



# Subclinical papillary tip calcifications in stone-naïve kidneys: micro-ct quantification, osteopontin expression, and associations with urinary and clinical risk factors

Ezel Aydoğ<sup>1,5</sup> · Havva Berber<sup>2</sup> · Mert Ocak<sup>3</sup> · Duygu Enneli<sup>2</sup> · Kaan Orhan<sup>4</sup> · Mehmet İlker Gökce<sup>1</sup>

Received: 31 August 2025 / Accepted: 3 December 2025

© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2025

## Abstract

Subepithelial papillary calcifications, encompassing both Randall's plaques (interstitial) and Randall's plugs (intraluminal), are established niduses for stone formation, yet their distribution and determinants in stone-naïve individuals remain poorly characterized. We hypothesized that subclinical papillary calcifications are present in non-stone-formers and associated with urinary and clinical risk factors similar to those in stone formers. In this retrospective cross-sectional study, we analyzed 50 patients undergoing nephrectomy for renal or upper urinary tract cancer (June 2019-January 2023). Patients with prior stone disease, calcium/vitamin supplementation, or metabolic disorders were excluded. High-resolution micro-computed tomography (micro-CT, 20 µm voxels) quantified mineral-to-parenchyma (M/P) ratios across papillary zones, and osteopontin (OPN) immunohistochemistry was performed on matched sections. Given the 20 µm spatial resolution limit of micro-CT, any papillary tip calcified lesion (PCL)  $\geq 1 \text{ mm}^3$  was considered clinically relevant; such lesions were present in 46% of kidneys. PCL+ patients were older, more frequently hypertensive, and exhibited lower urine volume (median 1200 vs. 1600 mL,  $p < 0.001$ ), lower urinary citrate ( $p = 0.002$ ), higher urine specific gravity ( $p = 0.001$ ), and elevated M/P ratios in the middle medullary zone ( $p = 0.003$ ). OPN staining was increased in loops of Henle in PCL+ cases and correlated with mineral burden. Multivariable analysis identified lower urine volume, higher urinary calcium, and hypertension as independent predictors of PCL (area under curve = 0.93). These findings indicate that PCLs are common in stone-naïve kidneys and are associated with specific urinary and systemic risk factors. Early interventions targeting urine chemistry and vascular health may influence mineral deposition and delay kidney stone development.

**Keywords** Randall's plaque · Kidney stones · Osteopontin · Micro-computed tomography · 24-hour urine

✉ Ezel Aydoğ  
ezelaydog@gmail.com

Havva Berber  
havva.berbermd@gmail.com

Mert Ocak  
mocak@ankara.edu.tr

Duygu Enneli  
dkankaya@ankara.edu.tr

Kaan Orhan  
knorhan@dentistry.ankara.edu.tr

Mehmet İlker Gökce  
migokce@ankara.edu.tr

<sup>1</sup> Department of Urology, Faculty of Medicine, Ankara University, Ankara, Turkey

<sup>2</sup> Department of Pathology, Faculty of Medicine, Ankara University, Ankara, Turkey

<sup>3</sup> Department of Anatomy, Faculty of Dentistry, Ankara University, Ankara, Turkey

<sup>4</sup> Department of Radiology, Faculty of Dentistry, Ankara University, Ankara, Turkey

<sup>5</sup> Faculty of Medicine, Ankara University, Hacettepe Mahallesi A. Adnan Saygun Caddesi No:35 Altındağ, Ankara 06230, Turkey

## Introduction

Kidney stones often originate from microscopic calcium phosphate deposits at the papillary tip that serve as attachment points for stone growth. These deposits include both subepithelial Randall's plaques (interstitial calcium phosphate beneath the urothelium) and intraluminal Randall's plugs (apatite within terminal collecting ducts). Although originally grouped together under Randall's early descriptions [1], contemporary research distinguishes these two based on location and pathogenesis [2]. In this study, we adopted a unified operational definition: any single papillary tip calcified lesion  $\geq 1 \text{ mm}^3$  in volume was considered a clinically relevant calcification. This definition includes both Randall's plaques and tubular plugs and will be referred as papillary tip calcified lesions (PCL) throughout this manuscript. While recognizing that our imaging and staining methods (20  $\mu\text{m}$  voxel micro-CT and H&E staining) do not permit definitive discrimination between Randall's plaques and plugs, this threshold-based definition standardizes lesion classification, and is consistent with volume estimates of individual plaques and plugs reported in the literature [3].

Endoscopic studies demonstrate that PCLs are present on most papillae in calcium stone formers, occurring in nearly 88–100% of idiopathic calcium oxalate (CaOx) patients, but are observed in only 27–43% of stone-naïve individuals [4]. This suggests that clinically silent mineralization may precede overt nephrolithiasis. However, the prevalence, distribution, and size of such calcifications in stone-naïve kidneys remain poorly defined, leaving a gap in our understanding of early stone pathogenesis in patients with no history of nephrolithiasis.

Metabolic factors, particularly hypercalciuria, low urine volume, and reduced urinary inhibitors such as citrate, have been implicated in PCL formation [5–7]. Systemic conditions, including hypertension, may also contribute through vascular or inflammatory pathways [8–10]. Yet, the relationship between urinary chemistry, clinical comorbidities, and PCL has not been systematically examined in stone-naïve patients.

High-resolution micro-computed tomography (micro-CT) provides a non-destructive three-dimensional view of mineral deposits in renal tissue, offering insights into its location and volume. Yet, few studies have used this modality in stone-naïve patients, and little is known about the relationship between PCL and individual urinary or clinical risk profiles in this group.

In this study, we used high-resolution micro-CT and histopathology to quantify PCL in stone-naïve nephrectomy specimens and to explore their associations with 24-hour urine composition and systemic factors.

## Methods

### Study design and patients

Data were prospectively collected and retrospectively analyzed from 50 consecutive patients who provided written informed consent. Institutional review board approval was obtained (approval no. i6-330-20). All patients underwent surgery for kidney or upper urinary tract cancer between June 2019 and January 2023.

Exclusion criteria included: history of other primary cancers ( $n=2$ ), supplementation with vitamin C/D/calcium ( $n=28$ ), prior stone disease ( $n=9$ ), hyperparathyroidism, hyperthyroidism, sarcoidosis, elevated vitamin D, bone disorders, distal renal tubular acidosis, medullary sponge kidney, primary or enteric hyperoxaluria, intestinal disorders, chronic pancreatitis, chronic diarrhea, lithogenic medications, recurrent urinary tract infections, gout, or cystinuria.

### Clinical and laboratory data

Demographic and clinical parameters, including sex, age, body mass index (BMI), diabetes, hypertension, atherosclerotic heart disease, and hyperlipidemia were recorded at enrollment. Preoperative blood samples, obtained  $\leq 7$  days before surgery, were analyzed for serum creatinine, potassium, and calcium.

### 24-Hour urine collection

All patients completed supervised 24-hour urine collections. Total volume, pH, and density were measured. Two 10 mL aliquots were preserved: one with 10 mg boric acid, the other with 4 mL 6 N HCl to prevent oxalate precipitation. Samples were refrigerated and analyzed for calcium, oxalate, citrate, uric acid and magnesium within 72 h.

### Micro-CT imaging and calcification analysis

Nephrectomy specimens were coronally sectioned and fixed in 10% buffered formalin. A 5–6 mm slab of non-tumor parenchyma was sampled, paraffin-embedded, and scanned using Skyscan 1275 micro-CT (Bruker, Belgium) at 20  $\mu\text{m}$  voxel size, 25 kV, 193  $\mu\text{A}$ . Images were reconstructed with NRecon v1.6.4.8. Using DataViewer v1.5.6.2, renal parenchyma was separated from paraffin; CTan v1.14.4.1 performed threshold-based segmentation to classify voxels as mineral vs. parenchyma.

The renal medulla was divided into three anatomical zones based on Hsi et al.: the basal zone (outer stripe of the outer medulla), the middle zone (inner stripe surrounding the loops of Henle), and the apex zone (papillary tip

**Fig. 1** Representative examples of OPN-IHC staining in proximal tubules. (a) Low, (b) medium, and (c) high OPN staining grades. Images were obtained from whole-slide scans at  $\times 40$  magnification. Scale bar = 50  $\mu\text{m}$  (all panels)

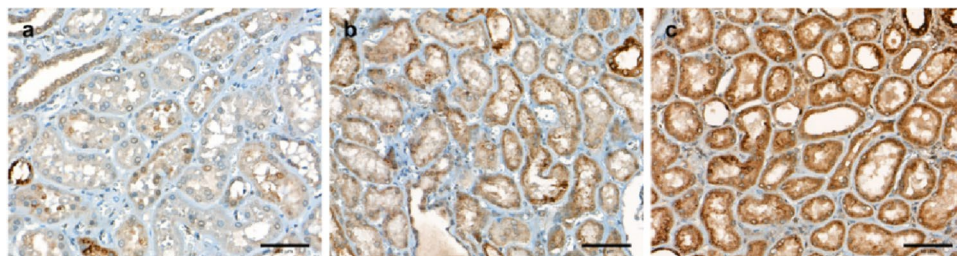
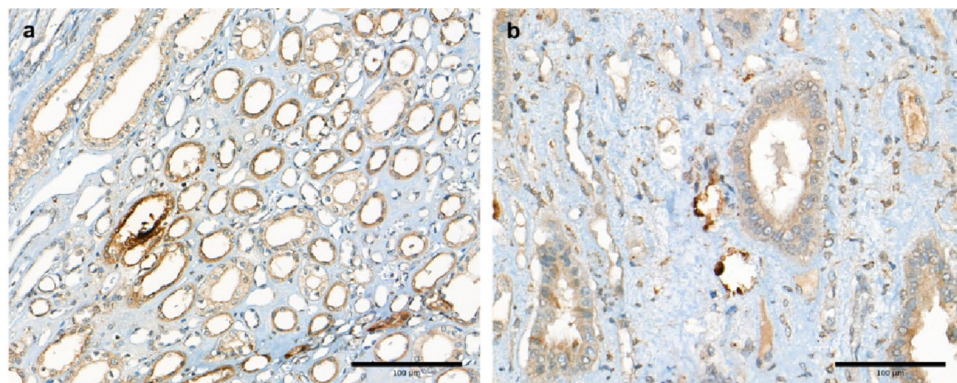


**Fig. 2** Representative examples of OPN-IHC staining in the loops of Henle. Panels illustrate cases showing interstitial and luminal extension of staining beyond tubular cells. Images were acquired from whole-slide scans at  $\times 30$  magnification. Scale bar = 100  $\mu\text{m}$  (all panels)



where Randall's plaques are localized) [11]. Mineral-to-parenchyma (M/P) ratios were calculated for each zone to quantify relative mineral burden. These measurements were analyzed in univariate exploratory analyses only and were not included as predictors in multivariable regression models.

Given the limitations of 20  $\mu\text{m}$  resolution (definitive differentiation would require voxel sizes  $< 5 \mu\text{m}$ ), we refer to any calcified structures detected in the apex zone as "papillary tip calcifications" (PTC's) without inferring specific anatomical origin (i.e., interstitial vs. intraluminal).

### Pathology examination

Two 4  $\mu\text{m}$  sections per block were stained with hematoxylin and eosin (H&E) and osteopontin (OPN) immunohistochemistry (IHC) (clone 7C5H12, Invitrogen) using Ventana Benchmark Ultra/XT systems. Whole-slide images were generated on 3D HISTECH P250 Flash 3 scanner (v3.0.2). Two blinded pathologists reviewed slides using Slide Viewer v2.5.

It should be noted that Yasue or von Kossa staining are considered the gold standard for definitive mineral identification and localization. In our study, standard H&E sections were evaluated for features consistent with mineral deposition using established histologic criteria [10, 12]. However, due to the limitations of H&E staining, distinguishing interstitial plaque from tubular plug was not reliably possible without mentioned mineral-specific stains.

### Quantitative OPN scoring

Two circular 4.9  $\text{mm}^2$  regions of interest were analyzed in the cortex and medulla per slide. OPN-positive tubules were matched with H&E findings and classified by nephron segment: proximal/distal tubules, loops of Henle, and collecting ducts. Proximal tubule staining intensity was graded 0 to +3, whereas collecting duct staining was classified as low or high. Distal tubule and loop of henle staining showed consistent severity; thus, a uniform classification was applied. Cases were further categorized based on interstitial or luminal extension beyond tubular cells. For each specimen, the number of tubules showing this extension was counted per 4.9  $\text{mm}^2$  area and classified as none (0), low ( $< 10$ ), or high ( $> 10$ ). Representative images illustrating staining grades and scoring criteria were compiled into Figs. 1, 2 and 3, and Fig. 4. OPN expression data were used for exploratory univariate analyses only and excluded from multivariable models.

### Statistical analysis

Analyses were conducted in R (v4.2). The primary endpoint was the presence of Randall's plaque. Normality of continuous variables was assessed using the Shapiro–Wilk test. Normally distributed data are presented as mean  $\pm$  standard deviation (SD) and compared using Student's t-test; non-normal data as median [interquartile range (IQR)] and compared using Mann–Whitney U. Categorical variables are reported as counts (%) and compared with chi-square or Fisher's exact tests.

**Fig. 3** Representative examples of OPN-IHC staining in the distal tubules. Panels demonstrate cases with interstitial and luminal extension of staining. Whole-slide images were scanned at  $\times 20$  magnification. Scale bar = 200  $\mu\text{m}$  (all panels)

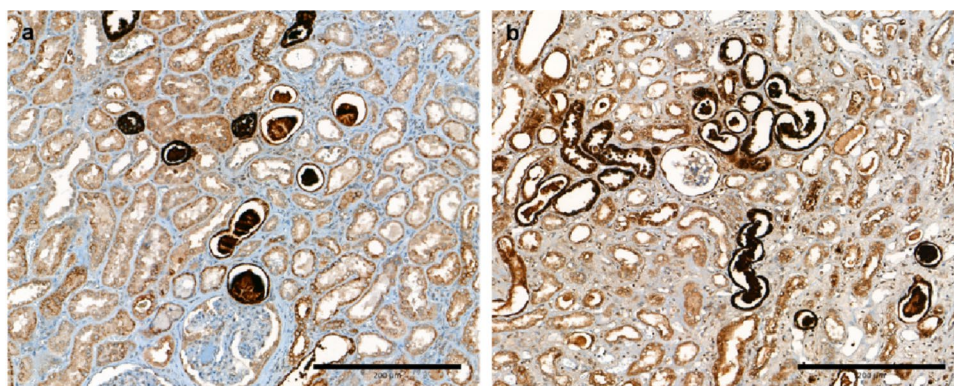
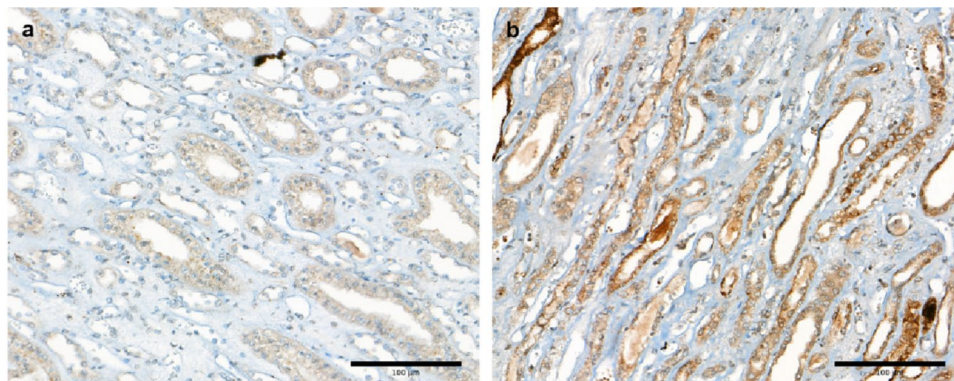


**Fig. 4** Representative examples of OPN-IHC staining in the collecting ducts. Panels show (a) low and (b) high OPN staining patterns. Images were obtained from whole-slide scans at  $\times 30$  magnification. Scale bar = 100  $\mu\text{m}$  (all panels)



Multicollinearity was checked using Pearson's correlation. Firth's penalized logistic regression was used to assess independent associations of 24-h urinary calcium, citrate, volume, pH, and hypertension with PCL positivity [13]. All predictors were included in the multivariable model regardless of univariate significance, as they were selected a priori based on established links to plaque formation [5–8, 14]. Odds ratios (OR) with 95% confidence intervals (CI) are reported. Model discrimination was evaluated via the area under the receiver operating characteristics (ROC) curve (AUC). Internal validation was performed by repeating the analysis using standard logistic regression. All tests were two-sided, with  $p < 0.05$  considered significant.

## Results

### Baseline demographics and urinary profiles

Among patients with and without PCL, age was significantly higher in the PCL+ group ( $65.2 \pm 10.3$  vs.  $58.1 \pm 12.0$  years,  $p = 0.028$ ). Hypertension was also more frequent among PCL+ patients (54.2% vs. 23.1%,  $p = 0.040$ ). Regarding urinary parameters, PCL+ cases exhibited lower urinary pH (median 6.30 vs. 6.90,  $p = 0.005$ ), higher urine specific gravity ( $1024.5 \pm 6.7$  vs.  $1018.0 \pm 7.1$ ,  $p = 0.001$ ), lower 24-hour urine volume (median 1200 mL vs. 1600 mL,  $p < 0.001$ ),

**Table 1** Baseline demographic and blood parameters of patients with and without papillary tip calcified lesions (PCLs)

| Variable                             | PCL-                | PCL+                | <i>p</i> -value |
|--------------------------------------|---------------------|---------------------|-----------------|
| Sex (Male=0, Female=1)               | 8 (30.8%)           | 10 (41.7%)          | 0.557           |
| Age (years)                          | $58.08 \pm 11.98$   | $65.21 \pm 10.29$   | 0.028           |
| Body Mass Index (kg/m <sup>2</sup> ) | $26.35 \pm 5.21$    | $27.40 \pm 3.98$    | 0.426           |
| Hypertension                         | 6 (23.1%)           | 13 (54.2%)          | 0.040           |
| Atherosclerotic Heart Disease        | 5 (19.2%)           | 8 (33.3%)           | 0.339           |
| Diabetes Mellitus                    | 4 (15.4%)           | 7 (29.2%)           | 0.314           |
| Hyperlipidemia                       | 4 (15.4%)           | 5 (20.8%)           | 0.721           |
| Serum Creatinine (mg/dL)             | 0.96<br>(0.91–1.08) | 1.06<br>(0.90–1.17) | 0.260           |
| Serum Potassium (mmol/L)             | $4.46 \pm 0.44$     | $4.60 \pm 0.30$     | 0.197           |
| Serum Calcium (mg/dL)                | $9.42 \pm 0.59$     | $9.50 \pm 0.43$     | 0.597           |

Values are presented as mean  $\pm$  SD for normally distributed variables, median [IQR] for skewed variables, and n (%) for categorical variables. P-values indicate comparisons between groups

and reduced urinary citrate levels ( $334.8 \pm 92.4$  mg vs.  $424.4 \pm 105.0$  mg,  $p = 0.002$ ).

No significant differences were observed for sex, BMI, diabetes, atherosclerotic heart disease, hyperlipidemia, serum creatinine, potassium, calcium, or 24-hour urinary calcium, oxalate, uric acid, and magnesium (all  $p > 0.05$ ). Detailed comparisons are summarized in Tables 1 and 2.

**Table 2** Twenty-four-hour urinary biochemical parameters in patients with and without papillary tip calcified lesions (PCLs)

| Variable                           | PCL-                      | PCL+                      | <i>p</i> -value |
|------------------------------------|---------------------------|---------------------------|-----------------|
| 24-hour Urine pH                   | 6.90 (6.70–7.20)          | 6.30 (5.77–6.73)          | 0.005           |
| 24-hour Urine Density              | 1017.96±7.07              | 1024.50±6.65              | 0.001           |
| 24-hour Urine Volume (mL)          | 1600.00 (1362.50–2012.50) | 1200.00 (1050.00–1362.50) | <0.001          |
| 24-hour Urinary Calcium (mg/day)   | 200.50 (107.25–275.25)    | 275.00 (162.75–302.75)    | 0.076           |
| 24-hour Urinary Oxalate (mg/day)   | 33.87±12.29               | 35.65±12.31               | 0.613           |
| 24-hour Urinary Citrate (mg/day)   | 424.35±105.00             | 334.79±92.37              | 0.002           |
| 24-hour Urinary Uric Acid (mg/day) | 564.04±112.47             | 537.58±140.64             | 0.469           |
| 24-hour Urinary Magnesium (mg/day) | 104.42±18.21              | 94.25±19.31               | 0.062           |

Values are presented as mean±SD for normally distributed variables and median [IQR] for skewed variables. P-values indicate comparisons between groups

**Table 3** Micro-CT derived M/P ratios across papillary zones in patients with and without papillary tip calcified lesions (PCLs)

| Variable               | PCL-                | PCL+                | <i>p</i> -value |
|------------------------|---------------------|---------------------|-----------------|
| M/P ratio: Basal Zone  | 6.95±2.98           | 8.45±3.33           | 0.101           |
| M/P ratio: Middle Zone | 16.60 (13.69–21.16) | 22.61 (19.11–23.66) | 0.003           |
| M/P ratio: Apex Zone   | 37.69 (29.82–46.61) | 73.21 (49.26–98.33) | <0.001          |
| M/P ratio: Total       | 17.13 (14.19–23.42) | 31.54 (19.18–36.40) | <0.001          |

Definitions of micro-CT M/P ratios and papillary zones are provided in the Methods section. Values are presented as mean±SD for normally distributed variables and median [IQR] for skewed variables. P-values indicate comparisons between groups

## Micro-CT and OPN-IHC findings

Micro-CT analysis revealed zone-specific differences in M/P ratios. In the middle zone, PCL+ cases showed significantly higher M/P ratios than PCL- cases (median 22.6, IQR 19.1–23.7 vs. 16.6, IQR 13.7–21.2;  $p=0.003$ ). In the basal zone, M/P ratios were slightly higher PCL+ cases ( $8.5\pm3.3$  vs.  $7.0\pm3.0$ ) but did not reach significance ( $p=0.101$ ). The apex zone showed the largest numerical difference; however, as this region includes the PCL itself, it lacks independent discriminative value and was excluded from comparative analyses. Distributions of M/P ratios across zones are presented in Table 3; Fig. 5. Representative micro-CT reconstructions appear in Fig. 6.

Pathological analysis was unavailable in two patients due to tissue-processing issues, excluding them from histological and OPN-IHC assessments. OPN-IHC staining in proximal tubules was evenly distributed between groups ( $p=0.622$ ). Among PCL- cases, 44.0% showed no staining, 40.0% low, 12.0% medium, and 4.0% high, while corresponding values for PCL+ cases were 60.9%, 21.7%, 13.0%, and 4.3%, respectively.

Loop of Henle OPN-IHC staining differed significantly ( $p=0.021$ ). In PCL- specimens, 36.0% showed no staining, 56.0% low, and 8.0% high, whereas PCL+ cases showed 9.1% no staining, 54.5% low, and 36.4% high.

Distal tubule OPN-IHC staining showed similar distributions between groups ( $p=0.706$ ). Among PCL- cases, 40.0% showed no staining, 28.0% low, and 32.0% high; PCL+ cases showed 34.8%, 21.7%, and 43.5%, respectively.

Collecting duct OPN-IHC staining also did not differ significantly ( $p=0.259$ ); low staining was observed in 52.0%

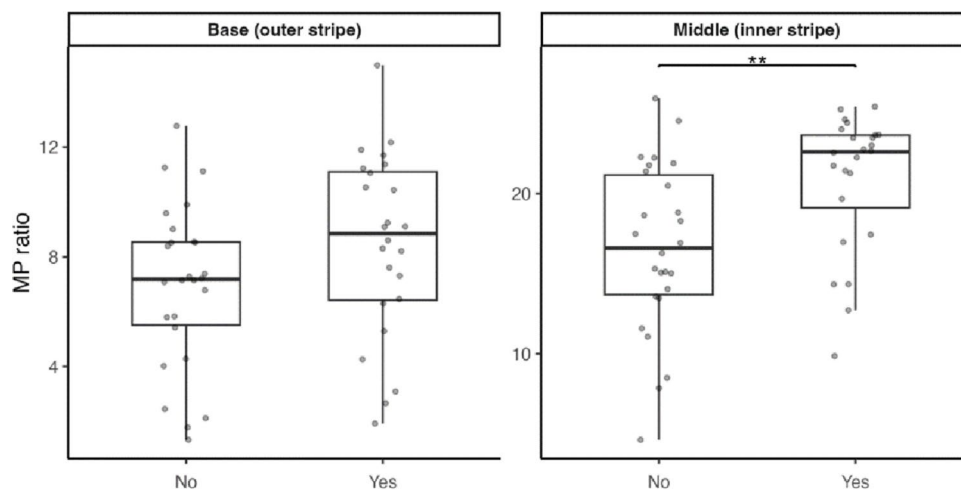


**Fig. 5** M/P volume ratios in basal and middle medullary zones, stratified by the presence of Papillary Tip Calcified Lesions (PCLs). In the basal zone (left), M/P ratios were slightly higher in PCL+ cases but did not reach statistical significance. In the middle zone (right), PCL+ cases showed significantly higher M/P ratios compared with

PCL- cases. Boxes represent the IQR, horizontal lines indicate medians, whiskers extend to  $1.5\times$  IQR, and dots represent outliers. P-values were calculated using the Wilcoxon rank-sum test; \*\* $p<0.01$ , blank=not significant. Boxplots were generated using R software (version 4.5.1; R Foundation for Statistical Computing, Vienna, Austria)

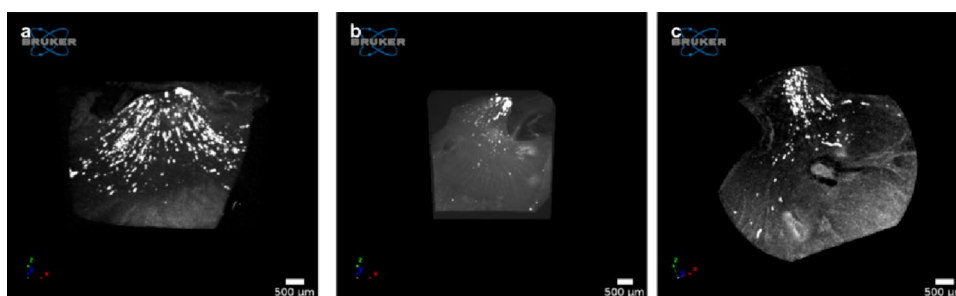


**Fig. 6** Representative micro-CT reconstructions of renal pyramids from patients with positive papillary tip calcified lesions (PCLs), illustrating varying degrees of proximal mineral accumulation. (a) A renal pyramid demonstrating a PCL at the papillary tip with marked proximal mineral deposition extending toward the inner medulla. (b)

A renal pyramid with a PCL but minimal proximal calcification, illustrating the heterogeneity of mineral distribution among PCL+ kidneys. (c) A renal pyramid showing a PCL with a moderate level of proximal mineral accumulation. Scale bar = 500 µm (all panels)

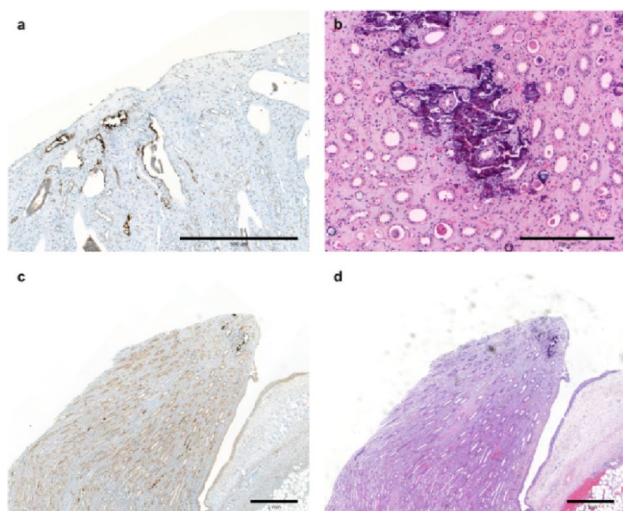


**Fig. 7** Representative histologic images demonstrating OPN expression and interstitial calcification in kidneys with positive Papillary Tip Calcified Lesions (PCLs). (a) OPN-stained papilla from a patient with PCL (10× magnification) highlighting strong OPN expression in loops of Henle surrounding the mineral area. Scale bar = 500 µm (b) Corresponding H&E-stained section from the same papilla (20× magnification) demonstrating extensive interstitial calcification. Scale bar = 200 µm (c) Another OPN-stained papilla (2× magnification) highlighting strong OPN expression in tubules adjacent to the plaque site. Scale bar = 1 mm (d) Corresponding H&E-stained Sect. (2× magnification) from the same region demonstrating interstitial calcification. Scale bar = 1 mm

of PCL- and 34.8% of PCL+ cases, whereas high staining occurred in 48.0% and 65.2%, respectively.

OPN-IHC demonstrated that OPN localizes to mineralized areas, coating apatite crystal surfaces, and is produced by loops of Henle and collecting duct cells adjacent to regions of high calcification (Fig. 7). A detailed summary of OPN-IHC staining distributions across nephron segments is provided in Table 4.

These findings suggest that increased mineral deposition relative to parenchyma begins in the middle medullary

**Table 4** OPN immunohistochemical staining grades across nephron segments in patients with and without papillary tip calcified lesions (PCLs)

| Variable                              | PCL-       | PCL+       | <i>p</i> -value |
|---------------------------------------|------------|------------|-----------------|
| <b>OPN Staining – Proximal Tubule</b> |            |            | 0.622           |
| None                                  | 11 (44.0%) | 14 (60.9%) |                 |
| Low                                   | 10 (40.0%) | 5 (21.7%)  |                 |
| Medium                                | 3 (12.0%)  | 3 (13.0%)  |                 |
| High                                  | 1 (4.0%)   | 1 (4.3%)   |                 |
| <b>OPN Staining – Loop of Henle</b>   |            |            | 0.021           |
| None                                  | 9 (36.0%)  | 2 (9.1%)   |                 |
| Low                                   | 14 (56.0%) | 12 (54.5%) |                 |
| High                                  | 2 (8.0%)   | 8 (36.4%)  |                 |
| <b>OPN Staining – Distal Tubule</b>   |            |            | 0.706           |
| None                                  | 10 (40.0%) | 8 (34.8%)  |                 |
| Low                                   | 7 (28.0%)  | 5 (21.7%)  |                 |
| High                                  | 8 (32.0%)  | 10 (43.5%) |                 |
| <b>OPN Staining – Collecting Duct</b> |            |            | 0.259           |
| Low                                   | 13 (52.0%) | 8 (34.8%)  |                 |
| High                                  | 12 (48.0%) | 15 (65.2%) |                 |

OPN expression scoring criteria are described in the Methods section. Values are presented as n (%). P-values indicate comparisons between groups

region in PCL+ patients, while basal zone changes remain less pronounced.

### Correlations between pathology and micro-CT findings

Spearman correlation analysis was performed to evaluate associations between micro-CT derived M/P ratios and OPN staining across nephron segments. No significant correlations were found between M/P ratios and staining in the proximal tubule, distal tubule, or collecting duct (all adjusted  $p > 0.2$ ).

In contrast, loop of Henle staining showed consistent positive correlations with M/P parameters. Strong associations

were observed with M/P Apex ( $\rho=0.573$ , adjusted  $p<0.001$ ) and M/P Total ( $\rho=0.577$ , adjusted  $p<0.001$ ). A moderate correlation was detected with M/P Middle ( $\rho=0.397$ , adjusted  $p=0.031$ ), while M/P Basal showed a weaker, non-significant trend ( $\rho=0.315$ , adjusted  $p=0.124$ ). It should be noted that apex and total M/P values inherently reflect the burden of PCL itself. This likely explains the particularly strong correlations observed between loop of henle staining and both apex and total M/P ratios.

### Multivariate analysis

Multicollinearity among the selected predictors (24-h urinary volume, urinary citrate, urinary pH, urinary calcium, and hypertension) was assessed using pairwise correlations and variance inflation factor (VIF) analysis. No strong correlations were observed (all coefficients  $<0.7$ ), and VIF values were  $<3$  for all predictors, indicating no problematic collinearity.

Firth's multivariable logistic regression included age, 24-hour urine volume, urinary citrate, urinary pH, urinary calcium, and hypertension. Lower urine volume, higher urinary calcium excretion, and hypertension were independently associated with existence of PCL. Each 100-mL increase in urine volume reduced the odds of PCL positivity by 21% (OR 0.79, 95% CI 0.58–0.98,  $p=0.028$ ). Urinary calcium excretion was positively associated (OR 1.11 per 10 mg, 95% CI 1.00–1.30,  $p=0.048$ ), while hypertension markedly increased risk (OR 5.78, 95% CI 1.12–46.57,  $p=0.035$ ). Age showed a nonsignificant trend toward increased risk (OR 2.12 per 10 years, 95% CI 0.91–8.56,  $p=0.094$ ). Urinary citrate and pH were not significantly associated with existence of PCL.

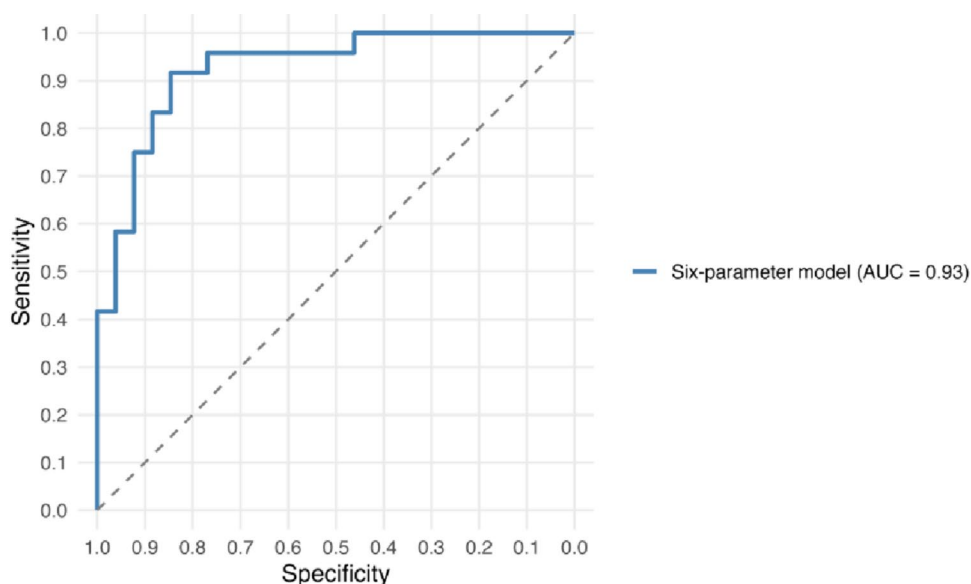
**Table 5** Multivariable logistic regression analyses for predictors of papillary tip calcified lesions (PCLs)

| Predictor                      | Firth logistic regression OR (95% CI) | <i>p</i> -value | Standard logistic regression OR (95% CI) | <i>p</i> -value |
|--------------------------------|---------------------------------------|-----------------|------------------------------------------|-----------------|
| 24-h urine volume (per 100 mL) | 0.79<br>[0.58–0.98]                   | 0.028           | 0.71<br>[0.51–0.98]                      | 0.035           |
| Urinary citrate (per 10 mg)    | 0.97<br>[0.87–1.06]                   | 0.468           | 0.96<br>[0.86–1.07]                      | 0.436           |
| Urinary pH (per 1 unit)        | 0.24<br>[0.02–1.41]                   | 0.116           | 0.13<br>[0.01–1.33]                      | 0.086           |
| Urinary calcium (per 10 mg)    | 1.11<br>[1.00–1.30]                   | 0.048           | 1.17<br>[1.00–1.36]                      | 0.048           |
| Hypertension (Yes vs. No)      | 5.78<br>[1.12–46.57]                  | 0.035           | 11.13<br>[1.22–101.95]                   | 0.033           |
| Age (per 10 years)             | 2.12<br>[0.91–8.56]                   | 0.094           | 3.22<br>[0.83–12.43]                     | 0.089           |
| Model AUC                      | 0.926                                 | –               | 0.931                                    | –               |

OR are reported per scaled units: 24-h urine volume per 100 mL, urinary calcium and citrate per 10 mg, age per 10 years, urinary pH per 1 unit, and hypertension as Yes vs. No. Outcome variable: presence of PCLs (Yes vs. No)

The model demonstrated strong discriminative ability, with an AUC of 0.926 for the Firth model and 0.931 for standard logistic regression, indicating excellent predictive performance. Effect sizes and *p*-values were consistent across both approaches, confirming the robustness of the findings. Full regression results are provided in Table 5, and the ROC curve is shown in Fig. 8.

**Fig. 8** ROC curve for the six-parameter multivariable model predicting existence of Papillary Tip Calcified Lesions (PCLs). The model demonstrated excellent discrimination with an AUC of 0.93



## Discussion

Our study extends prior observations by showing that even in patients without a history of nephrolithiasis, PCLs consistent with Randall's plaques can already be detected and are associated with established lithogenic factors. In our cohort, individuals with PCL were older and more likely to be hypertensive than those without. This parallels Stoller's cadaveric series, where hypertension was the only clinical correlate of papillary calcification, and is consistent with broader epidemiologic data linking stone disease with cardiovascular and vascular calcification risk [15]. For instance, Vermooten described extensive perivascular calcium deposition in the papillae that could "readily ulcerate through to form one of Randall's plaques", and noted a steep increase in plaque incidence with advancing age. Taken together, these findings suggest that vascular aging and hypertension may predispose to plaque formation even before clinical stone disease develops.

Urinary parameters also reflected a lithogenic profile. In univariate analysis, PCL + patients exhibited higher specific gravity, lower urine pH, reduced citrate, and lower 24-hour volume. This constellation is consistent with a supersaturation-driven environment. Prior studies have highlighted the same factors: Kuo et al. showed that hypercalciuria and reduced urine volume were the dominant predictors of plaque area [5], and other work in CaOx stone formers has confirmed associations with low urine output, low pH, and elevated urinary calcium [16]. Although Linnes et al. reported a paradoxical association between higher urinary citrate and plaque in stone formers [6], our stone-naïve cohort showed lower citrate in PCL + cases, possibly reflecting that citrate's protective role is overridden by concentrated urine and excess calcium at the earliest stages of plaque formation. Importantly, in multivariable analysis, each 100 mL reduction in urine volume and each 10 mg increase in calcium excretion independently predicted PCL positivity, underscoring the central importance of urine volume and calcium as drivers of mineral deposition.

High-resolution micro-CT enabled three-dimensional mapping of renal calcifications and demonstrated in PCL + patients that, mineral deposition is concentrated in the inner medulla and papillary apex, with the outer medulla and cortex largely unaffected. This distribution pattern suggests that calcifications predominantly follow the trajectory of the loops of Henle, rather than arising from cortical or outer medullary structures, supporting proposed mechanisms of Randall's plaque formation [12, 15, 17]. The elevated mineral density at the papillary apex primarily reflects the presence of the PCL itself. These findings are consistent with the model in which Randall's plaque pathogenesis begins with mineral deposition in the basement membranes of the loops

of Henle in the inner medulla and gradually extends toward the papillary tip, contrasting with alternative theories suggesting a different origin [11].

We observed strong OPN staining in the loops of Henle of PCL + kidneys, whereas staining in proximal, distal, and collecting segments was minimal. This distribution is consistent with OPN's known localization in healthy kidneys, where it is typically abundant within the loops of Henle and distal nephron segments [18]. Importantly, Evan et al. demonstrated that OPN localizes directly to Randall's plaques, coating apatite crystal surfaces and being produced by loops of Henle and collecting duct cells adjacent to plaque sites [16]. Our findings are consistent with this concept and are illustrated in Fig. 4.

Among all nephron segments examined, only the loops of Henle demonstrated significantly higher OPN staining in PCL + kidneys, based on our semiquantitative classification. This finding supports the hypothesis that plaques originate within the basement membranes of these segments [12, 15, 17]. Unified models of plaque pathogenesis propose that epithelial cells in these regions adopt an osteoblast-like phenotype, upregulating OPN and other bone-associated proteins to initiate apatite nucleation [2]. However, OPN is also recognized as a potent inhibitor of calcium crystal growth in vivo [19], suggesting that its expression may reflect a compensatory response to nascent mineral deposition rather than a purely pro-calcific process. Consistent with this, PCL + kidneys exhibited significantly higher M/P ratios in the middle medulla, and OPN staining in the loops of Henle correlated modestly with micro-CT-derived mineral burden in this region, while basal-zone associations were weaker and nonsignificant. Collectively, these findings suggest that increased OPN expression in the loops of Henle is linked to PCL formation and partially reflects overall mineral load. However, our results cannot determine whether OPN promotes plaque initiation, is upregulated in response to lesion expansion as a regulatory mechanism to limit growth, or reflects a combination of both processes.

Multivariable logistic regression demonstrated that low urine volume, elevated urinary calcium excretion, and hypertension were independent predictors of PCL positivity. These findings are consistent with established risk factors in stone-forming populations and corroborate prior work showing urine volume and calcium as independent predictors of plaque burden [5]. Khan and Canales proposed that hypercalciuria, reduced urinary citrate, and other lithogenic conditions induce renal epithelial cells to adopt an osteogenic phenotype, releasing matrix vesicles and bone-associated proteins such as OPN that facilitate plaque nucleation [2]. The strong association with hypertension ( $\approx$  five-fold higher odds) further supports the hypothesis that systemic vascular factors contribute to papillary calcification [15].

Although age showed a positive trend, it did not reach statistical significance after adjustment, likely reflecting its indirect contribution via cumulative exposure to these risk factors. Neither urine pH nor citrate remained significant in multivariable analysis, suggesting their effects are mediated primarily by concentrated urine and hypercalciuria.

Several methodological limitations warrant consideration. First, our micro-CT voxel size of 20  $\mu\text{m}$ , while suitable for quantifying overall mineral burden across the papillary regions, cannot definitively distinguish Randall's plaque (interstitial calcium phosphate deposits) from tubular plugs (intratubular calcifications). Recent reviews emphasize this distinction, as the two entities likely have different pathogenic origins and clinical implications [16]. True Randall's plaque represents interstitial deposits in the basement membranes of the loops of Henle and papillary interstitium, whereas tubular plugs are intraluminal calcifications within collecting ducts. Definitive differentiation would require either higher resolution imaging (voxel sizes  $< 5 \mu\text{m}$ ) or serial sectioning with mineral-specific histological stains. Second, we used H&E staining rather than Yasue or von Kossa staining, which are preferred for mineral visualization and localization. While basophilic areas consistent with calcification were observed in our histological sections, we cannot definitively exclude that some represent other basophilic structures rather than true mineral deposits. Third, we did not perform macroscopic photographic documentation to assess whether white plaque was visible on papillary surfaces (a key characteristic feature described in Randall's original observations). Therefore, our findings are best interpreted as characterizing papillary tip mineral deposits broadly, which may include both true Randall's plaque and tubular plugs, rather than definitively distinguishing between these entities. Lastly, the limited sample size was hard to avoid, since papillary tissue from stone-naïve individuals can only be ethically collected during nephrectomies performed for unrelated clinical reasons. Consequently, this brings several statistical limitations that are important to acknowledge, such as the wide confidence intervals seen for some predictors, like hypertension (OR: 5.78 [1.12–46.57]). These findings should be viewed as exploratory and hypothesis-generating rather than conclusive. Larger, independent studies will be essential to confirm these associations and produce more precise estimates.

Despite these limitations, this study has notable strengths. By focusing on stone-naïve individuals, we provide a unique characterization of early PCL formation in clinically normal kidneys. Micro-CT enabled unbiased three-dimensional quantification of calcifications across the entire papilla, avoiding sampling biases inherent to biopsy or endoscopic inspection. The use of a standardized semiquantitative OPN grading system further strengthened reproducibility and

allowed consistent comparisons across nephron segments. Stoller et al. reported subepithelial plaques in up to 57% of cadaveric kidneys; our findings extend these observations by showing that similar lesions can be quantified *in vivo* and associated with systemic and urinary risk factors.

## Conclusion

This study broadens the understanding of early Randall's plaque pathogenesis by characterizing subclinical PCLs in stone-naïve kidneys. We demonstrate that high urinary calcium, low urine volume, and hypertension drive mineral deposition before stone formation. We also identify OPN as a marker and potential mediator of this process. By combining high-resolution imaging and histology, we provide evidence that the mechanisms underlying mineral aggregation are already active in patients without stones. These findings bridge basic biology and clinical risk, suggesting that early interventions targeting urine chemistry and vascular health may influence mineral progression and delay stone formation.

**Acknowledgements** Funding: The research is supported by Ankara University - scientific research projects (BAP).

**Author contributions** E. Aydoğ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. H. Berber: Data curation, Formal analysis, Investigation, Methodology, Visualization. M. Ocak: Data curation, Formal analysis, Validation, Visualization. D. Enneli: Conceptualization, Supervision. K. Orhan: Data analysis, Conceptualization, Supervision. M. İ. Gökçe: Conceptualization, Project administration, Supervision, Writing – review and editing.

**Funding** The research is supported by Ankara University - scientific research projects (BAP).

**Data availability** Data are available from the corresponding author upon reasonable request and with permission from Ankara University.

## Declarations

**Competing interests** The authors declare no competing interests.

**Ethics approval** This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Ankara University (approval number: i6-330-20).

**Consent to participate** Informed consent was obtained from all individual participants included in the study.

## References

1. Randall A (1940) Papillary pathology as a precursor of primary renal calculus. *J Urol* 44:580–589. [https://doi.org/10.1016/S0022-5347\(17\)71305-5](https://doi.org/10.1016/S0022-5347(17)71305-5)
2. Khan SR, Canales BK (2015) Unified theory on the pathogenesis of Randall's plaques and plugs. *Urolithiasis* 43(Suppl 1):109–123. <https://doi.org/10.1007/s00240-014-0705-9>
3. Coe FL, Evan AP, Lingeman JE, Worcester EM (2010) Plaque and deposits in nine human stone diseases. *Urol Res* 38:239–247. <https://doi.org/10.1007/s00240-010-0296-z>
4. Low RK, Stoller ML (1997) Endoscopic mapping of renal papillae for randall's plaques in patients with urinary stone disease. *J Urol* 158:2062–2064. [https://doi.org/10.1016/s0022-5347\(01\)68153-9](https://doi.org/10.1016/s0022-5347(01)68153-9)
5. Kuo RL, Lingeman JE, Evan AP, Paterson RF, Parks JH, Bledsoe SB, Munch LC, Coe FL (2003) Urine calcium and volume predict coverage of renal papilla by randall's plaque. *Kidney Int* 64:2150–2154. <https://doi.org/10.1046/j.1523-1755.2003.00316.x>
6. Linnes MP, Krambeck AE, Cornell L, Williams JC Jr., Korinek M, Bergstralh EJ, Li X, Rule AD, McCollough CM, Vrtiska TJ, Lieske JC (2013) Phenotypic characterization of kidney stone formers by endoscopic and histological quantification of intrarenal calcification. *Kidney Int* 84:818–825. <https://doi.org/10.1038/ki.2013.189>
7. Sabaté Arroyo XA, Grases Freixedas F, Bauzá Quetglas JL, Guimerà García J, Pieras Ayala E (2020) Relationship of endoscopic lesions of the renal papilla with type of renal stone and 24 h urine analysis. *BMC Urol* 20:46. <https://doi.org/10.1186/s12894-020-00615-4>
8. Stoller ML, Low RK, Shami GS, McCormick VD, Kerschmann RL (1996) High resolution radiography of cadaveric kidneys: unraveling the mystery of randall's plaque formation. *J Urol* 156:1263–1266. [https://doi.org/10.1016/s0022-5347\(01\)65565-4](https://doi.org/10.1016/s0022-5347(01)65565-4)
9. Perre E Vd, Bazin D, Estrade V, Boudierlique E, Wissing KM, Daudon M, Letavernier E (2022) Randall's plaque as the origin of idiopathic calcium oxalate stone formation: an update. *C R Chim* 25:373–391. <https://doi.org/10.5802/crchim.102>
10. Khan SR (2022) Inflammation and injury: what role do they play in the development of randall's plaques and formation of calcium oxalate kidney stones? *CR Chim* 25:355–372. <https://doi.org/10.5802/crchim.93>
11. Hsi RS, Ramaswamy K, Ho SP, Stoller ML (2017) The origins of urinary stone disease: upstream mineral formations initiate downstream randall's plaque. *BJU Int* 119:177–184. <https://doi.org/10.1111/bju.13555>
12. Evan AP, Lingeman JE, Coe FL, Parks JH, Bledsoe SB, Shao Y, Sommer AJ, Paterson RF, Kuo RL, Grynpsas M (2003) Randall's plaque of patients with nephrolithiasis begins in basement membranes of thin loops of Henle. *J Clin Invest* 111:607–616. <https://doi.org/10.1172/jci17038>
13. van Smeden M, de Groot JA, Moons KG, Collins GS, Altman DG, Eijkemans MJ, Reitsma JB (2016) No rationale for 1 variable per 10 events criterion for binary logistic regression analysis. *BMC Med Res Methodol* 16:163. <https://doi.org/10.1186/s12874-016-0267-3>
14. Lo SK, Li IT, Tsou TS, See L (1995) Non-significant in univariate but significant in multivariate analysis: a discussion with examples. *Changeng Yi Xue Za Zhi* 18:95–101
15. Taylor ER, Stoller ML (2015) Vascular theory of the formation of Randall plaques. *Urolithiasis* 43(Suppl 1):41–45. <https://doi.org/10.1007/s00240-014-0718-4>
16. Evan AP, Coe FL, Rittling SR, Bledsoe SM, Shao Y, Lingeman JE, Worcester EM (2005) Apatite plaque particles in inner medulla of kidneys of calcium oxalate stone formers: osteopontin localization. *Kidney Int* 68:145–154. <https://doi.org/10.1111/j.1523-1755.2005.00388.x>
17. Evan AP, Coe FL, Lingeman J, Bledsoe S, Worcester EM (2018) Randall's plaque in stone formers originates in ascending thin limbs. *Am J Physiol Renal Physiol* 315:F1236–f1242. <https://doi.org/10.1152/ajprenal.00035.2018>
18. Xie Y, Sakatsume M, Nishi S, Narita I, Arakawa M, Gejyo F (2001) Expression, roles, receptors, and regulation of osteopontin in the kidney. *Kidney Int* 60:1645–1657. <https://doi.org/10.1046/j.1523-1755.2001.00032.x>
19. Mo L, Liaw L, Evan AP, Sommer AJ, Lieske JC, Wu XR (2007) Renal calcinosis and stone formation in mice lacking osteopontin, Tamm-Horsfall protein, or both. *Am J Physiol Renal Physiol* 293:F1935–1943. <https://doi.org/10.1152/ajprenal.00383.2007>

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.