ORIGINAL ARTICLE

Podocyturia in Fabry disease: a 10-year follow-up

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ABSTRACT

Background. Fabry disease (FD) is a rare X-linked disorder of sphingolipid metabolism that results in chronic proteinuric nephropathy. Podocytes are one of the most affected renal cells and play an important role in the development and progression of kidney disease. Detached podocytes found in urine (podocyturia) are considered as a non-invasive early marker of kidney injury; however, the dynamics of podocyte loss remains unknown.

Methods. In this 10-year follow-up study, podocyturia and other renal clinical data were evaluated in 39 patients with FD. From 2009 to 2019, podocyturia was assessed in 566 fresh urine samples from 13 male and 26 female FD patients using immunocytochemical detection of podocalyxin.

Results. Podocyturia (number of podocytes per 100 mL of urine) was found in 311/566 (54.9%) of the samples, more frequently (68.9 ± 21.9% versus 50.6 ± 25.9%; P = 0.035) and with higher values (364 ± 286 versus 182 ± 180 number of podocytes per gram of creatinine (Cr) in urine; P = 0.020) in males compared with females. The mean number of assessed samples for each patient was 14.5 (range 3–40) and the frequency of samples with podocyturia ranged from 0% to 100% (median 57%). Podocyturia was already present in 42.9% of patients <20 years of age and in 89.5% of normoalbuminuric patients. Podocyturia correlated with albuminuria (urine albumin:Cr ratio) (r = 0.20, P < 0.001) and a higher incidence and values of podocyturia were observed in patients with lower estimated glomerular filtration rate.
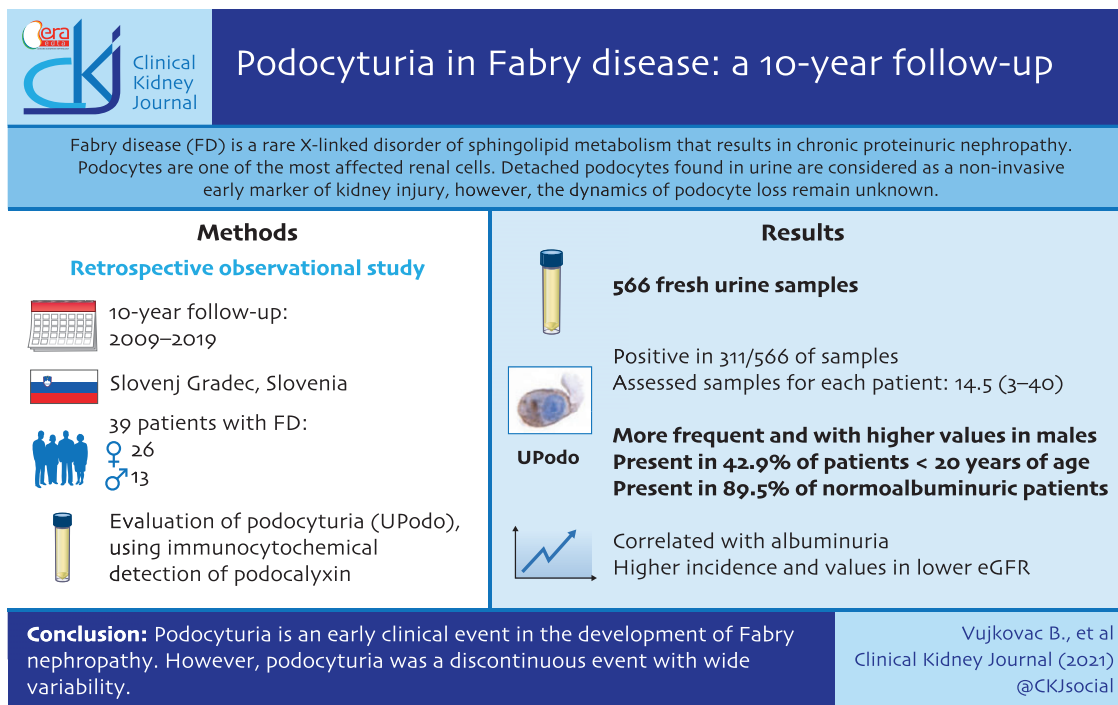
Conclusions. Our data demonstrated that podocyturia is an early clinical event in the development of nephropathy. In addition, we found podocyturia to be a discontinuous event with wide variability.

GRAPHICAL ABSTRACT

Podocyturia in Fabry disease: a 10-year follow-up

Fabry disease (FD) is a rare X-linked disorder of sphingolipid metabolism that results in chronic proteinuric nephropathy. Podocytes are one of the most affected renal cells. Detached podocytes found in urine are considered as a non-invasive early marker of kidney injury, however, the dynamics of podocyte loss remain unknown.

Methods

Retrospective observational study

10-year follow-up: 2009–2019

Slovenj Gradec, Slovenia

39 patients with FD:
♀ 26
♂ 13

Evaluation of podocyturia (UPodo), using immunocytochemical detection of podocalyxin

Results

566 fresh urine samples

UPodo

Positive in 311/566 of samples
Assessed samples for each patient: 14.5 (3–40)

More frequent and with higher values in males
Present in 42.9% of patients < 20 years of age
Present in 89.5% of normoalbuminuric patients

Correlated with albuminuria
Higher incidence and values in lower eGFR

Conclusion: Podocyturia is an early clinical event in the development of Fabry nephropathy. However, podocyturia was a discontinuous event with wide variability.

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Keywords: albuminuria, biomarkers, Fabry disease, immunocytochemistry, podocalyxin, podocyturia, proteinuria, renal function

INTRODUCTION

Fabry disease (FD; Online Mendelian Inheritance in Man 301500) is a rare and progressive X-linked inherited disorder of glyco-sphingolipid metabolism. As a result of deficient or absent lysosomal α-galactosidase A activity, globotriaosylceramide (Gb3) accumulates in cells, causing structural changes that lead to functional impairment. FD affects hemizygous males and heterozygous females with symptoms that range from very mild to severe. Disease presents with neurological pain, progressive proteinuric kidney disease and cardiomyopathy, as well as cerebrovascular symptoms and premature death. Disease progression can be slowed by early initiation of disease-specific treatment (DST), whether in the form of enzyme replacement therapy or pharmacological chaperone [1–3].

One of the hallmarks of FD is chronic proteinuric nephropathy, which is characterized by deposits of Gb3 and its deacylated derivative lyso-Gb3 in different renal cells and to a large extent in podocytes. Depositions seem to be age-dependent and progressive [4, 5]. Like most proteinuric glomerulopathies, including FD, the first morphologic sign of injury to podocytes is seen as a change in their size and architecture, mainly as reorganization of podocyte foot processes (i.e. foot process effacement) [6–8]. Continuing deposition of sphingolipids leads eventually to detachment and podocytes are lost in the urine (podocyturia). Recently it was shown that mechanisms of podocyte detachment are not just mechanical due to progressive accumulation of sphingolipid deposits, but lyso-Gb3 induces a stress to podocytes characterized by an increase in gene expression of components of the uPAR/αvβ3 integrin system, which plays an important role in podocyte detachment and podocyturia [9, 10]. Since podocytes are highly differentiated cells with a very limited potential of self-renewal, their loss is permanent [6, 11, 12]. Changes in the cytoskeleton of podocytes with foot process effacement and a decreased number or density of podocytes in the glomerular capillary tuft consequently result in alterations of the slit diaphragm, causing proteinuria [6, 8, 13], and in the long-term leads to segmental and global glomerulosclerosis [11, 14, 15], which are both common findings in the later stages of FD nephropathy [5, 16].

Currently albuminuria and proteinuria are commonly used biomarkers to clinically assess kidney injury in FD, but they are not sensitive enough for detecting early kidney injury and therefore it is not possible to initiate treatment at an earlier stage that would lead to better clinical outcomes [17]. Foot process effacement is one of the earliest changes in FD and can therefore be detected before proteinuria and loss of renal function become apparent, but it can only be assessed by invasive kidney biopsy [7]. Recent studies have shown that non-invasive podocyturia may also be a good marker of early kidney injury since podocytes were found in higher numbers in the urine of FD patients compared with healthy subjects even with normoalbuminuria and normoproteinuria [17–19].

Some previous cross-sectional studies have already demonstrated the potential prognostic value of podocyturia

in FD patients [10, 12, 17–22]. However, the dynamics of podocyte injury and loss remains unknown, therefore we evaluated podocyturia in relation to albuminuria/proteinuria and renal function in a Slovenian population of FD patients in a 10-year follow-up study using standard chromogen immunocytochemistry.

MATERIALS AND METHODS

Patients

This study was designed as a longitudinal retrospective observational study and in accordance with the principles of the Helsinki Declaration. The study was approved by the Ethics Committee of the General Hospital Slovenj Gradec (EC 202018) and informed consent was obtained from all subjects. Adult patients with FD who were treated and/or regularly monitored in the National Fabry Center at the General Hospital Slovenj Gradec between the years of 2009 and 2019 were invited to participate in the study. The criteria for inclusion were age >18 years and confirmed diagnosis of FD in accordance with current guidelines [2]. All patients had the classic form of FD, which was defined according to the literature [23]. Patient mutations are listed in Supplementary data, Table S1.

Laboratory measurements

At each visit, a selection of the following clinical measurements was performed. Serum creatinine (Cr) was measured using isotope dilution mass spectrometry and was reported as micromoles per litre (μmol/L). Renal function was calculated as estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease (CKD) Epidemiology Collaboration equation and expressed in millilitres per minute per body surface area (mL/min/1.73 m^2) [24]. GFR slope was calculated in patients where at least three values of serum Cr were available. Albuminuria and proteinuria were expressed as urine albumin/Cr and protein/Cr ratio (UACR and UPCR, respectively) in milligrams per gram (mg/g), which were measured from the second morning urine samples. Definitions for albuminuria and proteinuria and their categorization were used according to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines [25].

Podocyturia assessment

Podocyturia was assessed using immunocytochemical detection of podocalyxin-positive cells on slides prepared from fresh urine samples with a filter imprint technique where the whole sample was filtered through isopore polycarbonate membrane filters with 5-μm pores (Merck Millipore, Dublin, Ireland; TMTP04700) using the Microfil System (Millipore 3-Place Manifold MIAC03P01, MIHA WGO 72 funnels; Millipore Intertech, Bedford, MA, USA). Cells collected on the filter surface were immediately transferred to a pair of glass slides by the imprint technique and fixed with M-FIX spray fixative (3981; Merck, Darmstadt, Germany). Immunocytochemical staining with monoclonal antibody against podocalyxin (PHM5, Millipore MAB430; Millipore, Bayswater, VIC, Australia) was performed using the automated immunostaining system Ventana Benchmark XT (Roche Diagnostics, Rotkreuz, Switzerland) and the iView detection kit (Roche Diagnostics). The number of podocalyxin-positive cells per slide was counted by an experienced cytologist using a light microscope at magnification 200$\times$. Results were expressed as the number of podocytes per 100 mL of urine (UPodo/100 mL) and per gram of Cr in urine (UPodo/g Cr).

Statistical analysis

Data management and descriptive statistics were conducted using Stata version 15.1 (StataCorp, College Station, TX, USA). Quantitative variables were expressed as mean $\pm$ SD, except for age, UACR, UPCR and therapy duration, which, being not normally distributed, were expressed as median (range). Intergroup comparisons were analysed by the t-test and the chi-squared test. Bivariate correlation was performed by Spearman's rank correlation. The significance level was judged at $P < 0.05$.

RESULTS

Patient characteristics

In this longitudinal study, 39 FD patients with at least three podocyturia assessments in the period from 2009 to 2019 were included. Patient characteristics are outlined in Table 1. Individual patient data are available in Supplementary data, Table S1.

At baseline, 19 patients (48.7%) were normoalbuminuric with UACR <30 mg/g and 22 (56.4%) patients were normoproteinuric with UPCR <150 mg/g. In both groups, albuminuria and proteinuria were more frequent in males than in females (Table 1). Further details can be found in Supplementary data, Table S2.

Podocyturia in FD patients

Podocyturia was assessed in 566 urine samples of FD patients using immunocytochemical detection of podocalyxin. All samples where podocalyxin-positive cells were detected were considered as positive for podocyturia. Podocalyxin-positive cells exhibited a variable intensity of staining in the cytoplasm (weak, moderate and strong) as well as a variety of morphologies ranging from small to large cells, binucleated cells and cells with foamy cytoplasm (Figure 1), which is concordant with observations of other researchers [17, 26, 27]. Squamous cells, urothelial cells and renal tubular cells present in urinary samples of FD patients were consistently podocalyxin negative (Figure 1).

The mean number of assessed samples for each patient was 14.5 (range 3–40), with more assessments for patients on DST compared with those who were not on therapy (21.3 versus 5.7; $P < 0.001$) (Table 2).

All podocyturia measurements were expressed as the number of podocytes per 100 mL of urine (UPodo/100 mL) and per gram of creatinine in urine (UPodo/g Cr). There was a good correlation between both methods of expression ($r = 0.89$, $P < 0.001$) regardless of sex.

Podocyturia, in general, was higher in males than in females: 19.3 $\pm$ 16.0 versus 12.5 $\pm$ 9.0 UPodo/100 mL ($P = 0.096$) and 364 $\pm$ 286 versus 182 $\pm$ 180 UPodo/g Cr ($P = 0.020$), respectively (Table 2).

Podocyturia (UPodo/100 mL and UPodo/g Cr) correlated with UACR ($r = 0.20$, $P < 0.001$ and $r = 0.17$, $P < 0.001$, respectively) for both sexes (Figure 2). Podocyturia also correlated with UPCR ($r = 0.20$, $P < 0.001$ and $r = 0.18$, $P < 0.001$, respectively).

Podocyturia frequency and levels

Podocyturia was present in 311/566 (54.9%) samples and significantly more often in males than in females (68.9% versus 50.6%; $P = 0.035$). There was great variability in the level of podocyturia between samples for individual patients as well as in the frequency and levels of podocyturia between patients. There were only two female patients without podocyturia in all evaluated samples, while podocyturia was found in 11–100% of evaluated samples for

all other patients (Figure 3 and Supplementary data, Table S1). Two females without podocyturia had a relatively small number of evaluated samples (six and three), both were young (ages 23 and 38 years, respectively) and both were clinically asymptomatic and without any laboratory or imaging evidence of kidney disease.

Examples of podocyturia dynamics at the individual patient level are shown in Figure 4.

The frequency and levels of podocyturia for patients up to 40 years old were significantly connected to GFR slope. Patients with podocyturia present in ≥50% samples had a significantly lower GFR slope and higher levels of podocyturia compared with patients with podocyturia present in <50% of samples (Table 3).

Podocyturia in relation to other renal parameters

Podocyturia was present in 42.9% of samples from patients <20 years of age (males 50.0% versus females 40.0%) and was also present in 17 patients (89.5%) with normoalbuminuria as compared with all patients (100%) with albuminuria (UACR >30 mg/g).

Patients with albuminuria had a higher frequency of samples with podocyturia than patients with normoalbuminuria (66.4% versus 46.5%; P = 0.014) while the differences in the levels of podocyturia between these two groups were significant only when expressed as UPodo/g Cr (P = 0.023) (Supplementary data, Table S3). Podocyturia, expressed as UPodo/100 mL and UPodo/g Cr, was related negatively to eGFR (same for both parameters; $r = -0.16$, $P < 0.001$) for the entire study population and for females, while for males there was only a tendency to show correlation (P = 0.11 and P = 0.079, respectively). Levels of podocyturia increased with the progress of CKD, reaching highest levels at Stage 3 (Figure 5 and Supplementary data, Table S4).

Podocyturia was higher in the group of Fabry patients receiving DST, but was significant only when expressed as UPodo/g Cr (P = 0.048) (Table 4).

DISCUSSION

This longitudinal study of podocyturia in FD patients confirmed that the loss of podocytes is a variable and irregular phenomenon,
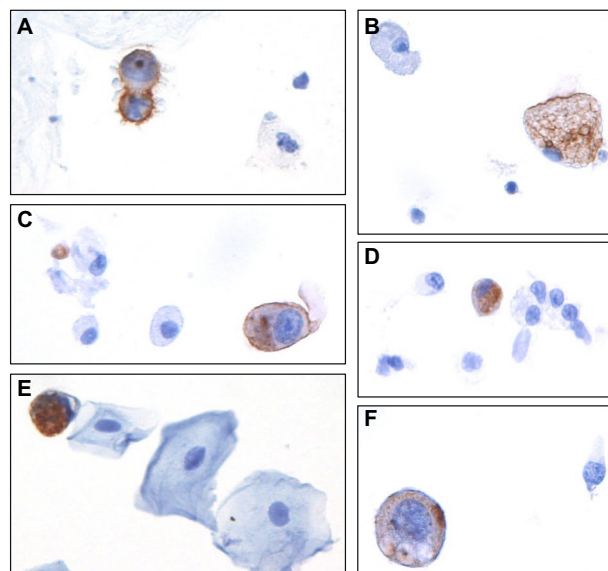

FIGURE 1: Urinary podocalyxin-positive cells (400×) surrounded by urothelial, squamous, and tubular cells (A–F). Bi-nucleated podocalyxin-positive cells (A), a podocalyxin-positive cell with foamy cytoplasm (B), and different sized of cells with variable intensity of podocalyxin staining (C–F).

as already suggested by Trimarchi [28]. Aside from variability in podocyturia between patients, which can be explained by the well-known heterogeneity of disease severity even between members of the same family [29], we found great variability and irregularity of podocyturia in sequential urinary samples of individual FD patients. Podocyturia was found at some point in almost all of our patients (94.9%), with the exception of two completely asymptomatic young females. Similar to Selvarajah *et al.* [20], we found podocytes in about half (54.9%) of all patient urine samples. Unfortunately we could not discern any pattern of podocyte loss dynamics, which possibly may be due to unscheduled podocyturia assessments at patient check-ups. Our observation indicates that serial analysis of podocyturia is necessary for estimation of

Table 1. Characteristics of Fabry patients included in the study

Characteristics	All	Males	Females	P-value
Patients, n	39	13	26	
Age at study entry (years), median (range)	40 (18–72)	40 (19–60)	41 (18–72)	0.626
Follow-up (months), median (range)	84 (21–128)	101 (21–128)	83.5 (21–126)	0.739
DST, n (%)	22	13 (100)	9 (34.6)	<0.001
ACEI/ARB, n (%)	24 (61.5)	10 (76.9)	14 (53.8)	0.163
eGFR (mL/min/1.73 m^2), mean ± SD	92.9 ± 28.8	85.2 ± 39.5	96.8 ± 21.6	0.242
GFR slope (mL/min/year), mean ± SD	−1.74 ± 2.41	−3.28 ± 1.73	−0.97 ± 2.35	0.003
UACR (mg/g), median (range)	36.0 (1–6434)	298.4 (1–1831)	18.8 (1–6434)	0.645
UPCR (mg/g), median (range)	122.1 (60–6591)	449.4 (80–2021)	113.9 (60–6591)	0.594
Normoalbuminuric,[a] n (%)	19 (48.7)	4 (30.8)	15 (57.7)	0.113
Normoproteinuric,[b] n (%)	22 (56.4)	4 (30.8)	18 (69.2)	0.022

[a]Normoalbuminuric: UACR <30 mg/g;
[b]Normoproteinuric: UPCR <150 mg/g.
ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.

Table 2. Podocyturia assessments in urine of Fabry patients

Characteristics	All	Males	Females	P-value
Samples, n	566	259	307	
Samples per patient (n), mean (range)	14.5 (3–40)	19.9 (5–36)	11.8 (3–40)	0.041
Samples with podocyturia, n (%)	311 (54.9)	165 (63.7)	146 (47.6)	
Samples with podocyturia (%) per patient, mean ± SD	56.7 ± 25.8	68.9 ± 21.9	50.6 ± 25.9	0.035
UPodo/100 mL, mean ± SD	14.7 ± 12.0	19.3 ± 16.0	12.5 ± 9.0	0.096
UPodo/g Cr, mean ± SD	243 ± 234	364 ± 286	182 ± 180	0.020

Mean UPodo/100 mL and UPodo/g Cr values refer to mean podocyturia per patient.

podocyte loss and better prediction of disease outcome, as already proposed by Riccio *et al.* [30]. However, the number and frequency of podocyturia assessments for reliable prediction of disease outcome remains to be determined in additional studies.

Our study confirmed that podocyturia is an early event and an early sign of kidney injury, as it was detected in almost half of the patients <20 years of age and in 90% of normoalbuminuric patients. The incidence and level of podocyturia were found to increase with the level of albuminuria/proteinuria, which are known as the best predictors of renal disease progression, as their magnitude increases with decreasing renal function [31]. Similarly, our data indicate that the level of podocyturia, as well as the incidence of podocyturia, might have predictive value for the progressive nature of kidney injury. The incidence of podocyturia increased as kidney function deteriorated and was highest in the later stages of kidney disease. However, the values of podocyturia increased with CKD stages, reaching a peak at Stage 3 and declined in later stages (4 and 5), which is concordant with a speculative model of podocyturia proposed by Trimarchi *et al.* [18]. Lower levels of podocyturia at later stages of CKD could indicate progressive loss of podocytes that

eventually leads to the development of podocytopaenia. Previous studies on the natural progression of Fabry nephropathy have shown that the onset of clinical signs of kidney injury usually appear at ~40 years of age [32, 33]. Our analysis in a subset of FD patients in the age group of up to 40 years of age indeed showed a significant connection between the incidence of podocyturia and renal disease outcome. Patients with a high incidence of podocyturia (≥50%) showed greater declines in GFR and eventually higher levels of podocyturia, while no difference was observed for UACR. This finding might have an important application in clinical practice for predicting which patients are at higher risk for disease progression. However, additional confirmation with a greater number of FD patients, counting males and females separately, would be necessary.

In our study, UACR and UPCR were strongly correlated with podocyturia in both sexes, as previously reported by some other authors [17, 21]. An inverse association was also observed between podocyturia and GFR. But correlation coefficients for UACR, UPCR and GFR were very low as a consequence of podocyturia variability. These results indicate that podocyturia is associated with disease progression and severity, although individual podocyturia measurements have very low predictive value regarding albuminuria/proteinuria and GFR.

Similar to other studies [17, 20], we did not observe any associations between DST treatment of FD patients and podocyturia. In contrast to the study of Trimarchi *et al.* [18], we found an even higher incidence and levels of podocyturia in treated compared with untreated patients. However, the results of this analysis in our group of FD patients are questionable, as treated patients were older and significantly more affected by the disease at the time of study entry. Moreover, sex distribution in both groups was uneven, as all males were treated in contrast to one-third of females. Finally, the majority of our patients (13/22) received DST many years before their first podocyturia assessment, therefore the effect of treatment on podocyturia in those patients could not be assessed. In addition, among nine patients who started with DST later, only one female with mainly heart involvement had at least three assessments before and after the DST initiation. As already stated by Fall *et al.* [17], objective evaluation of the connection between podocyturia and treatment would require additional
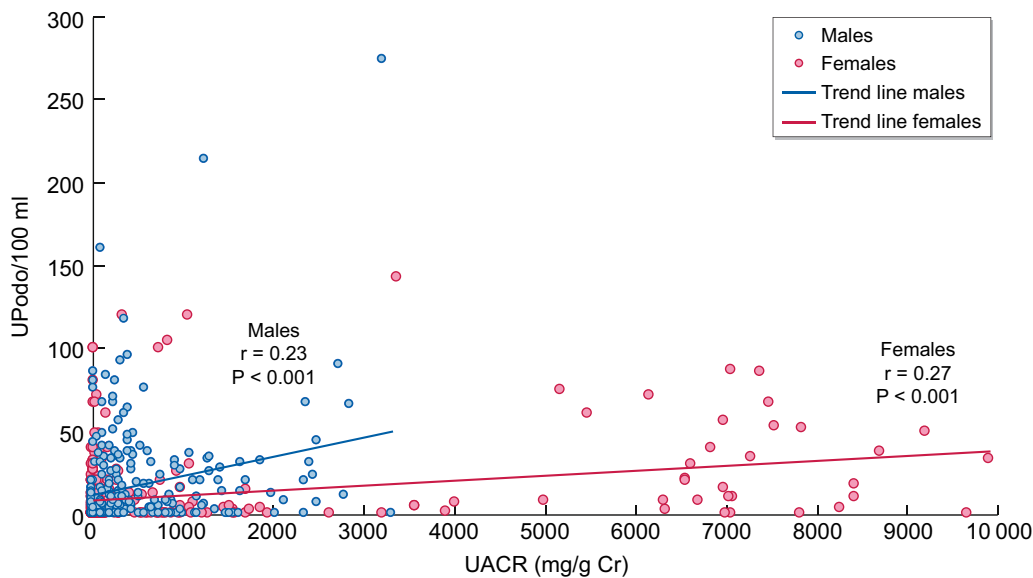

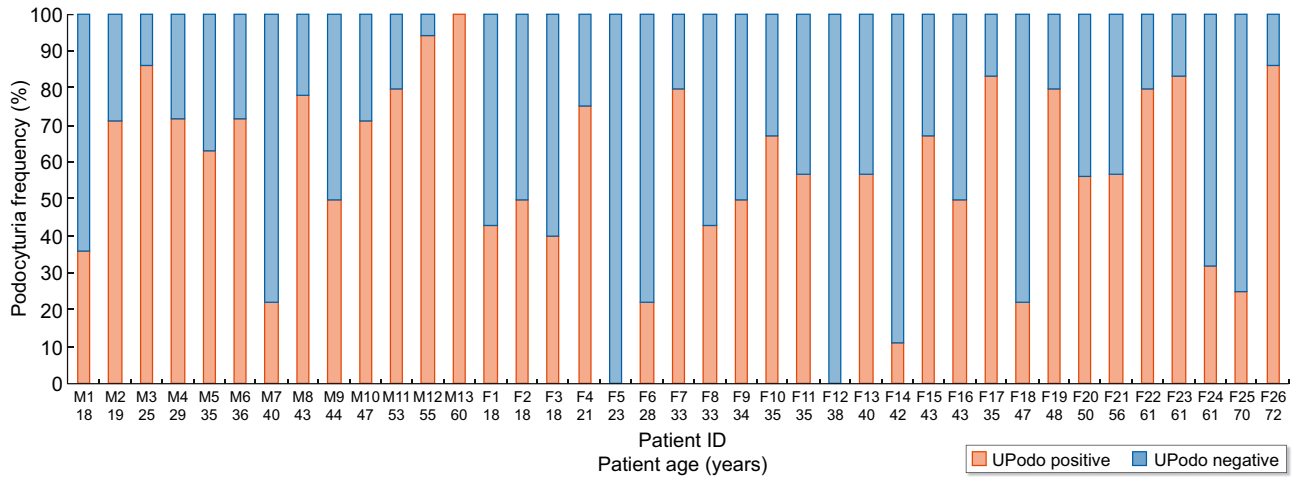**FIGURE 2:** Correlation between podocyturia (UPodo) and albuminuria (UACR) in Fabry patients.

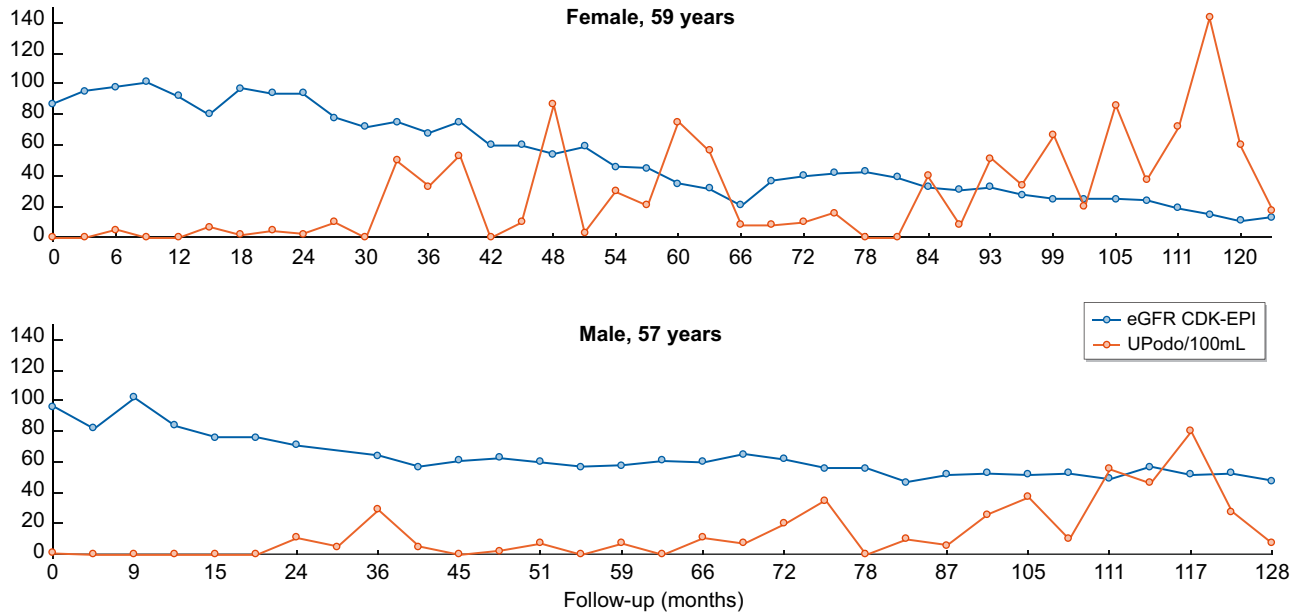FIGURE 3: Frequency of podocyturia in individual Fabry patients included in this study.

FIGURE 4: Dynamics of podocyturia (UPodo/100 mL) and eGFR for one male and one female Fabry patient.

Table 3. Renal parameters for Fabry patients up to 40 years old with high (≥50%) and low (<50%) frequency of podocyturia

Characteristics	Podocyturia <50%	Podocyturia ≥50%	P-value
Patients, *n* (M, F)	10 (3, 7)	10 (4, 6)	0.639
Age at study entry (years), median (range)	31.5 (18–40)	31.0 (18–35)	0.749
Samples, *n*	58	94	
Samples with podocyturia, %	24.1	63.2	<0.001
UACR (mg/g), median (range)	18.2 (4–294)	23.2 (1–315)	0.721
eGFR (mL/min/1.73 m^2), mean ± SD	110.2 ± 13.1	115.0 ± 8.7	0.344
GFR slope (mL/min/year), mean ± SD	−0.23 ± 2.36	−2.22 ± 1.55	0.039
DST, *n* (%)	3 (30.0)	3 (30.0)	1.000
UPodo/100 mL, mean ± SD	5.4 ± 6.4	19.8 ± 14.3	0.009
UPodo/g Cr, mean ± SD	83 ± 128	277 ± 247	0.041

F, female; M, male. Samples (*n*) correspond to all urine assessments for podocyturia. Mean UPodo/100 mL and UPodo/g Cr values refer to mean podocyturia per patient.
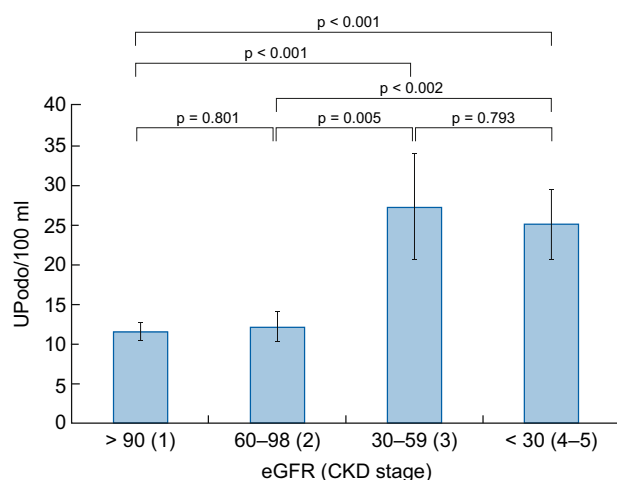
FIGURE 5: Podocyturia (UPodo/100 mL) in Fabry patients at different chronic kidney disease (CKD) stages.

Table 4. Podocyturia and renal parameters for patients with and without DST

Characteristics	DST	No DST	P-value
Patients, n (M, F)	22 (13, 9)	17 (0, 17)	<0.001
Age at study entry (years), median (range)	46.0 (19–70)	34.0 (18–72)	0.038
Follow-up (months), median (range)	110.75 (21–128)	78 (24–109)	0.263
Samples, n	469	97	
Samples with podocyturia, %	58.0	50.3	0.776
ACEI/ARB, n (%)	19 (86.4)	5 (29.4)	<0.001
eGFR (mL/min/1.73 m^2), mean $\pm$ SD	82.8 $\pm$ 32.9	106.0 $\pm$ 15.3	0.011
GFR slope (mL/min/year), mean $\pm$ SD	-2.28 ± 2.64	-0.66 ± 2.02	0.044
UACR (mg/g), median (range)	290.5 (1–6434)	16.6 (1–195)	0.046
UPodo/100 mL, mean $\pm$ SD	17.1 $\pm$ 14.0	11.7 $\pm$ 8.3	0.164
UPodo/g Cr, mean $\pm$ SD	307 $\pm$ 258	159 $\pm$ 172	0.048

Mean UPodo/100 mL and UPodo/g Cr values refer to mean podocyturia per patient.

longitudinal studies on younger patients without proteinuria and more consecutive podocyte assessments before treatment initiation followed by regular follow-ups.

The higher incidence and values of podocyturia in males than in females found in this study is concordant with the previously reported observation that males tend to have a higher disease burden than females [1].

The phenomenon of podocyturia is not limited to kidney disease and can be found in anyone without signs of kidney disease [34]. The frequencies and values of podocyturia in healthy individuals are lower than in patients with kidney diseases, and especially in patients with FD [17, 18]. We found podocyturia 7-fold more frequently and with higher values in FD patients than in a control group of 88 healthy individuals (52 females and 36 males, ages 20–80 years). Notably, podocyturia was found in 7 (8%) of these healthy individuals in a range of 1–20 UPodo/100 mL and with an average value of 0 ± 2 UPodo/100 mL [35].

Several studies already demonstrated that assessment of podocyturia could represent a non-invasive tool to detect subclinical kidney injury as well as a biomarker for monitoring progression and effect of treatment in various kidney diseases [6,

36–38]. However, the existing methods to assess podocyturia are not suitable for wide application in clinical settings, as these methods are time-consuming, expensive, laborious and not standardized. Moreover, the best urinary podocyte-specific markers have not yet been firmly established [18, 19, 30, 39]. Podocalyxin and synaptopodin, which were used as a target protein to detect podocyturia in Fabry patients [17, 18, 21, 39, 40], might be affected by the disease processes, as shown by some studies [41–43]. Podocytes with structural changes on podocalyxin or synaptopodin or with their reduced expression might remain undetected. Besides that, several other methodological factors might also affect successful detection of detached podocytes in urine, such as different clones of antibodies detecting specific parts on the podocalyxin or synaptopodin molecule, sample preparation, fixation procedures and detection systems used to demonstrate antibody binding site. In our study, podocalyxin was used as a target molecule to detect podocytes in the urine of Fabry patients, as it is considered the most robust and frequently used podocyte-specific marker [41]; moreover, our in-house validation confirmed effective detection of podocytes using the described method.

In the majority of podocyturia studies, immunofluorescence is used for the detection of different podocyte-specific markers [10, 17, 19, 44–46]. However, immunofluorescence requires a special microscope that is not widely available. Therefore, in this study, immunocytochemical detection of podocalyxin was used to assess podocyturia instead of immunofluorescence. Immunocytochemistry is a standard, well-established and mainly automated ancillary diagnostic method available in practically every cytological and histological laboratory. As slides with immunocytochemical reactions are permanent, the slides can be stored and evaluated repeatedly over many years. Our study showed that immunocytochemistry might facilitate a more affordable and feasible assessment of podocyturia.

In conclusion, our study clearly demonstrated that podocyturia is an early clinical event that is already present in young patients while they were still normoalbuminuric. The higher incidence of podocyturia in younger patients could present a useful biomarker to predict the worst outcomes. Podocyturia is associated with all important renal parameters of disease progression. The presence of high variability in the incidence and quantity of podocyturia requires serial analyses of repeated urine samples to correctly evaluate disease progression. Immunocytochemical detection of podocalyxin is a standardized and widely available method.

SUPPLEMENTARY DATA

Supplementary data are available at ckj online.

CONFLICT OF INTEREST STATEMENT

DATA AVAILABILITY STATEMENT

The data underlying this article are available in the article and in its supplementary material.

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