in *Fabp4*<sup>-/-</sup> mice subjected to glyoxylate-induced hyperoxaluria demonstrated that FABP4 deficiency markedly exacerbated both interstitial and intratubular CaOx crystal deposition compared with wild-type controls (111). This level C glyoxylate-model phenotype suggests a candidate protective role for FABP4 in experimental renal calcification; its contribution to human RP burden remains to be elucidated.

FABP4 exhibits a notable tissue-specific paradox: Although decreased renal expression promotes local calcification, elevated serum FABP4 levels are associated with the severity of vascular calcification (118), and FABP4 inhibition has been explored as a therapeutic strategy for cardiovascular disease (119). The inverse relationship suggests that circulating FABP4 reflects systemic metabolic disturbance (acting as a biomarker), while interstitial FABP4 functions as a local calcification suppressor (acting as a protective effector). The mechanistic basis of this dichotomy (intracellular lipid-chaperoning vs. extracellular signaling) has not been investigated, to the best of our knowledge.

*Dysglycemia and insulin resistance: SGLT2-associated pathways and other metabolic mechanisms.* A meta-analysis of cohort studies reported a 16% higher kidney-stone risk among participants with diabetes mellitus than among those without diabetes (RR=1.16); the pooled analysis did not consistently stratify diabetes by type (120). Gestational diabetes mellitus (GDM), a distinct form of hyperglycemia first recognized during pregnancy, has also been associated

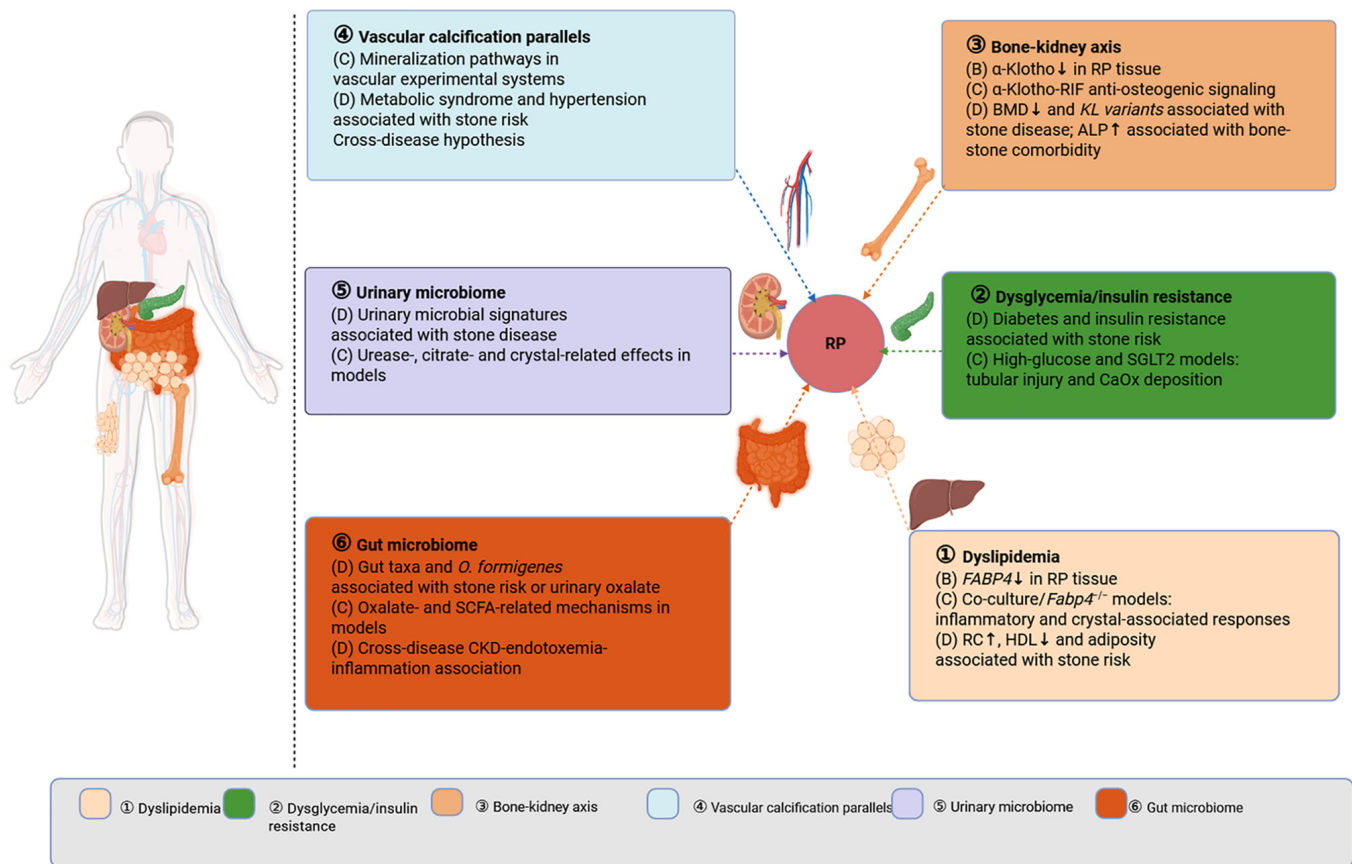


Figure 2. Hypothesized systemic and microbiome modifiers of the RP microenvironment. The schematic summarizes six candidate systemic and microbiome-related factors that may influence the renal papillary microenvironment: ① Dyslipidemia; ② dysglycemia/insulin resistance; ③ the bone-kidney axis; ④ vascular calcification-related mechanisms; ⑤ the urinary microbiome; and ⑥ the gut microbiome. Evidence levels are defined as described in Fig. 1. Dashed arrows indicate proposed associations rather than established causal effects on human RP formation or burden. RP, Randall's plaque; BMD, bone mineral density; CKD, chronic kidney disease; RC, remnant cholesterol; SCFA, short-chain fatty acid; SGLT2, sodium-glucose cotransporter 2.

with future kidney-stone risk (121), while Mendelian randomization supports a causal association between type 2 diabetes mellitus and kidney stones (122). The risk of kidney-stone formation is not confined to overt diabetes mellitus: Multiple insulin resistance indices, including the metabolic score for insulin resistance (METs-IR) (123), homeostatic model assessment of insulin resistance (HOMA-IR) (123) and triglyceride-glucose-body mass index (TyG-BMI) (123), were positively associated with both kidney-stone occurrence and recurrence in individuals without diagnosed diabetes, with each 1-unit increase in METs-IR corresponding to odds ratios of 1.020 for kidney-stone occurrence and 1.033 for recurrence, relative to a METs-IR value one unit lower (123). These data support insulin resistance as a metabolic correlate that may precede overt diabetes

A key route associating dysglycemia with renal crystal injury involves tubular epithelial stress in experimental systems (level C) (48,50,124). High-glucose exposure has been associated with increased apoptosis and reduced autophagic activity in HK-2 cells (level C) (48). In parallel, optineurin-enhanced mitophagy reduced mtROS accumulation and NLRP3 inflammasome signaling in high-glucose-treated murine renal tubular epithelial cells (level C) (50). These findings support a candidate tubular stress pathway; however, these findings do not establish high-glucose-induced pyroptosis or ferroptosis in the models cited in the present review, nor do these findings demonstrate

relevance to human RP. Anan *et al* (124) reported that SGLT2 silencing in HK-2 cells attenuated high-glucose-induced OPN and CD44 upregulation and reduced CaOx crystal adhesion. *In vivo*, phlorizin attenuated ethylene glycol-induced renal stone formation in rats and downregulated kidney injury molecule-1 and OPN without altering water intake or urine volume, whereas *Sglt2*<sup>-/-</sup> mice were resistant to glyoxylic acid-induced CaOx deposition (124). Therefore, level D stone associations and level C SGLT2-related experimental findings identify a candidate tubular injury pathway; however, whether SGLT2 inhibition alters human RP burden remains to be elucidated. In a distinct vascular model, high-glucose exposure promoted vascular smooth muscle cell calcification and senescence through BMF-AS1/BMF-associated signaling, providing cross-disease evidence that hyperglycemic stress can promote ectopic mineralization outside the kidney (125). However, these vascular findings do not establish a corresponding mechanism in human RP.

**Bone-kidney axis: Mineral homeostasis, ALP and  $\alpha$ -Klotho.** Kidney stone formers frequently exhibit lower bone mineral density (BMD) compared with non-stone controls, and clinical studies and reviews support an association between calcium stone disease and bone demineralization (126-128). Stone formers may also exhibit increased urinary calcium and altered bone-remodeling markers (129-132). In a cross-sectional

study, Zhu *et al* (133) reported an inverse association between BMD and kidney stones and identified that elevated ALP was independently associated with the coexistence of osteoporosis/osteopenia and kidney stones.

ALP hydrolyzes PPi in mineralizing systems, thereby reducing an endogenous inhibitor of HAP nucleation. ALP expression is also upregulated in osteogenically induced RIF cultures (66). Aged bone-matrix-derived extracellular vesicles have been reported to promote osteogenic phenotypic switching and vascular calcification in experimental systems (134). Whether bone-derived extracellular vesicles reach or influence the renal papillary interstitium remains to be elucidated.

$\alpha$ -Klotho serves a key role in the bone-kidney axis. Reduced circulating soluble  $\alpha$ -Klotho has been associated with CKD-related mineral and vascular abnormalities; however, methodological and clinical interpretation limitations remain (135). In a cross-sectional National Health and Nutrition Examination Survey (NHANES) analysis of middle-aged and older adults with diabetes mellitus (diabetes type was not stratified in the cited analysis), serum  $\alpha$ -Klotho levels were inversely associated with prevalent kidney stones (136). *KLOTHO* G395A polymorphism has been associated with kidney-stone susceptibility in a previous case-control study (137). In one study of RP-related human papillary tissue,  $\alpha$ -Klotho expression was reduced, and complementary cell experiments showed that tubular epithelial cell-derived  $\alpha$ -Klotho inhibited Wnt/ $\beta$ -catenin signaling and osteogenic differentiation in neighboring RIFs (35). Reduced  $\alpha$ -Klotho expression in RP-related tissue (level B) and  $\alpha$ -Klotho-RIF interactions in experimental systems (level C) support a potential association between anti-calcific signaling and the papillary interstitium. Whether systemic mineral homeostasis directly alters human RP burden remains to be investigated in future research.

The fibroblast growth factor 23 (FGF23)-parathyroid hormone (PTH)-vitamin D axis regulates CaP homeostasis through coordinated renal and intestinal effects. FGF23 and PTH decrease proximal tubular phosphate reabsorption and thereby promote phosphaturia; FGF23 suppresses renal 1 $\alpha$ -hydroxylase and 1,25-dihydroxyvitamin D production, whereas PTH stimulates vitamin D activation. Active 1,25-dihydroxyvitamin D, in turn, increases intestinal calcium and phosphate absorption (138). Dysregulation of the FGF23-Klotho-mineral axis is also implicated in CKD-associated mineral abnormalities and ectopic vascular calcification (139); however, it remains to be elucidated whether variation within this axis modifies kidney-stone risk or human RP burden independently of established metabolic abnormalities.

*Cross-disease parallels: Vascular calcification as a mirror of RP.* Metabolic syndrome and hypertension are associated with kidney-stone risk (140,141). Vascular calcification is considered primarily as a cross-disease mechanistic comparison rather than an established epidemiological predictor of stone disease in the present review. The molecular parallels between vascular calcification and RP are extensive enough to suggest overlapping disease mechanisms beyond what epidemiological co-occurrence alone would imply (detailed in section 3).

Vascular calcification and RP-related experimental systems share several candidate features, including osteogenic signaling, inflammatory pathways and altered calcification inhibitors (35,43,44,56,62,72-74,84,142,143); however, the evidence is heterogeneous and does not establish a shared causal mechanism in human RP. These cross-disease parallels nominate, rather than validate, potential therapeutic targets for future RP research. Furthermore, regarding causal directionality, it remains to be elucidated whether systemic vascular calcification drives renal interstitial calcification through circulating mediators or if both processes independently result from a shared metabolic insult.

*Sex hormone status and kidney stone risk.* A recent global meta-analysis estimated urolithiasis prevalence at 12.93% in men and 8.91% in women; however, the magnitude of the sex difference varied substantially according to population, diagnostic method and geographical region (144). In a population-based study from Rochester, the men-to-women incidence ratio for symptomatic nephrolithiasis decreased from 3.1 in 1970 to 1.3 in 2000 (level D) (145). In three large prospective cohorts, stone incidence remained higher in men than in women, with rates of 271 and 159 per 100,000 person-years, respectively; differences in lifestyle factors and urine chemistry provided partial explanations of the excess male risk (level D) (146). These epidemiological patterns alone do not establish a direct causal role for sex steroids. Clinical findings regarding estrogen status have also been reported to be inconsistent. Natural menopause was not independently associated with incident kidney stones in the Nurses' Health Study (147), whereas randomized Women's Health Initiative trials reported that estrogen therapy increased nephrolithiasis risk (148). Therefore, estrogen should not be described as uniformly protective in clinical stone disease. Furthermore, the mechanistic studies discussed below primarily focus on urinary solute metabolism, tubular CaOx crystal adhesion or injury and experimental crystal deposition; the findings do not establish that androgen or estrogen signaling alters the burden, mineral composition or molecular phenotype of morphologically verified human interstitial RP.

Circulating testosterone levels should not be treated as a direct surrogate for intrarenal androgen receptor (AR) activity. In a cross-sectional NHANES analysis, using the highest serum testosterone quartile as the reference, men in the lower three quartiles had higher adjusted odds of kidney stones (ORs, 1.375, 1.348 and 1.472 for the first, second and third quartiles, respectively), with the inverse association being most evident in men aged >40 years (level D) (149). A prospective controlled study of male patients with CaOx stones identified higher blood AR mRNA and plasma miR-185-5p levels, along with lower blood *CSF1* mRNA expression, compared with that in healthy controls; circulating androgen levels did not differ markedly between the groups (150). These non-RP human observations are associative and do not localize the pathway to renal papillary tissue.

Mechanistic evidence has been derived from COM-exposed renal tubular epithelial cells and experimental CaOx deposition models. In HK-2 and HKC-8 cells, AR bound an androgen-response element within the miR-185-5p promoter and increased miR-185-5p transcription; miR-185-5p

in turn directly targeted the 3'-untranslated region of *CSF1* mRNA, reducing *CSF1* expression and secretion. AR depletion increased *CSF1*-dependent macrophage recruitment, CD163/CD206-associated M2-like polarization and COM-crystal phagocytosis, whereas *CSF1* suppression or miR-185-5p restoration partially reversed these effects (level C) (96). Consistently, renal tubule-specific loss or pharmacological degradation of AR increased renal *CSF1* and M2-like macrophage accumulation and reduced CaOx deposition in glyoxylate-treated mice and hydroxy-L-proline-treated rats (level C) (96). This pathway aligns with independent evidence that *CSF1* deficiency reduces renal M2-like macrophages and increases experimental CaOx deposition, whereas recombinant *CSF1* or M2-macrophage transfer promotes crystal clearance (level C) (93). However, these systems model hyperoxaluria-associated intrarenal CaOx deposition rather than the initiation of chronic interstitial HAP plaque, and the AR-miR-185-5p-*CSF1* axis has not been demonstrated in morphologically verified human RP.

A mechanistic study reported two anatomically distinct ER $\beta$ -dependent effects in experimental CaOx deposition. In HepG2 hepatocytes, ER $\beta$  bound estrogen-response elements within the promoter of alanine-glyoxylate aminotransferase 1 (AGT1), increased AGT1 expression and reduced oxalate production. In oxalate-exposed HK-2 and HKC-8 renal tubular epithelial cells, ER $\beta$  suppressed NADPH oxidase 2 (NOX2) transcription, ROS and H<sub>2</sub>O<sub>2</sub> production and cell injury; NOX2 knockdown partially reversed the oxidative stress and injury responses caused by ER $\beta$  depletion. *In vivo*, genetic loss or pharmacological inhibition of ER $\beta$  increased urinary oxalate excretion, renal oxidative stress and CaOx deposition in glyoxylate-treated mice and hydroxy-L-proline-treated rats, whereas pharmacological NOX inhibition attenuated experimental crystal deposition (level C) (151). These findings support a dual hepatic AGT1 and renal tubular NOX2 mechanism in experimental CaOx disease. However, the renal systems model oxalate-exposed tubular injury and intrarenal CaOx deposition rather than early thin loop basement membrane HAP nucleation, and neither ER $\beta$  activity nor AGT1/NOX2 signaling has been demonstrated in morphologically verified human interstitial RP. Independently, 17 $\beta$ -estradiol exposure in MDCK renal tubular cells reduced CaOx crystal-binding capacity and promoted cell migration and tissue-healing phenotypes, providing additional experimental evidence that estrogen signaling can modify tubular crystal interactions (152).

Another candidate mechanism involves cross-talk between ER signaling, Wnt/ $\beta$ -catenin and fibroblast plasticity, although the available evidence is indirect and context-dependent. In established osteoblast-precursor cell lines and primary osteoblast progenitors, selective activation of kinase-mediated ER signaling stimulated Wnt and BMP2 pathways and promoted osteogenic differentiation, whereas conventional 17 $\beta$ -estradiol suppressed BMP2-induced osteoblast commitment (level C) (153). These opposing effects indicated that ER-Wnt interactions depend on the ligand, signaling mode and cellular context rather than exhibiting a uniformly pro- or anti-osteogenic effect of estrogen. In renal injury models, tubule-specific blockade of Wnt secretion reduced  $\beta$ -catenin activation, renal fibroblast proliferation and myofibroblast expansion, while tubule-derived Wnts promoted renal fibroblast activation

*in vitro* (level C) (154). Furthermore, in the context of RP, HK-2-derived  $\alpha$ -Klotho inhibited osteogenic differentiation of hRIFs by suppressing Wnt/ $\beta$ -catenin signaling (level C) (35), while activation of this pathway and reduced expression of its antagonists were observed in RP-related tissue (level B) (35). Therefore, these findings suggested that estrogen or ER signaling could modify epithelial-fibroblast Wnt communication or the osteogenic plasticity of RIFs. However, no study has directly investigated estrogen or ER pathway modulation in RP-derived RIFs, and ER-Wnt/ $\beta$ -catenin cross-talk has not been spatially demonstrated in morphologically verified human RP. This proposed association therefore remains a cross-model, hypothesis-generating inference.

## 5. Microbiome-metabolism axis

The microbiome may influence stone-associated biology through two proposed routes: Urinary microbial communities may modify local urinary chemistry and crystal behavior (155-165), whereas gut microbial communities may influence systemic oxalate handling and inflammatory signaling (166-172). Direct effects on human RP remain unproven. Notably, unlike the structural evidence for plaque-stone continuity and selected local osteogenic or immune observations described in section 3, the microbiome-interstitium link currently rests predominantly on associative data and inference rather than direct evidence in human RP. While a resident kidney microbiota that responds to antibiotics in a previous study (155) and the identification of multi-species bacterial networks that collectively regulate oxalate homeostasis (166) have provided a basis for mechanistic evidence, the key association with interstitial remodeling has not been investigated to date.

*Urinary microbiome: Local modulator of the pro-calcific niche.* The traditional assumption that urine from the healthy urinary tract is sterile has been challenged by expanded quantitative culture and sequencing studies demonstrating resident urinary microbial communities in individuals without clinical urinary tract infection (156-164). The concept of a urinary microbiome has recently been extended to the kidney parenchyma itself. Agudelo *et al* (155) reported that murine kidneys harbor a low-biomass, metabolically active microbiota in microniches between glomeruli and tubules. Cefazolin, a perioperative antibiotic extensively used in stone surgery, shifted the population away from uroprotective *Lactobacillus* species and toward pro-lithogenic *Enterobacteriaceae*; the effect was duration-dependent and reversible after cessation. *In vitro*, *Lactobacillus crispatus* inhibited CaOx crystallization, whereas stone-associated *Escherichia coli* promoted it. RNA sequencing and RNA fluorescence *in situ* hybridization imaging of human kidney tissue confirmed microbial signatures in both glomerular and tubular compartments (155). These findings raise the possibility that perioperative antibiotics may alter the renal microbiome in ways relevant to stone biology. Expanded quantitative urine culture and 16S ribosomal RNA sequencing studies have countered the dogma of sterile urine and identified resident urinary microbial communities in adults (156-164). In urinary stone disease, Zampini *et al* (165) reported urinary and gut dysbiosis in stone formers compared with controls, including altered

*Lactobacillus*- and *Enterobacteriaceae*-associated microbial community patterns; however, a direct effect on papillary interstitial RP remains to be investigated in the future.

Urease-producing bacteria can alkalize urine and favor phosphate precipitation (173). *In vitro*, selected *Enterobacteriaceae* can modify CaOx crystal interactions. *E. coli*-associated citrate depletion has also been proposed as a lithogenic mechanism (174). However, the direct influence of these microbial effects on papillary interstitial RP remains to be elucidated.

*Gut microbiome: Systemic regulator of mineral and oxalate homeostasis.* If the urinary microbiome acts locally, the gut microbiome influences stone risk distally, primarily through its effects on oxalate handling and systemic inflammation. Oxalate homeostasis is regulated by intestinal oxalotrophic bacteria, particularly *Oxalobacter formigenes*, which degrades dietary oxalate via formyl-CoA transferase and oxalyl-CoA decarboxylase (167-170). Reduced *O. formigenes* colonization has been associated with altered intestinal oxalate regulation and higher urinary oxalate in certain studies (167-170). However, these observations are associative and do not establish that antibiotic exposure or dietary change uniformly increases urinary oxalate through loss of *O. formigenes*.

Comparative gut microbiome profiling revealed extensive dysbiosis in stone formers. *Bacteroides* is ~3.4-fold more abundant in patients with kidney stones, while *Prevotella* is 2.8-fold more prevalent in controls (171). In a small pilot cohort study, *Eubacterium* abundance demonstrated an inverse trend with urinary oxalate, whereas *Escherichia* abundance exhibited an inverse trend with urinary citrate (171). These exploratory associations warrant validation in larger cohorts in the future.

Although much research has focused on *O. formigenes*, the broader gut microbial community may also contribute to intestinal oxalate metabolism and kidney-stone susceptibility (166,172). Miller *et al* (166) used metagenomic analysis to describe a network of bacterial taxa that co-occur with *O. formigenes* in non-stone-forming controls and are underrepresented in stone formers, suggesting that oxalate homeostasis depends on a consortium rather than a single species. A two-sample Mendelian randomization study reported no causal association between genus *Oxalobacter* and kidney stones, but identified protective effects for Actinomycetales, Clostridiaceae and Hungatella, and risk associations with *Haemophilus* and *Subdoligranulum* (172). Miller *et al* (166) suggested that community-level oxalate-degrading capacity, including genes such as *Oxc* and *Frc*, may be more informative than the presence of any single taxon.

Beyond oxalate degradation, the gut microbiome may influence the renal interstitium through systemic inflammatory routes, although this hypothesis has not been validated in an RP model. In a cross-disease context, CKD-mineral and bone disorder includes vascular calcification as a recognized complication (175), whereas circulating endotoxemia in CKD is associated with systemic inflammation and cardiovascular disease (176). The contribution of gut-derived inflammatory signals to papillary interstitial mineralization in stone formers remains inferential. Short-chain fatty acids (SCFAs), whose production depends on gut microbial composition, regulate macrophage polarization in multiple organs, and fecal SCFA

levels differ between stone formers and controls (177). Whether any of these gut-derived signals reach the renal papillary interstitium and modulate NLRP3 activation or M1/M2 macrophage balance remains to be elucidated. Future research is warranted to compare macrophage polarization and osteogenic markers in renal papillae of germ-free vs. conventionally housed mice under hyperoxaluric challenge.

## 6. Animal models: Strengths, limitations and emerging platforms

To date, no animal model fully recapitulates human RP. The persistent, progressive interstitial HAP deposition that characterizes RP in the multilobar human kidney has proved difficult to reproduce in rodent kidneys, whose unilobar anatomy and papillary organization differ markedly from those of humans (178). Nevertheless, the aforementioned models in the present review provide key insights into human RP, including current limitations and scope for future research.

*Chemically induced models: Hyperoxaluria and combined-hit models, in which two or more lithogenic or metabolic perturbations are applied together.* Chemically induced hyperoxaluria remains one of the most widely used strategies for experimentally inducing renal CaOx crystal deposition. Inducing agents, such as ethylene glycol and glyoxylic acid, are administered to experimental animals via dietary supplementation or injection (178-181). These protocols produce predictable hyperoxaluria and renal crystal deposition; however, the mineral phase and anatomical distribution of deposits depend on the inducing regimen (179-181). Single-agent hyperoxaluria models are limited by crystal transience: Deposits resolve upon cessation of the inducing stimulus, contrasting with the more persistent and progressive nature of human RP (178-181). This divergence restricts the utility of these models in studying chronic plaque maturation and limits their predictive validity for therapeutic durability.

Combined metabolic disruptions can increase renal crystal deposition in experimental models; however, the mineral phase, compartment and chronicity vary across protocols. Such models may approximate selected metabolic combinations observed in kidney stone disease; however, these models do not reproduce the full progressive interstitial plaque sequence of human RP (182,183). Therapeutically, induced models have identified druggable targets. The peroxisome proliferator-activated receptor- $\gamma$  (PPAR $\gamma$ ) agonist pioglitazone reduces glyoxylic acid-induced CaOx deposition via suppression of M1 macrophage polarization (179), while phlorizin attenuates ethylene glycol-induced renal stone formation in rats and *Sglt2* deficiency reduces glyoxylic acid-induced CaOx deposition in mice (124). These findings provide experimental proof-of-concept in targeting tubular injury and inflammatory responses; however, neither model reproduces human interstitial RP.

*Genetic models: Comparative analysis by phenotype.* The present review classifies the genetic and hereditary models according to the location and inducibility of their mineral phenotype (Fig. 3); Table SII summarizes the corresponding mechanisms and limitations. This grouping is intended to

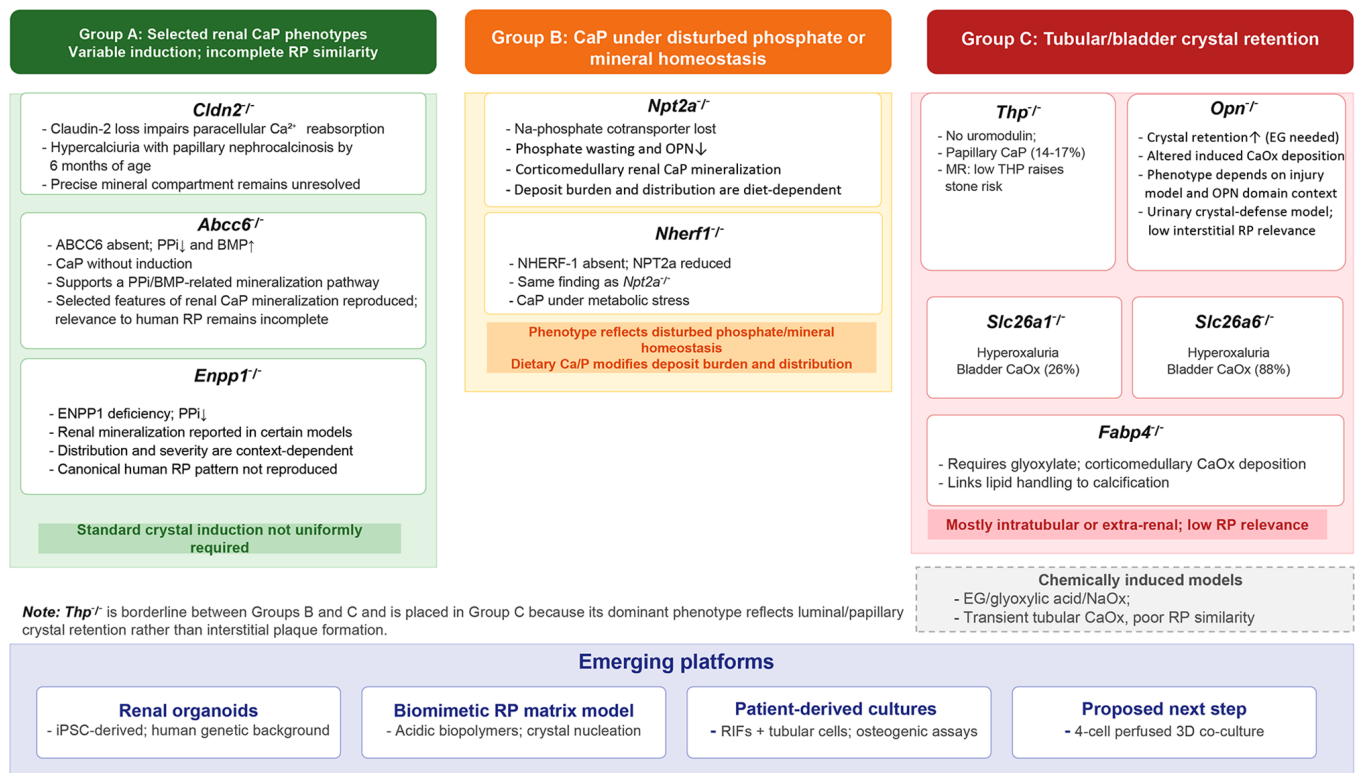


Figure 3. Genetic and hereditary animal models and emerging experimental platforms relevant to RP research. Models are classified according to the anatomical distribution and inducibility of mineral deposition rather than evidence strength. RP, Randall's plaque; CaOx, calcium oxalate; CaP, calcium phosphate; EG, ethylene glycol; ENPP1, ectonucleotide pyrophosphatase/phosphodiesterase 1; PPI, pyrophosphate; THP, Tamm-Horsfall protein; iPSC, induced pluripotent stem cell.

facilitate comparison of model phenotypes and should not be interpreted as an absolute biological classification. Certain models, particularly *Thp*<sup>-/-</sup>, are present near the boundaries between groups; therefore, the categories should not be viewed as absolute.

**Group A: Selected spontaneous renal CaP phenotypes.** These models develop renal CaP deposition without exogenous induction. The anatomical correspondence of these models to human RP remains incomplete and may differ across models. Claudin-2 (*Cldn2*)<sup>-/-</sup> mice lack the tight-junction protein CLDN2, impairing paracellular calcium reabsorption and causing hypercalciuria with papillary nephrocalcinosis by 6 months of age. The precise mineral compartment and similarity to progressive human interstitial plaque remain incompletely elucidated (184). ATP-binding cassette subfamily C member 6 (*Abcc6*)<sup>-/-</sup> mice lack ABCC6; ABCC6 deficiency reduces circulating PPI and is associated with spontaneous interstitial CaP deposition and BMP-related signaling (185,186). This finding supports a role for the PPI/BMP axis in this model, which is informative for PPI-dependent mineralization and BMP-related signaling (level C); however, it does not investigate an ROS-dependent mechanism of human RP. *Enpp1*<sup>-/-</sup> mice have reduced PPI and can develop ectopic mineralization involving the kidney (level C). However, the presence, distribution and severity of renal mineralization vary with the model background and experimental conditions, and the deposits reported in these studies do not establish the canonical thin loop basement membrane or progressive interstitial pattern of human RP (187-189).

**Group B: CaP deposition under disturbed phosphate or mineral homeostasis.** Sodium-dependent phosphate transport protein 2A (*Npt2a*)<sup>-/-</sup> mice (sodium-phosphate cotransporter deficiency) develop persistent corticomedullary CaP deposits postnatally, accompanied by hyperphosphaturia, hypophosphatemia and reduced urinary OPN (190-192). The burden and distribution of mineralization in this model can also be modified by dietary calcium and phosphorus (192). Na<sup>+</sup>/H<sup>+</sup> exchanger regulatory factor 1 (*Nherf1*)<sup>-/-</sup> mice phenocopy the *Npt2a*<sup>-/-</sup> model through scaffold protein-mediated downregulation of NPT2a, producing a hypercalciuric, hyperphosphaturic state with renal interstitial CaP deposition (193). Both models highlighted the role of phosphate transport dysfunction in interstitial calcification but required disruption of mineral homeostasis to induce the phenotype.

**Group C: Primarily tubular deposition or crystal retention modulators.** *Thp*<sup>-/-</sup> mice develop CaP crystal deposition in renal papillae in 14-17% of animals, with Mendelian randomization confirming that reduced urinary uromodulin increases human stone risk (108,194,195). *Opn*<sup>-/-</sup> mice exhibit altered induced CaOx deposition; the direction and distribution of the phenotype depend on the injury model and OPN domain context (88,196). These models primarily reveal the role of urinary protein defenses, not interstitial reprogramming. Solute carrier family 26 member 1 (*Slc26a1*)<sup>-/-</sup> and solute carrier family 26 member 6 (*Slc26a6*)<sup>-/-</sup> mice develop hyperoxaluria through sulfate transporter dysfunction, producing bladder CaOx stones in 26 and 88% of animals, respectively (197,198); the pathology of these models is predominantly intratubular and extra-renal, limiting RP relevance. *Fabp4*<sup>-/-</sup> mice

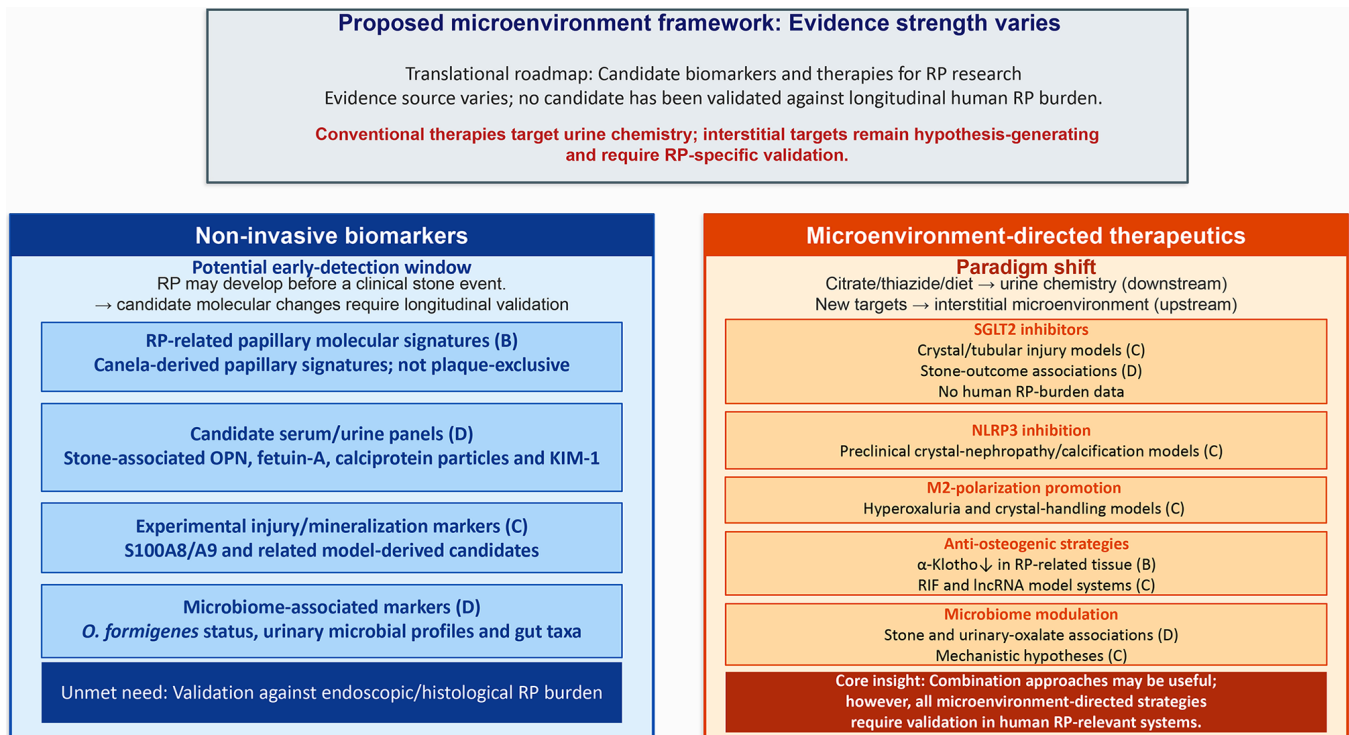


Figure 4. Proposed translational roadmap for biomarkers and microenvironment-directed therapeutic strategies in RP research. The left panel summarizes candidate non-invasive biomarkers for future RP detection and stratification. The right panel presents potential therapeutic approaches targeting the papillary interstitial microenvironment. Evidence levels are defined as described in Fig. 1. Rightward arrows (→) indicate proposed directional relationships or conceptual transitions and do not establish causality. None of the listed biomarkers or therapeutic strategies has been validated against longitudinal endoscopic or histological measurements of human RP burden; therefore, these approaches remain hypothesis-generating and require RP-specific validation. RP, Randall's plaque; OPN, osteopontin; KIM-1, kidney injury molecule-1; RIF, renal interstitial fibroblast; S100A8/A9, S100 calcium-binding proteins A8/A9; SGLT2, sodium-glucose cotransporter 2.

require glyoxylate induction to develop corticomedullary CaOx deposits (111); the principal value of this model lies in establishing the lipid metabolism-calcification association (section 4), not in recapitulating spontaneous RP.

**Emerging platforms: Organoids, biomimetic models and patient-derived systems.** The limitations of rodent models have promoted research using human-derived and biomimetic *in vitro* platforms. Patient-derived primary cultures of RIFs and tubular epithelial cells have contributed to the aforementioned osteogenic-differentiation studies (35,58,78), while biomimetic RP models using acidic biopolymer matrices have been developed to examine the effects of organic matrix components on crystal nucleation (199). Renal organoids and kidney-on-chip systems may provide additional human-background platforms; however, no available system recapitulates the complete adult papillary interstitial niche, including sustained medullary gradients and coordinated epithelial, interstitial, immune, vascular and ECM components, to date. These platforms should therefore be viewed as complementary tools rather than replacements for animal models in the future.

## 7. Unresolved questions and future directions

**Spatiotemporal initiation: Nucleation of the first crystal.** Available translational opportunities, stratified by biomarker readiness and therapeutic evidence level, are summarized in Fig. 4. Evan *et al* (19) localized the earliest RP mineral to

the basement membranes of the thin loops of Henle; however, the temporal coordination of immune cell recruitment, fibroblast activation and matrix remodeling at this site remains to be elucidated. In a previous study by Canela *et al* (22), the integration of single-nucleus RNA sequencing with spatial transcriptomics revealed that the injury signature in stone-bearing papillae, including immune activation, oxidative stress and ECM remodeling, extends beyond focal mineral deposits. This pattern is compatible with molecular remodeling beyond visible plaque but does not establish that such remodeling temporally precedes plaque formation. Furthermore, as noted in section 2, the dataset did not separately resolve interstitial from intratubular mineralization. Therefore, future studies should adopt a morphology-first, lesion-staged design rather than including further candidate pathways to an undifferentiated list. Based on established RP morphology and mineral-mapping studies (19,20,36,53,54), it may be proposed to classify serial papillary sections as non-mineralized tissue, early thin-loop basement-membrane HAP, expanding interstitial plaque, exposed plaque or intratubular plug before spatial profiling. Spatial transcriptomics combined with multiplexed protein imaging, such as multiplexed ion beam imaging by time of flight or co-detection by indexing, may then define cell identities, subcellular protein localization, cellular neighborhoods and distance-dependent molecular gradients around each lesion class (200,201). Comparisons may be proposed among plaque-adjacent cells, distance-matched cells from the same papilla, non-stone

controls and plug-dominant papillae, informed by prior papillary spatial profiling and emerging spatial multi-omics approaches (22,202,203), so that plaque-associated remodeling can be distinguished from generalized stone-related or intratubular injury (Fig. 4).

Candidate mechanisms should be evaluated as prespecified biological modules associated with the aforementioned framework described in section 3. A mechanotransduction-osteogenic module should assess PIEZO1 abundance, YAP/TAZ subcellular localization, local matrix properties and RUNX2-related output. A stress-resolution module should examine oxidative-stress signals along with autophagy- and mitophagy-associated proteins. However, fixed-tissue abundance of LC3- $\beta$ , sequestosome 1/p62, PINK1 or Parkin can identify spatially associated machinery but these proteins are unable to establish altered autophagic or mitophagic flux, which requires dynamic assays in viable *ex vivo* or experimental systems (204). A senescence-immune module should combine cell-cycle-arrest markers, loss of nuclear-envelope integrity, SASP mediators and macrophage phenotypes rather than defining senescence from a single p16 or p21 signal (100). Lastly, morphology-guided laser-capture microdissection could isolate plaque-adjacent RIFs and epithelial cells for targeted analysis of *KL* promoter methylation, DNMT activity, m<sup>6</sup>A abundance and transcript-specific m<sup>6</sup>A regulation. Emerging spatial methylome-transcriptome and spatial m<sup>6</sup>A technologies provide a potential route to retain the tissue microenvironment; however, neither platform has yet been validated in human papillary RP (202,203).

It may be proposed that a candidate mechanism should be considered plaque-associated when it is reproducibly enriched in defined cells adjacent to early interstitial HAP, varies with lesion stage or distance from the mineral and is unevenly present in non-mineralized or plug-dominant tissue. Such spatial associations could then be tested functionally in papillary cell co-cultures, organotypic tissue systems or plaque-relevant mineral-exposure models using pathway-specific disruption. Temporal inference could combine standardized endoscopic plaque mapping, clinically obtained papillary specimens, longitudinal biospecimens and subsequent plaque-growth or recurrence data, rather than depending on routine repeated papillary biopsies (19,22,202,203). This staged framework could distinguish a fixed sequence of events from a coupled network present at plaque initiation and from patient-specific entry into different nodes of the network.

**Pathway hierarchy: The dominant driver in a patient.** Oxidative stress, immune remodeling and osteogenic changes have been observed in RP-related tissue or experimental models (24,35,38,39,56,62), whereas dyslipidemia, dysglycemia and microbiome alterations are supported mainly by stone-related experimental or epidemiological evidence (111-124,155-174); however, the hierarchical relationship between these processes remains to be elucidated. To address whether lipid-induced inflammation and hyperglycemia-driven oxidative stress contribute independently or converge on pathways such as NLRP3, future studies could combine multicellular co-culture systems previously used to model metabolic inflammatory interactions (116,117) with controlled metabolic perturbation. Human cohorts could

additionally be stratified by metabolic phenotype and studied using matched endoscopic plaque quantification together with papillary multi-omics or spatial profiling (22,202,203) to identify candidate subgroups for targeted investigation. Another key question is whether these drivers can be ranked by importance, and if the associations established to date reflect causal relationships. Both of these issues warrant similar methodological advances in future studies.

**Model similarity.** The metabolic syndrome-stone association rests predominantly on cross-sectional and retrospective data. Mendelian randomization has strengthened the causal association for type 2 diabetes mellitus (122) but has not been systematically applied to individual metabolic-syndrome components (dyslipidemia and insulin resistance) or specific microbial taxa, to the best of our knowledge. Prospective cohort studies using serial endoscopic papillary assessment combined with longitudinal multi-omics profiling of urine, blood and stool are warranted to establish temporal precedence. Interventional studies (randomized trials of SGLT2 inhibitors, M2-promoting agents or microbiome-targeted therapies) with plaque burden as the primary endpoint would provide notable causal evidence.

The transient crystal deposition of chemically induced models and the single-gene focus of KO models capture only a fragment of multifactorial human RP. Next-generation models should incorporate multiple simultaneous targets. For example, crossing *Cldn2*<sup>-/-</sup> mice onto a high-fat diet background would combine hypercalciuria with metabolic syndrome and allow analysis of pathway interactions *in vivo*. Longitudinal intravital two-photon imaging of the rodent papilla could resolve the temporal sequence of cell death, immune infiltration and mineral nucleation that may not be captured by endpoint histology. For human-relevant platforms, papilla-on-chip devices incorporating primary patient-derived cells under medullary concentration gradients, combined with immune cell co-culture, represent the most promising path toward *in vitro* models that recapitulate the complexity of the human mineralization-permissive milieu. As a proposed next-generation human-relevant platform, a multicellular perfused 3D papillary co-culture incorporating papillary epithelial/tubular cells, RIFs and immune cells under medullary-like chemical gradients could enable controlled investigation of mineralization-associated cell-cell interactions.

## 8. Conclusion

RP formation likely involves a convergence of physico-chemical priming, epithelial injury responses, regulated cell death, osteogenic reprogramming and immune remodeling, all unfolding within a microenvironment where homeostasis is disrupted by local and systemic signals. However, the supporting evidence for this mechanism remains inconsistent. Observations of mineral continuity from interstitial HAP through papillary epithelial breach to the overlying CaOx stone provide the strongest structural foundation for the RP-to-CaOx stone pathway described in this review. Transcriptomic and molecular data from RP-bearing tissue have revealed various signatures; however, these remain associative and, in certain

datasets, confounded by coexistent ductal plugs. Cell and animal models have generated numerous candidate mechanisms; however, few models reproduce either the site-specific origin of calcification or the papillary interstitial microenvironment of human RP. Epidemiological findings associate systemic disorders with nephrolithiasis; however, direct effects on human RP burden remain to be elucidated.

Therefore, the integrated framework proposed in the present review should be interpreted as a set of verifiable hypotheses rather than a validated causal sequence. The potential of this framework lies in linking structural observations, tissue-level molecular findings, experimental models and clinical associations to specific questions that can be investigated in longitudinal human papillary studies and RP-relevant experimental platforms in the future. By considering RP as a renal interstitial microenvironmental disorder, it may lead to the potential development of biomarkers for early papillary remodeling and, ultimately, preventive strategies to treat CaOx stones that originate from RP in the future.

### Acknowledgements

Not applicable.

### Funding

The present review was supported by the National Natural Science Foundation of China [grant nos. 82500932 (to ZZ), 82170781 (to HC) and 82370771 (to FZ)], the Young Investigator Cultivation Initiative of the National Major Science and Technology Program for Chronic Disease Prevention and Control Research [grant no. 2024ZD0537600 (to ZZ)], the Youth Science Foundation of Xiangya Hospital [grant no. 2024Q20 (to ZZ)], the Youth Science Foundation of Hunan Province [grant no. 2025JJ60680 (to ZZ)], the China National Postdoctoral Program for Innovative Talents [grant no. BX20250266 (to ZZ)] and the China Postdoctoral Science Foundation [grant no. BHMS20252439 (to ZZ)].

### Availability of data and materials

Not applicable.

### Authors' contributions

JW conceived the structure of the present review, conducted the literature search and drafted the manuscript. ZL participated in the literature search and contributed in drafting sections 3 and 4 of the manuscript. ML contributed in drafting sections 5 and 6 of the manuscript and assisted with reference management. YW contributed in drafting section 4 of the manuscript and assisted with figure preparation. YC contributed to the critical revision of the manuscript for important intellectual content. HC contributed to the critical revision of the manuscript and provided supervision. FZ and ZZ conceived and designed the present review, supervised the work, critically revised the manuscript and were responsible for the overall content. All authors read and approved the final manuscript. Data authentication is not applicable.

### Ethics approval and consent to participate

Not applicable.

### Patient consent for publication

Not applicable.

### Competing interests

The authors declare that they have no competing interests.

### References

1. Khan SR, Pearle MS, Robertson WG, Gambaro G, Canales BK, Doizi S, Traxer O and Tiselius HG: Kidney stones. *Nat Rev Dis Primers* 2: 16008, 2016.
2. Kittanamongkolchai W, Vaughan LE, Enders FT, Dhondup T, Mehta RA, Krambeck AE, McCollough CH, Vrtiska TJ, Lieske JC and Rule AD: The changing incidence and presentation of urinary stones over 3 decades. *Mayo Clin Proc* 93: 291-299, 2018.
3. Shu X, Cai H, Xiang YB, Li H, Lipworth L, Miller NL, Zheng W, Shu XO and His RS: Nephrolithiasis among middle aged and elderly Urban Chinese: A report from prospective cohort studies in Shanghai. *J Endourol* 31: 1327-1334, 2017.
4. Rule AD, Lieske JC, Li X, Melton LJ III, Krambeck AE and Bergstralh EJ: The ROKS nomogram for predicting a second symptomatic stone episode. *J Am Soc Nephrol* 25: 2878-2886, 2014.
5. Randall A: The origin and growth of renal calculi. *Ann Surg* 105: 1009-1027, 1937.
6. Low RK and Stoller ML: Endoscopic mapping of renal papillae for Randall's plaques in patients with urinary stone disease. *J Urol* 158: 2062-2064, 1997.
7. Matlaga BR, Williams JC Jr, Kim SC, Kuo RL, Evan AP, Bledsoe SB, Coe FL, Worcester EM, Munch LC and Lingeman JE: Endoscopic evidence of calculus attachment to Randall's plaque. *J Urol* 175: 1720-1724; discussion 1724, 2006.
8. Miller NL, Gillen DL, Williams JC Jr, Evan AP, Bledsoe SB, Coe FL, Worcester EM, Matlaga BR, Munch LC and Lingeman JE: A formal test of the hypothesis that idiopathic calcium oxalate stones grow on Randall's plaque. *BJU Int* 103: 966-971, 2009.
9. Pless MS, Williams JC Jr, Andreassen KH, Jung HU, Osther SS, Christensen DR and Osther PJS: Endoscopic observations as a tool to define underlying pathology in kidney stone formers. *World J Urol* 37: 2207-2215, 2019.
10. Borofsky MS, Williams JC Jr, Dauw CA, Cohen A, Evan AC, Coe FL, Worcester E and Lingeman JE: Association between Randall's plaque stone anchors and renal papillary pits. *J Endourol* 33: 337-342, 2019.
11. Miller NL, Williams JC Jr, Evan AP, Bledsoe SB, Coe FL, Worcester EM, Munch LC, Handa SE and Lingeman JE: In idiopathic calcium oxalate stone-formers, unattached stones show evidence of having originated as attached stones on Randall's plaque. *BJU Int* 105: 242-245, 2010.
12. Williams JC Jr, Matlaga BR, Kim SC, Jackson ME, Sommer AJ, McAteer JA, Lingeman JE and Evan AP: Calcium oxalate calculi found attached to the renal papilla: Preliminary evidence for early mechanisms in stone formation. *J Endourol* 20: 885-890, 2006.
13. Winfree S, Weiler C, Bledsoe SB, Gardner T, Sommer AJ, Evan AP, Lingeman JE, Krambeck AE, Worcester EM, El-Achkar TM and Williams JC Jr: Multimodal imaging reveals a unique autofluorescence signature of Randall's plaque. *Urolithiasis* 49: 123-135, 2021.
14. Sherer BA, Chen L, Kang M, Shimotake AR, Wiener SV, Chi T, Stoller ML and Ho SP: A continuum of mineralization from human renal pyramid to stones on stems. *Acta Biomater* 71: 72-85, 2018.
15. Williams JC Jr, Lingeman JE, Coe FL, Worcester EM and Evan AP: Micro-CT imaging of Randall's plaques. *Urolithiasis* 43 (Suppl 1): S13-S17, 2015.

16. Gay C, Letavernier E, Verpont MC, Walls M, Bazin D, Daudon M, Nassif N, Stéphan O and de Frutos M: Nanoscale analysis of Randall's plaques by electron energy loss spectromicroscopy: Insight in early biomineral formation in human kidney. *ACS Nano* 14: 1823-1836, 2020.
17. Kuo RL, Lingeman JE, Evan AP, Paterson RF, Parks JH, Bledsoe SB, Munch LC and Coe FL: Urine calcium and volume predict coverage of renal papilla by Randall's plaque. *Kidney Int* 64: 2150-2154, 2003.
18. Wang X, Krambeck AE, Williams JC Jr, Tang X, Rule AD, Zhao F, Bergstralh E, Haskic Z, Edeh S, Holmes DR III, *et al*: Distinguishing characteristics of idiopathic calcium oxalate kidney stone formers with low amounts of Randall's plaque. *Clin J Am Soc Nephrol* 9: 1757-1763, 2014.
19. Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SB, Shao Y, Sommer AJ, Paterson RF, Kuo RL and Grynaps M: Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. *J Clin Invest* 111: 607-616, 2003.
20. Evan A, Lingeman J, Coe FL and Worcester E: Randall's plaque: Pathogenesis and role in calcium oxalate nephrolithiasis. *Kidney Int* 69: 1313-1318, 2006.
21. Sivaguru M, Saw JJ, Wilson EM, Lieske JC, Krambeck AE, Williams JC, Romero MF, Fouke KW, Curtis MW, Kear-Scott JL, *et al*: Human kidney stones: A natural record of universal biomineralization. *Nat Rev Urol* 18: 404-432, 2021.
22. Canela VH, Bowen WS, Ferreira RM, Syed F, Lingeman JE, Sabo AR, Barwinska D, Winfree S, Lake BB, Cheng YH, *et al*: A spatially anchored transcriptomic atlas of the human kidney papilla identifies significant immune injury in patients with stone disease. *Nat Commun* 14: 4140, 2023.
23. Khan SR, Canales BK and Dominguez-Gutierrez PR: Randall's plaque and calcium oxalate stone formation: role for immunity and inflammation. *Nat Rev Nephrol* 17: 417-433, 2021.
24. Taguchi K, Hamamoto S, Okada A, Unno R, Kamisawa H, Naiki T, Ando R, Mizuno K, Kawai N, Tozawa K, *et al*: Genome-wide gene expression profiling of Randall's plaques in calcium oxalate stone formers. *J Am Soc Nephrol* 28: 333-347, 2017.
25. Cifuentes Delatte L, Miñón-Cifuentes J and Medina JA: New studies on papillary calculi. *J Urol* 137: 1024-1029, 1987.
26. Daudon M, Bazin D and Letavernier E: Randall's plaque as the origin of calcium oxalate kidney stones. *Urolithiasis* 43 (Suppl 1): S5-S11, 2015.
27. Kim SC, Coe FL, Tinmouth WW, Kuo RL, Paterson RF, Parks JH, Munch LC, Evan AP and Lingeman JE: Stone formation is proportional to papillary surface coverage by Randall's plaque. *J Urol* 173: 117-119; discussion 119, 2005.
28. Strohmaier WL, Hörmann M and Schubert G: Papillary calcifications: A new prognostic factor in idiopathic calcium oxalate urolithiasis. *Urolithiasis* 41: 475-479, 2013.
29. Jaeger CD, Rule AD, Mehta RA, Vaughan LE, Vrtiska TJ, Holmes DR III, McCollough CM, Ziegelmann MJ, Herrera Hernandez LP, Lieske JC and Krambeck AE: Endoscopic and pathologic characterization of papillary architecture in struvite stone formers. *Urology* 90: 39-44, 2016.
30. Wiener SV, Ho SP and Stoller ML: Beginnings of nephrolithiasis: Insights into the past, present and future of Randall's plaque formation research. *Curr Opin Nephrol Hypertens* 27: 236-242, 2018.
31. Bushinsky DA: Nephrolithiasis: Site of the initial solid phase. *J Clin Invest* 111: 602-605, 2003.
32. Asplin JR, Mandel NS and Coe FL: Evidence of calcium phosphate supersaturation in the loop of Henle. *Am J Physiol* 270 (4 Pt 2): F604-F613, 1996.
33. Tiselius HG: The role of calcium phosphate in the development of Randall's plaques. *Urolithiasis* 41: 369-377, 2013.
34. Gorvin CM, Loh NY, Stechman MJ, Falcone S, Hannan FM, Ahmad BN, Piret SE, Reed AA, Jeyabalan J, Leo P, *et al*: Mice with a *Brd4* mutation represent a new model of nephrocalcinosis. *J Bone Miner Res* 34: 1324-1335, 2019.
35. Zhu Z, Ruan S, Jiang Y, Huang F, Xia W, Chen J, Cui Y, He C, Zeng F, Li Y, *et al*:  $\alpha$ -Klotho released from HK-2 cells inhibits osteogenic differentiation of renal interstitial fibroblasts by inactivating the Wnt- $\beta$ -catenin pathway. *Cell Mol Life Sci* 78: 7831-7849, 2021.
36. Coe FL, Evan AP, Worcester EM and Lingeman JE: Three pathways for human kidney stone formation. *Urol Res* 38: 147-160, 2010.
37. Rivera M, Cockerill PA, Enders F, Mehta RA, Vaughan L, Vrtiska TJ, Herrera Hernandez LP, Holmes DR III, Rule AD, Lieske JC and Krambeck AE: Characterization of inner medullary collecting duct plug formation among idiopathic calcium oxalate stone formers. *Urology* 94: 47-52, 2016.
38. Wu J, Liu M, Gao M, Li Y, Zhang Y, Tang L, Yu H, Liao Z, Cui Y, Zeng F, *et al*: Epigallocatechin gallate attenuates CaOx crystal-induced renal tubular injury to inhibit CaOx nephrolithiasis via GRP94/PI3K/AKT signaling. *Biomater Res* 29: 0271, 2025.
39. Li F, Shi K, Li S, Wei Y and Jia Z: Bioinformatics identifies key genes and potential therapeutic targets in the pathological mechanism of oxidative stress in Randall's plaque. *Sci Rep* 14: 31364, 2024.
40. Liu Q, Liu Y, Guan X, Wu J, He Z, Kang J, Tao Z and Deng Y: Effect of M2 macrophages on injury and apoptosis of renal tubular epithelial cells induced by calcium oxalate crystals. *Kidney Blood Press Res* 44: 777-791, 2019.
41. Xun Y, Zhou P, Yang Y, Li C, Zhang J, Hu H, Qin B, Zhang Z, Wang Q, Lu Y and Wang S: Role of Nox4 in high calcium-induced renal oxidative stress damage and crystal deposition. *Antioxid Redox Signal* 36: 15-38, 2022.
42. Khand FD, Gordge MP, Robertson WG, Noronha-Dutra AA and Hotherhall JS: Mitochondrial superoxide production during oxalate-mediated oxidative stress in renal epithelial cells. *Free Radic Biol Med* 32: 1339-1350, 2002.
43. Joshi S, Wang W, Peck AB and Khan SR: Activation of the NLRP3 inflammasome in association with calcium oxalate crystal induced reactive oxygen species in kidneys. *J Urol* 193: 1684-1691, 2015.
44. Chen Y, Yang S, Kong H, Wang Q, Chen S, Wang X, Chen L and Qi S: Oxalate-induced renal pyroptotic injury and crystal formation mediated by NLRP3-GSDMD signaling in vitro and in vivo. *Mol Med Rep* 28: 209, 2023.
45. Khan SR and Alli AA: Apoptosis, ferroptosis, necrosis, necroptosis and pyroptosis in the formation of calcium oxalate kidney stones. *Urolithiasis* 53: 153, 2025.
46. Anders HJ, Suarez-Alvarez B, Grigorescu M, Foresto-Neto O, Steiger S, Desai J, Marschner JA, Honarpisheh M, Shi C, Jordan J, *et al*: The macrophage phenotype and inflammasome component NLRP3 contributes to nephrocalcinosis-related chronic kidney disease independent from IL-1-mediated tissue injury. *Kidney Int* 93: 656-669, 2018.
47. Liang S, Wu YS, Li DY, Tang JX and Liu HF: Autophagy and renal fibrosis. *Aging Dis* 13: 712-731, 2022.
48. Lee WC, Chiu CH, Chen JB, Chen CH and Chang HW: Mitochondrial fission increases apoptosis and decreases autophagy in renal proximal tubular epithelial cells treated with high glucose. *DNA Cell Biol* 35: 657-665, 2016.
49. Lin Q, Li S, Jiang N, Shao X, Zhang M, Jin H, Zhang Z, Shen J, Zhou Y, Zhou W, *et al*: PINK1-parkin pathway of mitophagy protects against contrast-induced acute kidney injury via decreasing mitochondrial ROS and NLRP3 inflammasome activation. *Redox Biol* 26: 101254, 2019.
50. Chen K, Feng L, Hu W, Chen J, Wang X, Wang L and He Y: Optineurin inhibits NLRP3 inflammasome activation by enhancing mitophagy of renal tubular cells in diabetic nephropathy. *FASEB J* 33: 4571-4585, 2019.
51. Liu Y, Li D, He Z, Liu Q, Wu J, Guan X, Tao Z and Deng Y: Inhibition of autophagy-attenuated calcium oxalate crystal-induced renal tubular epithelial cell injury in vivo and in vitro. *Oncotarget* 9: 4571-4582, 2017.
52. Villalpando-Rodriguez GE and Gibson SB: Reactive oxygen species (ROS) regulates different types of cell death by acting as a rheostat. *Oxid Med Cell Longev* 2021: 9912436, 2021.
53. Evan AP, Coe FL, Rittling SR, Bledsoe SM, Shao Y, Lingeman JE and Worcester EM: Apatite plaque particles in inner medulla of kidneys of calcium oxalate stone formers: Osteopontin localization. *Kidney Int* 68: 145-154, 2005.
54. Evan AP, Coe FL, Lingeman JE and Worcester E: Insights on the pathology of kidney stone formation. *Urol Res* 33: 383-389, 2005.
55. Boskey AL and Posner AS: The role of synthetic and bone extracted Ca-phospholipid-PO4 complexes in hydroxyapatite formation. *Calcif Tissue Res* 23: 251-258, 1977.
56. Zhu M, Zeng F, Cui Y, Liu X and Chen H: Expression of matrix Gla protein and bone morphogenetic protein 2 in renal papillary tissues in patients with calcium oxalate kidney stones. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 42: 277-283, 2017 (In Chinese).
57. Khan SR, Rodriguez DE, Gower LB and Monga M: Association of Randall plaque with collagen fibers and membrane vesicles. *J Urol* 187: 1094-1100, 2012.

58. Mezzabotta F, Cristofaro R, Ceol M, Del Prete D, Priante G, Familiari A, Fabris A, D'Angelo A, Gambaro G and Anglani F: Spontaneous calcification process in primary renal cells from a medullary sponge kidney patient harbouring a GDNF mutation. *J Cell Mol Med* 19: 889-902, 2015.
59. Zhu Z, Cui Y, Huang F, Zeng H, Xia W, Zeng F, He C, Chen J, Chen Z, Chen H and Li Y: Long non-coding RNA H19 promotes osteogenic differentiation of renal interstitial fibroblasts through Wnt- $\beta$ -catenin pathway. *Mol Cell Biochem* 470: 145-155, 2020.
60. Zhu Z, Zhang X, Jiang Y, Ruan S, Huang F, Zeng H, Liu M, Xia W, Zeng F, Chen J, *et al*: NEAT1 functions as a key mediator of BMP2 to promote osteogenic differentiation of renal interstitial fibroblasts. *Epigenomics* 13: 1171-1186, 2021.
61. Zhu Z, Huang F, Jiang Y, Ruan S, Liu M, Zhang Y, Li Y, Chen J, Cui Y, Chen Z, *et al*: OLMALINC/OCT4/BMP2 axis enhances osteogenic-like phenotype of renal interstitial fibroblasts to participate in Randall's plaque formation. *Mol Med* 28: 162, 2022.
62. Zhu Z, Huang F, Gao M, Liu M, Zhang Y, Tang L, Wu J, Yu H, He C, Chen J, *et al*: Osteogenic-Like microenvironment of renal interstitium induced by osteomodulin contributes to Randall's plaque formation. *Adv Sci (Weinh)* 11: e2405875, 2024.
63. Haggitt RC and Pitcock JA: Renal medullary calcifications: A light and electron microscopic study. *J Urol* 106: 342-347, 1971.
64. Weller RO, Nester B and Cooke SA: Calcification in the human renal papilla: An electron-microscope study. *J Pathol* 107: 211-216, 1972.
65. Evan AP, Coe FL, Lingeman J, Bledsoe S and Worcester EM: Randall's plaque in stone formers originates in ascending thin limbs. *Am J Physiol Renal Physiol* 315: F1236-F1242, 2018.
66. Liu M, Liu Z, Huang F, Chen H, Yang Z and Zhu Z: A high-calcium environment induced ectopic calcification of renal interstitial fibroblasts via TFPI-2-DCHS1-ALP/ENPP1 axis to participate in Randall's plaque formation. *Urolithiasis* 52: 122, 2024.
67. Kuzan A, Wisniewski J, Maksymowicz K, Kobielarz M, Gamian A and Chwilkowska A: Relationship between calcification, atherosclerosis and matrix proteins in the human aorta. *Folia Histochem Cytobiol* 59: 8-21, 2021.
68. Zhao Y, Sun Z, Li L, Yuan W and Wang Z: Role of collagen in vascular calcification. *J Cardiovasc Pharmacol* 80: 769-778, 2022.
69. Probst S, Romanova N, Herbrechter R, Kern T, Bergmeier M, Lee WK and Thévenod F: Hyperosmolarity-induced activation of PIEZO1 engages detrimental calcium/oxidative stress signaling and adaptive catalase response in renal inner medullary collecting duct (mIMCD(3)) cells. *Biochim Biophys Acta Mol Cell Res* 1872: 120041, 2025.
70. Zhong G, Su S, Li J, Zhao H, Hu D, Chen J, Li S, Lin Y, Wen L, Lin X, *et al*: Activation of Piezo1 promotes osteogenic differentiation of aortic valve interstitial cell through YAP-dependent glutaminolysis. *Sci Adv* 9: eadg0478, 2023.
71. Wang L, Chennupati R, Jin YJ, Li R, Wang S, Günther S and Offermanns S: YAP/TAZ are required to suppress osteogenic differentiation of vascular smooth muscle cells. *iScience* 23: 101860, 2020.
72. Azuma M, Koyama D, Kikuchi J, Yoshizawa H, Thasinas D, Shiizaki K, Kuro-o M, Furukawa Y and Kusano E: Promoter methylation confers kidney-specific expression of the Klotho gene. *FASEB J* 26: 4264-4274, 2012.
73. Chen J, Zhang X, Zhang H, Lin J, Zhang C, Wu Q and Ding X: Elevated Klotho promoter methylation is associated with severity of chronic kidney disease. *PLoS One* 8: e79856, 2013.
74. Chen J, Zhang X, Zhang H, Liu T, Zhang H, Teng J, Ji J and Ding X: Indoxyl sulfate enhance the hypermethylation of klotho and promote the process of vascular calcification in chronic kidney disease. *Int J Biol Sci* 12: 1236-1246, 2016.
75. Li C, Liu L, Li S and Liu YS: N6-Methyladenosine in vascular aging and related diseases: Clinical perspectives. *Aging Dis* 15: 1447-1473, 2024.
76. Chen J, Ning Y, Zhang H, Song N, Gu Y, Shi Y, Cai J, Ding X and Zhang X: METTL14-dependent m6A regulates vascular calcification induced by indoxyl sulfate. *Life Sci* 239: 117034, 2019.
77. Li L, Cheng M, Jin J, Zhao Y, Bai W, Zhang D, Zhang S, Bai Y and Xu J: The m6A reader YTHDF2 protects vascular smooth muscle cells against the osteogenic differentiation through targeting Runx2. *Ren Fail* 47: 2488876, 2025.
78. He D, Wang S, Jia Z, Cui L, Lu Y, Hu H and Qin B: Calcium ions promote primary renal epithelial cell differentiation into cells with bone-associated phenotypes via transforming growth factor- $\beta$ 1-induced epithelial-mesenchymal transition in idiopathic hypercalciuria patients. *Mol Med Rep* 11: 2199-2206, 2015.
79. Priante G, Ceol M, Giancesello L, Furlan C, Del Prete D and Anglani F: Human proximal tubular cells can form calcium phosphate deposits in osteogenic culture: Role of cell death and osteoblast-like transdifferentiation. *Cell Death Discov* 5: 57, 2019.
80. Priante G, Quaggio F, Giancesello L, Ceol M, Cristofaro R, Terrin L, Furlan C, Del Prete D and Anglani F: Caspase-independent programmed cell death triggers Ca(2) PO(4) deposition in an in vitro model of nephrocalcinosis. *Biosci Rep* 38: BSR20171228, 2018.
81. He J, Cao Y, Zhu Q, Wang X, Cheng G, Wang Q, He R, Lu H, Weng Y, Mao G, *et al*: Renal macrophages monitor and remove particles from urine to prevent tubule obstruction. *Immunity* 57: 106-123.e7, 2024.
82. Lazarov T, Juarez-Carreño S, Cox N and Geissmann F: Physiology and diseases of tissue-resident macrophages. *Nature* 618: 698-707, 2023.
83. Dominguez-Gutierrez PR, Kwenda EP, Khan SR and Canales BK: Immunotherapy for stone disease. *Curr Opin Urol* 30: 183-189, 2020.
84. Heiss A, Eckert T, Aretz A, Richtering W, van Dorp W, Schäfer C and Jähnen-Dechent W: Hierarchical role of fetuin-A and acidic serum proteins in the formation and stabilization of calcium phosphate particles. *J Biol Chem* 283: 14815-14825, 2008.
85. Wang Q, Zhang J, Chen X, Sun F and Jiang K: Renal tubular epithelial cells treated with calcium oxalate up-regulate S100A8 and S100A9 expression in M1-polarized macrophages via interleukin 6. *Iran J Basic Med Sci* 26: 603-608, 2023.
86. Lund SA, Giachelli CM and Scatena M: The role of osteopontin in inflammatory processes. *J Cell Commun Signal* 3: 311-322, 2009.
87. Speer MY, McKee MD, Guldberg RE, Liaw L, Yang HY, Tung E, Karsenty G and Giachelli CM: Inactivation of the osteopontin gene enhances vascular calcification of matrix Gla protein-deficient mice: Evidence for osteopontin as an inducible inhibitor of vascular calcification in vivo. *J Exp Med* 196: 1047-1055, 2002.
88. Wesson JA, Johnson RJ, Mazzali M, Beshensky AM, Stietz S, Giachelli C, Liaw L, Alpers CE, Couser WG, Kleinman JG and Hughes J: Osteopontin is a critical inhibitor of calcium oxalate crystal formation and retention in renal tubules. *J Am Soc Nephrol* 14: 139-147, 2003.
89. Shirakawa K and Sano M: Osteopontin in cardiovascular diseases. *Biomolecules* 11: 1047, 2021.
90. Narisawa S, Yadav MC and Millán JL: In vivo overexpression of tissue-nonspecific alkaline phosphatase increases skeletal mineralization and affects the phosphorylation status of osteopontin. *J Bone Miner Res* 28: 1587-1598, 2013.
91. Kaleta B: The role of osteopontin in kidney diseases. *Inflamm Res* 68: 93-102, 2019.
92. Dominguez-Gutierrez PR, Kusmartsev S, Canales BK and Khan SR: Calcium oxalate differentiates human monocytes into inflammatory M1 macrophages. *Front Immunol* 9: 1863, 2018.
93. Taguchi K, Okada A, Kitamura H, Yasui T, Naiki T, Hamamoto S, Ando R, Mizuno K, Kawai N, Tozawa K, *et al*: Colony-stimulating factor-1 signaling suppresses renal crystal formation. *J Am Soc Nephrol* 25: 1680-1697, 2014.
94. Taguchi K, Okada A, Hamamoto S, Unno R, Moritoki Y, Ando R, Mizuno K, Tozawa K, Kohri K and Yasui T: M1/M2-macrophage phenotypes regulate renal calcium oxalate crystal development. *Sci Rep* 6: 35167, 2016.
95. Xi J, Chen Y, Jing J, Zhang Y, Liang C, Hao Z and Zhang L: Sirtuin 3 suppresses the formation of renal calcium oxalate crystals through promoting M2 polarization of macrophages. *J Cell Physiol* 234: 11463-11473, 2019.
96. Zhu W, Zhao Z, Chou F, Zuo L, Liu T, Yeh S, Bushinsky D, Zeng G and Chang C: Loss of the androgen receptor suppresses intrarenal calcium oxalate crystals deposition via altering macrophage recruitment/M2 polarization with change of the miR-185-5p/CSF-1 signals. *Cell Death Dis* 10: 275, 2019.
97. Yang X, Liu H, Ye T, Duan C, Lv P, Wu X, Liu J, Jiang K, Lu H, Yang H, *et al*: AhR activation attenuates calcium oxalate nephrocalcinosis by diminishing M1 macrophage polarization and promoting M2 macrophage polarization. *Theranostics* 10: 12011-12025, 2020.

98. Liu H, Yang X, Tang K, Ye T, Duan C, Lv P, Yan L, Wu X, Chen Z, Liu J, *et al*: Sulforaphane elicits dual therapeutic effects on Renal Inflammatory Injury and crystal deposition in calcium oxalate nephrocalcinosis. *Theranostics* 10: 7319-7134, 2020.
99. Yu L, Li G, Jin S, Su J and Li S: Identification of the core genes in Randall's plaque of kidney stone and immune infiltration with WGCNA network. *Front Genet* 14: 1048919, 2023.
100. Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, Campisi J, Collado M, Evangelou K, Ferbeyre G, *et al*: Cellular senescence: Defining a path forward. *Cell* 179: 813-827, 2019.
101. Luo C, Zhou S, Zhou Z, Liu Y, Yang L, Liu J, Zhang Y, Li H, Liu Y, Hou FF and Zhou L: Wnt9a promotes renal fibrosis by accelerating cellular senescence in tubular epithelial cells. *J Am Soc Nephrol* 29: 1238-1256, 2018.
102. Gong W, Luo C, Peng F, Xiao J, Zeng Y, Yin B, Chen X, Li S, He X, Liu Y, *et al*: Brahma-related gene-1 promotes tubular senescence and renal fibrosis through Wnt/ $\beta$ -catenin/autophagy axis. *Clin Sci (Lond)* 135: 1873-1895, 2021.
103. Zuccolo E, Badi I, Scavellio F, Gambuzza I, Mancinelli L, Macri F, Tedesco CC, Veglia F, Bonfigli AR, Olivieri F and Raucci A: The microRNA-34a-induced senescence-associated secretory phenotype (SASP) favors vascular smooth muscle cells calcification. *Int J Mol Sci* 21: 4454, 2020.
104. Yanagizawa R: A scanning and transmission electron microscopic study on the pelvic and papillary epithelia of the human and rat kidney. *Nihon Hinyokika Gakkai Zasshi* 78: 1792-1802, 1987 (In Japanese).
105. Liu M, Wu M, Gao M, Li Y, Yuan M, Liao Z, Liu Z, Wang Y, Cui Y, Chen J, *et al*: FASLG derived from fibroblasts in hydroxyapatite-rich microenvironment induces urothelial anoikis to trigger randall's plaque exposure. *Adv Sci (Weinh)* 13: e21605, 2026.
106. Evan AP, Bledsoe S, Worcester EM, Coe FL, Lingeman JE and Bergsland KJ: Renal inter-alpha-trypsin inhibitor heavy chain 3 increases in calcium oxalate stone-forming patients. *Kidney Int* 72: 1503-1511, 2007.
107. Sethmann I, Wendt-Nordahl G, Knoll T, Enzmann F, Simon L and Kleebe HJ: Microstructures of Randall's plaques and their interfaces with calcium oxalate monohydrate kidney stones reflect underlying mineral precipitation mechanisms. *Urolithiasis* 45: 235-248, 2017.
108. Mo L, Huang HY, Zhu XH, Shapiro E, Hasty DL and Wu XR: Tamm-Horsfall protein is a critical renal defense factor protecting against calcium oxalate crystal formation. *Kidney Int* 66: 1159-1166, 2004.
109. Liu J, Chen J, Wang T, Wang S and Ye Z: Effects of urinary prothrombin fragment 1 in the formation of calcium oxalate calculus. *J Urol* 173: 113-116, 2005.
110. Webber D, Radcliffe CM, Royle L, Tobiasen G, Merry AH, Rodgers AL, Sturrock ED, Wormald MR, Harvey DJ, Dwek RA and Rudd PM: Sialylation of urinary prothrombin fragment 1 is implicated as a contributory factor in the risk of calcium oxalate kidney stone formation. *FEBS J* 273: 3024-3037, 2006.
111. Taguchi K, Chen L, Usawachintachit M, Hamamoto S, Kang M, Sugino T, Unno T, Tzou DT, Sherer BA, Okada A, *et al*: Fatty acid-binding protein 4 downregulation drives calcification in the development of kidney stone disease. *Kidney Int* 97: 1042-1056, 2020.
112. Li G, Liang H, Hao Y, Huang Q, Shen X, Chen Y, Chen M, Xi J and Hao Z: Association between body fat distribution and kidney stones: Evidence from a US population. *Front Endocrinol (Lausanne)* 13: 1032323, 2022.
113. Yao L and Yang P: Relationship between remnant cholesterol and risk of kidney stones in U.S. Adults: A 2007-2016 NHANES analysis. *Ann Med* 56: 2319749, 2024.
114. Gao M, Liu M, Zhu Z and Chen H: The association of dyslipidemia with kidney stone: Result from the NHANES 2007-2020. *Int Urol Nephrol* 56: 35-44, 2024.
115. Kohjimoto Y, Sasaki Y, Iguchi M, Matsumura N, Inagaki T and Hara I: Association of metabolic syndrome traits and severity of kidney stones: results from a nationwide survey on urolithiasis in Japan. *Am J Kidney Dis* 61: 923-939, 2013.
116. Ichikawa J, Okada A, Taguchi K, Fujii Y, Zuo L, Niimi K, Hamamoto S, Kubota Y, Umemoto Y, Itoh Y, *et al*: Increased crystal-cell interaction in vitro under co-culture of renal tubular cells and adipocytes by in vitro co-culture paracrine systems simulating metabolic syndrome. *Urolithiasis* 42: 17-28, 2014.
117. Zuo L, Tozawa K, Okada A, Yasui T, Taguchi K, Ito Y, Hirose Y, Fujii Y, Niimi K, Hamamoto S, *et al*: A paracrine mechanism involving renal tubular cells, adipocytes and macrophages promotes kidney stone formation in a simulated metabolic syndrome environment. *J Urol* 191: 1906-1912, 2014.
118. Zhou S, Wang X, Shi J, Han Q, He L, Tang W and Zhang A: Serum fatty acid binding protein 4 levels are associated with abdominal aortic calcification in peritoneal dialysis patients. *Ren Fail* 43: 1539-1548, 2021.
119. Garaikoetxea M, Martín-Núñez E, Navarro A, Matilla L, Fernández-Celis A, Arrieta V, García-Peña A, Gainza A, Álvarez V, Sádaba R, *et al*: Targeting fatty acid-binding protein 4 improves pathologic features of aortic stenosis. *Int J Mol Sci* 23: 8439, 2022.
120. Aune D, Mahamat-Saleh Y, Norat T and Riboli E: Body fatness, diabetes, physical activity and risk of kidney stones: A systematic review and meta-analysis of cohort studies. *Eur J Epidemiol* 33: 1033-1047, 2018.
121. Mao Y, Hu W, Liu L and Liu Q: Association between gestational diabetes mellitus and future risk of kidney stones. *Front Public Health* 10: 843383, 2022.
122. Yuan S and Larsson SC: Assessing causal associations of obesity and diabetes with kidney stones using Mendelian randomization analysis. *Mol Genet Metab* 134: 212-215, 2021.
123. Yang YX, Xiang JC, Ye GC, Luo KD, Wang SG and Xia QD: Association of insulin resistance indices with kidney stones and their recurrence in a non-diabetic population: an analysis based on NHANES data from 2007-2018. *Ren Fail* 47: 2490203, 2025.
124. Anan G, Hirose T, Kikuchi D, Takahashi C, Endo A, Ito H, Sato S, Nakayama S, Hashimoto H, Ishiyama K, *et al*: Inhibition of sodium-glucose cotransporter 2 suppresses renal stone formation. *Pharmacol Res* 186: 106524, 2022.
125. Lin X, Xiang QY, Li S, Song WL, Wang YJ, Ni YQ, Zhao Y, Li C, Wang Y, Li HH, *et al*: BMF-AS1/BMF Promotes Diabetic Vascular Calcification and Aging both in vitro and in vivo. *Aging Dis* 14: 170-183, 2023.
126. Shavit I, Girkoglio D, Vijay V, Goldsmith D, Ferraro PM, Moomchhala SH and Unwin R: Vascular calcification and bone mineral density in recurrent kidney stone formers. *Clin J Am Soc Nephrol* 10: 278-285, 2015.
127. Krieger NS and Bushinsky DA: The relation between bone and stone formation. *Calcif Tissue Int* 93: 374-381, 2013.
128. Arrabal-Polo MA, Cano-García Mdel C, Canales BK and Arrabal-Martín M: Calcium nephrolithiasis and bone demineralization: Pathophysiology, diagnosis, and medical management. *Curr Opin Urol* 24: 633-638, 2014.
129. Arrabal-Polo MA, Arrabal-Martín M, de Haro-Munoz T, Lopez-Leon VM, Merino-Salas S, Ochoa-Hortal MA, Garrido-Gomez J, Lahoz-García C and Zuluaga-Gomez A: Mineral density and bone remodelling markers in patients with calcium lithiasis. *BJU Int* 108: 1903-1908; discussion 1908, 2011.
130. Caudarella R, Vescini F, Buffa A, Sinicropi G, Rizzoli E, La Manna G and Stefoni S: Bone mass loss in calcium stone disease: Focus on hypercalciuria and metabolic factors. *J Nephrol* 16: 260-266, 2003.
131. Arrabal-Polo MA, Arias-Santiago S, de Haro-Munoz T, Lopez-Ruiz A, Orgaz-Molina J, Gonzalez-Torres S, Zuluaga-Gomez A and Arrabal-Martín M: Effects of aminobisphosphonates and thiazides in patients with osteopenia/osteoporosis, hypercalciuria, and recurring renal calcium lithiasis. *Urology* 81: 731-737, 2013.
132. Alshara L, Batagello CA, Armanyous S, Gao T, Patel N, Remer EM and Monga M: The impact of thiazides and potassium citrate on bone mineral density evaluated by CT scan in stone formers. *J Endourol* 32: 559-564, 2018.
133. Zhu Z, Liu M, Zhang Y, Wu J, Gao M, Lei T, Huang F, Chen H and Wu M: Risk factors for the comorbidity of osteoporosis/osteopenia and kidney stones: A cross-sectional study. *Arch Osteoporos* 18: 128, 2023.
134. Wang ZX, Luo ZW, Li FX, Cao J, Rao SS, Liu YW, Wang YY, Zhu GQ, Gong JS, Zou JT, *et al*: Aged bone matrix-derived extracellular vesicles as a messenger for calcification paradox. *Nat Commun* 13: 1453, 2022.
135. Martín-Virgala J, Martín-Carro B, Fernández-Villabrille S, Ruiz-Torres MP, Gómez-Alonso C, Rodríguez-García M, Fernández-Martín JL, Alonso-Montes C, Panizo S, Cannata-Andía JB, *et al*: Soluble klotho, a potential biomarker of chronic kidney disease-mineral bone disorders involved in healthy ageing: Lights and shadows. *Int J Mol Sci* 25: 1843, 2024.

136. Xiao Y and Xiao Z: Association between serum klotho and kidney stones in US middle-aged and older individuals with diabetes mellitus: Results from 2007 to 2016 National health and nutrition survey. *Am J Nephrol* 54: 224-233, 2023.
137. Telci D, Dogan AU, Ozbek E, Polat EC, Simsek A, Cakir SS, Yeloglu HO and Sahin F: KLOTRO gene polymorphism of G395A is associated with kidney stones. *Am J Nephrol* 33: 337-343, 2011.
138. Ho BB and Bergwitz C: FGF23 signalling and physiology. *J Mol Endocrinol* 66: R23-R32, 2021.
139. Ren SC, Mao N, Yi S, Ma X, Zou JQ, Tang X and Fan JM: Vascular calcification in chronic kidney disease: An update and perspective. *Aging Dis* 13: 673-697, 2022.
140. Wong YV, Cook P and Somani BK: The association of metabolic syndrome and urolithiasis. *Int J Endocrinol* 2015: 570674, 2015.
141. Domingos F and Serra A: Metabolic syndrome: A multifaceted risk factor for kidney stones. *Scand J Urol* 48: 414-419, 2014.
142. Zebboudj AF, Imura M and Boström K: Matrix GLA protein, a regulatory protein for bone morphogenetic protein-2. *J Biol Chem* 277: 4388-4394, 2002.
143. Lu J, Xie S, Deng Y, Xie X and Liu Y: Blocking the NLRP3 inflammasome reduces osteogenic calcification and M1 macrophage polarization in a mouse model of calcified aortic valve stenosis. *Atherosclerosis* 347: 28-38, 2022.
144. Vera-Ponce VJ, Sanchez-Tamay NM, Ballena-Caicedo J, Zuzunaga-Montoya FE, De Carrillo CIG and Poemape Mestanza RL: Global prevalence of urolithiasis: A meta-analysis accounting for methodological heterogeneity. *Front Urol* 5: 1705953, 2025.
145. Lieske JC, Peña de la Vega LS, Slezak JM, Bergstralh EJ, Leibson CL, Ho KL and Gettman MT: Renal stone epidemiology in Rochester, Minnesota: An update. *Kidney Int* 69: 760-764, 2006.
146. Ferraro PM, Taylor EN and Curhan GC: Factors associated with sex differences in the risk of kidney stones. *Nephrol Dial Transplant* 38: 177-183, 2023.
147. Mattix Kramer HJ, Grodstein F, Stampfer MJ and Curhan GC: Menopause and postmenopausal hormone use and risk of incident kidney stones. *J Am Soc Nephrol* 14: 1272-1277, 2003.
148. Maalouf NM, Sato AH, Welch BJ, Howard BV, Cochrane BB, Sakhaee K and Robbins JA: Postmenopausal hormone use and the risk of nephrolithiasis: Results from the Women's Health Initiative hormone therapy trials. *Arch Intern Med* 170: 1678-1685, 2010.
149. Huang F, Li Y, Cui Y, Zhu Z, Chen J, Zeng F, Li Y, Chen Z and Chen H: Relationship between serum testosterone levels and kidney stones prevalence in men. *Front Endocrinol (Lausanne)* 13: 863675, 2022.
150. Elshal AM, Shamsoun H, Awadalla A, Elbaz R, Ahmed AE, El-Khawaga OY and Shokeir AA: Hormonal and molecular characterization of calcium oxalate stone formers predicting occurrence and recurrence. *Urolithiasis* 51: 76, 2023.
151. Zhu W, Zhao Z, Chou FJ, Zuo L, Liu T, Bushinsky D, Chang C, Zeng G and Yeh S: The protective roles of estrogen receptor  $\beta$  in renal calcium oxalate crystal formation via reducing the liver oxalate biosynthesis and renal oxidative stress-mediated cell injury. *Oxid Med Cell Longev* 2019: 5305014, 2019.
152. Peerapen P and Thongboonkerd V: protective cellular mechanism of estrogen against kidney stone formation: A proteomics approach and functional validation. *Proteomics* 19: e1900095, 2019.
153. Kousteni S, Almeida M, Han L, Bellido T, Jilka RL and Manolagas SC: Induction of osteoblast differentiation by selective activation of kinase-mediated actions of the estrogen receptor. *Mol Cell Biol* 27: 1516-1530, 2007.
154. Zhou D, Fu H, Zhang L, Zhang K, Min Y, Xiao L, Lin L, Bastacky SI and Liu Y: Tubule-Derived Wnts Are required for fibroblast activation and kidney fibrosis. *J Am Soc Nephrol* 28: 2322-2336, 2017.
155. Agudelo J, Chen X, Mukherjee SD, Nguyen JK, Bruggeman LA and Miller AW: Cefazolin shifts the kidney microbiota to promote a lithogenic environment. *Nat Commun* 15: 10509, 2024.
156. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, Fitzgerald M, Mueller ER, Schreckenberger P, Dong Q, Nelson DE and Brubaker L: Evidence of uncultivated bacteria in the adult female bladder. *J Clin Microbiol* 50: 1376-1383, 2012.
157. Siddiqui H, Nederbragt AJ, Lagesen K, Jeansson SL and Jakobsen KS: Assessing diversity of the female urine microbiota by high throughput sequencing of 16S rDNA amplicons. *BMC Microbiol* 11: 244, 2011.
158. Pearce MM, Hilt EE, Rosenfeld AB, Zilliox MJ, Thomas-White K, Fok C, Kliethermes S, Schreckenberger PC, Brubaker L, Gai X and Wolfe AJ: The female urinary microbiome: A comparison of women with and without urgency urinary incontinence. *mBio* 5: e01283-14, 2014.
159. Nienhouse V, Gao X, Dong Q, Nelson DE, Toh E, McKinley K, Schreckenberger P, Shibata N, Fok CS, Mueller ER, *et al*: Interplay between bladder microbiota and urinary antimicrobial peptides: Mechanisms for human urinary tract infection risk and symptom severity. *PLoS One* 9: e114185, 2014.
160. Lewis DA, Brown R, Williams J, White P, Jacobson SK, Marchesi JR and Drake MJ: The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front Cell Infect Microbiol* 3: 41, 2013.
161. Khasriya R, Sathiananthamoorthy S, Ismail S, Kelsey M, Wilson M, Rohn JL and Malone-Lee J: Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. *J Clin Microbiol* 51: 2054-2062, 2013.
162. Hilt EE, McKinley K, Pearce MM, Rosenfeld AB, Zilliox MJ, Mueller ER, Brubaker L, Gai X, Wolfe AJ and Schreckenberger PC: Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J Clin Microbiol* 52: 871-876, 2014.
163. Fouts DE, Pieper R, Szpakowski S, Pohl H, Knoblach S, Suh MJ, Huang ST, Ljungberg I, Sprague BM, Lucas SK, *et al*: Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. *J Transl Med* 10: 174, 2012.
164. Brubaker L, Nager CW, Richter HE, Visco A, Nygaard I, Barber MD, Schaffer J, Meikle S, Wallace D, Shibata N and Wolfe AJ: Urinary bacteria in adult women with urgency urinary incontinence. *Int Urogynecol J* 25: 1179-1184, 2014.
165. Zampini A, Nguyen AH, Rose E, Monga M and Miller AW: Defining dysbiosis in patients with urolithiasis. *Sci Rep* 9: 5425, 2019.
166. Miller AW, Choy D, Penniston KL and Lange D: Inhibition of urinary stone disease by a multi-species bacterial network ensures healthy oxalate homeostasis. *Kidney Int* 96: 180-188, 2019.
167. Mehta M, Goldfarb DS and Nazzari L: The role of the microbiome in kidney stone formation. *Int J Surg* 36 (Pt D): 607-612, 2016.
168. Sadaf H, Raza SI and Hassan SW: Role of gut microbiota against calcium oxalate. *Microb Pathog* 109: 287-291, 2017.
169. Ticinesi A, Nouvenne A, Chiussi G, Castaldo G, Guerra A and Meschi T: Calcium oxalate nephrolithiasis and gut microbiota: Not just a gut-kidney axis. A nutritional perspective. *Nutrients* 12: 548, 2020.
170. Ticinesi A, Milani C, Guerra A, Allegri F, Lauretani F, Nouvenne A, Mancabelli L, Lugli GA, Turrone F, Duranti S, *et al*: Understanding the gut-kidney axis in nephrolithiasis: An analysis of the gut microbiota composition and functionality of stone formers. *Gut* 67: 2097-2106, 2018.
171. Stern JM, Moazami S, Qiu Y, Kurland I, Chen Z, Agalliu I, Burk R and Davies KP: Evidence for a distinct gut microbiome in kidney stone formers compared to non-stone formers. *Urolithiasis* 44: 399-407, 2016.
172. Liu M, Zhang Y, Wu J, Gao M, Zhu Z and Chen H: Causal relationship between kidney stones and gut microbiota contributes to the gut-kidney axis: A two-sample Mendelian randomization study. *Front Microbiol* 14: 1204311, 2023.
173. Wang Z, Zhang Y, Zhang J, Deng Q and Liang H: Recent advances on the mechanisms of kidney stone formation (Review). *Int J Mol Med* 48: 149, 2021.
174. Barr-Beare E, Saxena V, Hilt EE, Thomas-White K, Schober M, Li B, Becknell B, Hains DS, Wolfe AJ and Schwaderer AL: The interaction between enterobacteriaceae and calcium oxalate deposits. *PLoS One* 10: e0139575, 2015.
175. Lalayiannis AD, Soeiro EMD, Moyses RMA and Shroff R: Chronic kidney disease mineral bone disorder in childhood and young adulthood: A 'growing' understanding. *Pediatr Nephrol* 39: 723-739, 2024.
176. McIntyre CW, Harrison LE, Eldehni MT, Jefferies HJ, Szeto CC, John SG, Sigrist MK, Burton JO, Hothi D, Korsheed S, *et al*: Circulating endotoxemia: A novel factor in systemic inflammation and cardiovascular disease in chronic kidney disease. *Clin J Am Soc Nephrol* 6: 133-141, 2011.

177. Liu Y, Jin X, Hong HG, Xiang L, Jiang Q, Ma Y, Chen Z, Cheng L, Jian Z, Wei Z, *et al*: The relationship between gut microbiota and short chain fatty acids in the renal calcium oxalate stones disease. *FASEB J* 34: 11200-11214, 2020.
178. Khan SR: Animal models of kidney stone formation: An analysis. *World J Urol* 15: 236-243, 1997.
179. Chen Z, Yuan P, Sun X, Tang K, Liu H, Han S, Ye T, Liu X, Yang X, Zeng J, *et al*: Pioglitazone decreased renal calcium oxalate crystal formation by suppressing M1 macrophage polarization via the PPAR- $\gamma$ -miR-23 axis. *Am J Physiol Renal Physiol* 317: F137-F151, 2019.
180. Khan SR, Shevock PN and Hackett RL: Urinary enzymes and calcium oxalate urolithiasis. *J Urol* 142: 846-849, 1989.
181. Khan SR: Crystal-induced inflammation of the kidneys: results from human studies, animal models, and tissue-culture studies. *Clin Exp Nephrol* 8: 75-88, 2004.
182. Khan SR and Glenton PA: Calcium oxalate crystal deposition in kidneys of hypercalciuric mice with disrupted type IIa sodium-phosphate cotransporter. *Am J Physiol Renal Physiol* 294: F1109-F1115, 2008.
183. Okamoto N, Aruga S, Tomita K, Takeuchi T and Kitamura T: Chronic acid ingestion promotes renal stone formation in rats treated with vitamin D3. *Int J Urol* 14: 60-66, 2007.
184. Curry JN, Saurette M, Askari M, Pei L, Filla MB, Beggs MR, Rowe PS, Fields T, Sommer AJ, Tanikawa C, *et al*: Claudin-2 deficiency associates with hypercalciuria in mice and human kidney stone disease. *J Clin Invest* 130: 1948-1960, 2020.
185. Letavernier E, Kauffenstein G, Huguet L, Navasolava N, Boudierlique E, Tang E, Delaitre L, Bazin D, de Frutos M, Gay C, *et al*: ABCC6 deficiency promotes development of Randall plaque. *J Am Soc Nephrol* 29: 2337-2347, 2018.
186. Blazquez-Medela AM, Guihard PJ, Yao J, Jumabay M, Lusis AJ, Boström KI and Yao Y: ABCC6 deficiency is associated with activation of BMP signaling in liver and kidney. *FEBS Open Bio* 5: 257-263, 2015.
187. Mackenzie NC, Zhu D, Milne EM, van 't Hof R, Martin A, Darryl Quarles L, Millán JL, Farquharson C and MacRae VE: Altered bone development and an increase in FGF-23 expression in *Enpp1*( $-/-$ ) mice. *PLoS One* 7: e32177, 2012.
188. Rutsch F, Ruf N, Vaingankar S, Toliat MR, Suk A, Höhne W, Schauer G, Lehmann M, Roscioli T, Schnabel D, *et al*: Mutations in *ENPP1* are associated with 'idiopathic' infantile arterial calcification. *Nat Genet* 34: 379-381, 2003.
189. Albright RA, Stabach P, Cao W, Kavanagh D, Mullen I, Braddock AA, Covo MS, Tehan M, Yang G, Cheng Z, *et al*: *ENPP1*-Fc prevents mortality and vascular calcifications in rodent model of generalized arterial calcification of infancy. *Nat Commun* 6: 10006, 2015.
190. Chau H, El-Maadawy S, McKee MD and Tenenhouse HS: Renal calcification in mice homozygous for the disrupted type IIa Na/Pi cotransporter gene *Npt2*. *J Bone Miner Res* 18: 644-657, 2003.
191. Caballero D, Li Y, Ponsetto J, Zhu C and Bergwitz C: Impaired urinary osteopontin excretion in *Npt2a* $^{-/-}$  mice. *Am J Physiol Renal Physiol* 312: F77-F83, 2017.
192. Li Y, Caballero D, Ponsetto J, Chen A, Zhu C, Guo J, Demay M, Jüppner H and Bergwitz C: Response of *Npt2a* knockout mice to dietary calcium and phosphorus. *PLoS One* 12: e0176232, 2017.
193. Evan AP, Weinman EJ, Wu XR, Lingeman JE, Worcester EM and Coe FL: Comparison of the pathology of interstitial plaque in human ICSF stone patients to NHERF-1 and THP-null mice. *Urol Res* 38: 439-452, 2010.
194. Serafini-Cessi F, Malagolini N and Cavallone D: Tamm-Horsfall glycoprotein: Biology and clinical relevance. *Am J Kidney Dis* 42: 658-676, 2003.
195. Jian Z, Yuan C, Xiong Z, Li H, Jin X and Wang K: Kidney function may partially mediated the protective effect of urinary uromodulin on kidney stone. *Urolithiasis* 51: 65, 2023.
196. Hamamoto S, Nomura S, Yasui T, Okada A, Hirose M, Shimizu H, Itoh Y, Tozawa K and Kohri K: Effects of impaired functional domains of osteopontin on renal crystal formation: Analyses of OPN transgenic and OPN knockout mice. *J Bone Miner Res* 25: 2712-2723, 2010.
197. Jiang Z, Asplin JR, Evan AP, Rajendran VM, Velazquez H, Nottoli TP, Binder HJ and Aronson PS: Calcium oxalate urolithiasis in mice lacking anion transporter *Slc26a6*. *Nat Genet* 38: 474-478, 2006.
198. Dawson PA, Russell CS, Lee S, McLeay SC, van Dongen JM, Cowley DM, Clarke LA and Markovich D: Urolithiasis and hepatotoxicity are linked to the anion transporter *Satl* in mice. *J Clin Invest* 120: 706-712, 2010.
199. Chidambaram A, Rodriguez D, Khan S and Gower L: Biomimetic Randall's plaque as an in vitro model system for studying the role of acidic biopolymers in idiopathic stone formation. *Urolithiasis* 43 (Suppl 1): S77-S92, 2015.
200. Keren L, Bosse M, Thompson S, Risom T, Vijayaragavan K, McCaffrey E, Marquez D, Angoshtari R, Greenwald NF, Fienberg H, *et al*: MIBI-TOF: A multiplexed imaging platform relates cellular phenotypes and tissue structure. *Sci Adv* 5: eaax5851, 2019.
201. Goltsev Y, Samusik N, Kennedy-Darling J, Bhate S, Hale M, Vazquez G, Black S and Nolan GP: Deep profiling of mouse splenic architecture with CODEX multiplexed imaging. *Cell* 174: 968-981.e15, 2018.
202. Lee CN, Fu H, Cardilla A, Zhou W and Deng Y: Spatial joint profiling of DNA methylome and transcriptome in tissues. *Nature* 646: 1261-1271, 2025.
203. Xiao Y, Bai Z, Zou Z, Ye C, Tao B, Zheng Z, Chen YM, Zou Z, Jiang L, Zhao L, *et al*: Spatially resolved m(6)A profiling using m(6)A-ARTR-DBiT. *Nat Methods* 23: 1318-1326, 2026.
204. Klionsky DJ, Abdel-Aziz AK, Abdelfatah S, Abdellatif M, Abdoli A, Abel S, Abeliovich H, Abildgaard MH, Abudu YP, Acevedo-Arozena A, *et al*: Guidelines for the use and interpretation of assays for monitoring autophagy (4th edition)(1). *Autophagy* 17: 1-382, 2021.



Copyright © 2026 Wang et al. This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0) License.