Proteomics Insights into Medullary Sponge Kidney Disease: Review of the Recent Results of an Italian Research Collaborative Network

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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 address this gap.

Summary: The aim of this review was 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 vesicle 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 \(\beta\), sphingomyelin, ephrins).

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 3–5% 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 and ETS variant transcription factor 5. 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, and 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 is 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 samples were 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 have been demonstrated on films taken at least 10
| Analysis          | Biological samples      | Comparison        | Proteins identified                                      | Gene name | Ref                      |
|-------------------|-------------------------|-------------------|----------------------------------------------------------|-----------|--------------------------|
| Proteomics        | Total urine             | MSK versus ICN    | **Upregulated in MSK:** Laminin subunit alpha 2*          | LAMA-2*   | Fabris et al. [16] 2017  |
|                   |                         |                   | Glypican-1                                               | GPC1                                               |
|                   |                         |                   | Plexin domain-containing protein 1                       | PLXDC1                                            |
|                   |                         |                   | Beta-hexosaminidase                                       | HEXA                                               |
|                   |                         |                   | Alpha-mannosidase                                         | MAN2B2                                            |
|                   |                         |                   | **Downregulated in MSK:** Mucin-1                        | MUC1                                               |
|                   |                         |                   | Twisted gastrulation protein homolog 1                   | TWSG1                                              |
|                   |                         |                   | Di-N-acetyl chitobiose                                    | 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                                             |
|                   | Urinary extracellular   | MSK versus ADPKD  | **Upregulated in urinary microvesicles of MSK:** Capping protein regulator and myosin 1 linker 3 | CARMIL3 | Bruschi et al. [40] 2019 |
|                   | vesicles                |                   | Zinc finger and SCAN domain-containing protein 32        | ZSCAN32                                           |
|                   |                         |                   | Osteopontin                                              | SPP1                                               |
|                   |                         |                   | Oxidized low-density lipoprotein receptor 1              | OLR1                                               |
|                   |                         |                   | **Upregulated in urinary exosomes of MSK:** Elongation factor 1-gamma | EEF1G |                                                        |
|                   |                         |                   | Matrilin-2                                               | MATN2                                              |
|                   |                         |                   | Semenogelin-2                                            | SEMG2                                              |
|                   |                         |                   | **Upregulated 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                                               |
|                   |                         |                   | **Upregulated in urinary exosomes of ADPKD:** Vacular protein sorting-associated protein | VPS4A |                                                        |
|                   |                         |                   | Prominin-1*                                              | PROM1*                                             |
|                   |                         |                   | Cellular repressor of E1A stimulated genes 1             | CREG1                                              |
|                   |                         |                   | Rabankynin-5                                             | ANKFY1                                              |
### Table 1 (continued)

| Analysis          | Biological samples | Comparison          | Proteins identified                                      | Gene name | Ref               |
|-------------------|--------------------|---------------------|-----------------------------------------------------------|-----------|-------------------|
| Proteomics        | Urinary extracellular vesicles | MSK versus ICN | Leucine-rich repeat transmembrane protein FLRT3 | FLRT3     |                   |
|                   |                    |                     | UDP-GlcNAc:betaGal beta-1,3-N-acetylglicosaminyltransferase 8 | B3GNT8    |                   |
|                   |                    |                     | DnaJ homolog subfamily B member 6 | DNAJ6     |                   |
|                   |                    |                     | Collagen alpha-1 | COL14A1    |                   |
|                   |                    |                     | von Willebrand factor A domain-containing protein 7 | VWA7      |                   |
|                   |                    |                     | Ankyrin repeat domain-containing protein 188 | ANKRD188  |                   |
|                   |                    |                     | N-acylthanolamine-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 monoxygenase | PAM       |                   |
|                   |                    |                     | Tetraspanin-9 | TSPAN9     |                   |
|                   |                    |                     | Solute carrier family 15 member 1 | hPEPT1-RF | Bruschi et al. [55] 2019 |
| **Upregulated in urinary microvesicles of MSK:** |                     |                     | FRAS1-related extracellular matrix protein 1 | CARMIL3 | Bruschi et al. [55] 2019 |
|                   |                    |                     | Zinc finger and SCAN domain-containing protein 32 | ZSCAN32   |                   |
|                   |                    |                     | Deoxyribonuclease-1-like 1 | DNASE1L1 |                   |
| **Upregulated in urinary exosomes of MSK:** |                     |                     | Proteasome subunit beta type-4 | PSMB4     |                   |
|                   |                    |                     | C4b-binding protein beta chain* | C4BPB*    |                   |
|                   |                    |                     | Ficolin-1* | FCN1*      |                   |
| **Downregulated 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      |                   |
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) has 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 upregulated 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.

Lamina-2, the role of LAMA-2 in cell polarization is noteworthy. 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].

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 previously linked to MSK or other cystic diseases, such as epithidymis-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 allows 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 100–1,000 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 segments 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 Normalizer 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 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 dif-
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 that 13 metabolites were 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 hypersecretion 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 pyrophosphatase phosphodiesterase 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 phosphodiesterase family and catalyzes the hydrolysis of choline-containing lysophospholipids to phosphocholine and monoacylglyc-
eride. 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 partial least squares discriminant analysis 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 downregulated proteins in MSK [57] (Fig. 1). Ephrin 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 [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 downregulation 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, ephrin 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 imaging).
urography) and reduce underdiagnosis due to noncon- 
trast 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 dis- 
ease (including complications). Finally, the cooperative 
network including clinicians, researchers, bioinformati-
cians, and biomolecular technicians has enabled us to test 
in nephrology an innovative collaborative approach use-
ful for studying other rare kidney disorders.

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

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