Kidney360

Enhanced SHP-1 Expression in Podocyturia Is Associated with Kidney Dysfunction in Patients with Diabetes

Farah Lizotte,[1] Stéphanie Robillard,[1] Nicolas Lavoie,[1] Marina Rousseau,[1] Benoit Denhez,[1] Julie Moreau,[1] Sarah Higgins,[2] Robert Sabbagh,[3] Anne-Marie Côté,[1,2] *and Pedro Geraldes*[1,4]

Key Points

- Diabetes-induced elevated expression of Src homology-2 domain-containing protein tyrosine phosphatase 1 (SHP-1) in podocytes is associated with glomerular sclerosis.
- Increased SHP-1 mRNA levels in urinary podocytes correlated with eGFR decline in patients with diabetes.
- Expression of SHP-1 in urinary podocytes may serve as a marker of glomerular disease progression in patients with diabetes.

Abstract

Background Diabetic kidney disease (DKD) remains the leading cause of end stage kidney disease worldwide. Despite significant advances in kidney care, there is a need to improve noninvasive techniques to predict the progression of kidney disease better for patients with diabetes. After injury, podocytes are shed in urine and may be used as a biologic tool. We previously reported that SHP-1 is upregulated in the kidney of diabetic mice, leading to podocyte dysfunction and loss. Our objective was to evaluate the expression levels of SHP-1 in urinary podocytes and kidney tissues of patients with diabetes.

Methods In this prospective study, patients with and without diabetes were recruited for the quantification of SHP-1 in kidney tissues, urinary podocytes, and peripheral blood monocytes. Immunochemistry and mass spectrometry techniques were applied for kidney tissues. Urinary podocytes were counted, and expression of SHP-1 and podocyte markers were measured by quantitative PCR.

Results A total of 66 participants (diabetic $n=48$, nondiabetic $n=18$) were included in the analyses. Diabetes was associated with increased SHP-1 expression in kidney tissues ($P=0.03$). Nephrin and podocin mRNA was not significantly increased in urinary podocytes from patients with diabetes compared with those without diabetes, whereas levels of SHP-1 mRNA expression significantly correlated with HbA1c and estimated glomerular filtration rate (eGFR). Additionally, follow-up (up to 2 years post recruitment) evaluation indicated that SHP-1 mRNA expression continued to increase with eGFR decline.

Conclusions Levels of SHP-1 in urinary podocytes may serve as an additional marker of glomerular disease progression in this population.

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Introduction

With poor kidney prognosis, significant medical costs, and high fatality potential, diabetes mellitus (DM) continues to be a major global health issue (1). DM remains the main cause of kidney complications that ultimately lead to ESKD (2,3). Diabetic kidney disease (DKD) is characterized by glomerular and tubular injury, gradual kidney function decline, increased blood pressure, and albuminuria, which in turn contribute to kidney failure (4–6). The current challenge is that not all patients living with diabetes develop kidney dysfunction, and the prediction of who will progress to ESKD is difficult. Several biomarkers currently available to predict DKD all have their shortcomings. Proteinuria can be measured to detect kidney damage using a variety of techniques, which include protein/creatinine ratio (PCR) and albumin/creatinine ratio (ACR) (7–10). However, a

[1]Research Center, Centre Hospitalier, Université de Sherbrooke, Québec, Canada
[2]Department of Medicine, Division of Nephrology, Université de Sherbrooke, Québec, Canada
[3]Department of Surgery, Université de Sherbrooke, Québec, Canada
[4]Department of Medicine, Division of Endocrinology, Université de Sherbrooke, Québec, Canada

Correspondence: Prof. Pedro Geraldes, Université de Sherbrooke, 3001 12e Ave Nord, Sherbrooke, Québec, Canada J1H 5N4. Email: Pedro.Geraldes@USherbrooke.ca

significant proportion of individuals with diabetes already presented kidney abnormalities despite the lack of proteinuria (11). Furthermore, since proteinuria also occurs in nondiabetic kidney diseases, it offers nonspecific measures. Due to their limitations, these clinical indicators have not been able to predict accurately among patients with diabetes who will develop kidney dysfunction (12–14). As a result, discovering novel biomarkers that will assist in the early identification and, ideally, early intervention of kidney disease to prevent ESKD progression is critically needed.

Podocytes are highly specialized epithelial cells that contribute significantly to the glomerular filtration barrier (15). Early stage DKD is characterized by a gradual decrease in podocyte quantity and foot process effacement and damage to the slit diaphragm induced by podocyte cell death and shedding of podocytes (16,17). Interestingly, analysis of kidney biopsies from individuals within the early stages of diabetes revealed a decrease in the number of podocytes before the appearance of kidney disease clinical indicators such as albuminuria. Therefore, of all morphologic changes, reduction in the number of glomerular podocytes seems to be the greatest predictor of DKD development (18–20). Several studies have reported that the assessment of podocytes in the urine (podocyturia) could be an effective biomarker of early glomerular injury (21,22). Podocyturia can be evaluated by numerous methods, including immunofluorescence of cultured podocytes (23,24), flow cytometry (25), mass spectrometry (26), and quantitative PCR (27). Thus, there is a strong interest in finding a unique protein product of urinary podocytes as a potential marker of accelerated podocyte injury to diagnose and forecast the progression of DKD noninvasively.

Our group has previously reported that a protein called Src homology-2 domain-containing protein tyrosine phosphatase 1 (SHP-1) has the unique property of deactivating the actions of insulin, nephrin, and other factors required for podocyte survival (28–30). SHP-1 is a member of the protein tyrosine kinase family, which controls important mechanisms during cell development and homeostasis (31). Our findings have shown that glomerular protein expression of SHP-1 is increased in diabetic mice and contributes to glomerulosclerosis by preventing the positive effects of insulin and nephrin signaling. In addition, we found that inhibition of SHP-1 activity in podocytes restored insulin and nephrin signaling pathways and prevented cell death (32). Interestingly, using a cohort of patients with type 1 diabetes (50 years Medalist Program), preliminary observations suggested that low levels of SHP-1 expression in isolated blood monocytes significantly correlated with the absence of kidney complications in patients with diabetes (33). However, this approach has never been performed in comparison with patients without diabetes. With these findings in mind, we hypothesized for this translational research that patients with diabetes will have higher levels of SHP-1 in urinary podocytes compared with patients without diabetes. Therefore, the purpose of this study was to explore whether elevated levels of SHP-1 in kidney tissues, urinary podocytes, and circulating monocytes are associated with kidney disease in patients with diabetes.

Materials and Methods

Study Design and Setting

This prospective observational study was conducted at the CIUSSS de l'Estrie–Centre Hospitalier Universitaire de Sherbrooke (CHUS) with the approval of the Research Ethics Board of the CHUS (REB #2016–1294). Participants (with diabetes [DM] or without diabetes [NDM]) were recruited after they had been approached by their doctor for their willingness to participate in this study. After meeting with the research coordinator during hospitalization or at the daily unit at the CHUS, all participants gave written informed consent before participation in the study. For follow-up analysis at 1–2 years after recruitment, participants with diabetes were asked to provide a urine sample to evaluate if quantitative PCR data are sustained over time. Both visits occurred between 2016 and 2019.

Study General Procedures

Urine and blood samples were collected to determine SHP-1 mRNA levels at recruitment and at the follow-up visit (between 1 and 2 years later according to the medical appointment). Kidney tissues were obtained from biopsies or after nephrectomy.

Participants

Inclusion criteria included being ≥18 years old, with or without kidney disease, and with or without kidney surgery or biopsy planned. Exclusion criteria were the presence of macroscopic hematuria or urinary tract infection, which can affect the quality of the urine sample.

Clinical Data Collection

At recruitment, demographic data, medical history and diabetes complications, medication, smoking status, BP, weight, and height were collected. Afterwards, the laboratory results of the blood and urine tests (serum creatinine and eGFR, albuminuria and proteinuria, glycated hemoglobin A1c, and lipid profile) were retrieved from the electronic medical records to be entered into the study database.

Urine Sample Collection

At least 40 ml of a urine sample was collected from each participant at recruitment and at the follow-up visit. Ten milliliters was transferred for analysis at the CHUS biochemistry laboratory for the ACR and PCR; albuminuria (g/L), proteinuria (g/L), and creatinine (mmol/L; Modular P Analyzer; Roche Diagnostics, Basel, Switzerland). Thirty milliliters was aliquoted (10 ml for immunofluorescence and 20 ml for quantitative PCR) and centrifuged for 7 minutes at 700 *g*. Subsequently, each centrifuged pellet containing the podocytes were analyzed using immunofluorescence of podocyte-specific marker and quantitative PCR as described below.

Blood Sample Collection

Two purple sample tubes were used to collect 8 ml of blood. One tube was sent to the biochemistry laboratory to obtain data such as serum creatinine and glycated hemoglobin. eGFR is reported by the laboratory using the CKD-

EPI formula. The other 4 ml tube was used for isolation of blood monocytes as described below.

Culture Cell and Immunofluorescence, Isolation of Human Blood Monocytes, Quantitative PCR, and Immunochemistry

Details are described in the Supplemental Material and have also been described previously (32,34,35).

Mass Spectrometry

Renal cortex proteins of three patients without and three with diabetes were solubilized in a 8 M urea/10 mM HEPES pH 8.0 lysis buffer. After BCA protein quantification, 50 μg of protein was used. DTT (5 mM final concentration) was incorporated, and samples were boiled for 2 minutes at 95°C followed by an incubation for 30 minutes at room temperature. Chloroacetamide was added to a final concentration of 7.5 mM and incubated in the dark for 20 minutes, and 150 μl of NH_4HCO_3 was added. Proteins were digested with 1 μg of trypsin overnight at 30°C. The samples were acidified with trifluoroacetic acid to reach a final concentration of 0.2%, and samples were cleaned by the ziptip method (cat. no. 87784; Pierce, Waltham, MA). Peptides were quantified and analyzed on nanoLC-MS/MS Orbitrap by the proteomics platform of the Université de Sherbrooke. Peptides were identified by the MaxQuant software and data processed with the Perseus software.

Study Size

In this translational research study, a sample size of 50 participants was initially planned to collect relevant clinical data and to conduct exploratory analyses to assess the pertinence of SHP-1 as a new biomarker in DKD, based in preclinical data on SHP-1 expression in mice.

Statistical Analyses

Statistical analyses were carried out by the Research Center of CHUS biostatistics service and conducted using IBM SPSS Statistics for Windows v26 (IBM Corp., Armonk, NY) for descriptive, comparative, and correlation analyses. All statistics were performed on valid data considering that missing data were excluded (see Supplemental Figure 1). Continuous variables are presented as mean±SD or median (interquartile range). Categorical variables are expressed as percentages and absolute numerical values. Normality of continuous variables was determined with the Shapiro Wilk's W test. A Wilcoxon test was performed when comparing continuous variables with repeated measures, followed by a Bonferroni correction in the case of multiple comparisons, whereas a Mann–Whitney *U* test was done to compare independent groups. Correlation analyses were performed using a Spearman test, with $\rho<0$ signifying a negative correlation and $\rho>0$ a positive correlation. $P<0.05$ was considered statistically significant.

Results

Characteristics of Participants at Recruitment and Follow-Up

A total of 84 participants were recruited to participate in the study (Supplemental Figure 1). Blood and urine samples were collected at the time of the visit for monocyte isolation and podocyturia. Due to insufficient quantities of RNA extracted, 18 participants were excluded. Thus, quantitative PCR of podocyte markers was performed for 66 participants. Regarding the immunofluorescence of podocin in cultured urinary podocytes, 15 samples were contaminated by bacteria. Therefore, podocyte count was performed for 51 participants. At follow-up, 20 participants with diabetes were assessed 1–2 years after entering the study to evaluate if quantitative PCR data are sustained

Table 1. Characteristics of participants without diabetes (NDM) and with diabetes (DM) at recruitment and follow-up visit

Characteristic	NDM	DM	*P* Value[a]	DM—Follow-Up
Participants (*n*)	18	48		15
Sex men/women	9/9	28/20	0.54	11/3
Age (years)	62 (54–74)	65 (56–69)	0.99	66 (58–76)
Active smoking	3 (16%)	8 (17%)	0.5	5 (29%)
HTN	10 (34%)	49 (88%)	<0.001	17 (100%)
ACEi-ARB	10 (34%)	49 (88%)	<0.001	17 (100%)
BP (mm Hg)	134±18/81±10	131±35/70±19	0.01	142±21/78±9
BMI (kg/m^2)	27 (25–35)	31 (28–38)	0.05	36 (30–38)
eGFR (ml/min per 1.73 m^2)	71 (52–94)	65 (44–93)	0.46	53 (37–75)
ACR (mg/mmol)	1.5 (0.5–3.2)	3.9 (1.1–19.8)	0.006	4.1 (1.3–30)
PCR (mg/mmol)	11.3 (11.3–11.3)	13.56 (11.3–41.81)	0.02	11.3 (11.3–62.15)
HbA1c (%)	5.5 (5.3–5.8)	7.8 (6.9–8.8)	<0.001	7.3 (6.8–8.9)
Years of diabetes	0	15 (10–20)	<0.001	15 (10–20)
SHP-1 mRNA levels	4 (1–9.4)	6 (2–19)	0.08	7.5 (3–20)

Data presented as mean±SD for normally distributed data and median (interquartile range) for non-normally distributed data. Chi-squared test was used for categorical variable such as sex and active smoking. *P* values were estimated by Mann–Whitney *U* test or Wilcoxon test wherever applicable with Bonferroni correction. HTN, hypertension; ACEi, angiotensin converting enzyme inhibitor; ARB, angiotensin receptor blocker; BMI, body mass index; ACR, albumin creatinine ratio; PCR, protein creatinine ratio; HbA1c, glycated hemoglobin A1c; SHP-1, Src homology-2 domain-containing protein tyrosine phosphatase 1.
[a]NDM versus DM at recruitment.

over time. Five were excluded due to an insufficient amount of RNA.

At recruitment, participants with diabetes were more hypertensive and more often on angiotensin converting enzyme inhibitor-angiotensin receptor blocker medication. They had a higher body mass index and a greater degree of kidney leakage assessed by ACR and PCR compared with those without diabetes (Table 1). However, eGFR measurements were similar in both groups. Characteristics of participants with diabetes who provided a urine sample 1–2 years after recruitment are presented in Table 1.

Main Results

Expression Levels of SHP-1 in Kidney Biopsies and Mass Spectrometry

Histology of kidney section was used to assess glomerular and podocyte injury. Mesangium expansion was quantified and increased by 166% in the glomeruli of patients with diabetes compared with those without diabetes ($P=0.03$; Figure 1, A and B). Podocyte count was measured using the podocyte nuclear marker WT-1. The number of WT-1-positive cells were significantly decreased by 47% ($P=0.002$) in patients with diabetes (Figure 1, C and D). Immunochemistry of SHP-1 expression was quantified in glomeruli of six patients without and six with diabetes. As shown in Figure 1, E–G, the protein and mRNA expression of SHP-1 was significantly elevated in the glomeruli of participants with diabetes compared with those without diabetes ($P=0.005$ and $P=0.02$). We performed mass spectrometry analyses on the kidney samples and observed that SHP-1 protein expression was increased by 2.26-fold in tissues of participants with diabetes (Figure 1H; $P=0.03$).

Podocyte Count and Quantitative PCR of SHP-1 in Urinary Podocytes

As podocyte detachment occurs after injury, podocyturia can be assessed with a variety of techniques. Our results indicated that diabetes was associated with enhanced average podocyte counts from 504 podocytes in the without diabetes group to 773 podocytes per millimole of creatinine in the with diabetes group (Figure 2, A and B; $P=0.44$). The association between podocyte count and eGFR was stronger in participants with diabetes (Figure 2C) compared with those without diabetes (Figure 2D). However, these analyses did not reach statistical significance.

Podocyturia was also evaluated by quantitative PCR of specific podocyte markers as an additional method (Supplemental Table 1. Interestingly, we observed a 2.9- and 1.9-fold increase in urinary expression of podocin and nephrin mRNA levels, respectively, in patients with diabetes. However, these augmentations did not reach statistical significance (Supplemental Figure 2). Excitingly, our data indicated that the diabetic group had higher amounts of SHP-1 mRNA levels in urinary podocytes compared with the nondiabetic group (Figure 3A; $P=0.04$). In addition, elevated urinary SHP-1 mRNA levels correlated with poor glycemic control (glycated hemoglobin A1c; Figure 3B; $P=0.05$) and the duration of diabetes (Figure 3C). More importantly, expression levels of SHP-1 mRNA in urinary podocytes negatively correlated with eGFR in the entire group (Figure 3D; $P=0.03$) and even more significantly in patients with diabetes (Figure 3E; $P=0.004$) but not in those without diabetes (Figure 3F). By contrast, SHP-1 mRNA levels did not statistically correlate with ACR and PCR (Supplemental Figure 3). Our data suggest that higher SHP-1 mRNA levels are associated with poor kidney function in the population with diabetes.

Urinary SHP-1 Expression Continues to Increase in Participants with Diabetes

To evaluate the association between SHP-1 levels and kidney dysfunction in DKD further, we followed with a subgroup of participants (with diabetes only) 1–2 years after enrollment. Figure 4A indicates that SHP-1 mRNA levels are statistically higher ($P<0.001$) in the urinary podocytes of patients with diabetes at their follow-up visit compared with their recruitment. Although not statistically significant ($P=0.25$) in this small number of participants, SHP-1 mRNA levels in urinary podocytes continue to correlate negatively with eGFR in this population (Figure 4B).

SHP-1 Expression in Monocytes

To follow the previous observation by our group that SHP-1 mRNA expression in circulating monocytes can potentially serve as a marker of vascular complications in diabetes including DKD (33), our data using isolated monocytes from our participants showed a trend of increased SHP-1 mRNA levels in participants with diabetes compared with those without diabetes (Supplemental Figure 4A; $P=0.09$). Despite indicating an inverse correlation between SHP-1 mRNA levels in monocytes and eGFR, this relationship was weaker and not statistically significant (Supplemental Figure 4B).

Discussion

Currently, kidney dysfunction associated with diabetes is identified with clinical indicators such as decreased eGFR and increased levels of creatinine and albuminuria, which are delayed in detecting early stage kidney injury (7). Therefore, a better understanding of which noninvasive techniques could detect early DKD would be advantageous from a clinical point of view for earlier treatment and preventing progression of ESKD. A previous study has indicated that elevated podocytes in the urine could associate with advanced tubular injuries in DKD (36). Our group has reported that SHP-1 is involved in long-term hyperglycemic memory and podocyte dysfunction, contributing to DKD progression (32,37). Our current study provides novel insight into the potential use of urinary SHP-1 mRNA expression as a marker of glomerular injury in diabetes.

Podocytes are glomerular epithelial cells, which play a critical role in maintaining the filtration barrier. Podocyte injury is observed in various glomerular disorders (38) and DKD (18,19). Because the presence of detached podocytes has been demonstrated to be more specific than proteinuria as a marker of active glomerular injury (39), detection of urinary podocytes could be a promising tool for glomerular injury prediction and DKD progression (27). One of the most used techniques to quantify urinary podocytes is immunofluorescence staining with podocyte markers (40). However, this method has several drawbacks mainly because it is time-consuming and operator dependent, and culturing urinary podocytes is frequently accompanied by

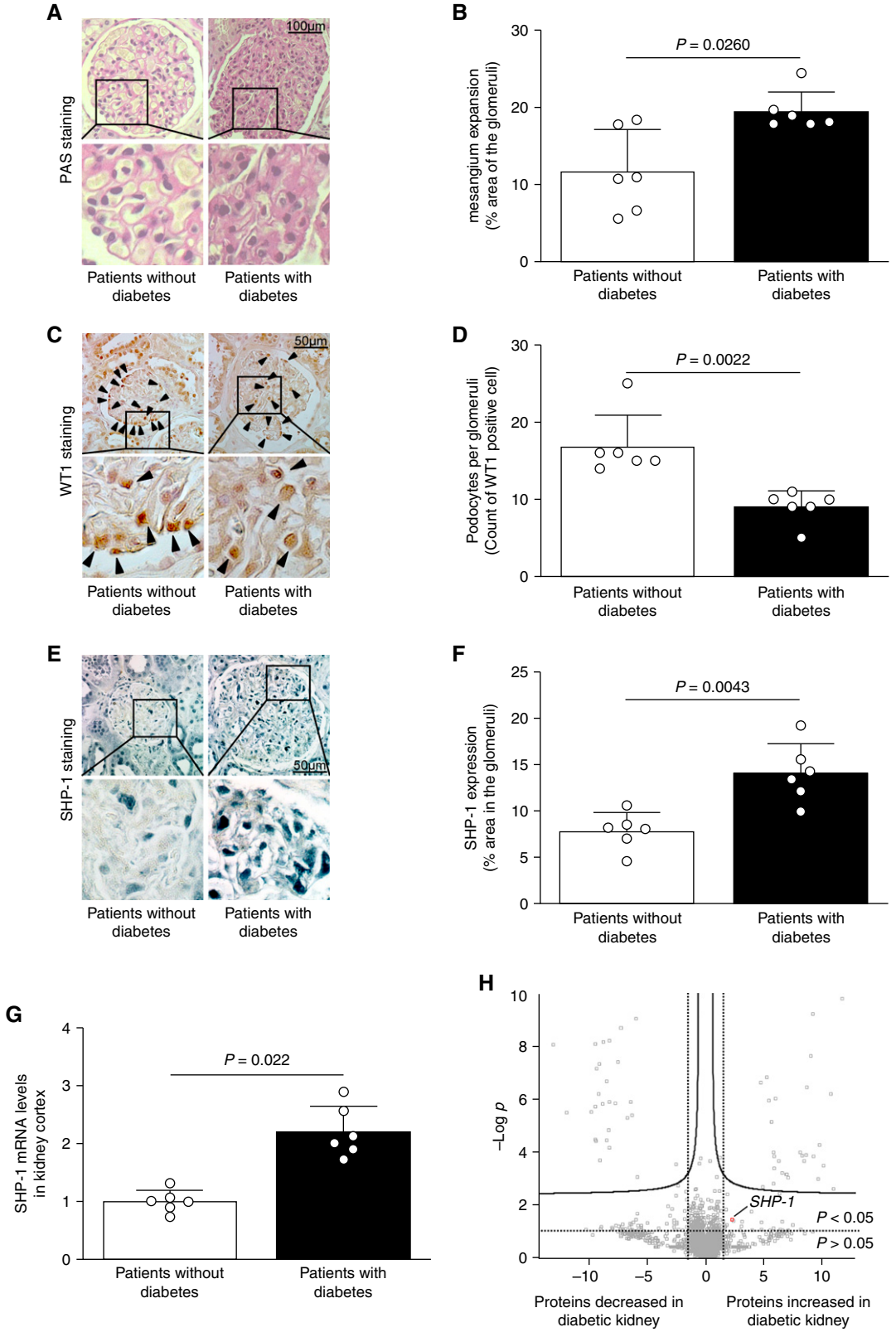

Figure 1. | **Src homology-2 domain-containing protein tyrosine phosphatase 1 (SHP-1) expression is increased in human diabetic glomeruli.** Kidney cross-sections stained with (A) periodic acid–Schiff, (C) WT-1, and (E) SHP-1 to quantify (B) mesangial expansion, (D) podocyte count, and SHP-1 expression by (F) immunohistochemistry and (G) quantitative PCR. (H) Mass spectrometry of human kidney biopsies/samples from patients with and without diabetes. Results are shown as mean±SD of six (A–G) and three (H) samples per group. Scale bar=50 μm.

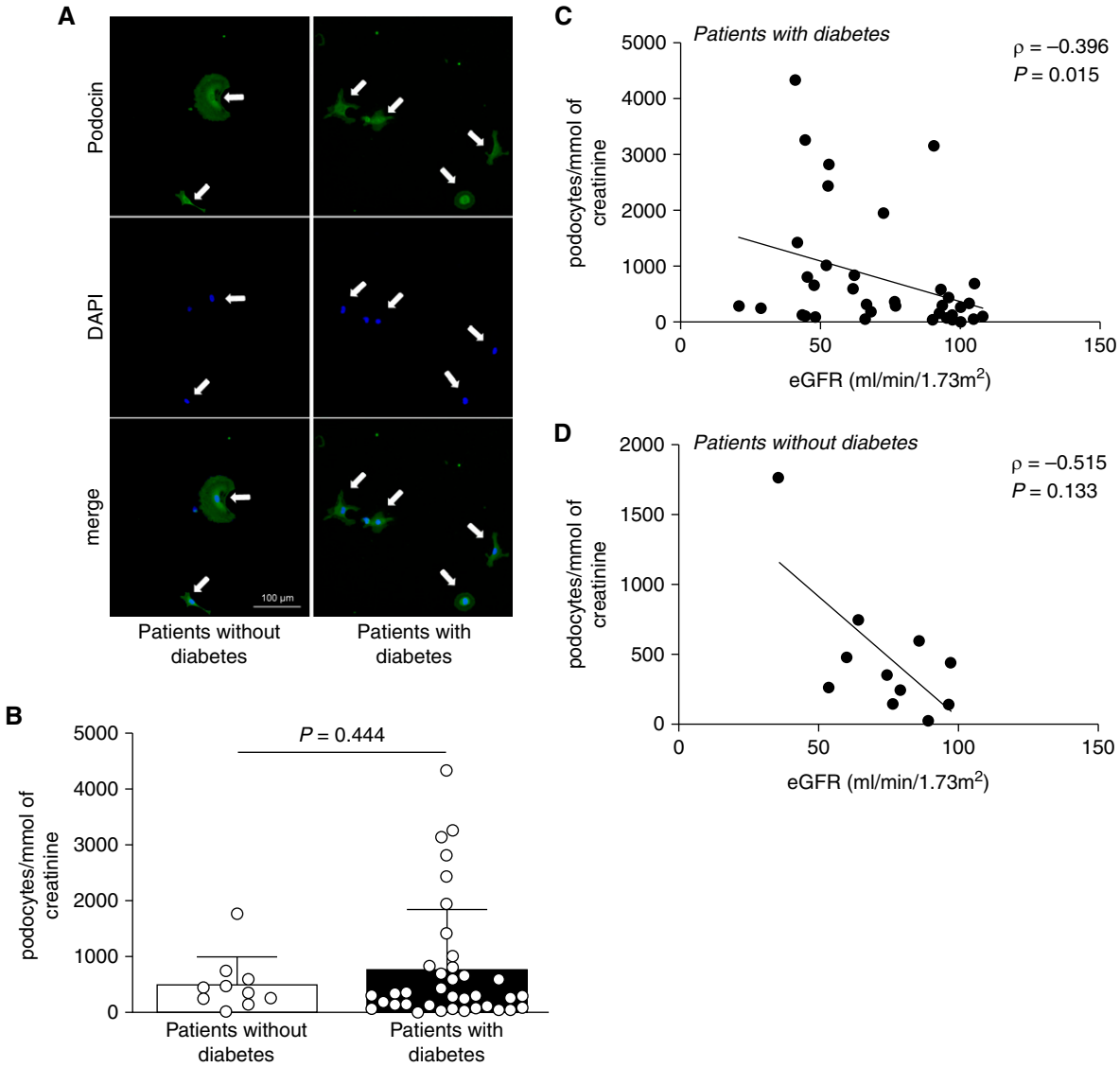

Figure 2. | **Urinary podocyte cell count in urinary samples in patients with and without diabetes.** (A) Immunofluorescence and (B) cell count per millimole (of creatinine) of urinary podocytes stained with podocin (green) and DAPI (blue). White arrows show live podocytes. Correlation between urinary podocyte cell count and eGFR in patients (C) with and (D) without diabetes. Results are shown as mean±SD (A and B) or Spearman correlation (C and D) of 11 nondiabetic patients and 40 diabetic patients.

bacterial and fungal contamination. In addition, not all the cells will attach to the cell culture dish, thus excluding podocytes that are dysfunctional or dead from the analysis (41). Therefore, cell culture and immunofluorescence techniques may not be suitable for the detection and quantification of podocyturia (42). Our data are in line with this statement. We observed an increased number of podocytes in the urine of patients with diabetes compared with those without diabetes. However, this elevation was not statistically significant potentially due to data variability, diabetes duration, presence, and severity of kidney dysfunction. Patients may be more susceptible to podocyte shedding during the early stage of DKD, and as kidney function declines, fewer podocytes are lost. In addition, some samples were not analyzed due to cell culture contamination.

Quantitative PCR allows the detection of specific podocyte markers such as podocalyxin, nephrin, synaptopodin, and podocin, which can be used as potential techniques of podocyturia diagnosis (24,27). Previous studies have reported that nephrin, podocin, and VEGF-A mRNA levels were enhanced in women with preeclampsia (43) and during the progression of human glomerular disease (21,44,45). In our study, we have also measured podocin and nephrin mRNA. Although we observed that both podocyte markers were elevated in patients with diabetes, results were not statistically significant and did not correlate with eGFR. It is possible that because its expression can be downregulated during stress conditions, nephrin mRNA levels appeared highly variable. Our group has previously demonstrated that elevated levels of SHP-1 (both

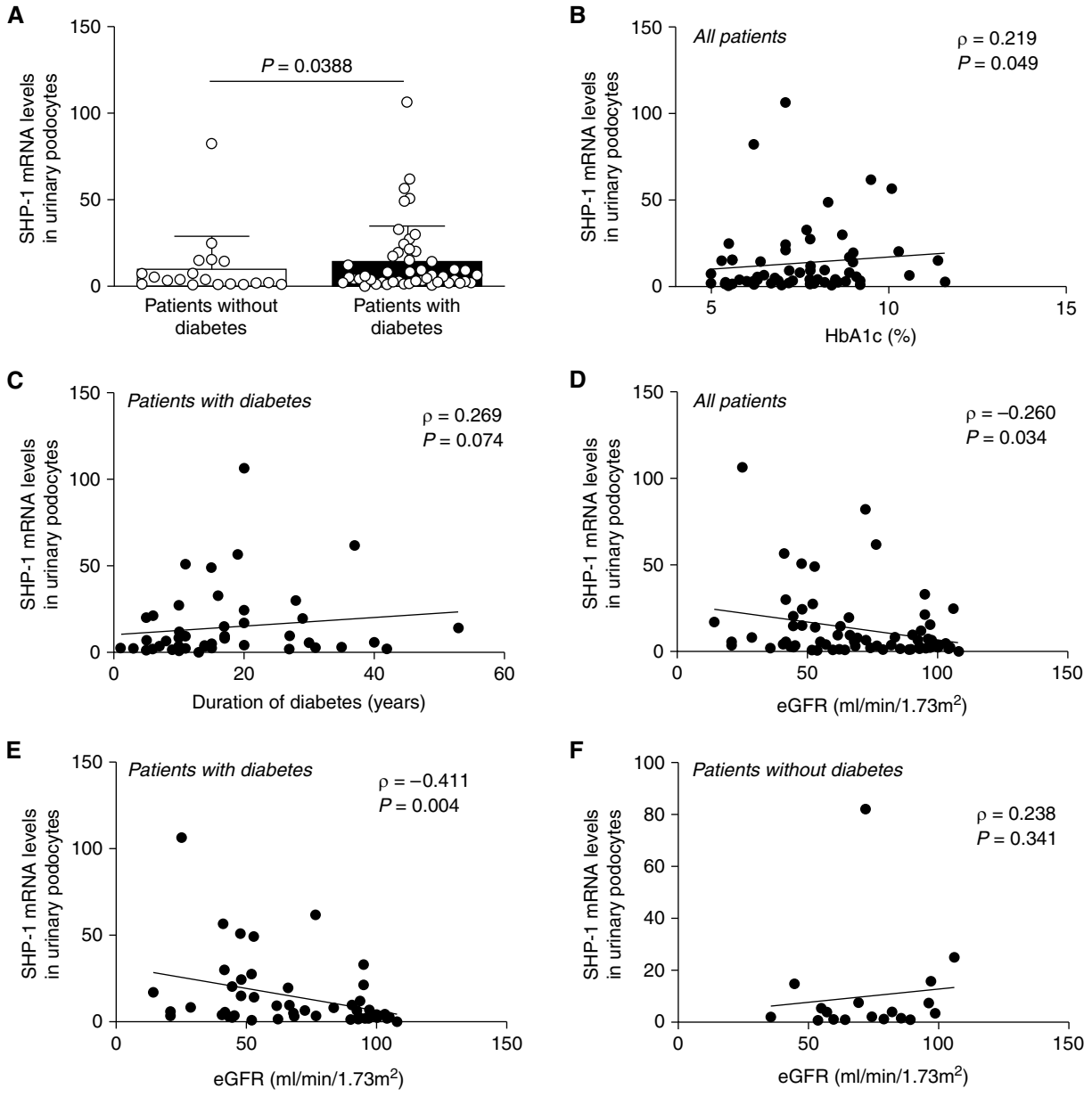

Figure 3. | **Urinary podocyte SHP-1 mRNA levels correlated with glycemic control, diabetes duration, and eGFR in patients with diabetes.** Expression of SHP-1 mRNA levels in (A) urinary podocytes and in correlation with (B) glycated hemoglobin A1c, (C) diabetes duration, and eGFR in (D) all participants, (E) patients with diabetes, and (F) patients without diabetes. Results are shown as mean±SD (A) or Spearman correlation (B–F) of 18 patients without diabetes and 48 patients with diabetes.

protein and gene expression) in podocytes were associated with glomerular dysfunction and pathology in diabetic mice (32). In addition, inhibition of SHP-1 activity and expression restored podocyte function, insulin actions, and prevented cell death caused by diabetes (28). Thus, to determine if SHP-1 could serve as a marker of podocyte injury in humans, samples were collected at the time of recruitment and 1–2 years later.

Encouraging findings emerged regarding the comparison of SHP-1 mRNA levels in urinary podocytes and the overall kidney function, but only in patients with diabetes. Our results indicate that reduction of eGFR in patients with diabetes is associated with an increase in mRNA levels of SHP-1 in urinary podocytes. In addition, there was also a positive trend between recruitment and follow-up analyses, suggesting that persistent increased SHP-1 expression seems to correlate with altered kidney function over time. Furthermore, there is a noticeable trend between the duration of diabetes and SHP-1 levels collected in urine, suggesting that the length of diabetes relates to the progression of kidney dysfunction (46). Similarly, it has been shown that SHP-1 expression persisted in podocytes and renal glomeruli of diabetic mice, despite glycemic control (32). Although there was no statistically significant evidence regarding the correlation between an increase in SHP-1 mRNA levels in monocytes between groups due to result

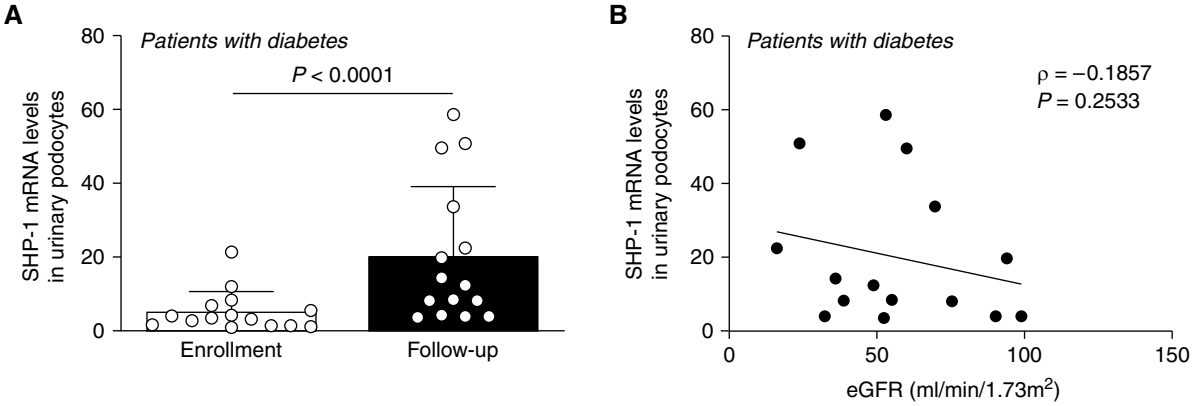

Figure 4. | SHP-1 expression continues to rise in patients with diabetes. Expression of SHP-1 mRNA levels in urinary podocytes from 15 participants with diabetes only (A) at enrollment, 1–2 years follow-up, and (B) in correlation with eGFR at follow-up. Results are shown as mean±SD (A) or Spearman correlation (B).

variability and number of participants, this does highlight the relationship between SHP-1 levels and vascular dysfunction.

Strengths and Limitations

Our study casts new light on the value of measuring gene expression through mRNA levels in podocyturia and solidifies the importance of translational research and exploring further investigations and techniques. Moreover, identification of noninvasive biomarkers to detect early onset kidney dysfunction in patients with diabetes may help physician investigations and reduce the need of performing invasive procedures such as renal biopsies (47).

Our previous research was conducted mainly within rodents because there is a lack of clinical evidence of SHP-1 in human disease. Consequently, in this present study, the sample size was determined to conduct exploratory analyses for feasibility investigation. The number of participants is relatively small for the analyses, which could potentially explain why some results did not reach statistical differences between patients with and without diabetes. Furthermore, the follow-up period was limited to 2 years, reducing the likeliness to reach statistical significance.

In conclusion, this study demonstrated that there is an inverse correlation between SHP-1 mRNA levels in urinary podocytes and eGFR, especially in participants with diabetes, and could be considered as an adjunct tool in future research for diagnostic criteria of early stage DKD.

Disclosures

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Author Contributions

A.-M. Côté, B. Denhez, F. Lizotte, J. Moreau, S. Robillard, M. Rousseau were responsible for validation; A.-M. Côté, B. Denhez, F. Lizotte, J. Moreau, S. Robillard, M. Rousseau, and R. Sabbagh were responsible for the methodology; A.-M. Côté and P. Geraldes were responsible for conceptualization and supervision; A.-M. Côté, S. Higgins, F. Lizotte, and M. Rousseau were responsible for visualization; A.-M. Côté, P. Geraldes, and M. Rousseau reviewed and edited the manuscript; P. Geraldes was responsible for funding acquisition and the investigation; P. Geraldes, N. Lavoie, and F. Lizotte wrote the original draft of the manuscript; F. Lizotte was responsible for the formal analysis and software; J. Moreau was responsible for project administration; and R. Sabbagh was responsible for resources.

Supplemental Material

This article contains the following supplemental material online at http://kidney360.asnjournals.org/lookup/suppl/doi:10.34067/KID.0002152022/-/DCSupplemental.

Supplemental Materials and Methods.

Supplemental References.

Supplemental Figure 1. Flow chart shows the number of participants who were recruited and included in the study.

Supplemental Figure 2. Urinary podocin and nephrin mRNA levels did not correlate with eGFR in patient with and without diabetes.

Supplemental Figure 3. Urinary podocyte SHP-1 mRNA levels did not correlate with ACR and PCR in patients with diabetes.

Supplemental Figure 4. Expression of SHP-1 in isolated monocytes.

Supplemental Table 1. Quantitative PCR primers.

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