Published in final edited form as:

J Pediatr Urol. 2015 August ; 11(4): 217.e1–217.e6. doi:10.1016/j.jpuro.2015.04.020.

Urine proteomic analysis in cystinuric children with renal stones

Larisa Kovacevic^{a,*}, Hong Lu^a, David S. Goldfarb^b, Yegappan Lakshmanan^a, and Joseph A. Caruso^c

^aDepartment of Pediatric Urology, Children's Hospital of Michigan, Detroit, MI, USA

^bNephrology Division, NYU Langone Medical Center, New York, NY, USA

^cProteomic Facility, Wayne State University, Detroit, MI, USA

Summary

Introduction—The gene mutations responsible for cystinuria do not fully explain kidney stone activity, suggesting that specific proteins may serve as promoters of cystine precipitation, aggregation, or epithelial adherence. In this study we assessed: (1) the differences in urinary proteins between children with cystinuria and kidney stones (CYS) and healthy controls (HC), with particular attention to the fibrosis-related proteins, and (2) the presence of diagnostic biomarkers for CYS.

Material and methods—We conducted a pilot study comparing individual urinary proteomes of two newly diagnosed children with CYS and two age- and gender-matched HC, using liquid chromatography-mass spectrometry. Relative protein abundance was estimated using spectral counting. Proteins of interest in both CYS and HC were selected using the following criteria: i) 5 spectral counts; ii) 2-fold difference in spectral counts; and iii) 0.05 *p* value for the Fisher's Exact test.

Discussion—This study demonstrates a different urinary polypeptide profile in two children with CYS compared with two HC. Of the 623 proteins identified by proteomic analysis, 180 exhibited at least a 2-fold increased relative abundance in CYS compared with HC. Of these, 39 were involved in response to stress, 26 in response to wounding, 21 in inflammatory response, 18 in immune response, and 4 in cellular response to oxidative stress. One-hundred and thirty-three proteins were found only in children with CYS, 33 of which met the selection criteria. Of these 33 unique proteins, six are known to be associated with fibrosis pathways (Table).

*Corresponding author: Children's Hospital of Michigan, Department of Pediatric Urology, 3901 Beaubien Blvd, 48201 Detroit, MI, USA, lkovacev@dmc.org.

Conflict of interest

None.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Accession number ^a	Protein	MW (kDa)	No. of assigned peptides ^b	No. of unique peptides ^b	Sequence coverage (%)
ACTG	Actin	42	202/117	52/37	70/51
VIME	Vimentin	54	10/9	8/5	21/14
HSP70	Heat shock 70 kDa protein	70	24/18	7/5	14/15
ITIH2	Inter-alpha-trypsin inhibitor	106	33/81	12/11	27/16
MMP9	Matrix metalloproteinase 9	78	26/15	9/7	18/12
PON1	Serum paraoxonase	40	23/74	9/14	48/55

^aHuman.

^bValues presented in each patient (Patient 1/Patient 2).

The major limitation of this study is the small number of samples that were analyzed. Validation using highly specific methods such as ELISA is needed.

Conclusion—We provide proteomic evidence of oxidative injury, inflammation, wound healing, and fibrosis in two children with CYS. We speculate that oxidative stress and inflammation may cause remodeling via actin and vimentin pathways, leading to fibrosis. Additionally, we identified ITIH and MMP-9 as potential diagnostic biomarkers and novel therapeutic targets in CYS. These proteins merit further investigation.

Keywords

Child; Cystinuria; Kidney calculi; Kidney stone; Nephrolithiasis; Proteomics; Renal aminoacidurias; Renal tubular transport; Inborn errors; Urolithiasis

Introduction

Cystinuria is an autosomal recessive disorder (SLC3A1 mutations) or autosomal dominant with incomplete penetrance (SLC7A9 mutations) characterized by impaired transport of cystine and dibasic amino acids in the proximal renal tubule [1]. It is caused by mutations in *SLC3A1*, the gene that encodes one of the two components of the neutral and basic amino acid transport protein, rBAT [2]. Mutations in *SLC7A9*, which encodes for the light component of the transporter, b⁰⁺AT, also causes cystinuria. However, these mutations cannot fully explain kidney stone activity. For example, not all patients with two mutated *SLC3A1* genes will form a stone. Additionally, patients with identical genotypes may have onset of stone disease at different ages and with different severity. Therefore, other factors contribute to kidney stone formation. These factors may include endogenous proteins that could serve as promoters or inhibitors of cystine precipitation, aggregation or epithelial adherence. Proteomics allows simultaneous examination of the patterns of multiple urinary proteins and has become one of the most promising tools in nephrology and urology [3,4].

Cystinuria frequently leads to large and recurrent kidney stones, and consequently there is a need for multiple procedures [5]. Renal function in patients with cystine kidney stones is reduced compared with those with non-cystine stones [6], but the cause of this decline is not

entirely clear. Biopsies of the papillae performed in patients with cystine kidney stones show interstitial fibrosis [7]. Therefore, preventive strategies and early diagnosis and treatment are crucial in this condition.

In this study we assessed i) the differences in the urinary proteins between children with cystinuria and kidney stones (CYS), and healthy controls (HC), with particular attention to the fibrosis-related proteins, and ii) the presence of diagnostic biomarkers for CYS.

Methods

Sample collection and preparation

We compared urinary proteomes of two newly diagnosed children with CYS and two age- and gender-matched HC, using liquid chromatography-mass spectrometry (LC-MS/MS). Both CYS children had normal kidney function, and were not on CYS treatment. Second morning midstream fresh urine samples were obtained in sterile cups, prepared within 3 hours of collection (centrifuged at 2500 rpm for 15 minutes) and stored as recommended by standardized protocols (developed by the Human Urine and Kidney Proteome Project, HUKPP, and the European Urine and Kidney Proteomics, EuroKUP Initiatives) until use. In the literature, different types of urine samples are used for proteomic studies, including first morning, second morning, random, or 24-hour urine collection [8]. However, there is no general consensus regarding the ideal urine sample. At first look, the 24-hour urine collection and the first morning urine appear to be the best specimens for the analysis of proteins; the 24-hour urine collection reflects the total amount of urinary proteins over a day, while the first morning urine avoids the protein fluctuation seen during a 24-hour period. However, several studies have shown that both these collections are less practical and that urine contamination may occur because of bacterial overgrowth, potentially affecting the urinary proteomics [9,10]. Therefore, we chose to perform the proteomic analysis on a second morning urine specimen.

Proteins in each sample were concentrated in a Centricon-type filter. Albumin and IgG were removed by anti-HAS/IgG resin (Sartorius). Protein concentration in each sample was measured by the BCA protein assay (Pierce). 30 µg of protein was separated by SDS-PAGE, the gel stained with Coomassie Blue, and the lanes cut into 12 slices per sample. Proteins were reduced, alkylated with iodoacetamide, and digested with trypsin (Promega) in-gel. Peptides were eluted with 0.1% formic acid and acetonitrile (1:1 by volume), then dried and stored at -80 °C.

LC-MS/MS

Dried peptides were resuspended in 5% acetonitrile/0.1% formic acid/0.005% trifluoroacetic acid and desalted on-line prior to separation by reverse phase chromatography. Peptides were identified and quantified using a LTQ-XL mass spectrometer (Thermo Fisher). Peak lists were generated with Proteome Discoverer (Thermo, version 1.3) and MS2 data were scored using Mascot (Matrix Science, version 2.4.0) against human protein sequences within the SwissProt database (20,255 entries) and simultaneously against a scrambled decoy database. Results were imported into Scaffold (Proteome Software, version 4.3) and re-

analyzed with X!Tandem against a subset protein database. Protein identifications were accepted if they achieved a 99% probability by the Peptide Prophet algorithm, and with at least two unique peptide identifications with 80% probability.

Proteomic data analysis

Relative protein abundance was estimated using spectral counting. This label-free method uses the number of spectra observed for each protein as a measure of relative abundance. Proteins of interest were selected using the following criteria: i) 5 spectral counts; ii) 2-fold difference in spectral counts; and iii) 0.05 *p* value for the Fisher's Exact test. Two-fold change in protein abundance was chosen as a threshold to be well above observed technical variations in MS experiments. Protein function was analyzed using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) online bioinformatics resource, and ReactomeFI application within Cytoscape (ver 3.1.1) was used to investigate protein-protein interaction networks.

The significant differentially expressed proteins were used to analyze the group differences for biological processes using Gene Ontology (GO) annotation via the DAVID online bioinformatics resource. DAVID uses a modified Fisher's Exact *p* value to determine whether a particular GO-term is over- or under-represented. The key nodes of proteins of interest were identified using Cytoscape.

Statistical analysis

The *p* value of each category included in the GO annotation was calculated using Fisher's Exact test. Statistical analyses were conducted with IBM SPSS version 20.

Results

Two CYS female children aged 15 (patient 1) and 7 years (patient 2), and two age- and gender-matched healthy controls were studied. The patient's baseline laboratory characteristics are shown in Table 1. At the time of the study, both patients had active stones as indicated by their renal and bladder ultrasound. Patient 1 had bilateral kidney stones with right staghorn calculus measuring 2.4 cm, while patient 2 had three stones at around 0.7 mm each in the right kidney.

Of the 623 proteins identified by proteomic analysis (supplementary data available upon request), 49 proteins were identified in controls only, and 133 in patient samples only (Fig. 1). One-hundred and eighty proteins met our cutoff limits, including at least 2-fold difference between CYS and HC. Of these, 94 proteins were upregulated in CYS, 39 of which were involved in response to stress, 26 in response to wounding, 21 in inflammatory response, 18 in immune response, and 4 in cellular response to oxidative stress (Fig. 2). Eight of the 21 proteins involved in inflammatory response were found to have a role in the classical and alternative complement pathways. Eighty-six proteins were downregulated in CYS, 26 of which were involved in cell adhesion. Eight of the 26 adhesion proteins consisted of cadherins and protocadherins.

One-hundred and thirty-three proteins were found only in children with CYS, 33 of which met the selection criteria. Of these 33 unique proteins, six are known to be involved in fibrosis pathways, and included actin, vimentin, heat shock 70 kDa protein, inter-alpha-trypsin-inhibitor heavy chain, matrix metalloproteinase-9, and serum paraoxonase.

We identified ceruloplasmin, neutrophil gelatinase-associated lipocalin, and vitamin D binding protein among the proteins with at least 2-fold increased abundance in CYS compared with HC (Table 2). Additionally, lower levels of osteopontin (5× lower), and uromodulin (3× lower) were found among the proteins with at least 2-fold decreased abundance in CYS compared with HC. Protein-protein interaction modeling of the 2-fold increased proteins in the CYS group revealed the complement pathway as being the most significant, with the HSP90 and 14–3–3 protein zeta being the key nodes in one local network, and complement component 3 in another (Fig. 3). Proteins in the cadherin signaling pathway were significantly over-represented in controls, with cadherin 1 identified as the key node (Fig. 4).

Discussion

In this study we found a different urinary polypeptide profile in two children with cystinuria and kidney stones compared with healthy controls. Urine samples from these two CYS children have increased levels of proteins involved in oxidative injury, inflammation, wound healing, and fibrosis pathways. We speculate that oxidative stress and inflammation may cause remodeling via actin and vimentin pathways, leading to fibrosis. This indicates that an aggressive approach in these children may be warranted. Indeed, biopsies of the renal papillae done in adults with cystinuria and kidney stones showed significant interstitial inflammation, fibrosis, and scarring, as well as glomerular injury [7]. These processes explain the lower glomerular filtration rate and the potential risk of losing one kidney reported in these patients [6,11].

Inflammation was reported in adults with calcium oxalate, calcium phosphate, and uric acid stones [12–14]. Complement membrane attack complex represents mediators of crystal-induced acute inflammation. The high numbers of immune response and inflammatory proteins found in our study suggest crystal-induced injury favoring crystal attachment and further renal epithelial damage. The release of free radicals and lipid peroxidation of the cell membrane appear to make a major contribution to the renal epithelial injury initiated by crystals [15,16]. Impaired tubular cells alter reabsorption of proteins, leading to their subsequent excretion in the urine [17].

Specific proteins have been shown to be involved in calcium oxalate crystal nucleation, growth, and aggregation, as well as crystal inhibition [17]. Proteomic analysis of urine allows identification of a large number of proteins and represents a non-invasive, sensitive, and reproducible tool for characterizing various kidney conditions including kidney stones [4,18]. The identified proteins in urine may represent biomarkers for prevention and early recognition of stones, allowing earlier intervention and avoiding complications such as kidney damage [19]. To date, few studies have been performed to compare the urinary proteome of adult stone-formers with that of non-stone formers, with all but one study done

in non-cystinuric patients. Among reported proteins, higher level of ceruloplasmin, and lower levels of uromodulin and osteopontin were found in the urine of adult non-cystinuric stone-formers [20,21]. In agreement with these studies, we found the same trend (upregulation of ceruloplasmin and downregulation of osteopontin and uromodulin) in CYS children. The significance of these urinary proteins in cystinuric patients is not yet clear. Osteopontin originates from renal tubular cells, and gets excreted when these cells are exposed to stress [22]. Osteopontin has been shown to inhibit calcium oxalate crystal formation *in vitro* [23], and participates in extracellular matrix remodeling and inhibition of apoptosis [24]. Uromodulin, also known as Tamm-Horsfall protein, is excreted by tubular cells, and has been shown to either inhibit or promote crystallization [25]. Ceruloplasmin is an acute phase protein, and plays a major role in copper transport. It has multiple cationic binding sites that may alter the solubility of crystals. Its definite role in stone formation has yet to be established.

Only one report exists in the literature regarding urinary proteomes in patients with cystinuria and kidney stones. This study recently reported a proteomic analysis of urinary exosomes in eight patients (six adults) with cystinuria and 10 controls [26]. Out of the 38 proteins overexpressed in cystinuric patients, they found 18 inflammatory proteins, pointing to the importance of inflammation in cystinuria. Additionally, this group identified proteins involved in wound healing and oxidative process, which is in agreement with our study.

The major limitation of this study is the small number of samples that were analyzed. The intra- and inter-individual variabilities of urine samples, and technical variabilities associated with mass spectrometry-based analyses mean that a large number of samples would be required to obtain the clearest picture of the proteomic alterations associated with disease [8]. Nonetheless, this was intended as a pilot study to generate hypotheses and identify novel biomarkers and intracellular pathways that will require further investigation. Validation using highly specific methods such as ELISA is needed. However, there is an assumption that a small group of core proteins that are associated with disease would remain constant, even in a small proteomic dataset. Indeed, the individual values of the studied proteins were pretty similar in both our patients, as seen in Table 2. We are at the discovery stage, with the goal of identifying members of this smaller group of core proteins, which would be tested in a more rigorous manner in a larger group of samples.

In conclusion, we provide proteomic evidence of oxidative injury, inflammation, wound healing, and fibrosis in two children with cystinuria and kidney stones. We speculate that oxidative stress and inflammation may cause remodeling via actin and vimentin pathways, leading to fibrosis. Additionally, we identified ITIH and MMP-9 as potential diagnostic biomarkers and novel therapeutic targets in CYS. These proteins merit further investigation.

Acknowledgments

Funding

This research is being funded by a grant from the Rare Kidney Stone Consortium (#U54DK083908), a member of the NIH Rare Diseases Clinical Research Network (RDCRN), supported through a collaboration between the National Center for Advancing Translational Sciences (NCATS), and the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). The Wayne State University Proteomics Core is supported through the

NIH Center Grant P30 ES 020957, the NIH Cancer Center Support Grant P30 CA 022453 and the NIH Shared Instrumentation Grant S10 OD 010700.

References

1. Chillarón J, Font-Llitjós M, Fort J, Zorzano A, Goldfarb DS, Nunes V, et al. Pathophysiology and treatment of cystinuria. *Nat Rev Nephrol*. 2010; 6(7):424–4. [PubMed: 20517292]
2. Pras E, Arber N, Aksentijevich I, Katz G, Schapiro JM, Prosen L, et al. Localization of a gene causing cystinuria to chromosome 2p. *Nat Genet*. 1994; 6(4):415–9. [PubMed: 8054985]
3. Thongboonkerd V. Proteomics and kidney stone disease. *Contrib Nephrol*. 2008; 160:142–8. [PubMed: 18401167]
4. Cadieux PA, Beiko DT, Watterson JD, Burton JP, Howard JC, Knudsen BE, et al. Surface-enhanced laser desorption/ionization-time of flight-mass spectrometry (SELDI-TOF-MS): a new proteomic urinary test for patients with urolithiasis. *J Clin Lab Analysis*. 2004; 18(3):170–5.
5. Dahlberg PJ, Van den Berg CJ, Kurtz SB, Wilson DM, Smith LH. Clinical features and management of cystinuria. *Mayo Clin Proc*. 1977; 52:533–2. [PubMed: 895195]
6. Assimios DG, Leslie SW, Ng C, Streem SB, Hart LJ. The impact of cystinuria on renal function. *J Urol*. 2002; 168:27–30. [PubMed: 12050485]
7. Evan AP, Coe FL, Lingeman JE, Shao Y, Matlaga BR, Kim SC, et al. Renal crystal deposits and histopathology in patients with cystine stones. *Kidney Int*. 2006; 69(12):2227–5. [PubMed: 16710357]
8. Thongboonkerd V. Practical points in urinary proteomics. *J Proteome Res*. 2007; 6(10):3881–90. [PubMed: 17824635]
9. Bottini PV, Ribeiro Alves MA, Garlipp CR. Electrophoretic pattern of concentrated urine: comparison between 24-hour collection and random samples. *Am J Kidney Dis*. 2002; 39(1):E2. [PubMed: 11774128]
10. Hoorn EJ, Pisitkun T, Zietse R, Gross P, Frokiaer J, Wang NS, et al. Prospects for urinary proteomics: exosomes as a source of urinary biomarkers. *Nephrology (Carlton)*. 2005; 10(3):283–90. [PubMed: 15958043]
11. Worcester E, Parks JH, Josephson MA, Thisted RA, Coe FL. Causes and consequences of kidney loss in patients with nephrolithiasis. *Kidney Int*. 2003; 64:2204–13. [PubMed: 14633144]
12. Khan SR, Canales BK. Genetic basis of renal cellular dysfunction and the formation of kidney stones. *Urol Res*. 2009; 37(4):169–80. [PubMed: 19517103]
13. Jou YC, Fang CY, Chen SY, Chen FH, Cheng MC, Shen CH, et al. Proteomic study of renal uric acid stone. *Urology*. 2012; 80(2):260–6. [PubMed: 22516363]
14. Khan SR. Crystal-induced inflammation of the kidneys: results from human studies, animal models, and tissue-culture studies. *Clin Exper Nephrol*. 2004; 8(2):75–8. [PubMed: 15235923]
15. Habibzadegah-Tari P, Byer KG, Khan SR. Reactive oxygen species mediated calcium oxalate crystal-induced expression of MCP-1 in HK-2 cells. *Urol Res*. 2006; 34(1):26–36. [PubMed: 16397773]
16. Thamilselvan S, Hackett RL, Khan SR. Lipid peroxidation in ethylene glycol induced hyperoxaluria and calcium oxalate nephrolithiasis. *J Urol*. 1997; 157(3):1059–63. [PubMed: 9072543]
17. Kumar V, Lieske JC. Protein regulation of intrarenal crystallization. *Current Opin Nephrol Hypertens*. 2006; 15(4):374–80.
18. Haubitz M, Wittke S, Weissinger EM, Walden M, Rupprecht HD, Floege J, et al. Urine protein patterns can serve as diagnostic tools in patients with IgA nephropathy. *Kidney Int*. 2005; 67(6):2313–20. [PubMed: 15882273]
19. Coon JJ, Zürlbig P, Dakna M, Dominiczak AF, Decramer S, Fliser D, et al. CE-MS analysis of the human urinary proteome for biomarker discovery and disease diagnostics. *Proteomics Clin Appl*. 2008; 10(2):964. [PubMed: 20130789]
20. Wright CA, Howles S, Trudgian DC, Kessler BM, Reynard JM, Noble JG, et al. Label-free quantitative proteomics reveals differentially regulated proteins influencing urolithiasis. *Mol Cell Proteomics*. 2011; 10(8):M110. [PubMed: 21474797]

21. Wai-Hoe L, Wing-Seng L, Ismaili Z, Lay-Harn G. Proteomics and detection of uromodulin in first-time renal calculi patients and recurrent renal calculi patients. *Appl Biochem Biotechnol.* 2009; 159(1):221–2. [PubMed: 19145410]
22. Nangaku M, Pippin J, Couser WG. Complement membrane attack complex (C5b-9) mediates interstitial disease in experimental nephrotic syndrome. *J Am Soc Nephrol.* 1999; 10:2323–31. [PubMed: 10541291]
23. Atmani F, Khan S. Role of urinary bikunin in the inhibition of calcium oxalate crystallization. *J Am Soc Nephrol.* 1999; 10(14):385–8.
24. Kazanecki CC, Uzwiak DJ, Denhardt DT. Control of osteopontin signaling and function by post-translational phosphorylation and protein folding. *J Cell Biochem.* 2007; 102:912–14. [PubMed: 17910028]
25. Knorle R, Schnierle P, Koch A, Buchholz N, Hering F, Seiler H, et al. Tamm-Horsfall glycoprotein: role in inhibition and promotion of renal calcium oxalate stone formation studied with Fourier-transform infrared spectroscopy. *Clin Chem.* 1994; 40:1739–43. [PubMed: 8070085]
26. Bourderioux M, Nguyen-Khoa T, Chhuon C, Jeanson L, Tondelier D, Walczak M, et al. A new workflow for proteomic analysis of urinary exosomes and assessment in cystinuria patients. *J Proteome Res.* 2015; 14:567–77. [PubMed: 25365230]

Figure 1.
Venn diagram representing the unique and common proteins identified in healthy control children compared to children with cystinuria and kidney stones. Concentrated urinary proteins were first separated by SDS-PAGE and then analyzed by LC-MS/MS.

GO Terms: Biological Process

Figure 2.

Gene ontology (GO) analysis of urinary proteins with relative decreased or increased abundance in children with cystinuria and kidney stones compared to healthy children. Proteins which were either i) only identified in either group, or ii) estimated to have at least a 2-fold difference in relative abundance were mapped against GO term lists to determine which biological processes were enriched. *, p -value < 0.05 .

Figure 3.

Protein-protein interaction network modeling of proteins with increased abundance in patients compared to healthy controls. In this representation, proteins are shown as nodes, and known interactions as edges. 'Key node' proteins with the highest number of interacting partners are colored yellow.

Abbreviations: ACTG1: Actin, Gamma 1; C3: Complement Component 3; C4BPA: Complement Component 4 Binding Protein, Alpha; C5: Complement Component 5; C6: Complement Component 6; C9: Complement Component 9; CALM1: Calmodulin; CFL1: Cofilin; CFB: Complement Factor B; CFH: Complement Factor H; GAPDH: Glyceraldehyde-3-Phosphate Dehydrogenase; HSP90AA1: Heat Shock Protein 90kDa Alpha, Class A Member 1; HSP90AA2: Heat Shock Protein 90kDa Alpha, Class A Member 2; HSPA1A: Heat Shock 70kDa Protein 1A; HSPA8: Heat Shock 70kDa Protein 8; PRDX1: Peroxiredoxin 1; PRDX2: Peroxiredoxin 2; TPI1: triosephosphate isomerase 1; YWHAZ: 14-3-3 protein zeta/delta; S100A11: S100 Calcium Binding Protein A11; S100P: S100 Calcium Binding Protein P; S100A8: S100 Calcium Binding Protein A8; S100A9: S100 Calcium Binding Protein A9; ANXA1: Annexin A1; PPIA: Peptidylprolyl Isomerase A; TUBA1B: Tubulin, Alpha 1b; TUBB: Tubulin, Beta Class I; LCN2: Lipocalin 2; MMP9: Matrix Metalloproteinase 9; PFN1: Profilin 1; ACTA1: Actin, Alpha 1, Skeletal Muscle¹; GC: Group-Specific Component (Vitamin D Binding Protein); HSPB1: Heat Shock 27kDa Protein 1; ENO1: Enolase 1, (Alpha); C4B: Complement Component 4B.

Figure 4.

Protein-protein interaction network modeling of proteins with decreased abundance in patients compared to healthy controls. In this representation, proteins are shown as nodes, and known interactions as edges. Cadherin 1 was identified as the 'Key node' protein in this analysis (in yellow).

Abbreviations: CDH1: Cadherin 1; CDH2: Cadherin 2; CDH11: Cadherin 11; CDH13: Cadherin 13; PCDHGC3: Protocadherin Gamma-C3; PCDHGC4: Protocadherin Gamma Subfamily C, 4; PCDHGC5: Protocadherin Gamma Subfamily C, 5; PCDHGA12: Protocadherin Gamma Subfamily A, 12; PVRL2: Poliovirus Receptor-Related 2 (Herpesvirus Entry Mediator B); AGRN: Agrin; CTTN: Cortactin

Table 1

Patient’s baseline laboratory characteristics

	sCr (mg/dL)	eGFR (mL/min/1.73m ²)	Prt/Cr	Ca/Cr	Cys 24 (mg/day)	SSCys	Capacity
Patient 1	0.7	121	0.23	0.10	522	1.12	-37
Patient 2	0.5	136	0.14	0.15	398	1.75	-123

sCr-serum creatinine; eGFR-estimated glomerular filtration rate; Prt/Cr-urinary protein/creatinine ratio; Ca/Cr-urinary calcium to creatinine ratio; Cys 24–24-hour cystine excretion (normal less than 75 mg/day); SSCys-cystine supersaturation (normal less than 0.1); Capacity-cystine capacity (normal-positive value; a negative value indicates supersaturation, goal of therapy >150 mg/L).

Table 2

Selected urinary proteins in two children with cystinuria and kidney stones with at least 2-fold increased or decreased abundance relative to healthy controls.

Accession Number*	Protein	MW (kDa)	No. of assigned peptides**	No. of unique peptides**	Sequence coverage (%)***	Patient/control ratio
CER U	Ceruloplasmin	122	411/349	48/48	48/43	4.63
NGAL	Neutrophil gelatinase-associated lipocalin	23	78/39	14/10	64/58	10.6
VTDB	Vitamin D-binding protein	53	279/223	34/35	80/78	3.1
OSTP	Osteopontin	35	21/13	4/3	12/12	0.14
UROM	Uromodulin	70	256/143	29/21	38/35	0.33

* Human;

** Values presented in each patient (Patient 1/Patient 2)

*** % of sequence that has been identified for each protein (Patient 1/Patient 2)