Kidney and Blood Pressure Research

Kidney Blood Press Res , DOI: 10.1159/000527195
Received: June 17, 2022
Accepted: September 16, 2022
Published online: October 20, 2022

Proteomics insights into medullary sponge kidney (MSK) disease: review of the recent results of an Italian research collaborative network.

Granata S, Bruschi M, Candiano G, Catalano V, Ghiggeri GM, Stallone G, Zaza G

ISSN: 1420-4096 (Print), eISSN: 1423-0143 (Online)
https://www.karger.com/KBR
Kidney and Blood Pressure Research

Review Article

Proteomics insights into medullary sponge kidney (MSK) disease: review of the recent results of an Italian research collaborative network.

Simona Granata[1,2], Maurizio Bruschi[3], Giovanni Candiano[3], Valeria Catalano[4], Gian Marco Ghiggeri[5], Giovanni Stallone[4] and Gianluigi Zaza[4]

[1] Department of Medical and Surgical Sciences, University of Foggia, Foggia, Italy
[2] Renal Unit, Department of Medicine, University/Hospital of Verona, Verona, Italy
[3] Laboratory of Molecular Nephrology, IRCCS Istituto Giannina Gaslini, Genova, Italy
[4] Nephrology, Dialysis and Transplantation Unit, University of Foggia, Foggia, Italy
[5] Division of Nephrology, Dialysis and Transplantation, IRCCS Istituto Giannina Gaslini, Genova, Italy

Short Title: Proteomics in medullary sponge kidney (MSK) disease

Corresponding Author:
Gianluigi Zaza
Nephrology, Dialysis and Transplantation Unit
Department of Medical and Surgical Sciences
University/Hospital of Foggia
Viale Pinto, 1
71122 Foggia, Italy
Tel: 0881732610
E-mail: gianluigi.zaza@unifg.it

Number of Tables: 1
Number of Figures: 1
Word count: 3104
Keywords: medullary sponge kidney; proteomics; extracellular vesicles; biomarkers.

Abstract

Background: Medullary sponge kidney (MSK) disease is a rare and neglected congenital condition typically associated with nephrocalcinosis/nephrolithiasis, urinary concentration defects and cystic anomalies in the precalyceal ducts that, although sporadic in the general population, is relatively frequent in renal stone formers. The physiopathologic mechanism associated with this disease is not fully understood and omics technologies may help to address this gap. **Summary:** The aim of this review is to provide an overview of the current state of the application of proteomics in the study of this rare disease. In particular, we focused on the results of our recent Italian collaborative studies that, analyzing the MSK whole and extracellular vesicles urinary content by mass spectrometry, have displayed the existence of a large and multifactorial MSK-associated biological machinery and identified some main regulatory biological elements able to discriminate patients affected by this rare disorder from those with idiopathic calcium nephrolithiasis and autosomal dominant polycystic kidney disease (including laminin subunit alpha 2, Ficolin 1, Mannan-binding lectin serine protease 2, Complement component 4-binding protein β, sphingomyelin, ephrines). **Key messages:** the application of omics technologies has provided new insights into the comprehension of the physiopathology of the MSK disease and identified novel potential diagnostic biomarkers that may replace in future expensive and invasive radiological tests (including CT) and select novel therapeutic targets potentially employable, whether validated in a large cohort of patients, in the daily clinical practice.

Introduction

Medullary sponge kidney (MSK) is a rare disease characterized by nephrocalcinosis/nephrolithiasis, urinary concentration defects, and cystic dilatation of papillary collecting ducts [1]. Although unusual in the general population, it is relatively frequent in renal stone formers. Approximately 3 to 5% of recurrent renal stone formers have MSK, although much larger proportion (more than 20%) have also been reported [2].

MSK is usually a benign disorder with mild symptoms mainly due to urinary tract infections and kidney stones (e.g., hematuria, fever, chills, and nausea), but in few cases (about 10%) it may induce chronic kidney impairment.

Several observations support the hypothesis that MSK could be a heritable disorder: its occurrence in childhood, the association with other developmental disorders (e.g., congenital hemihypertrophy and Beckwith-Wiedemann syndrome, horseshoe kidney, unilateral renal aplasia, contralateral congenital small kidney) [3–5] and the evidence of familial clustering of this disease with autosomal dominant inheritance, reduced penetrance and variable expressivity [6].

MSK disease pathogenesis could be related to mutations or polymorphisms of the glial cell line–derived neurotrophic factor (GDNF) and receptor tyrosine kinase (RET) genes, that hinder the branching morphogenesis of the developing kidney [7,8]. This condition may also be enhanced by a defective expression of other key regulators of kidney developmental process including hepatocyte nuclear factor 1β (HNF1B) [9], a transcription factor that controls endoderm development [10]. Molecular analysis demonstrated that HNF1B may act both upstream and downstream of RET signaling by directly regulating GDNF Family Receptor Alpha 1 (Gfra1) and ETS Variant Transcription Factor 5 (Etv5). Subsequently, HNF1B deletion may lead to massively mispatterned ureteric tree network, defective collecting duct differentiation and disrupted tissue architecture, which can induce cystogenesis [9].

All these findings have uncovered only a part of the specific MSK-associated biological machinery, additional studies are warranted to improve the comprehension of this complex disorder and to select diagnostic/prognostic biomarkers for introduction into clinical practice.

Currently, the most effective method for diagnosis of MSK disease is intravenous urography with contrast medium collection in papillary ducts that leads to a classic image of papillary blush or bouquets. However, its replacement with less invasive and accurate imaging techniques such as computed tomography (CT) has reduced the number of diagnoses of MSK over time, being unable to properly demonstrate the classic signs of this disease [1,11,12].

To study MSK disease omics technologies may offer the opportunity to uncover new mechanisms of the disease and to identify specific novel diagnostic targets. Additionally, omics technologies are hypothesis-generating and may provide the basis for other targeted research projects.

MSK-specific biological fingerprint identification: the main role of urine proteomics.

Urine proteomics is a technique that allows the identification of urinary excreted proteins/peptides in a specific stage of disease or treatment and the assessment of protein quantity, functions, and interactions [13].

The analysis of this easily collectible and stable biofluid (less prone to proteolytic degradation during and after sampling [13] compared to plasma/serum) may also allow the identification of key pathophysiological elements of a disease, the recognition of diagnostic/prognostic biomarkers and the selection of novel therapeutic targets.

Unfortunately, some barriers can limit urinary proteomics studies such as low concentrations of total protein, high concentrations of salts and other ingredients that hinder protein separation [14] and the availability of core facilities with high-performance technologies including mass spectrometry (mainly LC–MS/MS and MAL-DI-TOF/TOF), updated software and skilled personnel (including bioinformaticians, computational scientists, biologists) [15]. All these conditions and the cost of the analysis have significantly hindered the broad application of this technology in nephrology.

However, proteomics resulted a valuable methodology to study rare diseases including the MSK disease (Table 1).
Since MSK disease is relatively frequent in patients with idiopathic calcium nephrolithiasis (ICN), we decided to compare their urinary proteomic profiles in order to identify potential disease biomarkers to include in the clinical practice.
In all our proteomic studies (including the University of Verona, the University of Foggia and the Istituto Giannina Gaslini, Genova), the second morning urine was collected and centrifuged to remove cells, debris and organelle. Total urine or urinary-derived extracellular vesicles were then subjected to mass spectrometry. Whole-blood samples were collected in EDTA-coated tubes. The tubes were centrifuged (1,800×g for 10 min), and the plasma was extracted and aliquoted. Biological samples were collected from stable outpatients (with normal renal function). No patients enrolled in these proteomics studies were on pharmacological treatment. Control groups were matched for demographic characteristics and renal function. None of the patients enrolled in these studies was hospitalized and/or affected by obstructive nephropathy.
MSK was diagnosed according to our clinical protocols [16]. For the diagnosis of MSK papillary precalyceal ectasias has been demonstrated on films taken at least 10 min after injecting the contrast medium, with no compression maneuvers and no signs of obstruction.
ICN patients had normal serum creatinine and electrolyte concentrations, no endocrine or other disorders in addition to calcium stone disease and no evidence of obstructive nephropathy.
Additionally, although we cannot exclude the possible impact of comorbidities, the careful selection of patients enrolled (with normal renal function, no increment of inflammatory markers, negative urine culture and no signs of obstruction) have minimized results' biases and confounding factors in the proteomic analysis.
Differentially expressed proteins between MSK disease and ICN were detected using nonparametric Mann–Whitney U test. P values were adjusted for multiple testing using the Benjamini-Hochberg false discovery rate. A nonlinear Support Vector Machine (SVM) was used to distinguish between the 2 cohort patients and make a ranked protein list [16] including 16 proteins able to discriminate the two groups [16]. Among the selected proteins LAMA-2 (merosin), a well-described subunit of laminin, a family of at least 15 αβγ heterotrimeric proteins of extracellular matrix, representing a major component of the basement membrane, was significantly up-regulated in MSK compared to ICN and reached the highest degree of discrimination between the two study groups [17].
LAMA-2 by interacting with other extracellular matrix components is able to mediate the attachment, migration, and organization of cells into tissues during embryonic development [18,19] and it may also play a central role in cyst formation.
O'Brien et al., [19] using an in vitro model of kidney epithelial cell, showed that this protein plays a key role in the apical pole orientation during cyst formation. In particular, Rac1 (a GTPase belonging to the renin-angiotensin system superfamily of small guanosine triphosphate–binding proteins) mediates extracellular laminin assembly, and then assembled laminin directs the orientation of the apical pole. Contrarily, the lack of laminin leads to an inversion of the apical polarity.
Likewise, laminin may play a role in the cyst development in autosomal dominant polycystic kidney disease (ADPKD), the most common inherited renal disease due to germline and somatic PKD1 or PKD2 gene mutations. This clinical condition causes a rise to kidney symptoms, comprising cysts that gradually alter the kidney structure inducing fibrosis and nephrons' damage/lost.
In ADPKD, the basement membranes of the cysts are thickened with an aberrant increase in laminin-332 that contributes to the proliferation of cyst epithelial cells and cyst growth [20-22].
LAMA-2 could represent a suitable biomarker candidate, but further studies need to be accomplished in a large and multicenter cohort of patients. The role of LAMA-2 in cell polarization is noteworthy from a functional standpoint as well. It was then hypothesized that the various tubular dysfunctions observed in MSK patients could be the expression of anomalous tubular cell polarization and mistargeting of carriers [2].

Our analysis has identified additional proteins hyper-expressed in MSK patients but not linked to MSK or other cystic diseases, such as epididymis-specific alpha-mannosidase (MAN2B2), plexin domain–containing protein 1 (PLXDC1), beta-hexosaminidase (HEXA), and glypican-1 (GPC1). Among them, GPC1, a member of the family of heparan sulfate proteoglycans [23,24], stimulates the activity of growth factors such as fibroblast growth factor-2, vascular endothelial growth factor [25-28] thereby modulating the mechanisms of cellular growth, differentiation, and morphogenesis [24]. Its hyper-expression in MSK could suggest a possible increased cellular turnover/proliferation rate able to orchestrate cellular processes leading to cyst formation.
The cytoscape analysis of the proteins detected allow the recognition of the top biological processes that distinguish MSK disease from ICN: endocytosis, proteolysis, extracellular matrix organization, epidermal development, complement and coagulation cascades, tissue homeostasis, and the glycosaminoglycan catabolic process. This denotes the activation of biological processes (such as matrix remodeling and immune-inflammatory modulation) that make the kidney more prone to morphological modifications, leading to cyst formation and organ vulnerability.
This study also provided insight into the mechanism underlying kidney stone formation in MSK patients. Lithogenesis in MSK is related to hypercalciuria/hypocitraturia and urinary stasis in ductal cysts [29]. Apart from citrate, there is no information concerning the role of renal stone inhibitors and regarding macromolecular inhibitors of calcium lithogenesis in MSK patients. We found that the level of inter-alpha-trypsin inhibitor heavy chains 3 and 4 (the isoform-2) was considerably decreased in urine, suggesting a partial loss of the mechanism-contrasting stone formation in MSK patients (Fig. 1).

The proteomic analysis of extracellular vesicles revealed new biological factors potentially involved in the MSK disease.

Extracellular vesicles, such as microvesicles (diameter of 100–1000 nm) and exosomes (diameter of 30–100 nm), are membrane-enclosed particles released by most cell types under normal and pathological conditions [30-34]. Microvesicles are shed directly from the plasma membrane, while exosomes are produced by the fusion of multivesicular bodies with the plasma membrane.
These vesicles contain a great number of receptors, proteins, nucleic acids, and lipids, by which they may transfer much information to other cells [35] and induce cellular reprogramming and phenotypic modification [36].
Urinary extracellular vesicles are specific for different segment of the nephron [37,38] and, because of this characteristic, they may represent a large source of specific urinary biomarkers [38]. They may also be involved in kidney development and in the pathophysiology of several kidney diseases (including ADPKD). Proteomic analysis of urinary extracellular vesicles isolated from ADPKD (diagnosed according to the Ravine criteria [39]) and MSK revealed some differences in terms of the mechanism of cystogenesis between the 2 groups [40]. In this study aimed to find pathophysiological mechanisms differentiating the two diseases (in particular in the very early stage) and to identify specific diagnostic not invasive biomarkers, urinary extracellular vesicles were isolated by centrifugation and subjected to mass spectrometry by in-StageTip method. After normalization using the Normalyzer R-package with the LOESS-G method, mass spectrometry data were analyzed by unsupervised hierarchical clustering using multi-dimensional scaling with k means and Spearman correlation. Weighted gene co-expression network analysis, SVM learning and partial least squares discriminant analysis (PLS-DA) were then used to highlight the proteins discriminating the two groups of patients. Gene Ontology analysis identified the biochemical pathways in which the proteins identified are involved [40].
Urinary exosomes of ADPKD patients were abundant of proteins involved in the regulation of epithelial cell differentiation in kidney development, cell proliferation and extracellular matrix organization (factors promoting tissue remodeling and cystic development/enlargement). Prominin 1 (CD133), and the cellular repressor of E1A stimulated genes 1 (CREG1), as well as proteins required for matrix remodeling (ITIH5) and for salt secretion (GUCA2B or MAL) were more abundant in ADPKD.

None of the cited proteins were upregulated in MSK, suggesting a different mechanism of cystogenesis. The analysis of the urinary proteome of patients with MSK revealed the deregulation of several biological factors probably responsible of nephrolithiasis and systemic alterations (comprising bone mineralization defects) [41], defective capability to counteract oxidative stress/ischemia-induced neurological damages and bone metabolic alterations [41,42]. Bone mineralization defects and neurological damages could be also related to the reduced content of sphingomyelin in MSK patients, as revealed by metabolomics analysis performed in plasma samples isolated from MSK and ICN patients by using liquid chromatography combined with electrospray ionization tandem mass spectrometry (UHPLC–ESI-MS/MS). In this study raw mass spectrometry files were processed using XCMS software. For t-test analysis p-values were adjusted for multiple testing using the Benjamini-Hochberg false discovery rate. Statistical analysis identified 13 metabolites able to discriminate the two groups of patients with sphingomyelin reached the top level of discrimination [43]. Deregulation of sphingomyelin pathway could also sustain nervous system modifications related to genetic imbalance of the RET–GDNF [41,44,45].

More than 70% of MSK patients have bone mineralization defects in the absence of risk factors (i.e., menopause, hyperparathyroidism, hypercalciuria) [42]. This hyper-secretion of calcium may be due to the renal calcium-handling defect [2], absorptive hypercalciuria [46], and malfunctioning urinary acidification [47].

Interestingly, among the proteins involved in sphingomyelin metabolism, ectonucleotide pyrophophatasephosphodiesterase 6 (ENPP6) and osteopontin (SPP1) resulted, respectively, down- and high-regulated in urinary extracellular vesicles of MSK compared to ICN [43]. ENPP6 is an enzyme belonging to 7 phosphodiesterases family, catalyzes the hydrolysis of choline-containing lysophospholipids to phosphocholine and monoacylglyceride. It may be involved in numerous cell processes [48] in different organs/tissues (including heart, central nervous system and bone). In the kidney this protein, highly expressed in the luminal side of proximal tubule, contributes to the reabsorption of choline by hydrolyzing glycerophosphocholine in the primary urine [48]. ENPP6, then, regulating the metabolism of phosphocholine and enhancing inorganic phosphate levels [49] could cause nephrolithiasis [50].

SPP1, a phosphorylated acid glycoprotein, increases bone resorption by stimulating osteoclastogenesis [51,52] and in the kidney is involved in the normal physiological tubular machinery of the distal nephron. If increased in urine, it may reveal some kidney diseases, such as glomerulonephritis, nephroangiosclerosis, renal carcinoma and ADPKD.

The urinary hyper-expression of osteopontin has been reported also in the Autosomal Dominant Tubulointerstitial Kidney Disease (a disorder characterized by tubular cystic dilatation) [53] and in ADPKD [54] demonstrating a possible involvement in cyst onset/development.

The low levels of sphingomyelin in MSK patients, could be part of the adaptive response of the bone tissue remodeling induced by a negative calcium balance and high-renal tubular acidification.

A following published study of the proteomic profile of urine extracellular vesicles of MSK and ICN patients revealed a panel of 20 proteins able to discriminate these 2 groups [55]. In this study the protein content of microvesicles and exosomes was investigated by mass spectrometry, followed by weighted gene co-expression network analysis, SVM learning, and PLS-DA to select the most discriminative proteins.

Ficolin 1, Mannan-binding lectin serine protease 2 and Complement component 4-binding protein β, involved in the lectin complement pathway, exploited the discrimination between MSK and ICN. This revealed, for the first time, a possible role of complement in this rare disease. Particularly, the reduction of MASP2 (associated with the hyper-expression of C4BPB, a well-known inhibitor of the complement cascade) may reflect the physiological effort of the kidney to preserve renal function by minimizing the activation of the lectin pathway that may cause organ damage at the glomerular and tubule-interstitial level [56] (Fig.1).

An updated bioinformatic analysis of the same dataset revealed that several kinases could differentiate MSK from ICN and that 3 Ephrin receptors (EpHA1, EpHB3, and EpHB6) were the most significantly down-regulated proteins in MSK [57] (Fig.1).

Ephrins receptors and ephrins are expressed in almost all tissues of a developing embryo and are involved in a wide array of developmental processes such as cardio-vascular and skeletal development, axon guidance, modulation of cell adhesion, cell migration/fusion, and tissue patterning [58].
These biological processes, whether deregulated, may contribute to the pathophysiology of MSK disease. In fact, based on available literature, MSK could belong to congenital anomalies of the kidney and urinary tract (CAKUT) [1,4,42]. Its association with several developmental defects in other organs [1] suggests a defective embryogenesis [8,59,60].
Additionally, as recently reported [61], tyrosine kinases, and ephrin ligands may regulate kidney cytoarchitecture once development is completed.
Hence, the down-regulation of ephrins in MSK may represent a new potential mechanism involved in MSK pathogenesis and onset/development of systemic complications.

Conclusion

In the last few years, our national translational research network, taking advantage of our clinical collection of MSK cases and of innovative proteomics technologies, has provided new insights into the comprehension of the physiopathology of MSK.
These high-throughput studies have showed that several biological factors (mainly involved in cell proliferation/differentiation, matrix remodeling, innate immunity and organ fibrosis) may be part of a complex and previously unrecognized MSK-specific biological machinery and its complications. For example, the down-regulation of renal stone inhibitors, namely, inter-alpha-trypsin inhibitor heavy chains 3 and 4 (the isoform-2) has suggested a possible reduction of the mechanism contrasting stone formation in these patients.
Some of the identified biological hallmark of the disease (including Lamin-2, GPC-1, ephrins receptors) could turn to be in the future, whether they are validated in a larger patient' cohort, valuable and performing disease biomarkers and they can help clinicians to early identify these patients, avoiding time-consuming and costly tests. In fact, at the moment, no diagnostic biomarkers are available for clinical purposes and often this disease is undiagnosed and confused with other causes of nephrocalcinosis or papillary ductal plugging.
Urinary biomarkers, then, may avoid exposure of patients to radiation and/or nephrotoxic contrast media for medical imaging (e.g., intravenous urography and CT urography) and reduce underdiagnosis due to noncontrast CT scans.
Our data could select new therapeutic/pharmacological targets, facilitate drug discovery by pharmaceutical companies and allow drug makers to produce a therapy more targeted to this specific renal disease (including complications).
Finally, the cooperative network including clinicians, researchers, bioinformaticians and biomolecular technicians has enabled us to test in nephrology an innovative collaborative approach useful for studying other rare kidney disorders.

Statements

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Funding Sources
The authors received no funds for this work.

Author Contributions
Simona Granata, Maurizio Bruschi, Giovanni Candiano, Valeria Catalano and Gianluigi Zaza searched the literature and wrote the manuscript. Gian Marco Ghiggeri and Giovanni Stallone contributed to the literature analysis and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

References

1. Fabris A, Anglani F, Lupo A, Gambaro G. Medullary sponge kidney: state of the art. Nephrol Dial Transplant. 2013 May;28(5):1111-9.
2. Gambaro G, Feltrin GP, Lupo A, Bonfante L, D'Angelo A, Antonello A. Medullary sponge kidney (Lenarduzzi-Cacchi-Ricci disease): a Padua Medical School discovery in the 1930s. Kidney Int. 2006 Feb;69(4):663-70
3. Lambrianides AL, John DR. Medullary sponge disease in horseshoe kidney. Urology. 1987 Apr;29(4):426-7.
4. Gambaro G, Fabris A, Citron L, Tosetto E, Anglani F, Bellan F, et al. An unusual association of contralateral congenital small kidney reduced renal function and hyperparathyroidism in sponge kidney patients: on the track of the molecular basis. Nephrol Dial Transplant. 2005 Jun;20(6):1042-7.
5. Rommel D, Pirson Y. Medullary sponge kidney--part of a congenital syndrome. Nephrol Dial Transplant. 2001 Mar;16(3):634-6
6. Fabris A, Lupo A, Ferraro PM, Anglani F, Pei Y, Danza FM, et al. Familial clustering of medullary sponge kidney is autosomal dominant with reduced penetrance and variable expressivity. Kidney Int. 2013 Feb;83(2):272-7
7. Diouf B, Ka EH, Calender A, Giraud S, Diop TM. Association of medullary sponge kidney disease and multiple endocrine neoplasia type IIA due to RET gene mutation: is there a causal relationship? Nephrol Dial Transplant. 2000 Dec;15(12):2062-3.
8. Torregrossa R, Anglani F, Fabris A, Gozzini A, Tanini A, Del Prete D, et al. Identification of GDNF gene sequence variations in patients with medullary sponge kidney disease. Clin J Am Soc Nephrol. 2010 Jul;5(7):1205-10
9. Desgrange A, Heliot C, Skovorodkin I, Akram SU, Heikkilä J, Ronkainen VP, et al. HNF1B controls epithelial organization and cell polarity during ureteric bud branching and collecting duct morphogenesis. Development. 2017 Dec 15;144(24):4704-4719
10. Kolatsi-Joannou M, Bingham C, Ellard S, Bulman MP, Allen LIS, Hattersley AT, et al. Hepatocyte nuclear factor-1beta: new kindred with renal cysts and diabetes and gene expression in normal human development. J Am Soc Nephrol. 2001 Oct;12(10):2175-2180
11. Xiang H, Han J, Ridley WE, Ridley LJ. Medullary sponge kidney. J Med Imaging Radiat Oncol. 2018;62:93–94.
12. Gaunay GS, Berkenblit RG, Tabib CH, Blitstein JR, Patel M, Hoenig DM. Efficacy of multi-detector computed tomography for the diagnosis of medullary sponge kidney. Curr Uroly. 2017;11(3):139–143.
13. Kalantari S, Jafari A, Moradpoor R, Ghasemi E, Khalkhal E. Human Urine Proteomics: Analytical Techniques and Clinical Applications in Renal Diseases. Int J Proteomics. 2015;2015:782798.
14. Afkarian M, Bhasin M, Dillon ST, Guerrero MC, Nelson RG, Knowler WC, et al. Optimizing a proteomics platform for urine biomarker discovery. Mol Cell Proteomics. 2010 Oct;9(10):2195-204.
15. Aslam B, Basit M, Nisar MA, Khurshid M, Rasool MH. Proteomics: Technologies and Their Applications. J Chromatogr Sci. 2017 Feb;55(2):182-196
16. Fabris A, Bruschi M, Santucci L, Candiano G, Granata S, Dalla Gassa A, et al. Proteomic-based research strategy identified laminin subunit alpha 2 as a potential urinary-specific biomarker for the medullary sponge kidney disease. Kidney Int. 2017 Feb;91(2):459-468
17. Colognato H, Yurchenco PD. Form and function: the laminin family of heterotrimers. Dev Dyn. 2000 Jun;218(2):213-34
18. Hamill KJ, Kligys K, Hopkinson SB, Jones JC. Laminin deposition in the extracellular matrix: a complex picture emerges. J Cell Sci. 2009 Dec 15;122(Pt 24):4409-17.
19 O'Brien LE, Jou TS, Pollack AL, Zhang Q, Hansen SH, Yurchenco P, et al. Rac1 orientates epithelial apical polarity through effects on basolateral laminin assembly. Nat Cell Biol. 2001 Sep;3(9):831-8.
20. Shannon MB, Patton BL, Harvey SJ, Miner JH. A hypomorphic mutation in the mouse laminin alpha5 gene causes polycystic kidney disease. J Am Soc Nephrol. 2006 Jul;17(7):1913-22
21. Joly D, Berissi S, Bertrand A, Strehl L, Patey N, Knebelmann B. Laminin 5 regulates polycystic kidney cell proliferation and cyst formation. J Biol Chem. 2006 Sep 29;281(39):29181-9
22. Vijayakumar S, Dang S, Marinkovich MP, Lazarova Z, Yoder B, Torres VE, et al. Aberrant expression of laminin-332 promotes cell proliferation and cyst growth in ARPKD. Am J Physiol Renal Physiol. 2014 Mar 15;306(6):F640-54
23. Filmus J, Selleck SB. Glypicans: proteoglycans with a surprise. J Clin Invest. 2001 Aug;108(4):497-501
24. Fico A, Maina F, Dono R. Fine-tuning of cell signaling by glypicans. Cell Mol Life Sci. 2011 Mar;68(6):923-9
25. Gengrinovitch S, Berman B, David G, Witte L, Neufeld G, Ron D. Glypican-1 is a VEGF165 binding proteoglycan that acts as an extracellular chaperone for VEGF165. J Biol Chem. 1999 Apr 16;274(16):10816-22

26. Steinfeld R, Van Den Berghe H, David G. Stimulation of fibroblast growth factor receptor-1 occupancy and signaling by cell surface-associated syndecans and glypican. J Cell Biol. 1996 Apr;133(2):405-16.
27. Bonneh-Barkay D, Shlissel M, Berman B, Shaoul E, Admon A, Vlodavsky I, et al. Identification of glypican as a dual modulator of the biological activity of fibroblast growth factors. J Biol Chem. 1997 May 9;272(19):12415-21
28. Kleeff J, Ishiwata T, Kumbasar A, Friess H, Büchler MW, Lander AD, et al. The cell-surface heparan sulfate proteoglycan glypican-1 regulates growth factor action in pancreatic carcinoma cells and is overexpressed in human pancreatic cancer. J Clin Invest. 1998 Nov 1;102(9):1662-73.
29. Fabris A, Lupo A, Bernich P, Abaterusso C, Marchionna N, Nouvenne A, et al. Long-term treatment with potassium citrate and renal stones in medullary sponge kidney. Clin J Am Soc Nephrol. 2010 Sep;5(9):1663-8
30. Heijnen HF, Schiel AE, Fijnheer R, Geuze HJ, Sixma JJ. Activated platelets release two types of membrane vesicles: microvesicles by surface shedding and exosomes derived from exocytosis of multivesicular bodies and alpha-granules. Blood. 1999 Dec 1;94(11):3791-9.
31. Camussi G, Deregibus MC, Bruno S, Cantaluppi V, Biancone L. Exosomes/microvesicles as a mechanism of cell-to-cell communication. Kidney Int. 2010 Nov;78(9):838-48
32. Dear JW, Street JM, Bailey MA. Urinary exosomes: a reservoir for biomarker discovery and potential mediators of intrarenal signalling. Proteomics. 2013 May;13(10-11):1572-80
33. Salih M, Zietse R, Hoorn EJ. Urinary extracellular vesicles and the kidney: biomarkers and beyond. Am J Physiol Renal Physiol. 2014 Jun 1;306(11):F1251-9.
34. Panfoli I, Granata S, Candiano G, Verlato A, Lombardi G, Bruschi M, et al. Analysis of urinary exosomes applications for rare kidney disorders. Expert Rev Proteomics. 2020 Oct;17(10):735-749.
35. Mause SF, Weber C. Microparticles: protagonists of a novel communication network for intercellular information exchange. Circ Res. 2010 Oct 29;107(9):1047-57
36. Valadi H, Ekström K, Bossios A, Sjöstrand M, Lee JJ, Lötvall JO. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. Nat Cell Biol. 2007 Jun;9(6):654-9
37. Pisitkun T, Shen RF, Knepper MA. Identification and proteomic profiling of exosomes in human urine. Proc Natl Acad Sci U S A. 2004 Sep 7;101(36):13368-73
38. Moon PG, You S, Lee JE, Hwang D, Baek MC. Urinary exosomes and proteomics. Mass Spectrom Rev. 2011 Nov-Dec;30(6):1185-202
39. Pei Y, Obaji J, Dupuis A, Paterson AD, Magistroni R, Dicks E, et al. Unified criteria for ultrasonographic diagnosis of ADPKD. J Am Soc Nephrol. 2009 Jan;20(1):205-12
40. Bruschi M, Granata S, Santucci L, Candiano G, Fabris A, Antonucci N, et al. Proteomic Analysis of Urinary Microvesicles and Exosomes in Medullary Sponge Kidney Disease and Autosomal Dominant Polycystic Kidney Disease. Clin J Am Soc Nephrol. 2019 Jun 7;14(6):834-843
41. Fabris A, Bernich P, Abaterusso C, Marchionna N, Canciani C, Nouvenne A, et al. Bone disease in medullary sponge kidney and effect of potassium citrate treatment. Clin J Am Soc Nephrol. 2009 Dec;4(12):1974-9
42. Ria P, Fabris A, Dalla Gassa A, Zaza G, Lupo A, Gambaro G. New non-renal congenital disorders associated with medullary sponge kidney (MSK) support the pathogenic role of GDNF and point to the diagnosis of MSK in recurrent stone formers. Urolithiasis. 2017 Aug;45(4):359-362.
43. Granata S, Bruschi M, Deiana M, Petretto A, Lombardi G, Verlato A, et al. Sphingomyelin and Medullary Sponge Kidney Disease: A Biological Link Identified by Omics Approach. Front Med (Lausanne). 2021 May 26;8:671798
44. Ibáñez CF, Andressoo JO. Biology of GDNF and its receptors - Relevance for disorders of the central nervous system. Neurobiol Dis. 2017 Jan;97(Pt B):80-89.
45. Bouscary A, Quessada C, René F, Spedding M, Turner BJ, Henriques A, et al. Sphingolipids metabolism alteration in the central nervous system: Amyotrophic lateral sclerosis (ALS) and other neurodegenerative diseases. Semin Cell Dev Biol. 2021 Apr;112:82-91
46. O'Neill M, Breslau NA, Pak CY. Metabolic evaluation of nephrolithiasis in patients with medullary sponge kidney. JAMA. 1981 Mar 27;245(12):1233-6
47. Osther PJ, Mathiasen H, Hansen AB, Nissen HM. Urinary acidification and urinary excretion of calcium and citrate in women with bilateral medullary sponge kidney. Urol Int. 1994;52(3):126-30
48. Morita J, Kano K, Kato K, Takita H, Sakagami H, Yamamoto Y, et al. Structure and biological function of ENPP6, a choline-specific glycerophosphodiesterphosphodiesterase. Sci Rep. 2016 Feb 18;6:20995
49. Stewart AJ, Leong DTK, Farquharson C. PLA2 and ENPP6 may act in concert to generate phosphocholine from the matrix vesicle membrane during skeletal mineralization. FASEB J. 2018 Jan;32(1):20-25

50. Khan SR, Glenton PA, Backov R, Talham DR. Presence of lipids in urine, crystals and stones: implications for the formation of kidney stones. Kidney Int. 2002 Dec;62(6):2062-72.
51. McKee MD, Nanci A. Osteopontin: an interfacial extracellular matrix protein in mineralized tissues. Connect Tissue Res. 1996;35(1-4):197-205
52. Reinholt FP, Hultenby K, Oldberg A, Heinegård D. Osteopontin--a possible anchor of osteoclasts to bone. Proc Natl Acad Sci U S A. 1990 Jun;87(12):4473-5
53. Ricci P, Magalhães P, Krochmal M, Pejchinovski M, Daina E, Caruso MR, et al. Urinary proteome signature of Renal Cysts and Diabetes syndrome in children. Sci Rep. 2019 Feb 18;9(1):2225
54. Cowley BD Jr, Ricardo SD, Nagao S, Diamond JR. Increased renal expression of monocyte chemoattractant protein-1 and osteopontin in ADPKD in rats. Kidney Int. 2001 Dec;60(6):2087-96
55. Bruschi M, Granata S, Candiano G, Fabris A, Petretto A, Ghiggeri GM, et al. Proteomic Analysis of Urinary Extracellular Vesicles Reveals a Role for the Complement System in Medullary Sponge Kidney Disease. Int J Mol Sci. 2019 Nov 5;20(21):5517.
56. Salvadori M, Rosso G, Bertoni E. Complement involvement in kidney diseases: From physiopathology to therapeutical targeting. World J Nephrol. 2015 May 6;4(2):169-84
57. Bruschi M, Granata S, Petretto A, Verlato A, Ghiggeri GM, Stallone G, et al. A Comprehensive Proteomics Analysis of Urinary Extracellular Vesicles Identifies a Specific Kinase Protein Profile as a Novel Hallmark of Medullary Sponge Kidney Disease. Kidney Int Rep 2022; 7:1420–3
58. Fagotto F, Winklbauer R, Rohani N. Ephrin-Eph signaling in embryonic tissue separation. Cell Adh Migr. 2014;8(4):308-26
59. Hiltunen JO, Laurikainen A, Airaksinen MS, Saarma M. GDNF family receptors in the embryonic and postnatal rat heart and reduced cholinergic innervation in mice hearts lacking ret or GFRalpha2. Dev Dyn. 2000 Sep;219(1):28-39
60. Homma S, Oppenheim RW, Yaginuma H, Kimura S. Expression pattern of GDNF, c-ret, and GFRalphas suggests novel roles for GDNF ligands during early organogenesis in the chick embryo. Dev Biol. 2000 Jan 1;217(1):121-37
61. Weiss AC, Kispert A. Eph/ephrin signaling in the kidney and lower urinary tract. Pediatr Nephrol. 2016 Mar;31(3):359-71.

Figure Legends

Fig. 1. Representation of the main biological factors identified by proteomics able to discriminate medullary sponge kidney (MSK) from idiopathic calcium nephrolithiasis (ICN). Green arrows indicate proteins more abundant while red arrows indicate proteins less abundant in MSK patients compared to ICN patients. Abbreviations: MBL Associated Serine Protease 2 (MASP2); C4b-binding protein beta chain (C4BPB); Ephrin receptor A1 (EPHA1); Ephrin receptor B3 (EPHB3); Ephrin receptor B6 (EPHB6); Laminin subunit alpha 2 (LAMA-2); Glypican-1 (GPC-1).

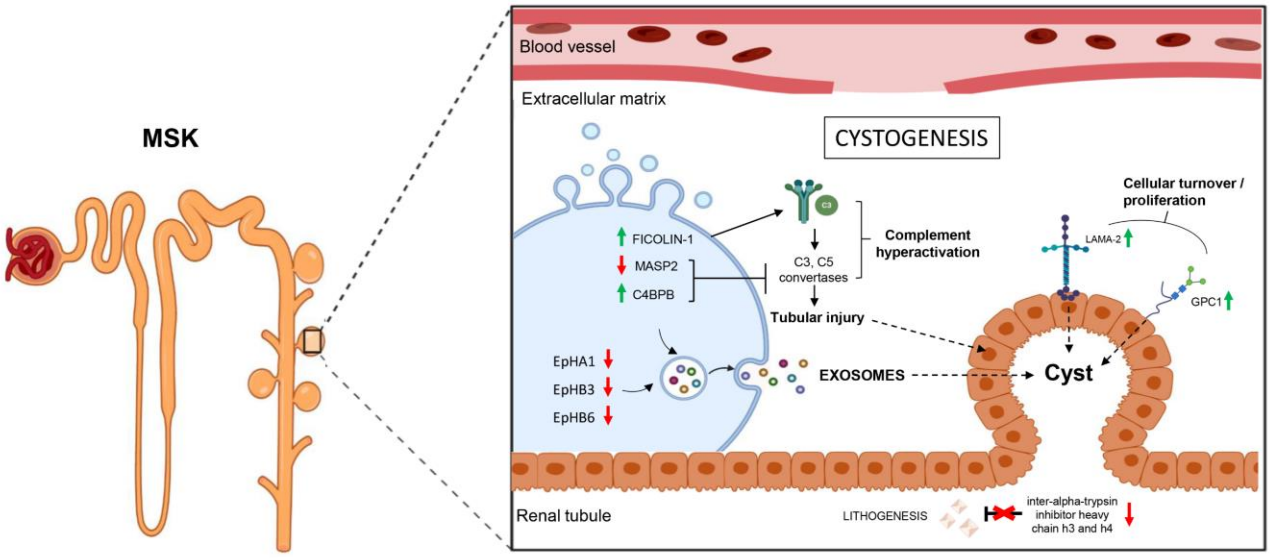

Table 1. List of proteins identified by omics studies discriminating MSK from controls.

Analysis	Biological samples	Comparison	Proteins identified	Gene name	*Ref*
Proteomics	Total urine	MSK vs ICN	**Up-regulated in MSK:**		*Fabris 2017 [16]*
			Laminin subunit alpha 2*	LAMA-2*	
			Glypican-1	GPC-1	
			Plexin domain containing protein 1	PLXDC1	
			Beta-hexosaminidase	HEXA	
			Alpha-mannosidase	MAN2B2	
			Down-regulated in MSK:		
			Mucin-1	MUC1	
			Twisted gastrulation protein homolog 1	TWSG1	
			Di-N-acetylchitobiose	CTBS	
			CMRF35-like molecule 2	CD300E	
			Calcyphosin	CAPS	
			Putative sodium-coupled neutral amino acid transporter 10	SLC38A10	
			Putative lipocalin 1-like protein	LCN1P1	
			Prostaglandin-H2 D-isomerase	PTGDS	
			Filamin-C	FLNC	
			cAMP-specific 3'-5'-cyclic phosphodiesterase 4D	PDE4D	
			CD99 antigen-like protein 2	CD99L2	
Proteomics	Urinary extracellular vesicles	MSK vs ADPKD	**Up-regulated in urinary microvesicles of MSK:**		*Bruschi 2019 [40]*
			Capping Protein Regulator And Myosin 1 Linker 3	CARMIL3	
			Zinc finger and SCAN domain-containing protein 32	ZSCAN32	
			Osteopontin	SPP1	
			Oxidized low-density lipoprotein receptor 1	OLR1	
			Up-regulated in urinary exosomes of MSK:		
			Elongation factor 1-gamma	EEF1G	
			Matrilin-2	MATN2	
			Semenogelin-2	SEMG2	
			Up-regulated in urinary microvesicles of ADPKD:		
			Bone marrow proteoglycan	PRG2	
			Guanylate cyclase activator 2B	GUCA2B	
			Dermatopontin	DPT	
			Mitochondrial enolase superfamily member 1	ENOSF1	
			Calsyntenin-3	CLSTN3	
			Dystroglycan	DAG1	
			Up-regulated in urinary exosomes of ADPKD:		
			Vacuolar protein sorting-associated protein	VPS4A	
			Prominin-1*	PROM1*	
			Cellular Repressor Of E1A Stimulated Genes 1	CREG1	
			Rabankyrin-5	ANKFY1	
			Leucine-rich repeat transmembrane protein FLRT3	FLRT3	
			UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 8	B3GNT8	
			DnaJ homolog subfamily B member 6	DNAJB6	
			Collagen alpha-1	COL14A1	
			von Willebrand factor A domain-containing protein 7	VWA7	
			Ankyrin repeat domain-containing protein 18B	ANKRD18B	
			N-acylethanolamine-hydrolyzing acid amidase	NAAA	
			C3 and PZP-like alpha-2-macroglobulin domain-containing protein 8	CPAMD8	
			Protocadherin Fat 4	FAT4	
			Alpha-mannosidase 2x	MAN2A	
			Zinc finger homeobox protein 3	ZFHX3	
			Leucine-rich repeat-containing protein 40	LRRC40	
			Myelin and lymphocyte protein	MAL	
			Inter-alpha-trypsin inhibitor heavy chain H5	ITIH5	

			Peptidyl-glycine alpha-amidating monooxygenase	PAM	
			Tetraspanin-9	TSPAN9	
			Solute carrier family 15 member 1	hPEPT1-RF	
Proteomics	Urinary extracellular vesicles	MSK vs ICN	**Up-regulated in urinary microvesicles of MSK:**		*Bruschi 2019 [55]*
			FRAS1-related extracellular matrix protein 1	CARMIL3	
			Zinc finger and SCAN domain-containing protein 32	ZSCAN32	
			Deoxyribonuclease-1-like 1	DNASE1L1	
			Up-regulated in urinary exosomes of MSK:		
			Proteasome subunit beta type-4	PSMB4	
			C4b-binding protein beta chain*	C4BPB*	
			Ficolin-1*	FCN1*	
			Down-regulated in urinary microvesicles of MSK:		
			Histamine N-methyltransferase	HNMT	
			Elongation factor Tu, mitochondrial	TUFM	
			Protein FAM179B	FAM179B	
			FRAS1-related extracellular matrix protein 1	FREM1	
			Dynactin subunit 1	DCTN1	
			Delta-1-pyrroline-5-carboxylate dehydrogenase, mitochondrial	ALDH4A1	
			Inter-alpha-trypsin inhibitor heavy chain H5	ITIH5	
			Mannan-binding lectin serine protease 2*	MASP2*	
			Leucine-rich repeat-containing protein 40	LRCC40	
			Cell adhesion molecule 1	CADM1	
			Down-regulated in urinary exosomes of MSK:		
			Sodium/potassium-transporting ATPase subunit alpha-4	ATP14A	
			Eosinophil peroxidase	EPX	
			Nesprin-1	SYNE1	
			Neuropilin-2	NRP2	
Metabolomics	Plasma	MSK vs ICN	**Down-regulated in plasma of MSK:**		*Granata 2021 [43]*
			Sphingomyelin(d18:0/20:0)*		
			PhosphatidylCholine(14:0/20:0)		
			PhosphatidylCholine(P-16:0/22:5(4z,7z,10z,13z,16z))		
			PhosphatidylCholine(P-18:1(11z)/22:6(4z,7z,10z,13z,16z))		
			LysoPE(0:0/24:0)		
			Citrulline		
			Up-regulated in plasma of MSK:		
			LysoPhosphatidylCholine(p7P-18:1(9z))		
			Ganglioside GA2(d18:1/16:0)		
			LysoPhosphatidylCholine(20:1(11z))		
			LysoPhosphatidylCholine(22:2(11z,14z))		
			DG(14:1(9z)/14:1(9z)/0:0)		
			LysoPC(22:5/(7z,10zz,13z,16z,19z))		
			2-Hexaprenyl-6-methoxy-1,4 benzoquinol		

*Proteins and/or metabolites selected for the validation of omics data.