Glomerular and Tubular Damage Markers in Individuals with Progressive Albuminuria

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Summary

Background and objectives Albuminuria is associated with risk for renal and cardiovascular disease. It is difficult to predict which persons will progress in albuminuria. This study investigated whether assessment of urinary markers associated with damage to different parts of the nephron may help identify individuals that will progress in albuminuria.

Design, setting, participants, & measurements Individuals were selected from a prospective community-based cohort study with serial follow-up and defined as "progressors" if they belonged to the quintile of participants with the most rapid annual increase in albuminuria, and reached an albuminuria ≥150 mg/d during follow-up. Patients with known renal disease or macroalbuminuria at baseline were excluded. Each progressor was matched to two control participants, based on baseline albuminuria, age, and sex. Furthermore, damage markers were measured in a separate set of healthy individuals.

Results After a median follow-up of 8.6 years, 183 of 8394 participants met the criteria for progressive albuminuria. Baseline clinical characteristics were comparable between progressors and matched controls (n=366). Both had higher baseline albuminuria than the overall population. Urinary excretion of the glomerular damage marker IgG was significantly higher in progressors, whereas urinary excretion of proximal tubular damage markers and inflammatory markers was lower in these individuals compared with controls. Healthy individuals (n=109) had the lowest values for all urinary damage markers measured.

Conclusions These data suggest that albuminuria associated with markers of glomerular damage is more likely to progress, whereas albuminuria associated with markers of tubulointerstitial damage is more likely to remain stable.

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Introduction

Albuminuria predicts cardiovascular events and renal function decline in patients with diabetes mellitus (1) and hypertension (2), in elderly individuals (3), and even in the general population (4–7). A spontaneous increase in albuminuria has been shown to be even more strongly associated with accelerated renal function decline and mortality in patients with diabetes (8) and with cardiovascular events in the general population (9).

Albuminuria is the result of two counteracting processes: glomerular leakage and tubular reabsorption. It has been argued that albuminuria (especially in the microalbuminuric range) is a marker of glomerular damage related to generalized atherosclerosis (10). However, albuminuria may also be explained by another process. According to the tubular hypoxia hypothesis (11), generalized atherosclerosis decreases blood flow in peritubular capillaries. This results in impaired oxygen diffusion and oxygen supply to tubular cells, leading to tubulointerstitial damage and interstitial fibrosis. In turn, these processes lead to impaired tubular albumin reabsorption.

Recently, it has become possible to measure markers in urine samples that represent damage to different parts of the nephron (12). These damage markers initially drew much attention as possible early predictors of AKI, but have also recently been reported as predictors for progressive CKD (13–16). In these studies, progressive CKD was defined as loss of GFR. As yet, no study has investigated whether progressive albuminuria is accompanied by a higher excretion of glomerular or tubular damage markers.

We therefore measured the excretion of several of these markers in urine samples of the following: individuals from the general population that had progressive albuminuria, control individuals matched for albuminuria, age, and sex, as well as a random set of healthy controls.

Materials and Methods

Study Population

This study was performed as a nested case-control study using information and samples obtained from individuals participating in the Prevention of Renal

and Vascular End Stage Disease (PREVEND) study. The PREVEND study is a prospective cohort study that investigates the association of albuminuria with renal and cardiovascular disease progression. Details of this study were published elsewhere (17). In summary, all inhabitants of the city of Groningen, The Netherlands, aged 28–75 years were sent a questionnaire and a vial to collect a first-morning-void urine sample. Of these participants, 40,856 responded (47.8%) and returned this vial to a central laboratory for urinary albumin assessment. From these participants, the PREVEND cohort was selected with the aim to create a cohort enriched for the presence of higher levels of albuminuria. After exclusion of participants with type 1 diabetes mellitus and pregnant women, all participants with a urinary albumin concentration of >10 mg/L (n=7768) were invited for the first screening round, of which 6000 participated. Furthermore, a randomly selected control group with a urinary albumin concentration of <10 mg/L (n=3394) was invited, of which 2592 participated. These 8592 participants constitute the actual PREVEND cohort and were screened at baseline and at follow-up screenings with approximately 3-year intervals.

The PREVEND study was approved by the medical ethics committee of our institution and conducted in accordance with the guidelines of the Declaration of Helsinki. All participants gave written informed consent.

Measurements and Definitions

At the baseline and follow-up screening rounds, all participants completed a questionnaire, underwent a physical examination, had blood drawn, and collected two 24-hour urine samples. Hypertension was defined as use of antihypertensive drugs, a systolic BP >140 mmHg, or a diastolic BP >90 mmHg. Diabetes mellitus was defined as use of glucose-lowering drugs, a fasting glucose >7 mmol/L (>126 g/dl), or a nonfasting glucose >11.1 mmol/L (>200 mg/dl). Hypercholesterolemia was defined as total cholesterol ≥6.0 mmol/L (≥234 mg/dl) or the use of lipid-lowering drugs.

Concentrations of cholesterol, glucose, and creatinine were measured using standard methods. GFR was estimated (eGFR) with the four-variable Modified Diet in Renal Disease formula. Urinary albumin concentration was determined in fresh urine samples by nephelometry (BNII; Dade Behring Diagnostic, Marburg, Germany). Urinary albumin excretion (UAE) is given as the mean of the two 24-hour UAEs.

Definition of Cases, Matched Controls, and Healthy Controls

For these analyses, individuals were excluded if at baseline they were known to have renal disease according to their questionnaire or if baseline albuminuria exceeded 300 mg/24 h (n=198), leaving 8394 participants. Change in albuminuria during follow-up was assessed as the last available albuminuria value during follow-up minus baseline albuminuria, divided by follow-up time in years. Participants were defined as having progressive albuminuria ("progressors") when they belonged to the 20% of individuals with the most rapid progression in albuminuria, and reached an albuminuria in excess of 150 mg/24 h during follow-up. These progressors were 1:2 randomly matched to controls, with matching criteria being baseline albuminuria (in 5 mg/24 h categories), exact age, and sex. Furthermore, for comparison, urinary markers were also measured in a random set of healthy controls selected in 2010 from hospital personnel that responded to an advertisement requesting volunteers (n=109), with healthy being defined as normoalbuminuria (<30 mg/24 h), normal eGFR (>60 ml/min per 1.73 m^2), normal BP (<140/90 mmHg), no history of cardiovascular and/or kidney disease, and no use of medication.

Measurement of Damage Markers

Urine and plasma samples were stored at −20°C and −80°C, respectively. After thawing, urine and plasma samples were vortexed and subsequently centrifuged. The supernatant was used for measurements. All urinary and plasma biomarkers were determined in one run. As markers of glomerular damage, we measured urinary IgG and IgG4 (18,19). The tubular injury markers investigated here reflect injury to different segments of the nephron. Kidney injury molecule-1 (KIM-1), N-acetyl-β-D-glucosaminidase (NAG), cystatin C, and β2-microglobulin (β2MG) were measured as markers for proximal tubular damage (20–22). As a marker of distal tubular damage, we measured heart-type fatty acid-binding protein (H-FABP) (23,24), whereas neutrophil gelatinase-associated lipocalin (NGAL) and monocyte chemoattractant protein-1 (MCP-1) were measured as markers of inflammation (25,26)

For quantification of IgG and IgG4, NGAL, β2MG, MCP-1, and H-FABP, we used direct sandwich-ELISAs as previously described (27). All samples were measured in duplicate in both urine and plasma. Fractional excretions were calculated as follows:

$$FE\ damage\ marker = ([marker]_u \times [creat]_p) / ([marker]_p \times [crea]_u)) \times 100\%,$$

where FE is fractional excretion, crea is creatinine, u is urinary, and p is plasma.

Statistical Analyses

Analyses were performed with SPSS software (version 18.0; SPSS Inc., Chicago, IL), and a two-sided P<0.05 was considered to indicate statistical significance. Parametric variables are expressed as the mean ± SD, whereas nonparametric variables are given as the median (25th and 75th percentiles). P values for differences in damage markers between progressors and controls were assessed using the Mann–Whitney test.

Correlations and corresponding P values are expressed as Spearman's correlation coefficients (ρ). Glomerular charge selectivity was calculated by dividing the fractional excretion of the positively charged total IgG by the fractional excretion of the negatively charged IgG4.

Several sensitivity analyses were performed. First, all damage marker concentrations were expressed per millimole of urinary creatinine instead of as 24-hour excretions. Second, we investigated whether a difference in prescription rate of BP, glucose, and lipid-lowering drugs during follow-up in matched controls versus progressors may

have influenced our results, because these drugs are known to influence the natural course of albuminuria. Third, we assessed the effect of raising the absolute threshold for albuminuria during follow-up to >300 mg/24 h instead of >150 mg/24 h.

Results

The selection of participants with progression in albuminuria (progressors) from the general PREVEND cohort is shown in Figure 1. From a total of 8394 participants at baseline, 183 met our criteria for defining progression in albuminuria. Data of four screening rounds were used for 111 of these individuals, data of three screening rounds were used for 44 individuals, and data of two screening rounds were used for 28 individuals. These progressors were 1:2 matched on baseline albuminuria, age, and sex to participants not selected as progressors (controls). Median follow-up was 8.6 (6.5–9.7) for progressors and 8.5 (6.2–9.5) for matched controls. Characteristics at baseline and their last follow-up visit are shown in Table 1 for both groups. Progressors were generally men (77%), aged 59 years (SD 11), and had a median UAE of 58 mg/24 h (33–106). At baseline, progressors and matched controls had by definition similar albuminuria, which was higher than in the overall cohort (both $P<0.001$). During follow-up, progressors had an increase in albuminuria from 58 (33–106) to 254 (188–410) mg/24 h ($P<0.001$), whereas albuminuria decreased slightly in matched controls from 52 (31–88) to 39 (17–80) mg/24 h ($P<0.001$). For comparison, the clinical characteristics are also given for the 109 randomly chosen healthy individuals (by definition no impaired kidney function [eGFR >60 ml/min per 1.73 m^2], elevated albuminuria [UAE <30 mg/24 h], hypertension, hypercholesterolemia, or diabetes mellitus) in whom urinary renal damage markers were measured (see below).

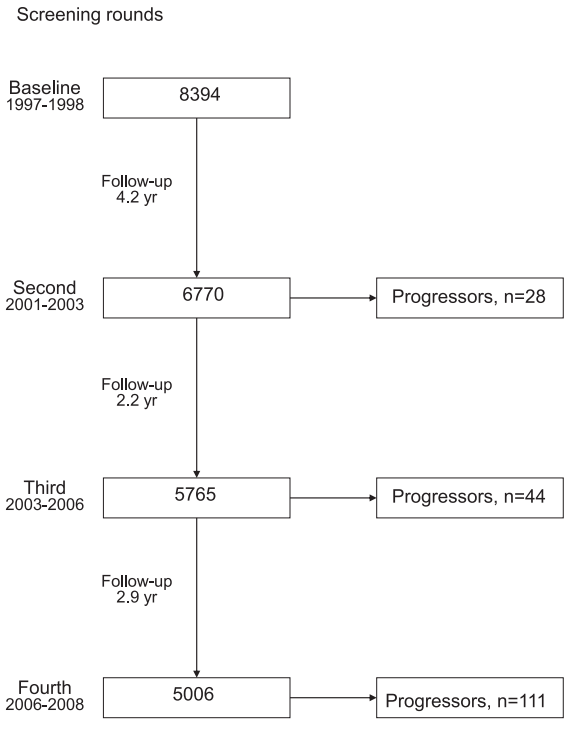

Figure 1. | Selection of participants with progressive albuminuria (*n*=183) from the Prevention of Renal and Vascular End Stage Disease cohort.

Urinary Excretion of Renal Damage Markers

Table 2 shows the urinary excretion of markers associated with damage to different segments of the nephron. As listed, the glomerular marker total IgG was significantly higher in progressors (1.29 versus 1.12 mg/24 h; $P<0.01$), whereas negatively charged IgG4 was similar in both groups. All proximal tubular markers, except cystatin C, and the general inflammation markers NGAL and MCP-1 were significantly lower in progressors compared with matched controls (NGAL, 5.5 versus 6.7 μg/24 h; MCP-1, 467 versus 741 ng/24 h) versus (KIM-1, 3.9 versus 7.4 μg/24 h; B2MG, 103 versus 169 μg/24 h; NAG, 0.01 versus 0.70 U/24 h; all $P<0.01$). In contrast, the distal tubular marker H-FABP tended to be higher in the progressors (3.4 versus 2.0 μg/24 h; $P=0.08$). For comparison, urinary excretion of markers associated with damage to different parts of the nephron is also given for a random set of healthy persons ($n=109$). In these healthy persons, urinary excretion of all markers was lower than in progressors ($P<0.001$ for all markers) and lower than in their albuminuria-matched controls ($P<0.001$ for all markers).

Fractional Excretions of Renal Damage Markers

To exclude that differences in plasma concentrations of the damage markers may have caused the differences in urinary excretion between progressors and matched controls, we also measured plasma concentrations of these damage markers and calculated their fractional excretions. As listed in Table 3, these findings corroborate the results observed with respect to urinary excretion.

Correlations between Markers

Table 4 shows correlations between all markers under investigation. Data are given separately for progressors and matched controls. As listed, almost all markers were significantly correlated to albuminuria and to each other.

Sensitivity Analyses

When all damage marker concentrations were expressed per millimoles of urinary creatinine, this yielded similar results as when 24-hour excretions were studied (data not shown). Furthermore, we investigated whether a difference in prescription rate of albuminuria-lowering drugs during follow-up in matched controls versus progressors may have influenced our results. When all participants were excluded that started with BP-, glucose-, and lipid-lowering drugs, 103 progressors and 195 matched controls remained. Using these strict criteria, essentially similar results were again obtained. In addition, when censoring for start of angiotensin converting-enzyme inhibitor/angiotensin receptor blocker treatment only, similar results were obtained. The same holds true when progressive albuminuria was defined *post hoc* as reaching the absolute threshold of >300 mg/24 h during follow-up instead of >150 mg/24 h. Again, both urinary and fractional

Table 1. Characteristics at baseline and last follow-up visit of participants with progressive albuminuria (progressors) and matched controls (matched for baseline albuminuria, age, and sex)

Characteristic	Healthy Participants (*n*=109)	Cohort (*n*=8394)	Progressors (*n*=183)		Matched Controls (*n*=366)		*P* Value
	Baseline	Baseline	Baseline	Last Follow-Up Visit	Baseline	Last Follow-Up Visit	
Age (yr)	38 (12)	50 (13)	59 (11)	67 (11)	59 (11)	67 (11)	0.88
Male	65 (60)	50	77	77	77	77	0.97
Follow-up (yr)	—	—	—	8.6 (6.5–9.7)	—	8.5 (6.2–9.5)	—
Smoking	—	38	40	23	36	18	0.39
Baseline history CVD	0	5	14	—	11	—	0.24
BMI (kg/m^2)	24.6 (2.8)	26.1 (4.2)	28.3 (4.1)	28.7 (4.8)	27.5 (4.1)	27.6 (4.8)	0.03
SBP (mmHg)	122 (12)	129 (20)	141 (21)	146 (23)	145 (23)	138 (20)	0.08
DBP (mmHg)	72 (8)	74 (10)	79 (9)	79 (10)	81 (10.6)	77 (9)	0.02
BP-lowering drugs	0	15	41	60	27	58	0.001
ACEi/ARB	0	5.5	14	—	36	—	0.36
CCA	0	—	14.6	—	8.7		0.08
Hypertension	0	33	66	78	65	69	0.78
Cholesterol (mmol/L)	—	218 (43)	226 (47)	191 (47)	230 (43)	191 (43)	0.07
Lipid-lowering drugs	0	6	14	38	11	35	0.42
Hypercholesterolemia	—	40	47	54	53	50	0.18
Glucose (mg/dl)	88 (82–95)	85 (77–92)	90 (83–101)	99 (88–117)	90 (83–103)	97 (86–110)	0.35
Glucose-lowering drugs	0	2	4	18	5	17	0.74
Diabetes mellitus	0	3	12	21	11	18	0.90
eGFR (ml/min per 1.73 m^2)	99 (13)	81 (14)	75 (17)	74 (24)	76 (14)	77 (19)	0.78
Albuminuria (mg/24 h)	7.7 (6.3–12.9)	9 (6–17)	58 (33–106)	254 (188–410)	52 (31–88)	39 (17–80)	0.24

Data are presented as the percentage, mean (SD), or median (25th–75th) percentile. *P* values are shown for difference in baseline values between progressors and controls. *P* values are calculated using the Mann–Whitney *U* test comparing baseline values in progressors versus matched controls. CVD, cardiovascular disease; BMI, body mass index; SBP, systolic BP; DBP, diastolic BP; ACEi, angiotensin converting-enzyme inhibitor; ARB, angiotensin II receptor blocker; CCA, calcium channel antagonist; eGFR, estimated GFR.

Table 2. Baseline urinary excretions of renal damage markers at baseline of participants with progressive albuminuria (progressors, *n*=183), their matched controls (*n*=366, matched for baseline albuminuria, age and sex), as well as healthy controls

Marker	Healthy Controls	Progressors	Matched Controls	*P* Value
Albumin (mg/24 h)	7.8 (6.4–10.4)	58 (33–106)	52 (31–88)	0.24
Glomerular				
IgG (mg/24 h)	0.2 (0.2–0.3)	1.29 (0.93–2.18)	1.12 (0.70–2.04)	<0.01
IgG4 (μg/24 h)	—	158 (0–239)	174 (0–290)	0.14
Proximal tubular				
KIM-1 (μg/24 h)	0.8 (0.4–1.2)	3.9 (1.9–7.0)	7.4 (3.2–15.0)	<0.001
CysC (μg/24 h)		0.01 (0.005–0.03)	0.02 (0.005–0.04)	0.24
β2MG (μg/24 h)	79.4 (49.1–121.3)	103 (39–395)	169 (58–641)	<0.01
NAG (U/24 h)	0.4 (0.3–2.4)	0.01 (0.00–0.26)	0.69 (0.00–1.86)	<0.001
Inflammatory				
NGAL (μg/24 h)	—	5.5 (2.7–9.6)	6.7 (3.9–11.4)	0.01
MCP-1 (ng/24 h)	273 (178–400)	467 (261–825)	741 (522–911)	<0.001
Distal tubular				
H-FABP (μg/24 h)	1.4 (1.0–2.2)	3.4 (0.8–7.2)	2.0 (5.9–7.3)	0.08

KIM-1, kidney injury molecule-1; CysC, cystatin C; β2MG, β2-microglobulin; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; MCP-1 monocyte chemoattractant protein-1; H-FABP, heart-type fatty acid-binding protein.

Table 3. Baseline fractional excretions of renal damage markers of participants with progressive albuminuria (progressors, *n*=183) and their matched controls (*n*=366, matched for baseline albuminuria, age, and sex)

Marker (%)	Progressors	Matched Controls	*P* Value
Glomerular			
IgG	8.0×10^{-5} ($5.0–15\times10^{-5}$)	5×10^{-5} ($2–16\times10^{-5}$)	<0.001
IgG4	1.1×10^{-4} ($0–4.0\times10^{-4}$)	1.0×10^{-4} ($0–4.2\times10^{-4}$)	0.59
Proximal tubular			
KIM-1	13.9 (0.9–1713)	1095 (5.2–4368)	<0.001
CysC	0.01 (0–0.02)	0.01 (0–0.02)	0.58
β2MG	0.04 (0.01–0.20)	0.07 (0.02–0.36)	0.03
NAG	1.2×10^{-3} ($0–3.1\times10^{-2}$)	55×10^{-3} (0–0.16)	<0.001
Inflammatory			
NGAL	0.04 (0.02–0.07)	0.05 (0.03–0.10)	<0.01
MCP-1	7.0 (3.6–11.3)	17.5 (11.1–28.5)	<0.001
Distal tubular			
H-FABP	1.31 (0.23–6.71)	0.69 (0.09–3.86)	0.03

Data are expressed as a percentage of creatinine excretion and are presented as the median (25th percentile–75th percentile). *P* values are calculated using the Mann–Whitney *U* test. KIM-1, kidney injury molecule-1; CysC, cystatin C; β2MG, β2-microglobulin; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; MCP-1 monocyte chemoattractant protein-1; H-FABP, heart-type fatty acid-binding protein.

excretion of IgG were significantly higher in progressors (*n*=75) compared with matched controls (*n*=150), whereas urinary and fractional excretion of all proximal tubular markers (except cystatin C) were lower (all $P<0.01$).

Discussion

In this study, we found that progress in albuminuria and the urinary excretion of the glomerular marker IgG and the distal tubular damage marker H-FABP were higher in participants compared with controls matched for baseline albuminuria, age, and sex. In contrast, the urinary excretion of most proximal tubular damage markers and general inflammation markers were lower in progressors. Furthermore, we showed that urinary excretion of all markers was lower in healthy individuals than in participants with progressive albuminuria as well as in their albuminuria-, age-, and sex-matched controls.

How do these data compare with the current literature? Recently, a number of studies have been published investigating whether urinary excretion of these damage markers is associated with progression of CKD. For instance, the glomerular damage marker IgG and several markers of the proximal tubule were found to predict the onset of ESRD or renal function decline in CKD patients (14,15,28,29) and renal transplant recipients (27). Similar findings have been obtained for the urinary excretion of the distal tubular marker H-FABP (30) and the inflammation markers NGAL and MCP-1 (16,31). It is important to note that in all of these studies, progression of CKD was

Table 4. Correlations between the 24-hour urinary excretions of albumin and the various damage markers in participants with progressive albuminuria (n=183) and matched controls (n=366)

Marker	Albumin	IgG Total	IgG4	KIM-1	CysC	B2MG	NAG	NGAL	MCP-1	H-FABP
Participants with progressive albuminuria										
Albumin		0.51 (<0.001)	0.42 (<0.001)	0.28 (<0.001)	0.45 (<0.001)	0.46 (<0.001)	0.24 (0.002)	0.38 (<0.001)	−0.05 (0.52)	0.50 (<0.001)
IgG total			0.42 (<0.001)	0.26 (<0.001)	0.40 (<0.001)	0.47 (<0.001)	−0.04 (0.62)	0.35 (<0.001)	0.08 (0.31)	0.36 (<0.001)
IgG4				0.26 (0.001)	0.40 (<0.001)	0.45 (<0.001)	0.18 (0.02)	0.28 (<0.001)	0.01 (0.87)	0.36 (<0.001)
KIM-1					0.47 (<0.001)	0.41 (<0.001)	0.08 (0.27)	0.19 (0.01)	0.34 (<0.001)	0.25 (0.001)
CysC						0.69 (<0.001)	0.12 (<0.001)	0.35 (<0.001)	0.13 (0.08)	0.51 (<0.001)
B2MG							0.14 (0.07)	0.51 (<0.001)	−0.08 (0.27)	0.60 (<0.001)
NAG								0.02 (0.76)	0.02 (0.80)	0.30 (<0.001)
NGAL									−0.14 (0.06)	0.35 (<0.001)
MCP-1										−0.08 (0.31)
Matched controls										
Albumin		0.36 (<0.001)	0.35 (<0.001)	0.30 (<0.001)	0.45 (<0.001)	0.48 (<0.001)	0.14 (0.01)	0.26 (<0.001)	0.07 (0.22)	0.33 (<0.001)
IgG total			0.39 (<0.001)	0.33 (<0.001)	0.33 (<0.001)	0.47 (<0.001)	0.22 (<0.001)	0.44 (<0.001)	0.07 (0.18)	0.31 (<0.001)
IgG-4				0.28 (<0.001)	0.37 (<0.001)	0.42 (<0.001)	−0.007 (0.89)	0.28 (<0.001)	0.20 (<0.001)	0.31 (<0.001)
KIM-1					0.39 (<0.001)	0.49 (<0.001)	0.11 (0.04)	0.20 (0.01)	0.34 (<0.001)	0.33 (0.001)
CysC						0.74 (<0.001)	0.18 (0.001)	0.24 (<0.001)	0.18 (0.001)	0.59 (<0.001)
B2MG							0.19 (<0.001)	0.45 (<0.001)	0.15 (0.003)	0.54 (<0.001)
NAG								0.31 (<0.001)	0.01 (0.83)	0.26 (<0.001)
NGAL									0.10 (0.05)	0.18 (0.001)
MCP-1										0.26 (<0.001)

Data are presented as Spearman's ρ and the corresponding P value. KIM-1, kidney injury molecule-1; CysC, cystatin C; β2MG, β2-microglobulin; NAG, N-acetyl-β-D-glucosaminidase; NGAL, neutrophil gelatinase-associated lipocalin; MCP-1 monocyte chemoattractant protein-1; H-FABP, heart-type fatty acid-binding protein.

expressed as a decrease in GFR. However, definition and staging of CKD is not only dependent on the level of GFR, but also on the level of albuminuria (32). An increase in albuminuria is generally an early sign of CKD progression, whereas a decrease in GFR mostly refers to a later phase of CKD progression. Higher levels of albuminuria, especially an increase in albuminuria, are associated with an increased risk for all-cause and cardiovascular mortality and with an increased risk for several kidney related outcomes (8,33–35). Little is known regarding how glomerular and tubular damage markers relate to progression in albuminuria. Kern *et al.* reported that urinary NAG predicted the occurrence of albuminuria in patients with diabetes mellitus (36). We are, to our knowledge, the first to investigate how a panel of markers associated with damage to different parts of the nephron relates to progression of albuminuria.

A priori we hypothesized that all damage markers would be higher, or at least equal, in participants that progress in albuminuria compared with participants that were matched for baseline albuminuria but had stable albuminuria during follow-up. We indeed found that the glomerular damage marker (IgG) was significantly higher in progressors. This suggests that damage to the glomerulus is especially associated with progression in albuminuria. Furthermore, because progressors in our study had higher urinary excretion and fractional excretion of IgG, but not of the neutrally charged IgG4, this suggests that loss of glomerular size selectivity is associated with progressive albuminuria, rather than loss of charge selectivity. This is corroborated by a higher total IgG/IgG4 index in progressors compared with controls. To our surprise, we found that markers associated with damage to the proximal tubule and markers associated with inflammation were significantly lower in participants with progressive albuminuria than in controls. In retrospect, we think that this may be explained by the fact that by design, controls were matched to progressors on baseline albuminuria. We chose this study design because *a priori* it seemed most suited to answer the question of whether it is possible to make a distinction between individuals with a given level of albuminuria that will progress or remain stable. Albuminuria is the result of two counteracting processes: glomerular filtration and tubular reabsorption. When controls have been selected to have similar albuminuria compared with progressors, and appear to have less glomerular loss of albuminuria, they should consequently have less tubular reabsorption of albumin. This may explain why we found proximal tubular damage markers to be higher in matched controls than in progressors. This finding, however, does not deny that individuals with progressive albuminuria also have damage to their proximal tubules. At baseline, progressors as well as their matched controls had more albuminuria than the overall cohort population. We and others have found that albuminuria is positively associated with the urinary excretion of markers indicating damage to the proximal tubule (37,38). This is corroborated in this study, because we found such an association in patients as well as controls (Table 4). Most importantly, we found that progressors as well as matched controls indeed had higher urinary excretion of all damage markers that were measured, including proximal tubule damage markers, compared with healthy individuals.

Our study has limitations that need to be mentioned. First, we found that for a given level of "total albuminuria," progressors had higher levels of IgG, whereas nonprogressors had higher levels of tubular proteinuria. It would be interesting to investigate whether tubular markers are associated with progression in albuminuria after adjusting for glomerular albuminuria. Our case-control study design, unfortunately, does not allow such an analysis, for which a cohort design is better suited. Second, albuminuria is known to show short-term fluctuations that are not due to real progression. For that reason, we choose strict criteria to define progression. Consequently, the participants that met our definition of progressive albuminuria showed an increase in albuminuria from 58 mg/24 h at baseline to 254 mg/24 h at the last follow-up visit. Such a rise in albuminuria is unlikely to be the result of random fluctuation, and suggests true progression in albuminuria. Third, our definition of progressive albuminuria is novel and based on a relative criterion (percent increase), as well as an absolute (threshold of 150 mg/d) criterion, in order to avoid defining participants as progressors that had a large percentage increase in albuminuria, but still clinically irrelevant albuminuria at the end of follow-up because baseline albuminuria was low (*e.g.*, increasing from baseline 1 mg/24 h to 5 mg/24 h at the end of follow-up). Every cut-off value to define clinically relevant albuminuria will be arbitrary, because the association of albuminuria and adverse health outcomes is continuous (39). Consequently, no clear cut-offs indicating increased risk exist. The threshold of 150 mg/24 h was chosen because it is compatible with clinically relevant albuminuria, and ensured a sufficient patient number for a case-control study design with multiple comparisons. Importantly, a *post hoc* analysis studying only the participants with progressive albuminuria that reached an albuminuria threshold >300 mg/24 h during follow-up (n=75) and comparing these individuals with matched controls (n=150) showed similar results. Fourth, all markers were measured in urine samples that have been stored at −20°C. It has been suggested that prolonged frozen storage influences urinary biomarker concentration (40). However, we recently showed that these markers can be measured from frozen samples with sufficient reliability, because marker concentrations measured in fresh urine samples and measured in these same samples, but after frozen storage, were in general significantly correlated. Ability to predict endpoints is therefore not harmed (41).

This study also has several strengths. First, we used 24-hour urine collection, which is the gold standard for assessing albumin and damage marker excretions. Other studies use spot urine, which induces more variation (42). Second, in contrast to most studies investigating the value of these markers in CKD, we measured not only one but a panel of markers, reflecting damage to different parts of the nephron. Third, our nested case-control study design, selecting individuals with a clinical relevant increase in albuminuria from a large cohort of 8592 persons with serial follow-up with respect to albuminuria during >9 years, renders a relatively large number of patients resulting in sufficient power to draw conclusions. Fourth, we investigated not only urinary excretion of these damage markers, but we also corrected for their plasma concentrations by

calculating fractional excretions. Importantly, this analysis yielded similar results. Lastly, we are one of the first to study progression in albuminuria as an endpoint. An increase in albuminuria is often the first sign of progressive CKD. Studies thus far have used decline in eGFR as endpoint. Of note, our results can therefore not be compared with results obtained in these latter studies.

What are the implications of this study? Our data suggest that individuals with albuminuria that is associated with glomerular damage are more likely to develop progressive albuminuria than persons with albuminuria that is associated with proximal tubular damage. However, we found considerable overlap in damage marker excretions between progressors and controls. It will therefore be difficult to predict which individual will progress in albuminuria using just one marker. Measuring a panel of markers might be more effective.

In conclusion, our data suggest that albuminuria associated with markers of glomerular damage is more likely to progress, whereas albuminuria associated with markers of tubulointerstitial damage is more likely to remain stable.

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Disclosures

None.

References

1. Mogensen CE: Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 310: 356–360, 1984
2. Wachtell K, Ibsen H, Olsen MH, Borch-Johnsen K, Lindholm LH, Mogensen CE, Dahlöf B, Devereux RB, Beevers G, de Faire U, Fyhrquist F, Julius S, Kjeldsen SE, Kristianson K, Lederballe-Pedersen O, Nieminen MS, Okin PM, Omvik P, Oparil S, Wedel H, Snapinn SM, Aurup P: Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: The LIFE study. *Ann Intern Med* 139: 901–906, 2003
3. Damsgaard EM, Frøland A, Jørgensen OD, Mogensen CE: Microalbuminuria as predictor of increased mortality in elderly people. *BMJ* 300: 297–300, 1990
4. Hillege HL, Fidler V, Diercks GF, van Gilst WH, de Zeeuw D, van Veldhuisen DJ, Gans RO, Janssen WM, Grobbee DE, de Jong PE; Prevention of Renal and Vascular End Stage Disease (PREVEND) Study Group: Urinary albumin excretion predicts cardiovascular and noncardiovascular mortality in general population. *Circulation* 106: 1777–1782, 2002
5. Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, Coresh J, Gansevoort RT; Chronic Kidney Disease Prognosis Consortium: Association of estimated glomerular filtration rate and albuminuria with all-cause and cardiovascular mortality in general population cohorts: A collaborative meta-analysis. *Lancet* 375: 2073–2081, 2010
6. van der Velde M, Halbesma N, de Charro FT, Bakker SJ, de Zeeuw D, de Jong PE, Gansevoort RT: Screening for albuminuria identifies individuals at increased renal risk. *J Am Soc Nephrol* 20: 852–862, 2009
7. Keane WF, Zhang Z, Lyle PA, Cooper ME, de Zeeuw D, Grunfeld JP, Lash JP, McGill JB, Mitch WE, Remuzzi G, Shahinfar S, Snapinn SM, Toto R, Brenner BM; RENAAL Study Investigators: Risk scores for predicting outcomes in patients with type 2 diabetes and nephropathy: The RENAAL study. *Clin J Am Soc Nephrol* 1: 761–767, 2006
8. Spoelstra-de Man AM, Brouwer CB, Stehouwer CD, Smulders YM: Rapid progression of albumin excretion is an independent predictor of cardiovascular mortality in patients with type 2 diabetes and microalbuminuria. *Diabetes Care* 24: 2097–2101, 2001
9. Brantsma AH, Bakker SJ, de Zeeuw D, de Jong PE, Gansevoort RT; PREVEND Study Group: Extended prognostic value of urinary albumin excretion for cardiovascular events. *J Am Soc Nephrol* 19: 1785–1791, 2008
10. El Nahas M: Cardio-Kidney-Damage: A unifying concept. *Kidney Int* 78: 14–18, 2010
11. Nangaku M: Chronic hypoxia and tubulointerstitial injury: A final common pathway to end-stage renal failure. *J Am Soc Nephrol* 17: 17–25, 2006
12. Waanders F, Navis G, van Goor H: Urinary tubular biomarkers of kidney damage: Potential value in clinical practice. *Am J Kidney Dis* 55: 813–816, 2010
13. van Timmeren MM, Vaidya VS, van Ree RM, Oterdoom LH, de Vries AP, Gans RO, van Goor H, Stegeman CA, Bonventre JV, Bakker SJ: High urinary excretion of kidney injury molecule-1 is an independent predictor of graft loss in renal transplant recipients. *Transplantation* 84: 1625–1630, 2007
14. Peters HP, Waanders F, Meijer E, van den Brand J, Steenbergen EJ, van Goor H, Wetzels JF: High urinary excretion of kidney injury molecule-1 is an independent predictor of end-stage renal disease in patients with IgA nephropathy. *Nephrol Dial Transplant* 26: 3581–3588, 2011
15. Branten AJ, du Buf-Vereijken PW, Klasen IS, Bosch FH, Feith GW, Hollander DA, Wetzels JF: Urinary excretion of beta2-microglobulin and IgG predict prognosis in idiopathic membranous nephropathy: A validation study. *J Am Soc Nephrol* 16: 169–174, 2005
16. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Campo S, Fazio MR, Nicocia G, Buemi M: Neutrophil gelatinase-associated lipocalin (NGAL) and progression of chronic kidney disease. *Clin J Am Soc Nephrol* 4: 337–344, 2009
17. Pinto-Sietsma SJ, Janssen WM, Hillege HL, Navis G, De Zeeuw D, De Jong PE: Urinary albumin excretion is associated with renal functional abnormalities in a nondiabetic population. *J Am Soc Nephrol* 11: 1882–1888, 2000
18. Nakamura Y, Myers BD: Charge selectivity of proteinuria in diabetic glomerulopathy. *Diabetes* 37: 1202–1211, 1988
19. Hemmelder MH, de Zeeuw D, de Jong PE: Measurement of glomerular charge selectivity in non-diabetic renal disease. *Nephrol Dial Transplant* 12[Suppl 2]: 57–62, 1997
20. Waanders F, van Timmeren MM, Stegeman CA, Bakker SJ, van Goor H: Kidney injury molecule-1 in renal disease. *J Pathol* 220: 7–16, 2010
21. Herget-Rosenthal S, Feldkamp T, Volbracht L, Kribben A: Measurement of urinary cystatin C by particle-enhanced nephelometric immunoassay: Precision, interferences, stability and reference range. *Ann Clin Biochem* 41: 111–118, 2004
22. Cruz DN, Goh CY, Haase-Fielitz A, Ronco C, Haase M: Early biomarkers of renal injury. *Congest Heart Fail* 16[Suppl 1]: S25–S31, 2010
23. Pelsers MM: Fatty acid-binding protein as marker for renal injury. *Scand J Clin Lab Invest Suppl* 241: 73–77, 2008
24. Maatman RG, Van Kuppevelt TH, Veerkamp JH: Two types of fatty acid-binding protein in human kidney. Isolation, characterization and localization. *Biochem J* 273: 759–766, 1991
25. Kuwabara T, Mori K, Mukoyama M, Kasahara M, Yokoi H, Saito Y, Yoshioka T, Ogawa Y, Imamaki H, Kusakabe T, Ebihara K, Omata M, Satoh N, Sugawara A, Barasch J, Nakao K: Urinary neutrophil gelatinase-associated lipocalin levels reflect damage to glomeruli, proximal tubules, and distal nephrons. *Kidney Int* 75: 285–294, 2009
26. Fornoni A, Ijaz A, Tejada T, Lenz O: Role of inflammation in diabetic nephropathy. *Curr Diabetes Rev* 4: 10–17, 2008
27. Nauta FL, Bakker SJ, van Oeveren W, Navis G, van der Heide JJ, van Goor H, de Jong PE, Gansevoort RT: Albuminuria, proteinuria, and novel urine biomarkers as predictors of long-term

allograft outcomes in kidney transplant recipients. *Am J Kidney Dis* 57: 733–743, 2011
28. Tofik R, Aziz R, Reda A, Rippe B, Bakoush O: The value of IgG-uria in predicting renal failure in idiopathic glomerular diseases. A long-term follow-up study. *Scand J Clin Lab Invest* 71: 123–128, 2011
29. Bazzi C, Petrini C, Rizza V, Arrigo G, Napodano P, Paparella M, D'Amico G: Urinary N-acetyl-beta-glucosaminidase excretion is a marker of tubular cell dysfunction and a predictor of outcome in primary glomerulonephritis. *Nephrol Dial Transplant* 17: 1890–1896, 2002
30. Hofstra JM, Deegens JK, Steenbergen EJ, Wetzels JF: Urinary excretion of fatty acid-binding proteins in idiopathic membranous nephropathy. *Nephrol Dial Transplant* 23: 3160–3165, 2008
31. Tam FW, Riser BL, Meeran K, Rambow J, Pusey CD, Frankel AH: Urinary monocyte chemoattractant protein-1 (MCP-1) and connective tissue growth factor (CCN2) as prognostic markers for progression of diabetic nephropathy. *Cytokine* 47: 37–42, 2009
32. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, Gansevoort RT, Kasiske BL, Eckardt KU: The definition, classification and prognosis of chronic kidney disease: A KDIGO Controversies Conference report. *Kidney Int* 80: 17–28, 2010
33. van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey A, de Jong P, Gansevoort RT, van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, Levey AS, de Jong PE, Gansevoort RT, Levey A, El-Nahas M, Eckardt KU, Kasiske BL, Ninomiya T, Chalmers J, Macmahon S, Tonelli M, Hemmelgarn B, Sacks F, Curhan G, Collins AJ, Li S, Chen SC, Hawaii Cohort KP, Lee BJ, Ishani A, Neaton J, Svendsen K, Mann JF, Yusuf S, Teo KK, Gao P, Nelson RG, Knowler WC, Bilo HJ, Joosten H, Kleefstra N, Groenier KH, Auguste P, Veldhuis K, Wang Y, Camarata L, Thomas B, Manley T; Chronic Kidney Disease Prognosis Consortium: Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. *Kidney Int* 79: 1341–1352, 2011
34. Astor BC, Matsushita K, Gansevoort RT, van der Velde M, Woodward M, Levey AS, Jong PE, Coresh J, Astor BC, Matsushita K, Gansevoort RT, van der Velde M, Woodward M, Levey AS, de Jong PE, Coresh J, El-Nahas M, Eckardt KU, Kasiske BL, Wright J, Appel L, Greene T, Levin A, Djurdjev O, Wheeler DC, Landray MJ, Townend JN, Emberson J, Clark LE, Macleod A, Marks A, Ali T, Fluck N, Prescott G, Smith DH, Weinstein JR, Johnson ES, Thorp ML, Wetzels JF, Blankestijn PJ, van Zuilen AD, Menon V, Sarnak M, Beck G, Kronenberg F, Kollerits B, Froissart M, Stengel B, Metzger M, Remuzzi G, Ruggenenti P, Perna A, Heerspink HJ, Brenner B, de Zeeuw D, Rossing P, Parving HH, Auguste P, Veldhuis K, Wang Y, Camarata L, Thomas B, Manley T; Chronic Kidney Disease Prognosis Consortium: Lower estimated glomerular filtration rate and higher albuminuria are associated with mortality and end-stage renal disease. A collaborative meta-analysis of kidney disease population cohorts. *Kidney Int* 79: 1331–1340, 2011
35. Gansevoort RT, Matsushita K, van der Velde M, Astor BC, Woodward M, Levey AS, de Jong PE, Coresh J; Chronic Kidney Disease Prognosis Consortium: Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts. *Kidney Int* 80: 93–104, 2011
36. Kern EF, Erhard P, Sun W, Genuth S, Weiss MF: Early urinary markers of diabetic kidney disease: A nested case-control study from the Diabetes Control and Complications Trial (DCCT). *Am J Kidney Dis* 55: 824–834, 2010
37. Nauta FL, Boertien WE, Bakker SJ, van Goor H, van Oeveren W, de Jong PE, Bilo H, Gansevoort RT: Glomerular and tubular damage markers are elevated in patients with diabetes. *Diabetes Care* 34: 975–981, 2011
38. Bolignano D, Lacquaniti A, Coppolino G, Donato V, Fazio MR, Nicocia G, Buemi M: Neutrophil gelatinase-associated lipocalin as an early biomarker of nephropathy in diabetic patients. *Kidney Blood Press Res* 32: 91–98, 2009
39. Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K, Gansevoort RT, Kasiske BL, Eckardt KU: The definition, classification, and prognosis of chronic kidney disease: A KDIGO Controversies Conference report. *Kidney Int* 80: 17–28, 2011
40. Brinkman JW, de Zeeuw D, Duker JJ, Gansevoort RT, Kema IP, Hillege HL, de Jong PE, Bakker SJ: Falsely low urinary albumin concentrations after prolonged frozen storage of urine samples. *Clin Chem* 51: 2181–2183, 2005
41. Nauta FL, Bakker SJ, Lambers Heerspink H, de Zeeuw D, van Oeveren W, Bilo H, de Jong PE, Gansevoort RT: Effect of frozen storage on urinary concentration of kidney damage markers. *Am J Kidney Dis* 59: 586–589, 2012
42. Witte EC, Lambers Heerspink HJ, de Zeeuw D, Bakker SJ, de Jong PE, Gansevoort R: First morning voids are more reliable than spot urine samples to assess microalbuminuria. *J Am Soc Nephrol* 20: 436–443, 2009

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