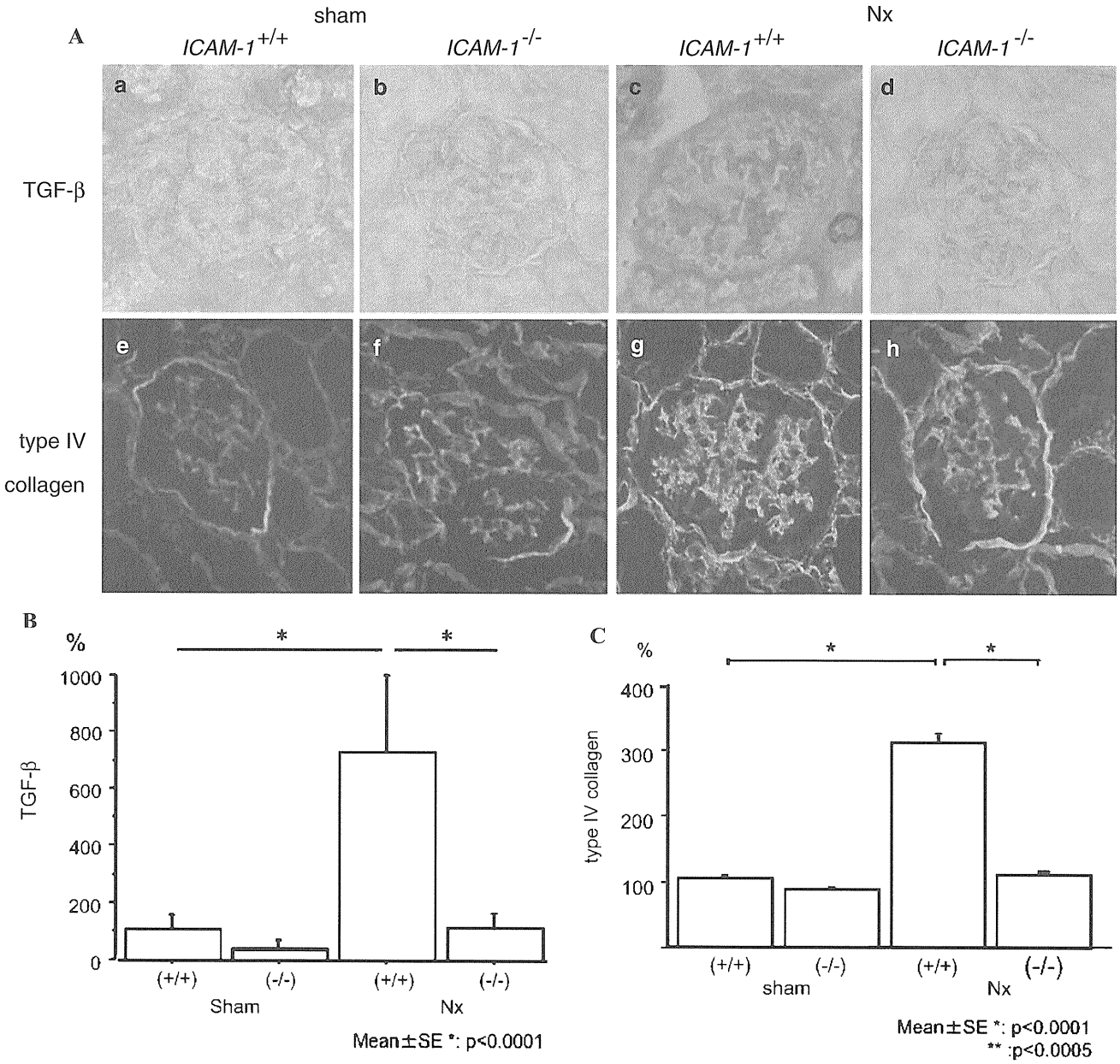

Fig. 3 Expression of TGF-β and type IV collagen in the mice glomeruli. **A** TGF-β (**a–d**) and type IV collagen (**e–h**) in the glomeruli of mice were visualized as described in the "Materials and methods". **B** TGF-β expression was prominent in the glomeruli of ICAM-$1^{+/+}$ Nx mice compared with ICAM-$1^{-/-}$ Nx mice. **C** Expression of type IV collagen was increased in the glomeruli of ICAM-$1^{+/+}$ Nx mice compared with ICAM-$1^{-/-}$ Nx mice. ×400. Data are mean ± SEM, *$P < 0.0001$, +/+, ICAM-$1^{+/+}$ mice; −/−, ICAM-$1^{-/-}$ mice

very early stages soon after the induction of diabetes [6] and subtotal nephrectomy [13]. These lines of evidences strongly suggest that ICAM-1 is a critical molecule for the infiltration processes of macrophages in several types of glomerular disorders.

The increased expression of ICAM-1 within the glomeruli is observed in various renal diseases and in experimental models [6, 10–15]. Thus, there are probably some common mechanisms for the induction of ICAM-1 expression in renal diseases. Recent studies have revealed several possible mechanisms of ICAM-1 induction in hyperfiltration-induced glomerular disorder. Nagel et al. [16] showed that shear stress upregulated the expression of ICAM-1 in cultured human vascular endothelial cells. Tsuboi et al. [17] also demonstrated that human umbilical vein endothelial cells increased their cell surface expression of ICAM-1 in response to flow loading. The expression of ICAM-1 can be also modulated by inflammatory

cytokines including tumor necrosis factor-α, interleukin-1, interferon-γ [18], and oxidative stress [19] as well as by shear stress.

In this study, we showed that amelioration of macrophage infiltration into the glomeruli of Nx group of ICAM-$1^{-/-}$ mice was associated with reduced mesangial matrix expansion (Fig. 2), production of TGF-β and accumulation of type IV collagen (Fig. 3) in the glomeruli, compared with the Nx group of ICAM-$1^{+/+}$ mice. To investigate the role of macrophages in the pathogenesis of glomerulosclerosis in a subtotal nephrectomized model, van Goor et al. [20] examined the effect of macrophage depletion using systemic X-irradiation on glomerular injury after 3/4 renal ablation in the rat. They demonstrated that the number of glomerular macrophages and semiquantitative scores for mesangial cellularity and mesangial matrix expansion were significantly lower in remnant glomeruli of X-irradiated rats compared to non-irradiated remnant kidney rats. This study provides the direct evidence that the macrophages mediate the progression of glomerular injury after renal ablation. Adding to the possible involvement of infiltrated macrophages in accelerated extracellular matrix (ECM) accumulation in glomeruli via in situ TGF-β production [21], Pawluczyk et al. [22] reported that the culture supernatant of macrophages stimulates mesangial cells to produce fibronectin in vitro. Together with our present results and other studies, we can speculate that the infiltrated glomerular macrophages involves in the induction of TGF-β secretion by mesangial cells associated with ECM overproduction and accumulation after renal ablation.

In conclusion, we have demonstrated that albuminuria, glomerular hypertrophy, and mesangial matrix expansion were ameliorated in subtotally nephrectomized ICAM-$1^{-/-}$ mice compared with that of ICAM-$1^{+/+}$ mice. These results suggest that ICAM-1 may play a critical role in the induction of glomerulosclerosis via recruitment of macrophages, the key participants in the progression of renal diseases.

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Conflict of interest The authors have declared that no Conflict of interest exists.

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Association between urinary angiotensinogen levels and renal and cardiovascular prognoses in patients with type 2 diabetes mellitus

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ABSTRACT

Aims/Introduction: Activation of the renin-angiotensin system (RAS) in the kidney plays an important role in renal function. The aim of this study was to investigate whether plasma and urinary angiotensinogen levels were associated with renal and cardiovascular prognosis in type 2 diabetic patients.

Materials and Methods: We measured plasma and urinary angiotensinogen levels in the observational follow-up cohort of 234 Japanese type 2 diabetic patients (144 with normoalbuminuria, 90 with albuminuria) enrolled between 1998 and 1999 and followed them up until the end of 2008. The associations of these markers with the annual decline in the estimated glomerular filtration rate (eGFR) and incidence of renal and cardiovascular composite endpoints (chronic hemodialysis, myocardial infarction, angina pectoris, stroke and cerebral hemorrhage) were evaluated.

Results: At baseline, urinary angiotensinogen levels correlated with urinary albumin-creatinine ratio, urinary β_2-microglobulin and inversely with eGFR. In contrast, plasma angiotensinogen levels correlated neither with these renal factors nor with urinary angiotensinogen levels. In the follow-up study (median duration: 9 years), urinary angiotensinogen, but not plasma angiotensinogen, correlated inversely with the annual change in eGFR ($r = -0.51$, $P < 0.001$). When patients were divided into four subgroups according to albuminuria and urinary angiotensinogen levels, patients with albuminuria and high urinary angiotensinogen levels showed a progressive decline of eGFR and a higher incidence of renal and cardiovascular composite endpoints.

Conclusions: These results suggest that the higher level of urinary angiotensinogen in type 2 diabetic patients with albuminuria is a high risk factor for worsening renal and cardiovascular complications. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00172.x, 2011)

KEY WORDS: Angiotensinogen, Diabetes mellitus, Glomerular filtration rate

INTRODUCTION

Diabetic nephropathy is a representative disorder of chronic kidney disease (CKD) and a leading cause of end-stage kidney disease (ESKD). This disorder is also associated with high morbidity and mortality of cardiovascular disease (CVD)[1–3]. Thus, prevention of development and progression of this disorder is of clinical importance to improve prognosis in diabetic patients.

Numerous clinical trials have documented that inhibition of the renin-angiotensin system (RAS) in diabetic patients can slow the progressive decrease in glomerular filtration rate (GFR) and reduce cardiovascular mortality and morbidity[4–6]. Based on clinical evidence, inhibition of the RAS is currently the first line treatment for diabetic nephropathy[7]. These results also support the concept that activation of RAS in diabetic patients is an important pathogenic mechanism of renal and cardiovascular complications[7]. However, despite the beneficial effects of RAS inhibition, all patients do not always show an improvement in the prognosis of these complications. Therefore, it is important to identify patients at higher risk of poor prognosis and a proper estimation of the status of intrarenal RAS activation may provide crucial information.

The kidney contains all components of the RAS pathway including the production of angiotensinogen[8]. Thus, the kidney can locally produce angiotensin II (AngII) by a mechanism independent of circulating AngII, known as the classical RAS pathway[8]. Intrarenally-produced AngII is reported to play an important role in renal hemodynamics and function as a paracrine factor[9].

We recently developed a direct method to quantify human plasma and urinary angiotensinogen levels using enzyme-linked

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immunosorbent assays (ELISA)[10]. Using this new method, we recently reported that urinary angiotensinogen may be a potential biomarker of the severity of CKD and intrarenal RAS status in hypertensive patients in the cross-sectional studies[11,12]. However, it is still unclear whether urinary and plasma angiotensinogen levels can be used to predict deterioration of renal function and the incidence of cardiovascular disease in a long longitudinal cohort. In the present study, we measured plasma and urinary angiotensinogen levels using our new ELISA method, in Japanese patients with type 2 diabetes who were enrolled in our observational follow-up study[2]. We then investigated whether these markers associate with renal and cardiovascular prognosis.

MATERIALS AND METHODS

Study Population and Samples

Japanese patients with type 2 diabetes mellitus were recruited from among participants who were registered in the Shiga Prospective Observational Follow-up Study between 1998 and 1999[2]. After obtaining written informed consent, each individual provided a spot urine sample and a fasting blood sample at baseline. The plasma and urine samples were kept at −80°C if not analyzed immediately. Based on the level of urinary albumin–creatinine ratio (UACR) at baseline, patients were classified as having normoalbuminuria (UACR < 30 mg/g Cr), microalbuminuria (30 ≤ UACR < 300 mg/g Cr), or overt proteinuria (UACR ≥ 300 mg/g Cr). Finally, 234 patients with normoalbuminuria (n = 144), microalbuminuria (n = 53) and overt proteinuria (n = 37) were enrolled and were followed up until the end of 2008 or the incidence of the renal and cardiovascular composite endpoints. In this study, patients with microalbuminuria and overt proteinuria were combined together into those with albuminuria (diabetic nephropathy). The participants underwent standardized clinical examination and biochemical tests annually, during the follow-up period. In this study, the values of HbA1c were presented in National Glycohemoglobin Standardization Program values according to the recommendations of the Japanese Diabetes Society[13]. The study protocol and informed consent procedure were approved by the Ethics Committee of Shiga University of Medical Science.

Measurement of Plasma and Urinary Angiotensinogen Levels

The concentrations of angiotensinogen in plasma and urine samples at baseline were measured with human angiotensinogen ELISA, as reported previously[10]. The sensitivity of this assay is >0.31 ng/mL. The intra- and inter-assay coefficients of variation were 4.4 and 4.3%, respectively. The urinary concentrations of creatinine were measured simultaneously by the enzymatic method. The urinary level of angiotensinogen was expressed in μg/g Cr.

Follow-up Evaluation

To evaluate deterioration of renal function, we assessed the annual decline in estimated GFR (eGFR). eGFR was calculated using the simplified prediction equation proposed by the Japanese Society of Nephrology[14]: eGFR (mL/min/1.73 m^2) = $194 \times [\text{age (years)}]^{-0.287} \times [\text{serum creatinine (mg/dL)}]^{-1.094} \times 0.739$ (for female). The serum concentration of creatinine was measured using the enzymatic method. The annual decline in eGFR over the course of the study was determined from the slope of the plot of all measurements of eGFR for each individual calculated by linear regression analysis and was expressed in mL/min/1.73 m^2/year.

We also investigated the incidence of the renal and cardiovascular composite endpoints, including myocardial infarction, angina pectoris, stroke and cerebral hemorrhage and initiation of chronic hemodialysis. Myocardial infarction was defined as a clinical presentation characterized by typical symptoms, electrocardiographic changes associated with an elevation of cardiac biomarkers and angiographic evidence of coronary thrombosis. Angina pectoris was defined as a history of typical chest pain and electrocardiographic changes compatible with ischemic heart disease or the detection of myocardial perfusion defects with exercise stress tests. Stroke and cerebral hemorrhage were defined as a persistent focal neurological symptom in which onset was sudden and was not due to trauma or a tumor and where the responsible lesion was detected by imaging studies.

Statistical Analysis

Data are expressed as mean ± SD or median (interquartile range). As compared between two groups, unpaired Student's t-test for continuous variables and chi-square test for categorical variables were applied. A comparison among three or more groups was performed by ANOVA with the Tukey-Kramer HSD test. Due to the skewed distribution, urinary angiotensinogen, UACR and urinary β_2-microglobrin (U-β_2MG) values were log-transformed before analysis. Pearson regression analysis was applied for analysis of the correlation between two variables, using logarithmic transformed values of non-normally distributed variables. A multivariate liner regression model was applied to evaluate the independency of factors that showed significant correlation in the univariate model. The cumulative incidences of renal and cardiovascular composite endpoints were estimated using Kaplan–Meier procedure and were compared by the log-rank test. The follow-up time was censored if any composite endpoint was observed or if the patient was unavailable for follow-up. Risk for renal and cardiovascular composite endpoint was evaluated by a Cox hazard regression model. A forward stepwise procedure was used to select explanatory variables with statistically significant effects on the time to the incidence of the endpoint. All analyses were performed by the SPSS software package (version 11; SPSS Inc., Chicago, IL, USA) and JMP for Windows (version 8.0.2; SAS Institute Inc, Cary, NC, USA). A two-sided P value <0.05 was considered statistically significant.

RESULTS

Baseline Clinical Characteristics

Table 1 lists the clinical characteristics of patients at baseline stratified by the stage of nephropathy. Gender, duration of diabetes, body mass index (BMI), HbA1c, systolic blood pressure

Table 1 | Clinical characteristics of the study subjects

	Normoalbuminuria	Albuminuria	*P*
Number	144	90	
Gender (male/female)	72/72	57/33	<0.05
Age (year)	60 ± 8	59 ± 9	n.s.
Duration of diabetes (year)	13 ± 8	16 ± 8	<0.01
Body mass index (kg/m^2)	23.1 ± 3.4	24.5 ± 3.7	<0.01
Waist to hip ratio	0.93 ± 0.08	0.95 ± 0.09	n.s.
HbA1c (%)	7.4 ± 0.8	7.9 ± 1.2	<0.01
Systolic blood pressure (mmHg)	135 ± 17	144 ± 19	<0.01
Diastolic blood pressure (mmHg)	77 ± 9	81 ± 10	<0.01
Taking RAS inhibitors (%)	16	31	<0.01
Past history of CVD (%)	13	20	n.s.
Total cholesterol (mg/dL)	213 ± 32	219 ± 37	n.s.
HDL-cholesterol (mg/dL)	60 ± 156	56 ± 15	n.s.
Triglycerides (mg/dL)	111 ± 32	135 ± 76	<0.05
Urinary ACR (mg/g Cr)	10 (7–15)	161 (61–672)	<0.05
Estimated GFR (mL/min/1.73 m^2)	81 ± 15	69 ± 26	<0.01
Urinary β_2-microglobulin (μg/g Cr)	114 (73–172)	188 (81–907)	<0.01

Data are mean ± SD for normally distributed continuous variables or median (25th–75th interquartiles) for skewed continuous variables. Albuminuria represents microalbuminuria and overt proteinuria. RAS, renin-angiotensin system; ACR, albumin-creatinine ratio; Cr, creatinine; GFR, glomerular filtration rate; CVD, cardiovascular disease.

(SBP), diastolic blood pressure (DBP), use of RAS inhibitors, triglyceride, UACR, eGFR and U-β_2MG were different between the normoalbuminuria and albuminuria groups.

Correlation Between Plasma Angiotensinogen Level and Various Parameters at Baseline

Plasma angiotensinogen levels were not different between two groups (normoalbuminuria: 24.7 ± 5.3, albuminuria: 24.1 ± 5.4 μg/mL). Univariate regression analysis showed weak correlations between plasma angiotensinogen levels and BMI, waist-hip ratio, SBP, total cholesterol, HDL-cholesterol and triglyceride, and no correlation with UACR, eGFR and U-β_2MG (Table 2). Plasma angiotensinogen levels were not different between patients treated with RAS inhibitors and those without them (24.4 ± 5.3 vs 24.6 ± 5.6 μg/mL, $P = 0.97$). Interestingly, plasma angiotensinogen levels were significantly higher in females than males (26.7 ± 5.3 vs 22.6 ± 4.6 μg/mL, $P < 0.001$). However, plasma angiotensinogen levels did not correlate with UACR, eGFR and U-β_2MG even when patients were analyzed separately according to gender.

Correlation Between Urinary Angiotensinogen Level and Various Parameters at Baseline

In contrast to plasma angiotensinogen, urinary angiotensinogen levels were higher in patients with albuminuria (62.0 μg/g Cr [interquartile range: 25.4–146.5]) than in those with normoalbuminuria (17.5 μg/g Cr [11.4–28.2], $P < 0.001$). Univariate regression analysis showed that urinary angiotensinogen levels correlated positively with UACR and U-β_2MG and inversely with eGFR (Table 2). Interestingly, there was no correlation between urinary angiotensinogen and plasma angiotensinogen ($r = 0.08$, $P = 0.21$). Urinary angiotensinogen levels were higher in patients treated with RAS inhibitors (38 μg/g Cr [19–133]) than those without (22 μg/g Cr [13–42], $P = 0.001$). However, this difference was probably due to the different prescription rate of RAS inhibitors in the two groups (normoalbuminuria: 16%, albuminuria: 32%). When urinary angiotensinogen levels were compared according to the stage of nephropathy, those in each stage were not different between patients treated with RAS inhibitors and those without. Unlike plasma angiotensinogen, the urinary angiotensinogen level in males was similar to that in females. Multiple regression analysis identified UACR and U-β_2MG as the independent and significant factors that correlated with urinary angiotensinogen levels.

Table 2 | Factors that correlated with plasma and urinary angiotensinogen levels in univariate analysis

Parameter	Plasma angiotensinogen		Urinary angiotensinogen	
	r	*P*	*r*	*P*
Age	−0.02	0.75	−0.07	0.31
Duration of diabetes	−0.10	0.11	0.24	<0.001
Body mass index	0.17	0.009	0.13	0.04
Waist to hip ratio	0.36	<0.001	0.10	0.15
HbA1c	0.10	0.14	0.26	<0.001
Systolic blood pressure	0.14	0.03	0.30	<0.001
Diastolic blood pressure	0.10	0.13	0.23	<0.001
Total cholesterol	0.35	<0.001	0.17	0.008
HDL-cholesterol	0.20	0.002	−0.12	0.06
Triglycerides	0.18	0.006	0.19	0.004
Urinary ACR	0.01	0.88	0.77	<0.001
Estimated GFR	0.04	0.59	−0.44	<0.001
Urinary β_2-microglobulin	−0.07	0.26	0.72	<0.001

Correlation was evaluated with the Pearson's correlation coefficient. The values of urinary angiotensinogen, urinary ACR and urinary β_2-microglobulin were log-transformed for the analysis because of their skewed distribution.
ACR, albumin-creatinine ratio; GFR, glomerular filtration rate.

Correlation Between Angiotensinogen Level and Annual Decline in eGFR

To explore the predictive role of plasma and urinary angiotensinogen levels for renal dysfunction, we investigated the correlation between each angiotensinogen and the annual change in eGFR during the follow-up period (median: 9 years, interquartile range: 6–10 years). As shown in Figure 1, urinary angiotensinogen, but not plasma angiotensinogen ($r = 0.00$, $P = 0.99$), correlated inversely with the annual change in eGFR ($r = -0.51$,

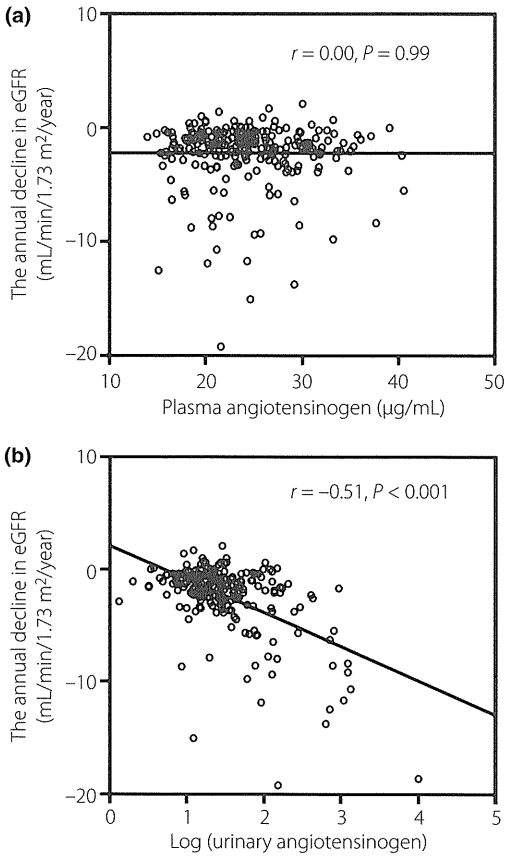

Figure 1 | Scatter diagram of the correlation between the annual decline in estimated glomerular filtration rate (eGFR) and (a) plasma angiotensinogen and (b) urinary angiotensinogen. Correlation was evaluated with the Pearson's correlation coefficient. Data are log-transformed values of urinary angiotensinogen.

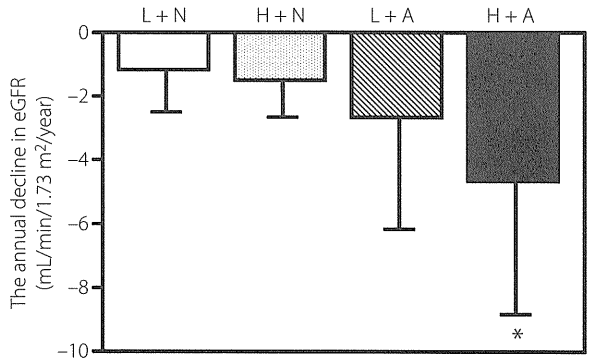

Figure 2 | Annual decline in estimated glomerular filtration rate (eGFR) during follow-up. Patients were divided into four groups using the median value of urinary angiotensinogen level (24.7 μg/g Cr) and the presence of albuminuria (>30 mg/g Cr). Patients with low levels of urinary angiotensinogen and normoalbuminuria (L + N, n = 97); patients with high levels of urinary angiotensinogen and normoalbuminuria (H + N, n = 47); patients with low levels of urinary angiotensinogen and albuminuria (L + A, n = 21) and patients with high levels of urinary angiotensinogen and albuminuria (H + A, n = 69). The respective annual decline in eGFR was: −1.2 ± 1.3, −1.4 ± 1.3, −2.7 ± 3.5 and −4.6 ± 4.2 mL/min/1.73 m^2/year. Data are mean ± SD. *P < 0.05 vs each other group (ANOVA with Tukey–Kramer HSD test).

P < 0.001). As other factors, the annual decline in eGFR correlated strongly with UACR (r = −0.65, P < 0.001) and correlated weakly with triglyceride (r = −0.28, P < 0.001), HDL-cholesterol (r = 0.15, P = 0.027), HbA1c (r = −0.22, P = 0.001), eGFR at baseline (r = 0.32, P < 0.001), BMI (r = −0.24, P < 0.001), SBP (r = −0.24, P < 0.001) and DBP (r = −0.23, P < 0.001).

Urinary Angiotensinogen and Renal Dysfunction in Patients with Albuminuria

Albuminuria is well known to be a risk factor for renal dysfunction and cardiovascular disease in patients with type 2 diabetes. Based on the strong correlation between urinary angiotensinogen and UACR, it was difficult to determine the specific role of each parameter in renal dysfunction. Therefore, to explore the clinical utility of measuring urinary angiotensiongen, we investigated the predictive effect of the combination of urinary angiotensinogen and albuminuria on deterioration of renal function. For this purpose, patients were divided into four groups according to the median value of urinary angiotensinogen levels (median cut-off values: 24.7 μg/g Cr) and the presence of albuminuria (>30 mg/g Cr). The eGFR at baseline (mL/min/1.73 m^2) was 80 ± 15 in those with low levels of urinary angiotensinogen and normoalbuminuria (L + N, n = 97), 84 ± 14 in those with high levels of urinary angiotensinogen and normoalbuminuria (H + N, n = 47), 82 ± 19 in those with low levels of urinary angiotensinogen and albuminuria (L + A, n = 21) and 66 ± 27 in patients with high levels of urinary angiotensinogen and albuminuria (H + A, n = 69). Among the four subgroups, the annual decline in eGFR during the follow-up was significantly greater in the H + A subgroup than other subgroups (P < 0.05 vs all other subgroup, Figure 2).

Urinary Angiotensinogen and Renal-Cardiovascular Outcomes in Patients with Albuminuria

Finally, we evaluated the association between urinary angiotensinogen at baseline and the incidence of renal and cardiovascular composite endpoints. A total of 58 patients experienced any of the composite endpoints (17 for chronic hemodialysis, 10 for myocardial infarction, 18 for angina pectoris, eight for stroke and five for cerebral hemorrhage). The incidence rate of this endpoint was higher in patients with high levels of urinary angiotensinogen than those with low levels of urinary angiotensinogen (36% vs 14%, χ^2 = 15.5, P < 0.001). Similarly, the incidence rate of this endpoint was higher in patients with albuminuria than those with normoalbuminuria (47% vs 11%, χ^2 = 37.6, P < 0.001). As shown in Figure 3, the cumulative incidence among the four subgroups was the highest in the H + A subgroup (log rank test: P < 0.001 for trend). Multivariate Cox proportional hazard regression model with the forward

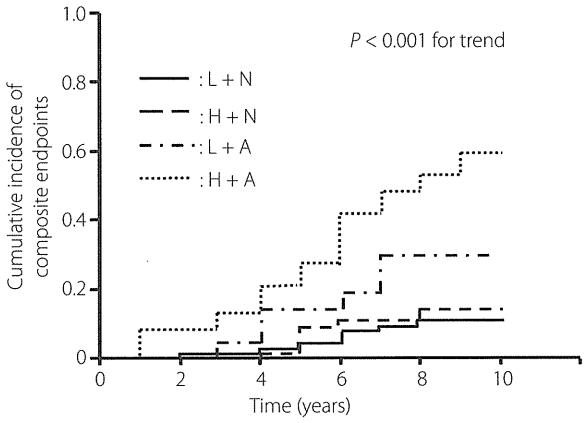

Figure 3 | Kaplan–Meier curves for cumulative incidence of renal and cardiovascular composite endpoints. Patients were divided into the four groups using the median value of urinary angiotensinogen level (24.7 μg/g Cr) and the presence of albuminuria (>30 mg/g Cr). Patients with low levels of urinary angiotensinogen and normoalbuminuria (L + N, $n = 97$); patients with high levels of urinary angiotensinogen and normoalbuminuria (H + N, $n = 47$); patients with low levels of urinary angiotensinogen and albuminuria (L + A, $n = 21$) and patients with high levels of urinary angiotensinogen and albuminuria (H + A, $n = 69$). Difference among the groups was tested by log rank test.

stepwise procedure identified four predictors of renal and cardiovascular outcomes: the combination of urinary angiotensinogen and albuminuria (adjusted odds ratio 4.5 [95% CI: 2.1–9.5] in H + A subgroup, 3.4 [1.2–9.3] in L + A subgroup and 1.6 [0.6–4.4] in H + N subgroup, 1.0 [reference] in L + N subgroup), age (1.04 [1.00–1.08]), eGFR at baseline (0.97 [0.96–0.98]) and past history of CVD (1.90 [1.06–3.41]).

DISCUSSION

In this study, analysis of baseline data showed that urinary angiotensinogen levels correlated with UACR and U-β_2MG and inversely with eGFR. In contrast, plasma angiotensinogen levels did not correlate with these factors or with urinary angiotensinogen levels. Furthermore, follow-up analysis indicated that patients with albuminuria and high levels of urinary angiotensinogen showed the progressive decline of renal function and the high incidence of renal-cardiovascular endpoints. These results suggest that the higher level of urinary angiotensinogen in type 2 diabetic patients with nephropathy is a high risk factor for worsening renal and cardiovascular complications.

In the present study, urinary angiotensinogen levels correlated closely with renal factors but did not correlate with plasma angiotensinogen levels. In contrast, plasma angiotensinogen levels correlated with various metabolic factors including BMI, waist-hip ratio and serum lipids, in agreement with the data of a previous report[15], but they did not correlate with renal factors. These results suggest that urinary and plasma angiotensinogen are produced by different sources and play different roles in renal function. Although angiotensinogen is produced and secreted by the liver, it is also produced in the kidney[9]. Previous studies have investigated whether circulating angiotensinogen is a source of urinary angiotensinogen. In hypertensive and normotensive rats infused human angiotensinogen, the circulating human angiotensinogen was not detectable in the urine, indicating limited glomerular permeability and/or tubular degradation of circulating angiotensinogen[16]. In the kidney under normal conditions, the expression of angiotensinogen is reported to localize in proximal tubular cells and angiotensinogen produced in proximal tubular cells is considered to be directly released into the renal tubular lumen[9]. Under diabetic conditions, the expression of angiotensinogen is reported to be enhanced in proximal tubular cells and to be also observed in mesangial cells[17,18]. Some human studies reported higher levels of urinary angiotensinogen in diabetic patients than in control subjects and patients with non-diabetic kidney diseases[11,19], whereas plasma angiotensinogen levels were similar in diabetic patients and control subjects[19]. Because the kidney contains all components of the RAS pathway, the enhanced expression of intrarenal angiotensinogen may lead to the intrarenal RAS activation. Thus, these results suggest that urinary angiotensinogen is produced locally in the kidney, but not from plasma, and its levels may associate with intrarenal RAS activation in diabetic patients.

In the present study, patients with high levels of urinary angiotensinogen, not plasma angiotensinogen, showed a greater decline in eGFR during the follow-up. A similar observation in patients with CKD documented the presence of higher urinary angiotensinogen levels in patients with low eGFR and patients with higher levels of urinary angiotensinogen showed increased risk of renal dysfunction during a mean follow-up period of 23 months[20]. Thus, urinary angiotensinogen is considered to be associated with the deterioration of renal function in patients with CKD including diabetic nephropathy.

Albuminuria is well known to be not only a predictor of progression to ESKD but also a risk factor for cardiovascular disease[1,2]. In this study, urinary angiotensinogen levels correlated closely with UACR as well as previous reports[12,21]. However, patients with albuminuria and higher levels of urinary angiotensinogen showed a progressive decline in eGFR and the high incidence of renal-cardiovascular endpoints than those with albuminuria and low levels of angiotensinogen. Thus, the increase of urinary angiotensinogen in patients with albuminuria may predict the patients at risk for worsening renal and cardiovascular complications.

What is the mechanism by which urinary angiotensinogen levels associate with worsening renal and cardiovascular complications? In this study, urinary angiotensinogen levels correlated with UACR and U-β_2MG. Transgenic mice overexpressing angiotensinogen in renal proximal tubular cells were reported to develop albuminuria, hypertension and renal injury[22]. The induction of diabetes with streptozotocin in these transgenic mice enhanced the aforementioned abnormal changes and induced apoptosis of renal proximal tubular cells[23]. Although diabetic nephropathy was traditionally considered to cause glomerular

damage primarily, it is now widely accepted that deterioration of renal function in diabetic patients correlates with the degree of tubulointerstitial fibrosis[24,25]. Thus, the enhanced expression of angiotensinogen in proximal tubular cells under diabetic conditions, which may correlate with urinary angiotensiongen levels, may cause the tubulointerstitial injury and, then, result in the decline in eGFR. Also the augmentation of urinary angiotensinogen is considered to lead to increased formation of AngII in the kidney[9]. Thus, the increase of urinary angiotensinogen may contribute to the development and progression of hypertension, which may associate with renal dysfunction and the incidence of cardiovascular disease. In the present study, urinary angiotenisinogen levels correlated with systolic and diastolic blood pressure as well as a previous report[12].

In this study, the data of clinical parameters including angiotensinogen were collected only at baseline. Thus, the time-dependent changes in these parameters during the follow-up were not evaluated. Also, the information regarding the use of RAS inhibitors during the follow-up period was not included in this study. Previous studies reported that RAS inhibitors were associated with reduction in urinary angiotensinogen levels[12,26]. In the present study, the levels of urinary angiotensinogen in patients treated with RAS inhibitors were not different from those without such treatment when data was analyzed separately according to the stage of nephropathy. In Japan, the prescription rate of RAS inhibitors in the past was much lower than that at present. Also, RAS inhibitors tended to be prescribed for patients who showed progression to the advanced stage of nephropathy or those at risk for cardiovascular disease. Thus, the present study does not provide conclusive data on the influence of RAS inhibitors on urinary angiotensinogen levels. Further studies are required to explore whether the reduction of urinary angiotensinogen level by any medication bring about improving renal and cardiovascular prognoses.

In conclusion, the present study demonstrated that urinary angiotensinogen levels correlated with progressive deterioration of renal function and the high incidence of renal-cardiovascular endpoints in patients with type 2 diabetes mellitus. These results suggest that higher levels of urinary angiotensinogen in patients with diabetic nephropathy are clinically useful to identify patients who are at high risk for worsening renal and cardiovascular complications. Also, the reduction of urinary angiotensinogen levels may be a new therapeutic index to prevent the worsening of renal and cardiovascular complications in diabetic patients with nephropathy.

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Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes. A collaborative meta-analysis of general and high-risk population cohorts

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Both a low estimated glomerular filtration rate (eGFR) and albuminuria are known risk factors for end-stage renal disease (ESRD). To determine their joint contribution to ESRD and other kidney outcomes, we performed a meta-analysis of nine general population cohorts with 845,125 participants and an additional eight cohorts with 173,892 patients, the latter selected because of their high risk for chronic kidney disease (CKD). In the general population, the risk for ESRD was unrelated to eGFR at values between 75 and 105 ml/min per 1.73 m^2 but increased exponentially at lower levels. Hazard ratios for eGFRs averaging 60, 45, and 15 were 4, 29, and 454, respectively, compared with an eGFR of 95, after adjustment for albuminuria and cardiovascular risk factors. Log albuminuria was linearly associated with log ESRD risk without thresholds. Adjusted hazard ratios at albumin-to-creatinine ratios of 30, 300, and 1000 mg/g were 5, 13, and 28, respectively, compared with an albumin-to-creatinine ratio of 5. Albuminuria and eGFR were associated with ESRD, without evidence for multiplicative interaction. Similar associations were found for acute kidney injury and progressive CKD. In high-risk cohorts, the findings were generally comparable. Thus, lower eGFR and higher albuminuria are risk factors for ESRD, acute kidney injury and progressive CKD in both general and high-risk populations, independent of each other and of cardiovascular risk factors.

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KEYWORDS: acute kidney injury; albumin-to-creatinine ratio (albuminuria); dipstick (proteinuria); eGFR (kidney function); ESRD (end-stage renal disease); meta-analysis

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This is the third in a series of four manuscripts to report the results of collaborative meta-analyses of estimated GFR (eGFR) and albuminuria on outcomes of chronic kidney disease (CKD) undertaken by the CKD Prognosis Consortium. These analyses were undertaken in conjunction with the 2009 Controversies Conference sponsored by Kidney Disease Improving Global Outcomes (KDIGO) to evaluate the current definition and classification of CKD and proposed alternatives.[1] The report of the Consensus Conference is included in this issue of Kidney International.[2]

Widespread implementation of the definition and classification of CKD, as proposed by Kidney Disease Outcomes Quality Initiative (KDOQI) in 2002 and subsequently endorsed by KDIGO in 2004, has promoted increased attention to CKD in clinical practice, research, and public health.[3–6] It has also generated substantial debate about the appropriateness of recommending the same GFR thresholds for people of all ages, the optimal level of albuminuria for diagnosing kidney damage, and about the value of the 5-stage classification system based on eGFR without consideration of albuminuria.[7–11] It was the position of KDOQI and KDIGO that a comprehensive analysis of mortality and kidney outcomes according to eGFR and albuminuria was needed to answer key questions underlying the debate.[1,2]

Until recently, most of the data on kidney outcomes were from studies of patients with later stages of CKD rather than from general population cohorts or cohorts at increased risk for CKD.[12–14] Reports from the general population and high-risk cohorts focused mainly on all-cause and cardiovascular mortality,[15–20] with fewer data available on kidney outcomes.[19–22] In this manuscript, we describe a collaborative meta-analysis of nine general population and eight high-risk cohorts. The outcomes reported in this manuscript include kidney failure treated by dialysis or transplantation (end-stage renal disease (ESRD)) or coded on the death certificate. In addition, we also included acute kidney injury, because it is

increasingly recognized as a major cause for[23] and consequence of CKD,[24] and kidney disease progression, based on fast eGFR decline (progressive CKD), because of its clinical importance and potential to lead to ESRD or other complications.

Other papers in this series deal with all-cause and cardiovascular mortality in general population cohorts and high-risk cohorts.[25,26] This report describes the kidney outcomes from these cohorts. A fourth manuscript reports mortality and kidney outcomes in CKD cohorts.[27] *A priori* we hypothesized that both eGFR and albuminuria would be associated with these outcomes, independent of traditional cardiovascular risk factors and independent of each other, and despite inclusion of diverse study populations.

RESULTS

Study and population characteristics

Of the nine general population cohorts (845,125 subjects), five had data on albumin-to-creatinine ratio and four on dipstick. Of the eight high-risk cohorts (173,892 subjects), five had data on albumin-to-creatinine ratio and three on dipstick (Table 1). Acronyms and abbreviations for studies included in the current report are given in Supplementary Web appendix Table S1 online. Subjects in the high-risk cohorts were more often male, and these cohorts had a higher prevalence of cardiovascular risk factors than did the general population cohorts. Moreover, the high-risk cohorts generally had a lower eGFR and higher albumin-to-creatinine ratio. Not all cohorts had data on all kidney outcomes. There were a total of 2179, 4939, and 11,144 participants who developed ESRD, acute kidney injury, and progressive CKD, respectively. The incidence rates for the kidney outcomes were two- to sixfold higher in the high-risk cohorts compared with the general population cohorts (1.83 versus 0.31 for ESRD, 4.88 versus 2.21 for acute kidney injury, and 18.44 versus 7.55 events per 1000 person-years for progressive CKD, respectively) (Supplementary Web appendix Tables S1–4 online, respectively). A total of 13.7% of the subjects of general population cohorts with albumin-to-creatinine ratio data had CKD according to the current definition (eGFR <60 ml/min per 1.73 m^2 or albumin-to-creatinine ratio $\geqslant 30$ mg/g) (Supplementary Web appendix Table S5 online). This subgroup accounted for 88.6% of ESRD events (Supplementary Web appendix Table S6 online), 61.5% of acute kidney injury events (Supplementary Web appendix Table S7 online), and 76.7% of subjects with progressive CKD (Supplementary Web appendix Table S8 online).

Independent continuous associations of eGFR and albuminuria with kidney outcomes

Pooled hazard ratios of ESRD according to eGFR and albuminuria adjusted for each other and covariates in the general population cohorts and the high-risk cohorts are shown in Figure 1. ESRD risk was relatively constant between an eGFR of 75 and 120 ml/min per 1.73 m^2, and was exponentially greater at lower eGFR. In the general population cohorts, eGFR risk association with ESRD showed hazard ratios at eGFR 60, 45, and 15 ml/min per 1.73 m^2 of 3.69 (2.36–5.76), 29.3 (19.5–44.1), and 454.9 (112.4–1840.2), respectively. The relationship of albumin-to-creatinine ratio to the relative risk of ESRD was monotonic on the log–log scale, without threshold effects. As compared with albumin-to-creatinine ratio 5 mg/g, hazard ratios for ESRD at albumin-to-

Table 1 | Characteristics of included studies

	N	Age, year	Male, %	Black, %	CVD, %	HT, %	HC, %	DM, %	Smoking, %	eGFR, ml/min per 1.73 m^2	ACR, mg/g	FU, Year	ESRD, *n*	AKI, *n*	pCKD, *n*
General population cohorts with ACR data													**147**	**427**	**173**
ARIC	11,408	62.8	44.2	22.2	8.6	47.6	34.5	16.7	14.9	82.5	3.7	8.0	92	363	—
AusDiab	11,240	51.5	44.9	0	8.3	32.7	70.6	8.4	15.5	78.9	4.9	5.0	—	—	72
CHS	3230	78.0	40.2	15.9	29.3	50.1	31.0	14.7	7.6	79.4	8.8	7.6	—	64	—
HUNT2	9525	62.0	44.8	0	22.5	82.5	61.3	17.6	19.7	83.8	7.5	10.5	55	—	—
MESA	6728	62.2	47.2	27.5	0.0	44.8	9.0	12.6	13.0	81.2	5.3	4.7	—		101
General population cohorts with dipstick data													**713**	**3438**	**4624**
AKDN UDIP	690,680	47.4	45.1	NA	1.8	20.2	NA	6.1	NA	80.9	—	2.3	478	3438	4475
Beaver Dam	4926	62.0	43.9	0	14.8	50.5	53.9	10.3	19.7	76.2	—	11.6	—	—	149
Okinawa 83	6659	51.9	39.5	NA	NA	NA	NA	3.8	NA	73.9	—	16.8	61	—	—
Okinawa 93	93,234	54.6	43.6	NA	NA	NA	NA	4.7	NA	77.3	—	6.9	174	—	—
High-risk cohorts with ACR data													**740**	**1074**	**4935**
ADVANCE	11,140	65.8	57.5	NA	32.2	82.2	33.0	100	15.1	78.2	15.9	4.8	59	—	822
AKDN ACR	67,406	55.5	56.8	NA	5.0	46.8	NA	49.0	NA	76.8	11.1	2.3	191	1013	1572
ONTARGET	25,620	66.4	73.3	2.5	92	NA*	NA*	37.5	12.6	73.6	52.2	4.5	162	61	1914
Pima	6341	26.4	45.4	0	NA	12.9	4.2	20.4	27.8	144	11.9	13.5	328	—	273
TRANSCEND	5926	66.9	57	1.8	92.5	NA*	NA*	35.7	9.8	71.7	25.3	4.6	—	—	354
High-risk cohorts with dipstick data													**579**	—	**1412**
CARE	4098	58.6	87.2	3.2	100	82.9	79.0	14.2	16.1	71.9	—	4.8	—	—	124
Hawaii	40,210	59.0	50.4	NA	17.0	NA	NA	48.0	13.6	71.5	—	2.4	331	—	1288
MRFIT	12,851	46.2	100	31.3	0.0	62.3	57.1	3.1	63.7	79.7	—	21.6	248	—	—

Abbreviations: ACR, albumin-to-creatinine ratio; AKI, acute kidney injury; CVD, cardiovascular disease; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; FU, duration of follow-up; HC, hypercholesterolemia; HT, hypertension; NA, not available; pCKD, progressive chronic kidney disease.
NA* in ONTARGET and TRANSCEND, respectively, a history of hypertension was reported by 69 and 76%, and statin use by 62 and 55%.

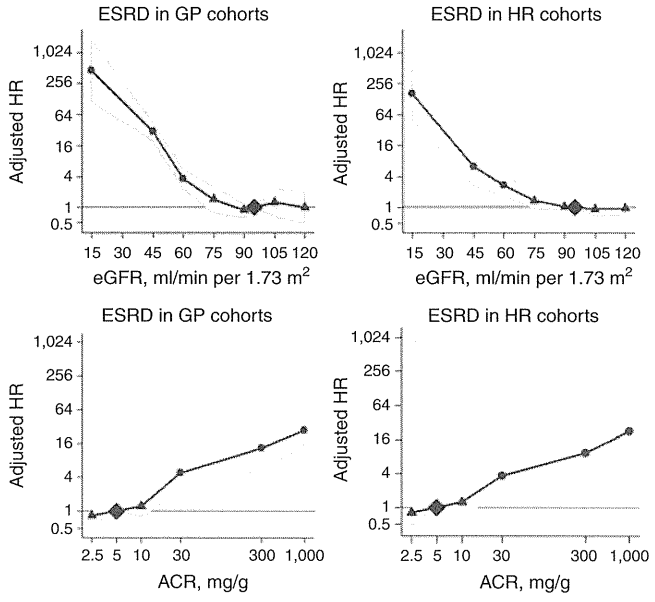

Figure 1 | Pooled hazard ratios (95% confidence interval) for ESRD according to spline eGFR (upper panels) and albumin-to-creatinine ratio (lower panels), adjusted for each other and for age, sex, and cardiovascular risk factors (continuous analyses). Reference categories are eGFR 95 ml/min per 1.73 m^2 and albumin-to-creatinine ratio 5 mg/g or dipstick negative or trace. Left panels show results for general population cohorts, and right panels for high-risk cohorts. Dots represent statistical significance, triangles represent non-significance, and shaded areas are 95% confidence interval. ACR, albumin-to-creatinine ratio; AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GP cohorts, general population cohorts; HR, hazard ratio; HR cohorts, high-risk cohorts.

Table 2 | General population cohorts

	Albumin-to-creatinine ratio (mg/g) or dipstick (classes)				
	<10 Negative	10–29 Trace	30–299 (1+)	⩾300 (⩾2+)	All
ESRD					
eGFR ml/min per 1.73 m^2					
>105			0.13	0.75	
90–104	0.04		0.05	0.57	0.06
75–89			0.11	2.35	
60–74			0.27	2.66	
45–59	0.12	0.77	1.44	5.13	0.34
30–44	1.03	1.55	9.15	27.07	4.02
15–29	9.05	19.50	37.69	128.4	42.99
All	0.09		1.61	14.9	0.31
Acute kidney injury					
eGFR ml/min per 1.73 m^2					
>105			3.55	7.57	
90–104	0.98		3.04	5.73	1.14
75–89			3.45	5.86	
60–74			6.46	13.77	
45–59	4.73	13.10	21.40	36.08	6.48
30–44	24.49	42.53	52.09	76.62	32.65
15–29	69.66	65.82	92.93	109.6	81.37
All	1.69		10.15	26.26	2.21
Progressive CKD					
eGFR ml/min per 1.73 m^2					
>105			1.56	12.60	
90–104	2.02		2.72	7.02	2.48
75–89			5.25	25.21	
60–74			16.80	47.50	
45–59	23.91	31.91	63.61	135.1	28.78
30–44	37.53	54.60	82.27	177.5	55.37
15–29	33.12	55.36	82.08	178.9	77.14
All	5.62		25.93	89.59	7.55

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease.
Unadjusted incidence rates (per 1000 patient-years) for ESRD, acute kidney injury, and progressive CKD. Shaded areas make up the combined reference groups.

creatinine ratios of 30, 300 and 1000 mg/g were 4.87 (2.30–10.3), 13.4 (5.49–32.7), and 28.4 (14.9–54.2), respectively. These patterns for ESRD in the high-risk cohorts were similar to the general population cohorts (Figure 1). The patterns for acute kidney injury and progressive CKD were generally similar to the patterns for ESRD, although less steep (Supplementary Web appendix Figures S1, S2 online).

Interactions

The multiplicative interaction between eGFR and albuminuria was significant for ESRD in only 1 out of 8 cohorts, for acute kidney injury in 3 out of 5 cohorts, and for progressive CKD in 4 out of 11 cohorts (Supplementary Web appendix Table S9 online). Significant interaction between eGFR and age was found for ESRD in only 1 out of 9 cohorts, for acute kidney injury in 3 out 5 cohorts, and for progressive CKD in 4 out of 11 cohorts (Supplementary Web appendix Table S9 online). Age interactions tended to show lower hazard ratios at older age, but a similar pattern of the associations of eGFR and albumin-to-creatinine ratio with the various kidney outcomes (Supplementary Web appendix Tables S10–12 online). The eGFR × albumin-to-creatinine ratio interaction can be visually assessed in graph 2. At low eGFR, the hazard ratio of higher albumin-to-creatinine ratio tended to be less than at high eGFR for ESRD as well as for acute kidney injury, but not for progressive CKD.

Joint associations of eGFR and albuminuria with kidney outcomes

As the albumin-to-creatinine ratio and the dipstick cohorts showed similar relationships between eGFR and albuminuria with ESRD, these two type of cohorts were combined to increase power for investigation of the joint associations of eGFR and albuminuria with kidney outcomes, both in general population and in high-risk cohorts (Supplementary Web appendix Figure S3 online). Table 2 shows unadjusted incidence rates of the three kidney outcomes for general population cohorts. Pooled hazard ratios/odds ratios for ESRD, acute kidney injury, and progressive CKD of the 21 categories of eGFR and albuminuria for the general population cohorts are shown in Tables 3 and 4. Low eGFR showed a similar association with risk across all levels of albuminuria, and high albuminuria showed a similar association with risk across all levels of eGFR, indicating multiplicative independent risk for kidney outcomes. At severely reduced eGFR values (15–29 ml/min per 1.73 m^2),

Table 3 | General population cohorts

	Albumin-to-creatinine ratio (mg/g) or dipstick (classes)				
	<10 Negative	10–29 Trace	30–299 (1+)	⩾300 (⩾2+)	All
ESRD					
eGFR ml/min per 1.73 m²					
>105			7.8 (1.7–35.9)	18.1 (4.3–75.9)	
90–104	Ref		11.3 (2.7–47.7)	19.7 (5.8–66.5)	Ref
75–89			3.8 (1.2–12.3)	48.1 (28.1–82.3)	
60–74			7.4 (3.6–15.2)	67.2 (40.1–113)	
45–59	5.2 (3.3–8.0)	21.8 (12.0–39.6)	40.3 (23.5–69.2)	147 (98.7–219)	9.6 (7.0–13.2)
30–44	55.5 (36.0–85.6)	74.1 (29.3–187)	293 (199–433)	763 (563–1035)	98.1 (61.8–156)
15–29	433 (239–787)	1044 (524–2077)	1056 (572–1948)	2286 (1114–4695)	573 (241–1362)
All	Ref		12.0 (7.9–18.1)	72.1 (43.0–121)	
Acute kidney injury					
eGFR ml/min per 1.73 m²					
>105			2.7 (0.9–8.5)	8.4 (5.1–13.8)	
90–104	Ref		2.4 (1.1–5.2)	5.8 (3.7–9.2)	Ref
75–89			2.5 (1.9–3.4)	4.1 (2.8–5.9)	
60–74			3.3 (2.6–4.1)	6.4 (5.0–8.2)	
45–59	2.2 (2.0–2.5)	4.9 (3.3–7.3)	6.3 (4.8–8.4)	5.9 (2.4–14.5)	2.6 (2.2–3.1)
30–44	7.3 (6.5–8.2)	10.2 (5.9–17.5)	12.4 (10.2–15.2)	19.6 (16.5–23.2)	7.9 (7.1–8.7)
15–29	16.8 (14.0–20.2)	16.8 (11.3–25.1)	21.4 (16.5–27.8)	28.8 (23.7–35.1)	16.7 (14.7–18.9)
All	Ref		2.5 (1.7–3.7)	6.0 (4.5–8.0)	
Progressive CKD					
eGFR ml/min per 1.73 m²					
>105			0.7 (0.7–0.8)	3.0 (0.4–23.7)	
90–104	Ref		0.9 (0.4–2.1)	3.3 (0.5–23.3)	Ref
75–89			1.9 (0.6–5.6)	5.0 (0.9–27.1)	
60–74			3.2 (1.4–7.5)	8.1 (5.2–12.8)	
45–59	3.1 (1.6–6.0)	4.0 (1.9–8.8)	9.4 (3.7–23.7)	56.6 (4.2–767.6)	3.9 (1.9–7.8)
30–44	3.0 (1.2–7.5)	19.1 (19.0–19.2)	14.9 (2.8–78.5)	22.2 (4.8–103.6)	3.7 (1.1–12.3)
15–29	4.0 (3.9–4.0)	11.7 (11.6–11.9)	21.0 (4.5–99.5)	7.7 (2.9–20.6)	7.9 (3.0–21.2)
All	Ref		3.1 (2.5–3.8)	11.2 (5.8–21.5)	

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; Ref, reference.
Pooled adjusted hazard ratios (95% confidence interval) for ESRD and acute kidney injury, and pooled adjusted odds ratios (95% confidence interval) for progressive CKD. Shaded areas make up the combined reference groups.

Table 4 | General population cohorts

	Albumin-to-creatinine ratio (mg/g) or dipstick (classes)				
	<10 Negative	10–29 Trace	30–299 (1+)	⩾300 (⩾2+)	All
ESRD, younger than 65 years of age					
eGFR ml/min per 1.73 m²					
>105			12.4 (2.3–66.8)	28.6 (6.5–127)	
90–104	Ref		14.2 (3.3–61.0)	13.8 (1.9–101.2)	Ref
75–89			5.8 (1.4–24.2)	65.2 (37.3–114)	
60–74			5.6 (2.0–15.7)	87.3 (32.3–236)	
45–59	3.1 (1.1–8.3)	31.8 (14.3–70.5)	55.4 (29.6–103)	261 (112–610)	9.5 (5.6–15.9)
30–44	101 (54.8–187)	293 (69.3–1236)	272 (107–693)	828 (443–1545)	110 (49.6–245)
15–29	999 (493–2023)	3897 (1717–8845)	2398 (1247–4609)	5081 (2736–9435)	1281 (556–2952)
All	Ref		13.7 (8.8–21.3)	124 (60.2–257)	
ESRD, older than 65 years of age					
eGFR ml/min per 1.73 m²					
>105			0.0 (0.0–∞)	0.0 (0.0–∞)	
90–104	Ref		0.0 (0.0–∞)	0.0 (0.0–∞)	Ref
75–89			0.0 (0.0–∞)	0.0 (0.0–∞)	
60–74			6.6 (1.6–27.2)	18.8 (5.3–67.1)	
45–59	3.4 (1.6–7.2)	9.6 (3.8–24.4)	16.4 (5.9–45.9)	41.4 (8.0–215)	4.5 (3.0–6.8)
30–44	11.5 (6.0–22.1)	18.1 (3.83–85.9)	90.8 (48.3–171)	268 (157–458)	42.1 (28.7–61.7)
15–29	131 (62.7–274)	115 (33.8–389)	413 (222–768)	1071 (645–1779)	186 (92.9–372)
All	Ref		10.3 (6.0–17.8)	47.5 (27.2–82.9)	

Abbreviations: eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; Ref, reference.
Pooled adjusted hazard ratios (95% confidence interval) for ESRD subdivided for age groups <65 and >65 years of age. Shaded areas make up the combined reference groups.

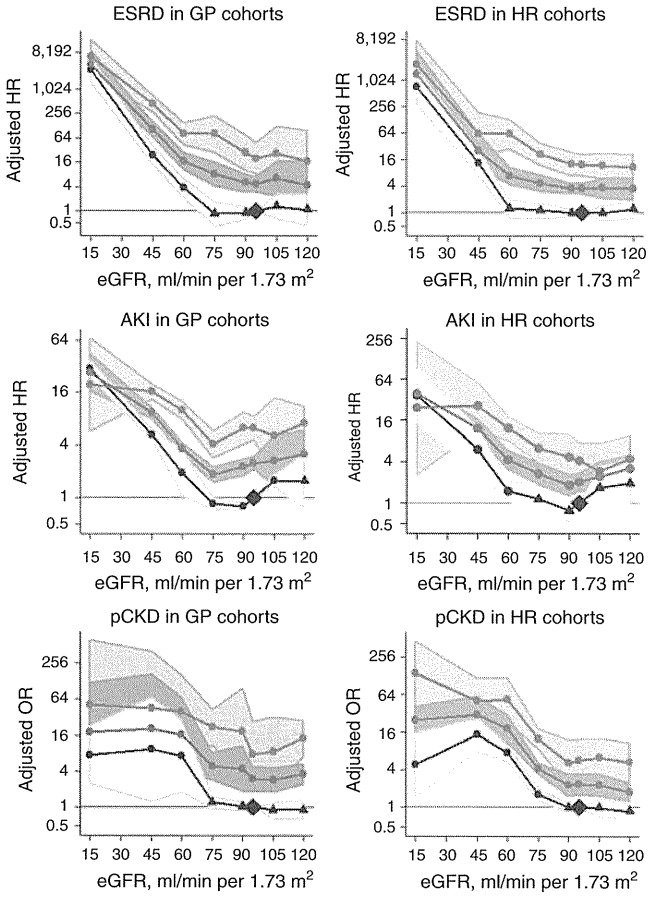

Figure 2 | Pooled adjusted hazard ratios or odds ratios (95% confidence interval) for ESRD (upper panels), acute kidney injury (middle panels), and progressive chronic kidney disease (lower panels) according to eGFR and albuminuria based on continuous models with eGFR (splines), albuminuria (log-linear albumin-to-creatinine ratio or categorical dipstick), and their interaction terms. Hazard ratios are adjusted for age, sex, and cardiovascular risk factors. Reference category is eGFR 95 ml/min per 1.73 m^2 plus albumin-to-creatinine ratio 5 mg/g or dipstick negative or trace. Left panels shows results for general population cohorts, and right panels for high-risk cohorts. Dots represent statistical significance, triangles represent non-significance, and shaded areas are 95% confidence interval. In this figure, albuminuria is treated categorically. Black lines and blue shading represent an albumin-to-creatinine ratio <30 mg/g or dipstick negative or trace, green lines and green shading an albumin-to-creatinine ratio 30–299 mg/g or dipstick 1+, and red lines and red shading an albumin-to-creatinine ratio ⩾300 mg/g or dipstick ⩾2+. AKI, acute kidney injury; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; GP cohorts, general population cohorts; HR, hazard ratio; HR cohorts, high-risk cohorts; OR, odds ratio; pCKD, progressive chronic kidney disease.

the risk associated with higher albuminuria was attenuated. The patterns were much steeper (that is, risk increased more rapidly with increasing albuminuria) for ESRD than for acute kidney injury and progressive CKD (Tables 3 and 4). Figure 2 shows the continuous analyses (allowing interaction) of the hazard ratios/odds ratios of eGFR and albuminuria for ESRD, acute kidney injury, and progressive CKD, respectively.

Table 5 | High-risk cohorts

	Albumin-to-creatinine ratio (mg/g) or dipstick (classes)				
	<10 Negative	10–29 Trace	30–299 (1+)	⩾300 (⩾2+)	All
ESRD					
eGFR ml/min per 1.73 m^2					
>105			1.22	6.52	
90–104	0.22		0.39	5.00	0.45
75–89			0.30	4.56	
60–74			0.36	7.77	
45–59	0.25	0.36	1.65	13.38	1.44
30–44	1.56	2.42	4.33	29.80	7.35
15–29	1.57	12.78	20.93	133.0	60.98
All	0.31		1.41	25.72	1.83
Acute kidney injury					
eGFR ml/min per 1.73 m^2					
>105			2.99	5.54	
90–104	1.41		3.35	5.43	2.25
75–89			3.09	9.92	
60–74			6.06	13.73	
45–59	2.28	8.00	13.42	29.03	8.07
30–44	11.20	17.76	36.70	52.09	27.63
15–29	25.74	48.66	69.90	104.7	73.94
All	2.33		9.08	26.59	4.88
Progressive CKD					
eGFR ml/min per 1.73 m^2					
>105			4.43	27.52	
90–104	5.51		5.75	14.44	7.97
75–89			8.59	30.90	
60–74			19.01	68.77	
45–59	23.75	37.88	57.67	147.1	43.84
30–44	33.55	35.35	64.99	160.3	65.65
15–29	12.44	43.16	58.43	209.3	103.3
All	10.40		25.96	105.0	18.44

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease.
Unadjusted incidence rates (per 1000 patient-years) for ESRD, acute kidney injury, and progressive CKD. Shaded areas make up the combined reference groups.

Similar data are given for cohorts at high risk for CKD (Tables 5, 6 and 7). The patterns for ESRD were less steep in the high-risk cohorts (Table 6) compared with the general population cohorts (Table 3), whereas the patterns for acute kidney injury and progressive CKD were similar in the general population cohorts and high-risk cohorts.

Joint associations of eGFR and albuminuria with kidney outcomes per age group

The overall incidence rates for the kidney outcomes were three- to ninefold higher in the subgroup of subjects with age ⩾65 years compared with the subgroup with age <65 years (Supplementary Web appendix Tables S2–4 online, respectively). Pooled hazard ratios for ESRD of the 21 categories of eGFR and albuminuria according to age group are shown in Table 4 for the general population cohorts and in Table 5 for the high-risk cohorts. The general pattern of higher risk for a lower eGFR independent of albuminuria level and of a higher albuminuria independent of eGFR level was observed in both age groups. However, in general, relative hazards were smaller among participants ⩾65 years of age than among participants <65

Table 6 | High-risk cohorts

	Albumin-to-creatinine ratio (mg/g) or dipstick (classes)				
	<10 Negative	10–29 Trace	30–299 (1+)	⩾300 (⩾2+)	All
ESRD					
eGFR ml/min per 1.73 m²					
>105			1.1 (0.8–1.6)	2.0 (0.9–4.5)	
90–104	Ref		2.3 (1.0–5.4)	10.0 (2.1–47.2)	Ref
75–89			1.7 (0.9–3.3)	17.3 (4.0–74.9)	
60–74			3.1 (1.8–5.3)	32.2 (11.8–87.8)	
45–59	2.7 (1.7–4.3)	3.8 (1.9–7.5)	14.5 (6.3–33.1)	55.5 (17.9–173)	5.7 (1.7–4.3)
30–44	23.4 (11.0–49.5)	33.4 (12.9–86.4)	56.0 (20.0–157)	139.8 (35.6–549)	27.4 (11.0–49.5)
15–29	32.6 (4.3–249)	308 (97.0–979)	387 (86.9–1725)	462.7 (31.6–6780)	166 (52.4–524)
All	Ref		4.3 (2.6–7.1)	38.1 (15.6–93.5)	
Acute kidney injury					
eGFR ml/min per 1.73 m²					
>105			2.2 (1.2–4.2)	3.8 (1.2–12.0)	
90–104	Ref		2.1 (1.3–3.4)	3.4 (1.4–8.3)	Ref
75–89			1.8 (1.3–2.5)	5.2 (3.2–8.6)	
60–74			2.8 (1.4–5.6)	6.3 (4.3–9.2)	
45–59	1.7 (1.2–2.5)	3.5 (2.6–4.7)	6.6 (5.2–8.5)	13.0 (9.7–17.3)	3.0 (2.5–3.5)
30–44	8.0 (5.4–11.8)	7.5 (5.3–10.6)	14.3 (11.2–18.3)	26.9 (12.3–58.8)	10.6 (5.2–21.9)
15–29	12.3 (5.4–27.8)	1.6 (0.0–∞)	25.3 (18.2–35.3)	13.7 (0.0–∞)	16.8 (13.5–20.9)
All	Ref		2.7 (2.2–3.4)	7.4 (5.5–9.8)	
Progressive CKD					
eGFR ml/min per 1.73 m²					
>105			0.6 (0.5–0.8)	4.7 (0.3–69.4)	
90–104	Ref		0.9 (0.7–1.2)	3.5 (0.5–26.0)	Ref
75–89			1.0 (0.8–1.1)	3.5 (2.5–5.0)	
60–74			2.8 (1.3–6.1)	9.3 (6.0–14.4)	
45–59	3.0 (2.1–4.4)	4.8 (3.7–6.2)	10.1 (4.9–20.8)	31.4 (16.1–61.5)	4.7 (3.3–6.8)
30–44	3.3 (2.7–4.1)	3.4 (2.5–4.7)	9.8 (6.3–15.3)	68.7 (57.6–81.9)	6.4 (4.3–9.7)
15–29	0.5 (0.4–0.7)	3.1 (1.2–7.7)	9.4 (5.3–16.6)	38.6 (15.7–94.8)	8.9 (4.8–16.7)
All	Ref		2.2 (1.9–2.7)	9.9 (6.7–14.5)	

Abbreviations: CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; Ref, reference.
Pooled adjusted hazard ratios (95% confidence interval) for ESRD and acute kidney injury, and pooled adjusted odds ratios (95% confidence interval) for progressive CKD. Shaded areas make up the combined reference groups.

Table 7 | High-risk cohorts

	Albumin-to-creatinine ratio (mg/g) or dipstick (classes)				
	<10 Negative	10–29 Trace	30–299 (1+)	⩾300 (⩾2+)	All
ESRD, younger than 65 years of age					
eGFR ml/min per 1.73 m²					
>105			1.1 (0.8–1.7)	1.4 (0.9–3.6)	
90–104	Ref		2.6 (1.0–6.9)	10.5 (2.0–55.3)	Ref
75–89			1.7 (0.8–3.8)	16.3 (2.3–119)	
60–74			4.0 (2.0–7.7)	39.0 (10.3–148)	
45–59	2.4 (1.4–4.2)	5.3 (2.3–12.2)	16.9 (4.7–60.5)	66.9 (20.1–222)	7.0 (4.3–11.6)
30–44	15.9 (1.9–133)	73.6 (20.5–264)	90.9 (27.6–299)	161 (26.3–989)	33.9 (14.6–78.9)
15–29	#	656 (172–2507)	792 (210–2982)	998 (105–9455)	223 (69.9–709)
All	Ref		4.5 (2.4–8.5)	43.8 (16.4–117)	
ESRD, older than 65 years of age					
eGFR ml/min per 1.73 m²					
>105			0.0 (0.0–∞)	20.6 (2.4–173)	
90–104	Ref		0.0 (0.0–∞)	15.5 (2.0–122)	Ref
75–89			1.9 (0.6–5.9)	16.2 (3.1–84.6)	
60–74			1.7 (0.6–4.7)	20.7 (9.4–45.8)	
45–59	2.8 (1.1–7.2)	1.8 (0.5–6.4)	10.0 (5.5–18.1)	31.2 (10.9–89.5)	3.8 (2.5–5.8)
30–44	16.1 (6.7–38.8)	18.1 (7.5–43.6)	24.3 (9.3–63.4)	92.7 (46.3–186)	20.7 (14.0–30.6)
15–29	25.0 (3.2–196)	175 (42.5–718)	125 (43.0–363)	506 (158–1620)	146.6 (46.3–464)
All	Ref		4.1 (2.5–6.8)	43.3 (13.0–145)	

Abbreviations: #, insufficient number of events for reliable estimates; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; Ref, reference.
Pooled adjusted hazard ratios (95% confidence interval) for ESRD subdivided for age groups <65 and >65 years of age. Shaded areas make up the combined reference groups.

years of age (Supplementary Web appendix Table S10 online). Similar findings were obtained for acute kidney injury (Supplementary Web appendix Table S11 online) and progressive CKD (Supplementary Web appendix Table S12 online).

Heterogeneity

eGFR × albumin-to-creatinine ratio categories with significant heterogeneity are shown in the Supplementary Web appendix Table S10–12 online. Quantitative heterogeneity, rather than qualitative heterogeneity, was observed in several categories, reflecting numerical differences in the hazard ratios between cohorts, but the direction of the risk was similar in all cohorts (increased risk with lower eGFR categories and with higher albuminuria categories). However, in all cohorts, the direction of the risk was similar (increased risk with lower eGFR categories and with higher albuminuria categories). Moreover, significant heterogeneity was limited to the lowest eGFR and the highest albuminuria categories. There was no significant heterogeneity in the groups with eGFR of 45–60 ml/min per 1.73 m^2 and in the groups with microalbuminuria (albumin-to-creatinine ratio 30–299 mg/g or dipstick 1+), either in the general population or in the high-risk population.

Meta-regression analysis was performed to test whether the association between eGFR and albumin-to-creatinine ratio with outcomes differed by the proportion of diabetic participants within each high-risk cohort. The proportion of diabetic participants was not significantly associated with the hazard ratio for ESRD associated with eGFR (45 versus 95 ml/min per 1.73 m^2; $P=0.58$) or albumin-to-creatinine ratio (30 versus 5 mg/g; $P=0.31$). Likewise, the proportion of diabetic participants was not significantly associated with the hazard ratio for progressive CKD associated with eGFR ($P=0.57$) or albumin-to-creatinine ratio ($P=0.96$). There were too few cohorts with sufficient events to allow similar meta-regression models for acute kidney injury.

DISCUSSION

In this collaborative meta-analysis of nine general population and eight high-risk cohorts, including a total of more than 1 million subjects, we found that lower eGFR and higher albuminuria were associated with a higher risk for ESRD, independent of each other and independent of traditional CVD risk factors. A similar association of eGFR and albuminuria was found with the risk for acute kidney injury and for progressive CKD, although the relative hazards were higher for ESRD.

The risk for ESRD based on eGFR and albuminuria have been reported in a limited number of follow-up studies from general population cohorts.[20,22,28–30] The current meta-analysis confirms these studies and extends the generalizability of these data to other populations worldwide. Furthermore, our collaborative meta-analysis includes 2201 ESRD outcomes, substantially more than the number of events in reports of individual studies, thereby allowing evaluation of the independent and joint associations of eGFR and albuminuria with this outcome. In addition, we included data on acute kidney injury and progressive CKD, other kidney disease outcomes of clinical and epidemiologic interest.

We found similar patterns in studies that had data on albumin-to-creatinine ratio and in the studies that only had semiquantitative information available on dipstick proteinuria. These findings suggest that measurement of dipstick proteinuria is useful for risk stratification, despite being a less precise measure of albuminuria. This is of importance considering the lower cost of dipstick compared with albumin-to-creatinine ratio measurement. However, studies directly comparing dipstick testing with more accurate albuminuria measurements are needed to investigate sensitivity, specificity, and negative and positive predictive value to make definite recommendations for screening. Also, it is important to bear in mind that most studies had measured albuminuria only once, thus raising questions regarding reproducibility and chronicity of albuminuria. However, the finding that a single urine test has significant prognostic implication strengthens the conclusion that albuminuria is an important risk factor. In addition, a single test may underestimate rather than overestimate the risk associated with albumin-to-creatinine ratio because of regression dilution bias.[31]

The general pattern of a graded increase in relative risk for the various kidney outcomes with higher albuminuria and lower eGFR was observed in both cohorts at high risk for CKD as well as cohorts derived from the general population. Although the absolute incidence of ESRD was higher in the high-risk population compared with the general population, the increase in relative hazards for a lower eGFR and a higher albuminuria was more pronounced in the general population than the high-risk population. The consistency of our findings in both cohorts with albumin-to-creatinine ratio and dipstick proteinuria data, in both general population and high-risk cohorts, and in both continuous and categorical models for eGFR and albumin-to-creatinine ratio, demonstrates the robustness of our findings. The finding of only quantitative, but not qualitative heterogeneity, and that heterogeneity was not observed in the categories of most clinical interest, that is, eGFR 45–60 ml/min per 1.73 m^2 and albumin-to-creatinine ratio 30–299 mg/g or dipstick >1+, further underscores the strengths of our observations. Of note, our meta-regression analyses showed that the associations of eGFR and albuminuria with adjusted hazard rates for ESRD and acute kidney injury outcomes were not related to the proportion of diabetic subjects included in the various high-risk cohorts. This provides no evidence for the assumption of some investigators that diabetic and non-diabetic kidney disease should be regarded as separate entities.

The statistical code that was sent to the participating cohorts rendered output that did not permit computation of a meta-analytic result for interactions. However, Tables 3 and 4 show that the pattern of higher relative hazards for ESRD for a lower eGFR and for a higher albuminuria is less steep in subgroups older than ⩾65 than in those <65 years of

age. The relationship of higher albuminuria with higher unadjusted incidence rate of ESRD is comparable for both age groups, but less steep with lower eGFR in the elderly when compared with the young (Supplementary Web appendix Table S3 online). The less steep relationship with lower eGFR needs to be balanced against the higher incidence rates in the older subgroup. Although in elderly the increase in adjusted relative risk with lower eGFR is less than in the young, the increase in unadjusted incidence rates is higher. The age–eGFR interaction will be studied in depth in later analyses by the CKD Prognosis Consortium.

The observed relative risk increase for ESRD with lower eGFR is more pronounced than the relative risk increase for all-cause and cardiovascular mortality, as described separately.[24] The hazard ratios for ESRD at eGFR 60, 45, and 15 ml/min per 1.73 m^2 were 3.69 (2.36–5.76), 29.3 (19.5–44.1), and 454.9 (112.4–1840.2), respectively, compared with 1.16 (1.04–1.30), 1.49 (1.28–1.72), and 3.18 (2.45–4.14), respectively, for all-cause mortality.[25] Interestingly, the increase in relative risk for higher albuminuria is also substantially higher for ESRD compared with all-cause mortality, with hazard ratios for ESRD at albumin-to-creatinine ratio 30, 300, and 1000 mg/g of 4.87 (2.30–10.3), 13.4 (5.49–32.72), and 28.4 (14.9–54.2), respectively, compared with 1.16 (1.08–1.25), 1.51 (1.34–1.70), and 2.15 (1.80–2.58), respectively, for all-cause mortality.[25] For kidney outcomes, eGFR and albumin-to-creatinine ratios were the strongest risk factors examined, often stronger than age, which differs from all-cause mortality and cardiovascular mortality where age is the dominant factor. The higher relative risks for kidney outcomes than for mortality likely reflect a greater specificity of association of eGFR and albumin-to-creatinine ratio with these outcomes. The implications of the more steep relationship of low eGFR and high albuminuria with relative risk for ESRD than for mortality should be considered in view of the relative low incidence rates of the kidney outcomes. Lastly, these data are not consistent with the suggestion by others that microalbuminuria is only a marker for increased CVD risk,[11] as it also indicates substantially increased risk for all kidney outcomes examined.

A strength of this pooled analysis is that it includes data on acute kidney injury and progressive CKD as well as on ESRD. A disadvantage of limiting study of kidney outcomes to only ESRD is that it will predispose to identification of low eGFR values as the most important risk predictor, as the decision to start renal replacement therapy is for a large part based on eGFR. For clinical practice, however, it is also important to identify risk predictors in subjects with relatively preserved renal function, who may benefit from early initiation of therapies to slow progression of CKD, thereby delaying or even preventing ESRD and other complications. Therefore, incident acute kidney injury and progressive CKD were studied as earlier kidney outcomes than ESRD. For acute kidney injury, the International Classification of Diseases hospital discharge code 584 was adopted as defining criterion. For progressive CKD, different definitions have been used in the literature. Our definition required loss of eGFR of more than 2.5 ml/min per 1.73 m^2 per year ($\sim$3–5 times faster than the rate of renal function decline in the general population[21,30]) and a final eGFR during follow-up of $\leqslant$45 ml/min per 1.73 m^2 (as it is widely acknowledged that this threshold is of clinical significance). Such a combination of a relative decrease and an absolute threshold has been used before in epidemiological studies[32] to increase specificity with a recognized loss of sensitivity. Of note, the weaker associations of eGFR and albuminuria for progressive CKD in comparison with the two other kidney outcomes can be partially explained by misclassification of the outcome and regression to the mean.

Some limitations of this meta-analysis should be mentioned. First, we included only a relatively limited number of cohorts, and measurements of serum creatinine and albuminuria were not centrally standardized across these cohorts. The present analysis, however, is to the best of our knowledge the largest and most comprehensive assessment of the relation between eGFR, albuminuria, and kidney outcomes yet performed. Second, no data on treatment effects could be taken into account. Thus, it cannot be excluded that the observed associations are influenced by the start of specific treatments. However, if such treatment were effective in preventing kidney disease progression, then it would be expected to lead to an underestimation of the true relative risk of low eGFR and high albuminuria for these outcomes. Finally, we used a restrictive definition of progressive CKD, and alternative definitions should be explored.

What do these findings mean for the current debate on the definition and classification of CKD? First, as albuminuria is a risk factor for kidney outcomes independent of eGFR and conventional cardiovascular risk factors, this suggests that albuminuria could be used for risk stratification at each level of eGFR. A lack of multiplicative interaction means that albuminuria has a similar relative risk at normal and low eGFR. However, the baseline risk is higher at lower eGFR, and hence the attributable risk will be higher at lower eGFR for the same relative risk. Furthermore, as the risk for kidney outcomes is higher for subjects with macroalbuminuria ($\geqslant$300 mg/g) than for subjects with microalbuminuria (30–299 mg/g), it seems prudent to define not only one, but several thresholds for albuminuria to indicate increased risk for kidney outcomes. Second, our finding that risk for kidney outcomes is substantially higher in subjects with eGFR 30–45 ml/min per 1.73 m^2 as compared with 45–60 ml/min per 1.73 m^2 suggests that it may be appropriate to subdivide the present stage 3 CKD into two stages, as has been proposed by others.[33] Our finding of increased relative risk for all three kidney outcomes for eGFR below 60 ml/min per 1.73 m^2 and albuminuria (albumin-to-creatinine ratio $>$30 mg/g or dipstick $>$trace) are consistent with the current thresholds for the definition of CKD. Some have suggested age-specific thresholds, arguing that lower eGFR at older age is a reflection of ageing[11] and less associated with

risk for adverse outcomes.[34,35] Although we found a less steep pattern of risk for kidney outcomes with lower eGFR in older subjects compared with younger subjects, the pattern of incidence rates was similar in older and younger subjects. These data do not provide clear-cut evidence for the use of age-specific eGFR thresholds to define CKD. In general, decisions about the threshold levels for decreased GFR and albuminuria to define and classify CKD should consider the prevalence and absolute risk of decreased eGFR and albuminuria, as well as relative risk.

In conclusion, our data show that both albuminuria and eGFR are associated with all three kidney outcomes, independent of each other and cardiovascular risk factors. There was no evidence of multiplicative interaction between eGFR and albuminuria. These findings provide a quantitative basis for including these two kidney measures for risk stratification, and CKD definition and staging.

MATERIALS and METHODS

Search strategy and study selection

In August 2009, we performed a systematic review of the available literature to retrieve all general population cohorts that might have information on the relation between eGFR and/or albuminuria versus kidney outcomes. Details of the search strategy can be found elsewhere.[25] To be eligible for inclusion, studies had to meet the following criteria: (1) prospective, general population-based cohort study, (2) information at baseline on eGFR as well as albuminuria levels, (3) at least 1000 subjects included, (4) information on at least one of the three kidney outcome measures, and (5) a minimum of 50 events for that outcome measure. The reason to require a minimum sample size is to ensure sufficient out comes in the reference cell. Ultimately, 21 general population cohorts met these eligibility criteria and were willing to cooperate, of which 9 had data on kidney outcomes.[20,28,36–42]

We also included cohorts of individuals selected because of high risk of CKD, including patients with cardiovascular disease risk factors (such as hypertension and diabetes) or a history of cardiovascular disease, because screening for CKD is recommended in these groups. However, the associations between eGFR and/or albuminuria and kidney outcomes may differ between high-risk populations and the general population. We analyzed eight high-risk cohorts that met the same eligibility criteria as the general population cohorts.[20,29,31,43–47]

Study variables

In each cohort, subjects were subdivided according to eGFR and albuminuria. GFR was estimated using the abbreviated Modification of Diet in Renal Disease Study equation.[48] Each participating study was asked to standardize their serum creatinine to Isotope Dilution Mass Spectrometry traceable methods, but calibration methods were not uniform. As recommended in clinical practice guidelines,[3,33] albuminuria was assessed as the urine albumin-to-creatinine ratio. If first morning voids were not available, spot urine samples or samples from 24 h urine collections were used. In studies in which no quantitative albuminuria measurements were available, data on urine protein-to-creatinine ratio[47] or dipstick testing for proteinuria[20] were collected. eGFR and albuminuria were measured at the onset of cohort studies.

Besides eGFR and albuminuria, information on demographic factors and cardiovascular risk factors were obtained to compare baseline characteristics of the different cohort studies and to adjust for confounding in multivariable models. Cardiovascular disease history was defined as a history of myocardial infarction, bypass grafting, percutaneous coronary intervention, heart failure, or stroke. Hypertension was defined as systolic blood pressure $\geqslant 140$ mm Hg or diastolic blood pressure $\geqslant 90$ mm Hg or use of antihypertensive medication. Hypercholesterolemia was defined as total cholesterol > 5.0 mmol/l in the case of a positive history of cardiovascular disease and as > 6.0 mmol/l in the case of a negative history of cardiovascular disease. Diabetes mellitus was defined as fasting glucose $\geqslant 7.0$ mmol/l or non-fasting glucose $\geqslant 11.1$ mmol/l or use of glucose-lowering drugs. Smoking habit was dichotomized as current versus not current smoking.

Definition of kidney outcome measures

ESRD was defined as start of renal replacement therapy or death coded as because of kidney disease other than acute kidney injury. Acute kidney injury was defined as ICD-9 code 584 as primary or additional discharge code. Progressive CKD was defined as an average annual decline in eGFR during follow-up of at least 2.5 ml/min per 1.73 m^2 per year and a last eGFR value being less than 45 ml/min per 1.73 m^2, independent of the level of baseline eGFR. The average annual decline in eGFR was calculated as last available eGFR minus baseline eGFR divided by follow-up time (in years, minimum two) between the two observations.

Statistical analysis

Our primary objective was to evaluate the associations of eGFR and albuminuria, independently and jointly, on kidney outcome measures. To maximize uniformity and minimize bias, investigators from the cohort studies were invited to collaborate in a pooled analysis following an *a priori* analytic plan using standard statistical code that was provided by the analytic team of the CKD Prognosis Consortium. All analyses were conducted using Stata version 10 or 11 (Stata Corp, College Station, TX), SAS version 9 (SAS Institute, Cary, NC), or R version 2.9.2 (R Foundation for Statistical Computing, Vienna, Austria). All data classification was performed separately by analytic teams at the John Hopkins Institute for Public Health, Baltimore, USA (KM, JC, and BCA) and the University Medical Center Groningen, Groningen, the Netherlands (MvdV, PEdJ, and RTG), and differences were resolved by consensus.

For each study, a table was generated providing baseline study characteristics. Cox proportional hazard models were used to estimate the hazard ratios for ESRD and acute kidney injury, and logistic regression analysis to estimate odds ratios

for progressive CKD. These analyses were adjusted for age, sex, race, and cardiovascular risk factors. Cardiovascular risk factors taken into account were cardiovascular disease history, smoking status, diabetes mellitus, systolic blood pressure, and serum total cholesterol. The independent continuous association of eGFR and of albuminuria with risk for kidney outcomes was evaluated after adjusting for each other and for CVD risk factors. eGFR and albumin-to-creatinine ratio were modeled using linear splines with knots at 45, 60, 75, 90, and 105 ml/min per 1.73 m^2 and 10, 30, and 300 mg/g, respectively. eGFR splines were also adjusted for albuminuria (adjusted to an albumin-to-creatinine ratio of 5 mg/g and dipstick negative), whereas albuminuria splines were also adjusted for eGFR. For the continuous albuminuria splines, only cohorts that had albumin-to-creatinine ratio data were taken into account. eGFR 95 ml/min per 1.73 m^2 and albumin-to-creatinine ratio 5 mg/g were treated as the reference points. These points were chosen, as they reflect the anticipated low-risk groups. Interactions between eGFR and both albuminuria and age were evaluated by likelihood-ratio tests in individual studies, with albuminuria and age treated as continuous variables.

For each outcome variable, information was generated for the joint association of eGFR and albuminuria with kidney outcomes. Eight eGFR categories were defined: <15, 15–29, 30–44, 45–59, 60–74, 75–89, 90–104, and $\geqslant 105$ ml/min per 1.73 m^2. These 15 ml/min per 1.73 m^2 categories were chosen to correspond to current CKD stages 1–5 and to evaluate whether these stages require subdivision. For albumin-to-creatinine ratio, we defined four categories: <10, 10–29, 30–299, and $\geqslant 300$ mg/g. These categories were chosen to correspond to current definitions for microalbuminuria and macroalbuminuria, and to evaluate whether the normoalbuminuria category should be subdivided. When information on albumin-to-creatinine ratio was lacking, we used information on dipstick proteinuria. As it has been shown that the majority of subjects with a dipstick trace have high-normal albuminuria, dipstick 1+ microalbuminuria, and dipstick $\geqslant 2+$ macroalbuminuria,[49] we defined four dipstick categories as: negative, trace, 1+, and $\geqslant 2+$. We tested whether combining cohorts with data on albumin-to-creatinine ratio and cohorts with data on dipstick proteinuria were valid. Unlike the mortality analyses,[24,25] there were insufficient kidney outcomes in the 'optimal' reference cell (eGFR 90–104 ml/min per 1.73 m^2 and albumin-to-creatinine ratio <10 mg/g) for the current analyses. Therefore, eGFR $\geqslant 60$ ml/min per 1.73 m^2 and albumin-to-creatinine ratio <30 mg/g or dipstick negative/trace were chosen as the reference cell, as present guidelines classify this group as being free of CKD. For all of the 25 eGFR $\times$ albumin-to-creatinine ratio categories, information was obtained on the distribution of subjects and the distribution of incident events. For each study, the unadjusted incidence rate per 1000 person-years was calculated for each category. Hazard ratios or odds ratios were estimated with adjustment for the aforementioned cardiovascular risk factors. We conducted complementary analyses where eGFR and albumin-to-creatinine ratio were modelled continuously using the same statistical models and adjustments. These models were parameterized with eGFR = 95 ml/min per 1.73 m^2 and albumin-to-creatinine ratio = 5 mg/g or eGFR = 95 ml/min per 1.73 m^2 and dipstick = negative/trace as the reference point (hazard ratio or odds ratio = 1.0).

Pooled unadjusted incidence rates were obtained by weighting the individual studies by the number of subjects per category. Pooled estimates of the adjusted hazard ratios and odds ratios, with 95% confidence interval, were obtained from meta-analyses of random effects. Heterogeneity was estimated using the χ^2-test for heterogeneity and the I^2 statistic.[50] Meta-analyses were conducted separately for general population cohorts and high-risk cohorts. As there were few participants (0.1%) with eGFR <15 ml/min per 1.73 m^2, we only report results for participants with eGFR $\geqslant 15$ ml/min per 1.73 m^2. *A priori* it was considered that age could be an important effect modifier, and hence results were also produced for age <65 and $\geqslant 65$ years. This age subdivision was chosen, as guidelines advise to screen for CKD in subjects $\geqslant 65$ years of age.

In all analyses, a P-value of <0.05 was considered to indicate statistical significance.

DISCLOSURE
All the authors declared no competing interests.

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