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Original Article

Comparative proteomic profiling of uric acid, ammonium acid urate, and calcium-based kidney stones

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Abstract: Introduction: Kidney stone matrix proteins may help explain cellular mechanisms of stone genesis. However, most existing proteomic studies have focused on calcium oxalate stones. Here, we present a comparative proteomic analysis of different kidney stone types. Methods: Proteins were extracted from the stones of patients undergoing percutaneous nephrolithotomy (PCNL). Approximately 20 µg of protein was digested into tryptic peptides using filter aided sample preparation, followed by liquid chromatography tandem-mass-spectrometry using an EASY-nLC 1200 and Orbitrap Fusion Lumos mass spectrometer. A standard false discovery rate cutoff of 1% was used for protein identification. Stone analysis was used to organize stone samples into similar groups. We selected the top 5% of proteins based on total ion intensities and used DAVID and Ingenuity Pathway Analysis to identify and compare significantly enriched gene ontologies and pathways between groups. Results: Six specimens were included and organized into the following four groups: 1) mixed uric acid (UA) and calcium-based, 2) pure UA, 3) pure ammonium acid urate (AAU), and 4) pure calcium-based. We identified 2,426 unique proteins (1,310-1,699 per sample), with 11-16 significantly enriched KEGG pathways identified per group and compared via heatmap. Based on number of unique proteins identified, this is the deepest proteomic study of kidney stones to date and the first such study of an AAU stone. Conclusions: The results indicate that mixed UA and calcium-based kidney stones are more similar to pure UA stones than pure calcium-based stones. AAU stones appear more similar to pure calcium-based stones than UA containing stones and may be related to parasitic infections. Further research with larger cohorts and histopathologic correlation is warranted.

Keywords: Nephrolithiasis, proteins, proteomics, uric acid, calcium oxalate, ammonium acid urate

Introduction

Kidney stone disease (KSD) is one of the most common pathologies encountered in urologic practice, with an estimated prevalence of 8.8% and estimated incidence of 0.9% in the United States [1, 2]. KSD is often a recurrent illness and has an estimated annual cost of over two billion dollars to the US healthcare system [3]. Sequelae of KSD include severe pain, kidney damage, and urinary tract infections. Yet, despite the associated economic and medical burden, the pathogenesis of KSD remains poorly understood.

Kidney stones are predominantly composed of inorganic salts, and accordingly pathologic excesses and shortages of various urinary electrolytes are known to promote stone formation. Furthermore, urinary stasis has also been implicated in stone formation. However, these two mechanisms alone do not fully explain the pathogenesis of kidney stone disease. A small but significant portion of the stone mass is attributable to organic compounds (matrix), the majority of which is protein (stone matrix proteins, SMPs) [4]. It has long been postulated that proteins may form a stone scaffold which dictates the physical characteristics of the

stone. Furthermore, the cellular origins and functions of the proteins within kidney stones may provide insights into the pathophysiologic process of stone genesis.

One of the historical challenges in studying SMPs has been isolating proteins from the tightly bound protein-crystalline complex found in kidney stones. However recent advances in mass spectrometry (MS) have improved our ability to study SMPs. Since the late 2000s several groups have published on the use of MS to better define the kidney stone proteome [5-9]. In 2008, Canales et al. published on the use of MS to analyze the protein content of seven calcium oxalate monohydrate (COM) stones. They were able to identify 68 distinct proteins, many of which are known inflammatory proteins prompting them to postulate that inflammation plays a critical role in kidney stone genesis. Additionally, they identified 2% sodium dodecyl sulfate as an efficient buffer for solubilizing SMPs for MS analysis [6]. In the same year Merchant et al. and Chen et al. published similar analyses on an additional fifteen calcium stones, bringing the known proteome to over 100 distinct proteins [7, 8]. Recent advances in MS have enabled proteomics to identify and quantify over a thousand unique proteins in human kidney stones [5-18]. Yet, the expansion in our knowledge of the proteome, exact role of SMPs in kidney stone formation remains unclear.

In the United States, calcium-containing stones represent approximately 70% of kidney stones [19]. Accordingly, much of the existing literature on kidney stone proteomics has focused on calcium-containing stones. Such studies have implicated inflammatory and phagocytosis pathways [15, 20, 21]. The second most common kidney stone type is uric acid containing stones, representing approximately 20% of kidney stones in the United States [19]. Uric acid containing stones can be pure uric acid or mixed with calcium oxalate. The third, and least common type of uric acid-based stones are ammonium acid urate (AAU) stones. The literature on uric acid stone proteomics is sparse, with the largest study being a single case series of five pure uric acid stone formers [8]. In the present study, we aim to compare proteomic profiles of different stone types including AAU stones, mixed uric acid/calcium stones, pure uric acid stones, and pure calcium stones to gain insight into potential differences in their pathogenesis. To the best of our knowledge, this is the first proteomic analysis to include an AAU stone and one of the first comparative analyses of different kidney stone types.

Methods

Patient recruitment and stone procurement

The present study was prospectively performed and received IRB approval (#15-00552). Patients were recruited from the practices of two high-volume endourologists at an academic tertiary care center in a large urban setting. Consecutive adult (age ≥ 18 years) patients undergoing percutaneous nephrolithotomy (PCNL) surgery for kidney stone disease were recruited. Exclusion criteria were pregnancy, inability to provide informed consent, presence of existing nephrostomy tube, and planned miniPCNL (sheath size ≤ 18 Fr). We aimed to enroll six patients for this initial hypothesis-generating study. Upon enrollment, patients underwent routine PCNL surgery with renal access obtained through a combination of ultrasonography and fluoroscopy.

Upon intraoperative identification, kidney stones were extracted. Kidney stones were either extracted intact, or if stone size precluded intact extraction through our access sheath, stones were fragmented using the Swiss LithoClast® Trilogy Lithotripter (Boston Scientific, USA), and then extracted. Upon extraction, several of the stones/stone fragments were placed in cryovials and a member of the research team promptly placed the specimens in a -80°C freezer to prevent protein degradation. The remaining stone specimens were subjected to routine stone analysis via Fourier-transform infrared spectroscopy (Labcorp, USA). Patient demographics including medical comorbidities were recorded for each patient. Given the prospective nature of this study, stone composition remained unknown until after patient enrollment and stone procurement.

Protein extraction

Stone specimens were ground to a fine powder using a mortar and pestle and then added to

Table 1. Patient demographics

Patient ID	Age	Gender	Known Family History of Kidney Stone Disease	Known Comorbidities
1	43	M	No	Hypertension, Diabetes Mellitus, hyperlipidemia
2	60	M	No	Obesity, hyperlipidemia, BPH, bladder stones
3	53	F	Yes	Hypertension, Diabetes Mellitus, obesity
4	58	F	No	Vertigo, hypertension, anxiety, gout
5	54	M	No	Hypertension, obstructive sleep apnea, gastro-esophageal reflux disease, hyperlipidemia
6	64	M	No	None

Laemmli buffer. This suspension was then heated to 100°C for 15 minutes and centrifuged at 21,130×g for 15 minutes. The supernatant was then isolated and protein concentration within the supernatant was measured using Pierce 660 nm protein assay (Thermo Scientific, USA). About 20 µg protein was digested into tryptic peptides using filter-aided sample preparation (FASP), followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) using an EASY-nLC 1200 connected to an Orbitrap Fusion Lumos mass spectrometer (Thermo Scientific, USA), essentially as we have previously described [22-24]. Briefly, 4 µL of peptide solution containing about 1.5 µg tryptic peptides was loaded onto a 2-cm trap column and then separated on a 50-cm EASY-Spray analytical column heated to 55°C, using a 3-h gradient at the flow rate of 250 nL/min. Tryptic peptides were ionized by an EASY-Spray ion source (Thermo Scientific), and mass spectra were collected in a data-dependent acquisition manner.

Proteomic and statistical analysis

The acquired RAW files were searched against the Uniprot_Human protein sequence database, which contains 96,996 protein sequences (released on 04/24/2020), with MaxQuant (v1.5.5.1) [25]. For database searching, cysteine carbamidomethylation was set as a fixed modification, and methionine oxidation as well as acetylation of the protein N-terminus were used as variable modifications. The maximum mass tolerances were 4.5 ppm for precursor ions and 0.5 Da for fragment ions. For label-free quantification, the "Match between runs" function was enabled.

A standard false discovery rate cutoff of 1% was applied for the identification of peptide spectrum matches, peptides, and proteins. Stone samples were organized into groups based on similar stone analysis results. We then selected the top 5% of proteins based on total ion intensities and used Database for Annotation, Visualization, and Integrated Discovery (DAVID) and Ingenuity Pathway Analysis (IPA) to identify significantly enriched gene ontologies and pathways within each group.

Results

In total, six patients' stone samples were included in this pilot analysis. Patient demographics and comorbidities are reported in **Table 1**. Stone samples were organized into the following four groups based on stone analysis results: mixed uric acid/calcium (Group 1, two patients), pure uric acid (Group 2, one patient), AAU (Group 3, one patient), and pure calcium-based (Group 4, two patients). A total of 2,426 unique proteins were identified with a range of 1,310 to 1,699 per individual patient's sample (**Tables 2** and S1). By stone analysis grouping, number of proteins identified ranged from 1,317-1,978 (**Table 3**). A total of 896 proteins were common to all four groups and 666 proteins were common to all six samples. Significantly enriched gene ontologies identified by DAVID and IPA analyses of top 5% of proteins in each group are presented in **Figure 1** as a Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway heatmap, **Figure 2** as a Gene Ontology Biological Process (GOBP) heatmap, and **Figure 3** as a Gene Ontology Cellular Component (GOCC) heatmap. Complete results are shown in Tables S2, S3 and S4. Additionally, all significant KEGG pathways in each group and associated genes are presented in **Table 4**.

In our proteomic analysis of the AAU stone, significantly enriched KEGG pathways included those related to amino acids biosynthesis, car-

Table 2. Number of proteins identified in and stone analysis of each stone sample

Patient ID	Total Number of Proteins Identified	Stone Analysis	Analysis Group
1	1,538	COM 20%, UA 80%	Mixed UA and calcium (group 1)
2	1,605	COD 90%, COM 5%, HA 5%	Pure calcium stones (group 4)
3	1,317	AAU 100%	Pure AAU stones (group 3)
4	1,498	UA 100%	Pure UA stones (group 2)
5	1,699	UA 90%, COM 10%	Mixed UA and calcium (group 1)
6	1,310	COM 35%, COD 60%, HA 5%	Pure calcium-containing stones (group 4)

Table 3. Number of proteins identified in each stone analysis group

Stone Analysis Group	Total Number of Proteins Identified
Group 1: Mixed UA and calcium	1,978
Group 2: Pure UA stones	1,498
Group 3: Pure AAU stones	1,317
Group 4: Pure calcium-containing stones	1,773

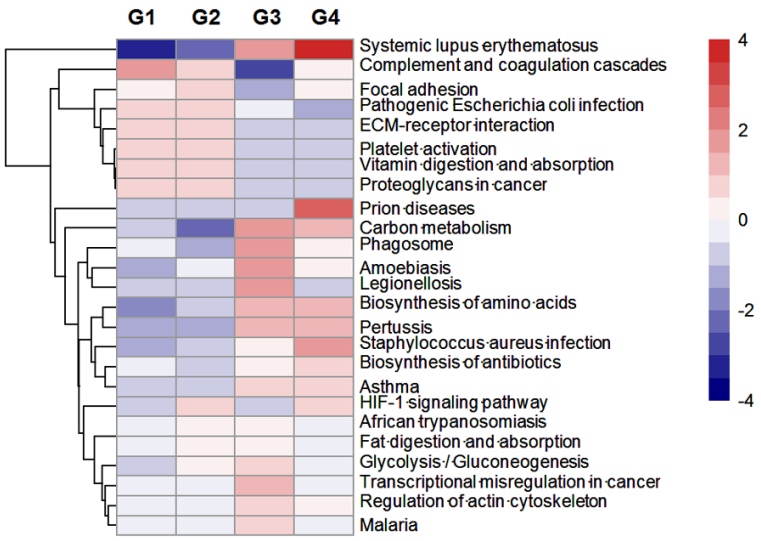

Figure 1. Heatmap of the top 25 significantly enriched KEGG pathways. Color key represents row-scaled -$\log_{10}$(P) values.

bon metabolism, glycolysis/gluconeogenesis, vitamin digestion/absorption, and fat digestion/absorption. We also found that several parasitic infectious pathways were identified including amoebiasis, African trypanosomiasis, and malaria. Mixed calcium and uric acid stones (group 1) appear to have a more similar proteomic profile to pure uric acid stones (group 2) than to pure calcium-based stones (group 4). Indeed, KEGG pathways implicated in both pure UA and mixed UA-calcium stones include ECM-receptor interactions, platelet activation, proteoglycans in cancer, and vitamin digestion and absorption. None of the aforementioned pathways were noted in AAU or pure calcium-based stones. On the other hand, the AAU stone (group 3) had similarity to pure calcium containing stones (group 4) in that it shared similar intensities of the systemic lupus erythematosus, carbon metabolism, biosynthesis of amino acid, pertussis, and asthma pathways. A noteworthy pathway is the complement and coagulation cascades pathway as this was the pathway with the greatest number of unique proteins across all groups representing > 20% of unique gene products in each stone.

Discussion

The proteins within human kidney stones are suspected to play an important role in stone genesis and advances in MS have given rise to the study of human kidney stone proteomics. Indeed, since the late 2000s there have been several publications by independent groups on the use of MS to better define the kidney stone proteome [5-18]. However, kidney stone compositions are heterogeneous, and the majority of studies to date have focused on calcium oxalate stones, with only a single dedicated study on the uric acid stone proteome [8]. In the present study, we have conducted a comparative analysis on a small set of heterogeneous

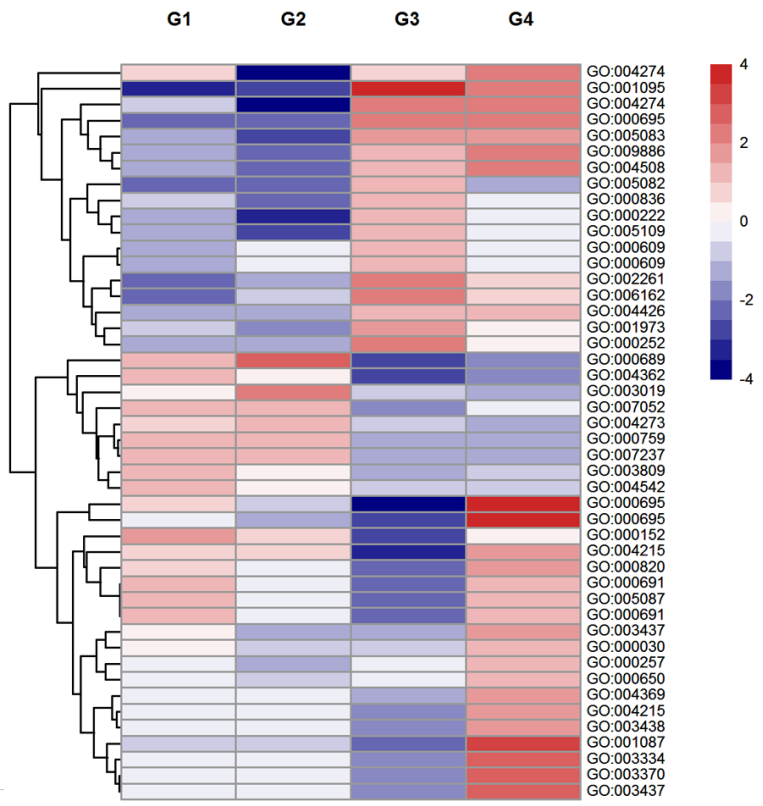

Figure 2. Heatmap of significantly enriched gene ontology biological processes (GOBP). Color key represents row-scaled -$\log_{10}(P)$ values.

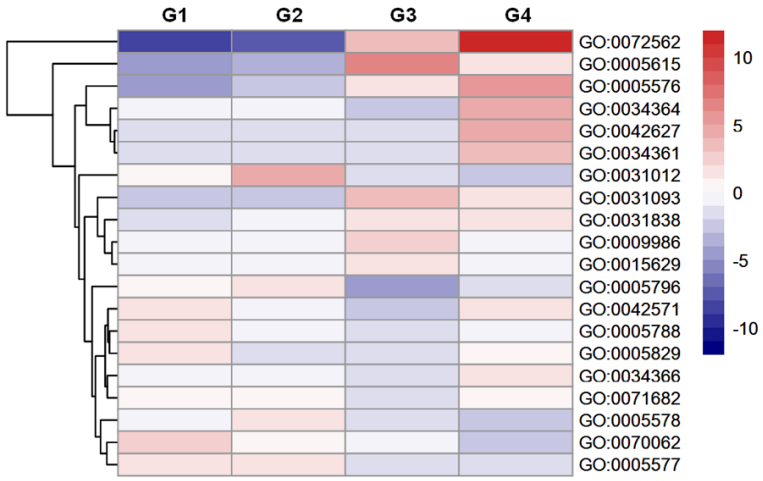

Figure 3. Heatmap of significantly enriched gene ontology cellular components (GOCC). Color key represents row-scaled -$\log_{10}(P)$ values.

stones. We have also performed the first known proteomic analysis of an AAU stone that enabled the deepest proteomic profiling of kidney stones to date with 2,426 unique proteins identified [26].

The AAU proteome

Ammonium acid urate (AAU) urinary stones are uncommon in industrialized nations compared to developing nations, particularly sub-Saharan Africa. Malnutrition, particularly dietary deficiency of inorganic phosphates may contribute, but UTIs and laxative abuse are also causative [27]. We found that significantly enriched KEGG pathways included several metabolic pathways. These findings are intuitive given the known association between malnutrition and AAU stones. However, we also found that several enriched parasitic infectious pathways suggesting that in addition to metabolic processes, parasitic infectious processes may also be involved in AAU stone genesis. If confirmed, it may help explain the disease epidemiology as many of these parasitic infections are more common in developing nations. Interestingly, AAU stones had more enriched pathways in common with pure calcium-based stones than uric acid containing stones despite being biochemically related to uric acid.

Comparative analysis of different stone types

The enhanced KEGG pathways in pure uric acid stones had little overlap with pure calcium-based stones, suggesting significantly different pathogenic pathways for these difference stone types. Indeed, the biological pathways implicated on comparative analysis suggest that uric acid containing stone genesis may be more of an extracellular process compared to the genesis of pure calcium-based stones as ECM-receptor interactions, platelet activation, and compliment and

Table 4. KEGG pathways and associated genes identified in each stone analysis group

Stone Analysis Group	KEGG Term	N	%	Genes
Mixed UA and Calcium				
	hsa04610: Complement and coagulation cascades	24	20.00	FGB, FGA, SERPINA1, CFH, SERPIND1, F10, SERPINC1, SERPINF2, CFI, F11, FGG, C4BPA, PLG, F2, C8B, KNG1, SERPINA5, C3, C4B, PLAU, C9, SERPING1, MASP2, CFB
	hsa05150: Staphylococcus aureus infection	8	6.67	C4B, C3, CFH, CFI, FGG, MASP2, PLG, CFB
	hsa04977: Vitamin digestion and absorption	4	3.33	CUBN, APOA1, APOA4, APOB
	hsa04512: ECM-receptor interaction	5	4.17	VTN, COL6A1, FN1, AGRN, HSPG2
	hsa05130: Pathogenic Escherichia coli infection	4	3.33	TUBA1B, WASL, TUBB4B, ACTG1
	hsa04510: Focal adhesion	7	5.83	VTN, EGF, COL6A1, FN1, FLNA, TLN1, ACTG1
	hsa01130: Biosynthesis of antibiotics	7	5.83	TPI1, CAT, AKR1A1, PGK1, PGLS, GAPDH, ASS1
	hsa01200: Carbon metabolism	5	4.17	TPI1, CAT, PGK1, PGLS, GAPDH
	hsa00010: Glycolysis/Gluconeogenesis	4	3.33	TPI1, AKR1A1, PGK1, GAPDH
	hsa01230: Biosynthesis of amino acids	4	3.33	TPI1, PGK1, GAPDH, ASS1
	hsa05133: Pertussis	4	3.33	C4B, C3, SERPING1, C4BPA
	hsa05143: African trypanosomiasis	3	2.50	HBB, APOA1, HBA1
Pure UA				
	hsa04610: Complement and coagulation cascades	23	20	FGB, FGA, SERPINA1, CFH, SERPIND1, SERPINC1, SERPINF2, CFI, F11, FGG, C4BPA, PLG, F2, C8B, KNG1, SERPINA5, C3, C4B, PLAU, C9, SERPING1, MASP2, CFB
	hsa05150: Staphylococcus aureus infection	8	6.96	C4B, C3, CFH, CFI, FGG, MASP2, PLG, CFB
	hsa04977: Vitamin digestion and absorption	4	3.48	CUBN, APOA1, APOA4, APOB
	hsa00010: Glycolysis/Gluconeogenesis	5	4.35	TPI1, AKR1A1, PGK1, ENO1, GAPDH
	hsa01230: Biosynthesis of amino acids	5	4.35	TPI1, PGK1, ENO1, GAPDH, ASS1
	hsa05322: Systemic lupus erythematosus	6	5.22	C4B, C3, HIST1H4A, HIST2H3A, C9, C8B
	hsa04512: ECM-receptor interaction	5	4.35	VTN, COL6A1, FN1, AGRN, HSPG2
	hsa05130: Pathogenic Escherichia coli infection	4	3.48	TUBA1B, WASL, TUBB4B, ACTG1
	hsa04510: Focal adhesion	7	6.09	VTN, EGF, COL6A1, FN1, FLNA, TLN1, ACTG1
	hsa05133: Pertussis	4	3.48	C4B, C3, SERPING1, C4BPA
	hsa04611: Platelet activation	5	4.35	FGB, FGA, FGG, TLN1, ACTG1
	hsa05143: African trypanosomiasis	3	2.61	HBB, APOA1, HBA1

Pure AAU				
	hsa04610: Complement and coagulation cascades	21	17.80	FGB, FGA, SERPINA1, CFH, SERPIND1, SERPINC1, SERPINF2, FGG, C4BPA, PLG, F2, KNG1, SERPINA5, C3, C4B, C8G, C9, SERPING1, MASP2, A2M, CFB
	hsa05150: Staphylococcus aureus infection	9	7.63	C4B, C3, ITGAM, CFH, ITGB2, FGG, MASP2, PLG, CFB
	hsa05322: Systemic lupus erythematosus	11	9.32	C4B, HIST1H2BN, C3, HIST1H4A, HIST2H3A, C8G, HIST1H3A, C9, CTSG, ELANE, HIST2H2AC
	hsa01230: Biosynthesis of amino acids	7	5.93	PKM, TPI1, PGK1, ENO1, ALDOA, TKT, GAPDH
	hsa05133: Pertussis	7	5.93	C4B, C3, ITGAM, CFL1, ITGB2, SERPING1, C4BPA
	hsa01200: Carbon metabolism	8	6.78	PKM, TPI1, CAT, PGK1, ENO1, ALDOA, TKT, GAPDH
	hsa00010: Glycolysis/Gluconeogenesis	6	5.08	PKM, TPI1, PGK1, ENO1, ALDOA, GAPDH
	hsa05146: Amoebiasis	7	5.93	SERPINB1, ITGAM, C8G, C9, ITGB2, FN1, CTSG
	hsa04145: Phagosome	8	6.78	C3, TUBA1B, ITGAM, ITGB2, TUBB4B, MPO, CORO1A, ACTG1
	hsa05134: Legionellosis	5	4.24	EEF1A1, C3, ITGAM, ITGB2, HSPA1B
	hsa04810: Regulation of actin cytoskeleton	8	6.78	ITGAM, GSN, CFL1, ITGB2, FN1, PFN1, F2, ACTG1
	hsa01130: Biosynthesis of antibiotics	8	6.78	PKM, TPI1, CAT, PGK1, ENO1, ALDOA, TKT, GAPDH
	hsa04977: Vitamin digestion and absorption	3	2.54	APOA1, APOA4, APOB
	hsa05202: Transcriptional misregulation in cancer	6	5.08	ITGAM, HIST2H3A, HIST1H3A, MPO, MMP9, ELANE
	hsa05310: Asthma	3	2.54	PRG2, RNASE3, EPX
	hsa05143: African trypanosomiasis	3	2.54	HBB, APOA1, HBA1
Pure calcium-containing				
	hsa04610: Complement and coagulation cascades	23	19.17	FGB, C1QB, FGA, SERPINA1, CFH, SERPIND1, SERPINC1, SERPINF2, FGG, C4BPA, PLG, F2, C8B, KNG1, C3, C4B, C5, C9, SERPING1, MASP2, A2M, CFB, C1QC
	hsa05150: Staphylococcus aureus infection	10	8.33	C4B, C1QB, C3, C5, CFH, FGG, MASP2, PLG, CFB, C1QC
	hsa05322: Systemic lupus erythematosus	13	10.83	C1QB, HIST1H2BN, C8B, HIST2H2AC, C3, C4B, HIST1H4A, C5, HIST2H3A, C9, CTSG, ELANE, C1QC
	hsa01230: Biosynthesis of amino acids	7	5.83	PKM, TPI1, PGK1, ENO1, TKT, GAPDH, ASS1
	hsa05133: Pertussis	7	5.83	C4B, C1QB, C3, C5, SERPING1, C4BPA, C1QC
	hsa01200: Carbon metabolism	8	6.67	G6PD, PKM, TPI1, CAT, PGK1, ENO1, TKT, GAPDH
	hsa05020: Prion diseases	5	4.17	C1QB, C5, C9, C8B, C1QC
	hsa01130: Biosynthesis of antibiotics	9	7.50	G6PD, PKM, TPI1, CAT, PGK1, ENO1, TKT, GAPDH, ASS1
	hsa00010: Glycolysis/Gluconeogenesis	5	4.17	PKM, TPI1, PGK1, ENO1, GAPDH
	hsa04145: Phagosome	6	5.00	C3, TUBA1B, TUBB4B, MPO, CORO1A, ACTG1
	hsa04977: Vitamin digestion and absorption	3	2.50	APOA1, APOA4, APOB
	hsa04810: Regulation of actin cytoskeleton	7	5.83	GSN, EGF, RAC2, FN1, PFN1, F2, ACTG1
	hsa05146: Amoebiasis	5	4.17	C9, FN1, HSPB1, CTSG, C8B
	hsa05310: Asthma	3	2.50	PRG2, RNASE3, EPX
	hsa05143: African trypanosomiasis	3	2.50	HBB, APOA1, HBA1

coagulation cascade pathways were more active in uric acid-based stones, and these are predominantly extracellular processes. Furthermore, on our comparative proteomic analysis we found mixed uric acid/calcium-based stones to be more similar to pure UA stones than pure calcium-based stones, despite the mixed stones in our study being predominantly calcium. One possible explanation for this finding is that in a mixed calcium oxalate-uric acid stone, calcium crystals are more likely the "innocent bystander" that gets incorporated into the stone in the urinary collecting system based on urinary concentrations of calcium, oxalate, and citrate instead of what is the traditional mechanism of calcium oxalate stone formation which involves calcium oxalate and phosphate crystal formation and deposition in the renal tubules [28, 29]. This would challenge the current understanding of the composition of mixed stones-that calcium oxalate is the primary stone that forms in the interstitial and collecting ducts then once exposed to the urine in the collecting system, uric acid gets deposited into the stone. One must consider however that, per our hypothesis, by raising urine pH or deceasing urinary uric acid via xanthine oxidase inhibitors, we could prevent these mixed stones by preventing the formation of the uric acid crystal nidus that serves as a "scaffold" for these stones to form.

The complement and coagulation cascades pathway were the most significantly enriched pathway in each stone group (Table S2). We suspect that this is in part due to contamination from non-stone related blood products integrated into the stone specimen either at the time of surgical extraction or during stone growth via micro trauma to the collecting system from the actual stone. Though great care was taken to ensure each stone was free of obvious blood products, contamination is inevitable. However, importantly, on the comparative KEGG pathway heatmap, we see that the complement and coagulation cascade pathways were relatively more important for uric acid containing stones as compared to pure calcium-based stones and the AAU stone, suggesting that these pathways may actually play a role in stone genesis rather than simply be "bystanders".

Clinical perspective

Abstractly speaking, kidney stone disease is similar to thromboembolic vascular disease. Consider Virchow's Triad for vascular thrombosis: stasis of blood flow, hypercoagulability, and endothelial injury. Urinary stone disease similarly is caused by urinary stasis and an imbalance in pro-lithogenic and anti-lithogenic factors resulting in a "hyperlithogenic" state. Yet these two factors alone do not fully explain who will develop kidney stone stones. Indeed, there has long been suspected to be a third factor, akin to the "endothelial injury", known to promote vascular thrombosis. In vascular thrombotic disease, the three elements of Virchow's Triad hold different degrees of weight for the different types of thrombotic illnesses. For example, deep venous thrombosis is known to be heavily influenced by hematologic stasis while arterial plaque disease is more driven by endothelial injury secondary to hyperlipidemia. Like vascular thrombosis, kidney stone disease is heterogeneous, and proteins identified within uric acid stones differed substantially from those within calcium-containing stones. Given that calcium-containing stones appeared to involve more proteins implicated in intracellular processes, cellular injury may play a greater role in this stone type. Ultimately a better definition of the exact cellular mechanism of stone formation may guide a definitive means for stone prevention, akin to the statin for coronary artery disease.

Study limitations and future directions

Our study has several limitations. First, the small sample size. Indeed, each group had only 1 or 2 stone specimens and thus the generalizability of our findings is limited. Calcium-based stones are themselves a heterogeneous entity and contain varying percentages of calcium oxalate monohydrate, calcium oxalate dihydrate, and calcium phosphate. Second, we did not perform proteomic analysis on urine specimens from our patients and in turn some of the proteins identified within the stones may be "bystander" proteins. Third, we performed DAVID and IPA analyses on the top 5% of proteins in each group, but abundance of a protein may not necessarily correlate to its biological significance. Thus, potentially significant proteins may have been excluded from our analysis.

Despite these limitations, the present study provides significant evidence in the context of kidney stone proteomics with over 2,400 unique proteins identified and compared bet-

ween heterogeneous stone types. Additionally, we have performed the first ever proteomic study of an AAU stone. Ultimately, kidney stone proteomics should guide histopathologic studies in the kidney tissues of stone formers to help definitively translate proteomic findings into tangible mechanistic data. Indeed, further study with renal tissue biopsy samples from stone formers for histopathologic correlation to proteomic findings is warranted, and the likely avenue for future studies.

Conclusions

Based on the present proteomic analysis, mixed uric acid/calcium-based kidney stones are more similar to pure uric acid stones than pure calcium-based stones. This suggests that in these mixed stones, calcium components are more likely to be the "innocent bystanders" that get incorporated onto a uric acid scaffold. Alternatively, these differences may be related to the microenvironment (i.e., intracellular space vs extracellular space) in which stones form, that mixed UA/calcium-based stones and pure UA stones form in a similar microenvironment. Regarding AAU stones, parasitic infections may play a role in stone genesis as several unique parasitic pathways were identified and these stones have a known epidemiologic prediction for areas where these infections are more common. Further research involving larger patient cohorts and histopathologic correlation is warranted to validate and confirm these findings.

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Disclosure of conflict of interest

None.

Abbreviations

UA, Uric Acid; KSD, Kidney stone disease; SMPs, Stone matrix proteins; MS, mass spectrometry; AAU, ammonium acid urate; PCNL, undergoing percutaneous nephrolithotomy; FASP, filter-aided sample preparation; LC-MS/MS, liquid chromatography tandem mass spectrometry; DAVID, Database for Annotation, Visualization, and Integrated Discovery; IPA, Ingenuity Pathway Analysis; KEGG, Kyoto Encyclopedia of Genes and Genomes; GOBP, Gene Ontology Biological Process; GOCC, Gene Ontology Cellular Component.

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