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Stone former urine proteome demonstrates a cationic shift in protein distribution compared to normal

Ann M Kolbach-Mandel⁴, Neil S Mandel^{3,4,6}, Brian R. Hoffmann⁵, Jack G. Kleinman⁴, and Jeffrey A Wesson^{1,2,4}

²Nephrology Section/Consultant Care Division, Zablocki VA Medical Center, 5000 W. National Avenue (111K), Milwaukee, Wisconsin 53295

³Mandel International Stone and Molecular Analysis Center, Zablocki VA Medical Center, 5000 W. National Avenue, Milwaukee, Wisconsin 53295

⁴Division of Nephrology/Department of Medicine, Medical College of Wisconsin, 9200 W Wisconsin Avenue, Milwaukee, WI 53226

⁵Division of Cardiology/Department of Medicine, Biotechnology and Bioengineering Center, Cardiovascular Center, Medical College of Wisconsin, 9200 W Wisconsin Avenue, Milwaukee, WI 53226

Abstract

Many urine proteins are found in calcium oxalate stones, yet decades of research have failed to define the role of urine proteins in stone formation. This urine proteomic study compares the relative amounts of abundant urine proteins between idiopathic calcium oxalate stone forming and non-stone forming (normal) cohorts to identify differences that might correlate with disease. Random mid-morning urine samples were collected following informed consent from 25 stone formers and 14 normal individuals. Proteins were isolated from urine using ultrafiltration. Urine proteomes for each sample were characterized using label-free spectral counting mass spectrometry, so that urine protein relative abundances could be compared between the two populations. A total of 407 unique proteins were identified with the 38 predominant proteins accounting for >82% of all sample spectral counts. The most highly abundant proteins were equivalent in stone formers and normals, though significant differences were observed in a few moderate abundance proteins (immunoglobulins, transferrin, and epidermal growth factor), accounting for 13% and 10% of the spectral counts respectively. These proteins contributed to a cationic shift in protein distribution in stone formers compared to normals (22% vs. 18%, $p = 0.04$). Our data showing only small differences in moderate abundance proteins suggest that no single protein controls stone formation. Observed increases in immunoglobulins and transferrin suggest increased inflammatory activity in stone formers, but cannot distinguish cause from effect in stone formation. The observed cationic shift in protein distribution would diminish protein

¹Corresponding Author: Jeffrey A. Wesson, MD, PhD, Department of Veterans Affairs Medical Center, 5000 W National Avenue (111K), Milwaukee, WI, 53295, USA, jwesson@mcw.edu, Phone (414) 384-2000 ext 42828, Fax (414) 383-9333.

⁶Dr. Mandel is a Senior Research Career Scientist in the VA

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charge stabilization, which could lead to protein aggregation and increased risk for crystal aggregation.

Keywords

kidney calculi; calcium oxalate; proteomics; urine; osteopontin

Introduction

Historically, urinary proteins have been a primary focus of research regarding kidney stone genesis. Early scientific reports such as Tamm and Horsfall's discovery of uromodulin (UROM) in human urine[1] and Boyce's careful analysis of the 3% protein matrix component in kidney stones[2] began the collection of a large body of knowledge on specific proteins in urine and in stone matrix.[3] Key urinary proteins that have been extensively studied include osteopontin,[4, 5] albumin,[6] UROM,[7, 8] bikunin,[9] and others.[10] Each of these negatively charged proteins has been shown to inhibit various crystallization processes (i.e., nucleation, growth, aggregation, and cell attachment) *in vitro*. Albumin is thought to have a prominent role in stone formation, based on its presence in most stones. [11] UROM is thought to either inhibit or promote crystal aggregation depending on its level of glycosylation.[12, 13] More recently, mass spectrometry-based proteomic reports of calcium stone matrix have identified >1,000 proteins in the stone matrix.[14–17] Additional studies have implicated immune and fibrotic proteins based on the prevalence of these classes of proteins detected within stone matrix.[18, 19] However, these earlier studies failed to define either individual proteins or groups of urine proteins that cause stone formation or serve as biomarkers for disease.

Our recent studies of protein interactions with calcium oxalate (CaOx) crystals have demonstrated that mixtures of anionic and cationic proteins at nearly equal proportions induced CaOx crystal aggregation, which correlated with protein aggregate formation.[20, 21] Furthermore, de-glycosylated UROM induced crystal aggregation when mixed with CaOx crystals in the appropriate proportions, which correlated with self-aggregation at physiologically relevant protein and salt concentrations.[12] Removing glycosyl side chains reduced the number of anionic charges on UROM, so both of these examples illustrated the link between reduced net negative charge in the mixture and protein destabilization in solution (aggregation), which correlated with CaOx crystal aggregation. The observed correlation between protein aggregate formation and CaOx crystal aggregation is likely to be critical to stone formation, because addition of preformed crystals to stone aggregates has been demonstrated repeatedly.[22–24] Since most urine proteins are anionic and demonstrate similar CaOx crystal interactions, stone formation will likely be driven by highly abundant rather than trace urinary proteins, or possibly the summation of contributions from many urine proteins in the mixture.

In this study, we compare the proteomes observed in random, mid-morning urine samples between idiopathic CaOx stone formers (as defined by the absence of predisposing medical conditions or ingestions)[25] and a matching control (normal) population, using label-free

spectral counting mass spectrometry (MS) methods and subsequent bioinformatics analysis to identify possible differences in abundant urine proteins. No differences were observed in the most abundant individual proteins, though small differences were detected in some moderate abundance proteins. These differences highlighted several inflammatory proteins. While this work cannot distinguish cause from effect regarding their role in stone formation, the stone former proteome demonstrated a cationic shift (lower net negative charge) compared to normal, which could correlate with increased risk for protein aggregation and stone formation.

Results

Demographic data for our study populations (14 normal (N) and 25 recurrent, idiopathic CaOx stone forming (SF) individuals) are shown in Table 1 along with some standard urine composition data based on ion chromatography analysis of samples acquired for this study. Other data were extracted from their clinic records including stone analysis results, as well as routine blood and 24 hour urine data. Stone analysis data were available in our hospital records for 20 of 25 stone formers, and all samples were reported to be mixtures of CaOx (typically mixtures of monohydrate and dihydrate phases) with other calcium salts; 19 with calcium phosphate and 1 with calcium carbonate. The average CaOx content for these 20 patients was $80 \pm 30\%$, including 3 patients where the calcium phosphate phase was dominant. Among stone formers with multiple stone analyses reported, 3 of 4 demonstrated both oxalate dominant and phosphate dominant stones at different time points. Stone activity was rated in each patient as “few” for 1 to 3 recurrent stone passage events or “many” for > 3 events at the time of recruitment. Stone analysis data are summarized in Supplementary Data (S1). Routine blood chemistry data were available for all 25 stone forming patients and showed that all had normal renal function and normal serum electrolytes, including calcium and bicarbonate levels. At least one 24 hour urine collection for stone risk assessment (results from either Litholink® or Urorisk® kits) had been obtained on 24 of 25 stone formers, with 22 patients having 2 or more such collections (57 total urine results; maximum = 5 in one patient). Patients demonstrating urinary risk for CaOx stone formation were identified from these data using the following the definitions: hypercalciuria >300mg/day calcium excretion, hyperoxaluria >45mg/day oxalate excretion, and hypocitraturia <320mg/day citrate excretion (see Supplementary Data, S2). In patients with two or more analyses, most patients were inconsistently categorized by these urinary excretion definitions (6 of 10 for hypercalciuria; 4 of 6 for hyperoxaluria, and 4 of 6 for hypocitraturia), similar to inconsistencies noted in defining their stone type in repeat stone analysis.

Our study populations were well matched and show the expected “middle” age demographic of CaOx stone disease, though skewed toward women compared to the expected male disease predominance reflecting the typical female gender bias in most clinical follow up. Standard urine analyses for total protein content and small ion composition were performed on each urine sample; demonstrating similar levels for creatinine concentration, protein/creatinine, and calcium content, but significantly higher oxalate concentration in the SF population compared to the normal population (see Table 1). No differences were noted for other urine anion and cation measurements (see Supplementary Data, S3). The oxalate concentrations for both groups were within the accepted normal range, despite the observed

difference between the groups. Differences in random urine values between stone forming individuals correlated well with their 24 hour urine data from the clinical laboratory for both calcium and oxalate levels. The SF population exhibited a trend toward higher protein concentrations than the N group, but both groups were well within normal range for mean protein/creatinine values. Both populations were otherwise in good health; though about 32% of SF patients were being treated for hypertension and 24% were being treated for hyperlipidemia (see S3). Most stone formers had no other medical diagnoses.

The urine proteomes were measured using MS. Prior proteomic studies have shown that using label-free spectral counting (SC), as applied here, is a robust measure of protein abundance in a sample given that proper exclusion criteria are applied and adequate spectral counts have been acquired (> 1000 SC).[26, 27] After applying the stringent filtering criteria defined in Methods (see also S4. Complete Methods) to ensure data quality, a total of 407 proteins were identified from these 39 urine samples (see Supplementary Data S5), and they were then sorted by relative abundance and number of times detected in each population (incidence). The average sample contained 3200 spectral counts and identified 80 individual proteins. Table 2 summarizes the 38 proteins that were both moderate to high abundance (>0.5 %SC) and high incidence ($>75\%$ of either population), including their accession numbers and standard abbreviations. These proteins accounted for 82% and 85% of the %SC in the N and SF groups, respectively. The most abundant urine proteins and most moderate abundance proteins (accounting for 72 %SC) did not differ between the two populations, including UROM, OSTP, ALBU and other proteins previously thought to be critical to stone formation. Only six proteins from Table 2 demonstrated significantly different abundance in the SF population compared to the N population ($p<0.05$). IGHA1, IGHA2, IGHG1, IGLC and TRFE were significantly increased, while EGF was significantly decreased in the SF population. These proteins accounted for about 13% of the SF proteome but only 10% of the N proteome. Certainly, the large variances in mean abundances likely limited our ability to identify individual protein differences, but most of these variations arose from differences between individuals in this population study. Variations in mean abundances were much smaller when comparing multiple samples from a single individual (tested in a few individuals).

The remaining lower abundance proteins are summarized in Supplementary Data (S6. %SC), and they have been divided into three groups defined as “lesser” ($0.2\text{--}0.5$ %SC; $> 50\%$ incidence), “low” ($0.1\text{--}0.2$ %SC; $20\text{--}50\%$ incidence) and the “trace” (< 0.1 %SC; $< 10\%$ incidence) in abundance and frequency of detection. The “lesser” abundant protein classification included 40 proteins (10 %SC in the N and 9 %SC in the SF). The “low” abundant proteins included 73 proteins (5 %SC in N and 4 %SC in SF), and “trace” proteins included 252 proteins (3 %SC in N and 3 %SC in SF). Statistical comparisons were not made for proteins in the “lesser”, “low”, and “trace” categories, due to the low reliability of these data (near the detection limit for our method, leading to inconsistent observations). The primary focus of our discussion will be on the dominant proteins listed in Table 2. Many of these proteins are known to interact strongly with CaOx crystals and should overwhelm possible stone interactions due to lower abundance proteins.[4–10]

If individual proteins do not define stone disease mechanisms, then composite properties need to be examined. Since mechanistic studies of crystal aggregation have suggested a role for protein aggregation in stone formation, the relative risk for the protein aggregation within the mixture should be compared for both hydrophobicity and net negative charge density, either of which could contribute to protein aggregation. The GRAVY (Grand Average of hydropathicity) score, a predictor of hydrophobicity, was not different between SF and N proteomes. Comparing the relative quantities of anionic and cationic proteins in the two populations is probably the simplest method for defining change in net negative charge density. Using $pI > 6.5$ to define polycations, SF samples contained a significantly larger cationic protein fraction than the N samples, 22 vs. 18 %SC ($p < 0.04$; see Figure 1). This result agrees with an empirical assessment of net negative charge using colloidal titration that showed a 25% reduction in net negative charge for SF macromolecules compared to normal (unpublished data). This comparison is limited, however, by the lack of post-translational modification data for proteins identified by MS in this study, since most post-translational modifications (i.e., phosphorylation or glycosylation) would increase the number of negative charges and shift the isoelectric point (pI) lower.

The cationic shift in protein distribution can be visualized by plotting a histogram of relative abundance vs pI for each 0.5 pI unit protein grouping (Figure 2). A clear trend is observed in this representation, where SF urine demonstrated systematically reduced abundance of strong polyanions ($pI < 6.0$) with increased abundance of neutral and polycationic proteins ($pI > 6.5$) compared to normal. Proteins between pI 6.5 and 7 were significantly increased in SF urine samples ($p = 0.04$) largely due to increases in IGLC and TRFE. The composite grouping of proteins between 6.5 and 8 was also significantly increased in patient samples ($p = 0.01$) and accounted for nearly 90% of the cationic proteins detected in the urine samples in both populations. In addition to previously noted proteins, PTGDS and IgGamma immunoglobulins contributed to the cationic shift, though differences in their individual abundances were not significant.

Discussion

Before considering the ramifications of our findings in stone research, we should compare our protein abundance observations to prior work. Unfortunately, sample preparation methods strongly influence the apparent abundance of certain proteins. In this report, samples were desalted against a dilute salt solution to protect against losses of proteins through aggregation. In particular, UROM is known to aggregate at higher salt concentrations,[28, 29] which likely contributes to the highly variable results for this protein in prior work. Certainly efforts to standardize urine proteomic methods described variable protein results when precipitation methodology is employed,[30] and some studies explicitly exclude certain fractions of the total proteome.[31] For studies seeking to enumerate the proteins that can be found in urine, these limitations can be tolerated, but only relatively few proteomic studies provide useful comparisons to this report mapping overall protein abundance.

Our data demonstrated the expected finding that albumin and UROM were the dominant proteins in the mixture with significantly smaller amounts of other proteins present. Only

one prior study (Nagaraj, et. al.) reported urine protein abundance data and sample variance in a similar manner for seven normal individuals.[32] Our results were similar to those reported by Nagaraj, et. al. for both relative abundance and level of variance for most moderate and high abundance proteins in Table 2, except for UROM. We observed 12 ± 6 %SC for UROM in our study, while they reported only 1.3%. They did not provide data on salt or creatinine concentrations in their samples, so it is possible that a significant quantity of UROM may have been lost in their initial centrifugation step due to pre-existing aggregation. Their reported values for other proteins (i.e., ALBU - 25.4%, KNG1 - 4.3%, and APOD - 2.7%) agreed well with our observations, especially when we adjusted the UROM concentration within our set to match the 1.3 %SC concentration they reported. Other proteins with appropriate comparison data include IGHA1, where our data were consistent with normal baseline measures reported,[33, 34] and A1AT, where similar concentrations were reported in hypertensive individuals with normal kidney function.[35] Other studies have manipulated samples to enhance lower abundance proteins, by first depleting their samples of high abundance proteins, such as ALBU, UROM and immunoglobulins, which makes comparison with our data problematic.[36, 37]

Previous studies have described specific urine protein differences between stone forming and normal populations,[31, 38] but they were generally biased to only selected proteins or a restricted subset of the proteome with mixed results. A recent comprehensive proteomic study of stone former and normal patient urines conducted by Wright et al. obtained their samples by isoelectric focusing but examined only anionic proteins (pI 4–7) by MS analysis. Their results suggested an important role for carbohydrate metabolism and ceruloplasmin in stone formation,[31] but this observation was not supported by our study. Bergsland, et. al. screened 24 hour urine samples from 50 normals and 50 calcium oxalate stone formers for eight specific urine proteins using antibody detection methods.[38] They found no difference in the relative excretion rates of ALBU, UROM, AMBP and calgranulin A (S10A8), matching our findings for the first three proteins that were among the most abundant proteins detected. S10A8 did not appear to differ in our report (0.2% in SF and 0.3% in N), but its abundance and incidence were too low for reliable statistical analysis. Our observations were quantitatively similar to a previous report showing 0.1 to 0.3% calgranulin.[39] Bergsland, et al. did report differences in AMBP, prothrombin fragment 1, CD59, and Calgranulin B between stone formers and normals, including some gender specific differences. Our study contained reliable data for only AMBP, which did not differ between SF and N. The other three proteins were not detected with adequate sensitivity in our study to reliably compare their relative abundances. We also note that subgroup analysis in our study did not reveal any gender specific differences.

Regarding the implications of our urine proteomics data to stone disease, the overall absence of differences in highly abundant proteins between our SF and normal populations virtually eliminates the possibility that stone disease would be uniquely defined by the presence or absence of a single protein. In particular, abundant proteins previously thought to regulate stone disease, like UROM and OSTP, were found at similar levels in both populations, these proteins would be competing for putative crystal binding sites with any other potential protein regulators of stone formation. Consequently, the influences on stone formation from these strongly interacting, abundant proteins would likely overwhelm any differences due

lower abundance single proteins, as well as post-translational modification differences in single proteins, which were not measured in this study. Significant differences in protein relative abundances were observed in only a handful of low to moderate abundance proteins, including immunoglobulins, TRFE, and EGF. A few additional proteins appeared to be increased in SF urine, if we based our comparison on protein concentrations ($\mu\text{g protein/mg creatinine}$) rather than relative abundances, notably A1AT, A1BG, and A2GL. Even adding these 3 additional proteins to the set of proteins differing between SF and N, the difference in their combined contribution to protein mass (12.0% in N and 16.8% in SFU) was still small compared to the abundant urine protein mass.

The heterogeneity of the stone former population with respect stone phenotype must be examined more closely, since mixing phenotypes may obscure real differences between stone formers and normals. Indeed, somewhat larger variations in protein relative abundances were apparent in our stone former population for the abundant proteins (Table 2), where standard deviations were larger in stone formers for 24 proteins, but larger in normals for only 7 proteins, with the remaining 7 proteins demonstrating identical standard deviations. Subgroup analysis was performed by separating the stone formers into a CaOx dominant group (17 patients with mean CaOx content of $85 \pm 12\%$; M/F = 9/8) and an “Others” group including the 5 patients with no reported stone analysis and 3 patients with phosphate dominant stones. In comparing these subgroups to the complete stone former population, no significant proteomic differences were found between the CaOx dominant and all stone formers. The “Others” subgroup contained somewhat less APOD than either the total or CaOx dominant stone former populations ($3.1 \pm 1.6\%$ in all stone formers vs $2.2 \pm 0.8\%$ in the “Others” subgroup), but all other abundant proteins were statistically similar within these groupings. Even the lower abundance proteins appeared to be similar between the 3 groups. Not surprisingly, only subtle differences were noted in the identities of proteins that were statistically different from the normal proteome distribution in either subgroup compared to the result above using the total stone former population. Since the resulting groups were no longer appropriately matched for gender, we feel that these differences are more likely to be the result of statistical artifact from grouping smaller numbers of patients than from true physiological differences.

A second form of heterogeneity in this group of idiopathic CaOx stone formers is encapsulated in the differences in urine chemistry normally used to define risk categories in the clinical management of stones; specifically, hypercalciuria, hyperoxaluria, and hypocitraturia. Among these 25 stone formers, we identified 10 (40%) hypercalciuric patients (M/F=6/4), 6 (24%) hyperoxaluric patients (M/F=4/2), 6 (24%) hypocitraturic patients (M/F=2/4), 4 (16%) of these patients exhibited multiple risk factors (1 with all 3 risk factors; M/F=3/1), and 8 (32%) with all normal findings (M/F=2/6). This distribution of risk factors was similar to that reported by Levy et al.[40] though our population showed somewhat fewer hypercalciurics and somewhat more hyperoxalurics than the earlier report. As noted with the stone composition assignments, most patients in these groups were inconsistently categorized by their urine chemistry with only 3 hypercalciurics, 2 hyperoxalurics, and 2 hypocitraturics consistently exceeding their respective cutoffs for inclusion in each group. Subgroup analyses using groups defined by these cutoff values were attempted with similar results to those seen above for stone composition. Only subtle

changes in the identities of proteins that differed from the normal proteome distribution were seen in comparing results from these subgroups to the observations above using the total stone former cohort. Consequently, we have concluded again that these differences were more likely attributable to statistical artifact from grouping smaller numbers of patients rather than true physiologic differences in these subgroups, especially since we have lost matching between the pools. While patients with these various urine phenotypes may exhibit unique proteomic differences, our results suggest either that a much larger pool of patients will be required, or that patients exhibiting more extreme deviations from normal must be studied. We will restrict further discussions to the comparison of the full populations.

We note that the proteins exhibiting differences in this study are linked to a single metabolomics network (ingenuity.com) connecting immune response, synthesis of reactive oxygen species and chronic inflammation (see Supplementary Data S7). Earlier studies have reported immunoglobulins in stone matrix,[14, 15, 18] and immune-complement and inflammation response proteins have been associated with stone disease.[14, 19, 41] Similarly, fibrotic proteins associated with the injury and healing processes have been detected in stone matrix in several reports,[14, 18] supporting the conclusion that stone formers have underlying kidney disease.[42] Not surprisingly, many other disease linkages have been reported with these same proteins (i.e., TRFE with diabetes and hypertensive,[35, 43] A1BG with bladder cancer,[44] A2GL with appendicitis,[45] IGLC with renal tubule function assessment,[46] and IgGamma class immunoglobulins with diabetes and hypertension).[34] The inclusion of blood related proteins (i.e. A1AT and TRFE) also provided a conceptual conundrum, because hematuria is commonly associated with stone disease,[16] though no samples tested were positive for blood by urine dipstick.

Our data cannot differentiate cause from effect in stone disease for this group of proteins. The studies mentioned above showing correlations between increased immunoglobulins and other disease states suggest that this altered distribution may be a consequence of stone disease, rather than a cause. On the other hand, composite measures of N and SF proteomes did reveal a clear cationic shift in the protein distribution of SF. While the choice of $pI > 6.5$ was arbitrary, the SF protein distribution demonstrated an overall reduction in polyanions and increased content of polycations compared to normal (see Figure 2). Notably, 3 of the 5 proteins that were significantly increased in abundance in SF had $pI > 6.5$, while the lone protein found in decreased abundance had $pI < 6.5$. This systematic cationic shift would undermine the electrostatic stabilization of the protein mixture, potentially fostering protein aggregation in urine and subsequent stone formation.

We and others have shown that protein aggregation can contribute to crystal aggregation.[12, 20, 21, 23, 24, 29] Observations of stone morphology over many years have shown that aggregation of preformed crystals with an existing stone must play a significant role in stone growth.[47] Unfortunately, the absence of data on post-translational modifications in this study prevents us from testing for the loss of anionic side chains (i.e., glycosyl or phosphoryl), as suggested in our earlier work,[12] so our data are insufficient to truly distinguish between the two prior protein aggregation models; the electrostatically driven association between strong polyanions and strong polycations[20, 21] versus the inherent solution instability of weak polyanions in salt solutions.[12] Nevertheless, we conclude that

the observed cationic shift in protein distribution suggests a role for protein aggregation in stone formation. Definitive confirmation of this hypothesis will require data from other experiments, since this study cannot define either the origin of these proteomic shifts or their role in stone formation.

Conclusion

We compared the abundant urine proteomes between CaOx stone formers and normals. The absence of differences in highly abundant proteins argues against the correlation of stone formation with any single protein, particularly since many abundant proteins that did not differ between stone formers and normal are known to interact strongly with CaOx crystals. Significant differences were found in only a handful of relatively low abundance inflammatory and immune proteins that have been associated with stone formation in other studies, though this study cannot distinguish between cause and effect in stone formation. However, the observed cationic shift in SF suggests a role for protein aggregation in stone formation that will need to be confirmed through other experimental approaches.

Concise Methods

Research study participation

Patients with established or reported CaOx stone formation were recruited from our Stone Clinic population and from the community, excluding patients with any of the established metabolic conditions with known risk for CaOx stones as defined in the recent consensus statement (such as hyperparathyroidism, sarcoidosis, calcium handling disorders, excessive intake of supplements, chronic diarrhea from surgical or medical disease, renal transport disorders or medullary sponge kidney).[25] These idiopathic CaOx stone formers were entered into our research study on the influence of urine macromolecules on crystal aggregation following informed consent (VA-IRB approved study: 9305-01P).

Urine sample collection and initial characterization

Freshly voided random urine samples were collected, centrifuged briefly (1,000g x 5 minutes) and ultrafiltered (10 kDa, 10mM NaCl). The recovered urine macromolecules (UM) were stored at -80C. Urine dipsticks were used as a preliminary urine diagnostic tool to screen for hematuria (Multistix 10SG, Siemens, Malvern, PA).

Urine Anion and Cation Measurement

Urine anion, cation, and creatinine concentrations were determined using ion chromatography (Dionex) as described earlier.[48]

Protein Assays

Initial urine protein concentrations were determined using a pyrogallol red based protein assay (Sigma-Aldrich, Milwaukee, WI). Isolated UM samples were assayed using a commercially available protein dye reagent (Biorad). Both assays used bovine serum albumin as a standard.

Mass spectrometry-based proteomics (MS)

MS analyses were performed at the Medical College of Wisconsin Mass Spectrometry Facility, Milwaukee, WI. Equivalent samples (20 µg) of UM were lyophilized and in-gel trypsin digested prior to loading on the ThermoFinnigan LTQ Mass Spectrometer (MSⁿ capability) coupled to an Eksigent nanoHPLC system.[26] Capillary columns (10 cm x 75 µm) packed with 3 µm Magic C18AQ particles (Michrom-Bruker, Auburn, CA) were used for nano-HPLC separation. Two technical replicates were run for each sample. All spectra were searched against the human Uniprot database and all analyses of results were processed using Visualize software.[49, 50] Filters were applied to the datasets, including exclusion of common contaminants and redundant proteins, a peptide probability <0.85 (5% FDR), and a peptide count >2. The total spectral counts in the filtered dataset must be >1000 for sample inclusion. Relative protein abundance was then defined as percent total spectral counts for each given sample. See supplementary data for complete MS analysis details.

Statistical analysis

All data are reported as mean values ± standard deviation, using unpaired Welch's t-test to report statistical significance (p<0.05).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Distribution of anionic and cationic urine proteins

The relative protein abundance data were sorted on the basis of isoelectric point grouping based on $pI < 6.5$ (anionic) and $pI > 6.5$ (cationic). The SFU samples have a significantly greater cationic protein fraction 22 ± 6 %SC vs. 18 ± 5 %SC ($p=0.04$).

Figure 2. Isoelectric point (pI) histogram

The relative protein abundance data was sorted on the basis of pI and represented in 0.5 pI increments for normal (N; light gray) and stone former urine (SF; dark gray) samples. The pI region 6.5 to 7 was significantly increased in SF samples (9 ± 3 vs. 7 ± 3 %SC; * $p = 0.04$). The combined pI region of 6.5 to 8 accounted for 16 ± 4 %SC in NU and 20 ± 5 %SC in SFU; ($¥ p = 0.01$).

Table 1

Patient Demographics

	n (Female)	Age, years	Urine Protein, mg/L	Creatinine, mg/dL	Prot/Creat, mg/g	Ca, mg/dL	Ox, mg/dL	Calc Ca/Cr, mg/g	Calc Ox/Cr, mg/g
N	14 (9)	41	100	110	110	13	1.7	120	21
	SD	14	40	70	40	8	0.5	50	12
SF	25 (15)	46	140	110	140	12	2.9	130	32
	SD	13	80	70	80	9	1.4	70	19
p =		0.294	0.034	0.996	0.066	0.812	0.001	0.588	0.023

Table 2

Predominant Urine Proteins (demonstrating both high incidence and abundance).
 38 proteins were detected in greater than 75% of either population (11 of 14 NU; 19 of 25 SF) and constitute >82% of the total spectral count of the urine proteome.

Access #	Protein Description	Abbr.	pI	N	Average %SC	SF	Average %SC	p =	N	SF
									n	n
Q9P117	Albumin	ALBU	5.97	20 ± 6	20 ± 9	0.933	14	25		
P07911	Uromodulin (Tamm-Horsfall glycoprotein)	UROM	5.07	12 ± 6	13 ± 7	0.768	14	25		
P02760	AMBIP protein (α-1-microglobulin; Inter-α-trypsin inhibitor light chain (Bikunin)).	AMBIP	5.98	3.6 ± 1.0	3.0 ± 1.6	0.139	14	25		
P41222	Prostaglandin-H2D-isomerase (β-trace)	PTGDS	7.34	3.6 ± 1.6	3.8 ± 1.6	0.747	14	25		
P05090	Apolipoprotein-D	APOD	5.07	3.2 ± 1.5	3.1 ± 1.6	0.832	14	25		
P01042	Kininogen-1	KNG1	6.41	3.2 ± 0.9	3.2 ± 1.3	0.900	14	25		
Q08380	Galectin-3-binding protein	LG3BP	5.14	2.7 ± 1.2	2.3 ± 1.3	0.323	14	25		
P10451	Osteopontin	OSTP	4.38	2.6 ± 1.5	2.4 ± 1.0	0.590	14	25		
P01876	IgAlpha-1 chain C	IGHA1	6.14	2.0 ± 0.8	2.8 ± 1.3	0.013	14	25		
Q6EMK4	Vasorin	VASN	7.01	1.5 ± 0.5	1.5 ± 0.6	0.658	14	25		
P02787	Transferrin	TRFE	6.76	1.2 ± 0.9	1.9 ± 1.1	0.040	14	25		
P01133	Epidermal growth factor (Urogastrone)	EGF	5.61	2.8 ± 1.1	2.0 ± 1.1	0.038	14	24		
Q96FE7	Phosphoinositide-3-kinase-interacting protein 1	P3IP1	4.93	1.3 ± 0.7	1.1 ± 0.6	0.546	14	24		
Q8N177	α-1-antichymotrypsin	AACT	5.36	0.8 ± 0.4	0.9 ± 0.5	0.622	14	24		
Q16208	CD44 antigen	CD44	5.16	0.9 ± 0.4	0.6 ± 0.5	0.090	14	23		
P10909	Clusterin	CLUS	5.95	0.7 ± 0.5	0.6 ± 0.5	0.665	14	23		
P05155	Plasma protease C1 inhibitor	IC1	6.16	1.1 ± 0.5	0.9 ± 0.6	0.169	14	21		

Access #	Protein Description	Abbr.	pI	N Average %SC	SF Average %SC	p =	N	SF
P10253	Lysosomal alpha-glucosidase	LYAG	5.68	0.9 ± 0.6	0.7 ± 0.7	0.365	14	20
P01009	α-1-antitrypsin	A1AT	5.40	1.5 ± 0.8	1.8 ± 0.6	0.231	13	25
P01877	IgAlpha-2 chain C	IGHA2	5.78	1.3 ± 0.7	1.9 ± 1.0	0.025	13	24
P01857	IgGamma-1 chain C	IGHG1	7.95	1.1 ± 0.8	1.9 ± 1.2	0.021	13	24
P25311	Zinc-α-2-glycoprotein	ZA2G	5.62	1.3 ± 1.1	1.6 ± 1.5	0.537	13	22
Q14624	Inter-α-trypsin inhibitor heavy chain H4	ITIH4	6.58	0.9 ± 0.4	0.9 ± 0.5	0.955	13	22
P05154	Plasma serine protease inhibitor	IPSP	9.08	0.8 ± 0.5	0.6 ± 0.5	0.323	13	21
P01833	Polymetric-immunoglobulin receptor	PIGR	5.61	0.5 ± 0.4	0.3 ± 0.3	0.083	13	18
P01842	IgLambda chain C	IGLC	6.62	1.1 ± 0.8	2.8 ± 1.8	0.0002	12	24
P04217	α-1B-glycoprotein	A1BG	5.63	0.5 ± 0.5	0.9 ± 0.6	0.091	12	23
P98164	Low-density lipoprotein receptor-related protein 2 (Megalin)	LRP2	4.91	0.7 ± 0.5	0.6 ± 0.6	0.581	12	22
Q9UNN8	Endothelial protein C receptor	EPCR	6.73	1.0 ± 0.8	0.7 ± 0.7	0.294	12	21
P00790	Pepsin A	PEPA	4.18	0.6 ± 0.4	0.4 ± 0.3	0.133	12	20
P06396	Gelsolin	GELS	5.96	0.7 ± 0.8	1.0 ± 1.0	0.344	12	19
P06870	Kallikrein-1	KLK1	4.64	1.0 ± 0.8	0.8 ± 0.8	0.556	12	18
P01834	IgKappa chain C	IGKC	5.63	1.6 ± 1.2	1.8 ± 1.2	0.718	11	22
P08571	Monocyte differentiation antigen CD14	CD14	5.90	0.8 ± 0.5	0.7 ± 0.6	0.458	11	21
P02750	Leucine-rich α-2-glycoprotein	A2GL	6.52	0.5 ± 0.5	0.8 ± 0.7	0.205	11	18
P98160	Basement membrane-specific heparan sulfate proteoglycan (Perlecan)	PGBM	6.13	0.5 ± 0.4	0.4 ± 0.5	0.469	11	16
P15144	Aminopeptidase N	AMPN	5.33	0.7 ± 0.7	0.3 ± 0.4	0.067	11	15

Access #	Protein Description	Abbr.	pI	N Average %SC	SF Average %SC	d	n	n	SF
P01859	IgGamma-2 chain C	IGHG2	7.30	0.8 ± 0.9	1.3 ± 0.9	0.133	9	23	

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