Research: Complications

Differences in risk factors for the onset of albuminuria and decrease in glomerular filtration rate in people with Type 2 diabetes mellitus: implications for the pathogenesis of diabetic kidney disease

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Abstract

Aims   To determine differences in predictors of albuminuria and decreased estimated GFR in Japanese people with Type 2 diabetes mellitus without chronic kidney disease.

Methods   This single-centre observational cohort study involved 1802 Japanese people with Type 2 diabetes with normoalbuminuria and estimated GFR $\geq$ 60 ml/min/1.73 m^2 (740 women; mean $\pm$ SD age 58 $\pm$ 12 years). Two separate outcomes were evaluated: onset of albuminuria ($\geq$ 30 mg/g creatinine, albuminuria cohort; n = 1655) and decrease in estimated GFR (< 60 ml/min/1.73 m^2; estimated GFR cohort; n = 1777). A Cox proportional hazards model was used to identify significant predictors for each outcome.

Results   During a median follow-up period of 6.9 years for the albuminuria cohort and 8.0 years for the estimated GFR cohort, 181 and 316 individuals reached the respective outcome. The 5-year cumulative incidence of albuminuria was 8.3%, and that of decreased estimated GFR was 10.4%. In the multivariate Cox model, greater urinary albumin-to-creatinine ratio, presence of diabetic retinopathy and higher HbA$_{1c}$ levels were associated with both outcomes. Unique risk factors for onset of albuminuria were male gender and higher uric acid levels; those for decreased estimated GFR were older age, greater systolic blood pressure, and lower baseline estimated GFR and HDL cholesterol levels.

Conclusions   Identification of both common and distinct predictive factors for onset of albuminuria and decreased estimated GFR support the hypothesis that both common and distinct pathophysiological mechanisms are involved in the development of these two manifestations of chronic kidney disease in diabetes.

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Introduction

Diabetic kidney disease is the leading cause of chronic kidney disease worldwide, reflecting the global increase in the prevalence of Type 2 diabetes mellitus. Diabetic kidney disease has been traditionally diagnosed based on the presence of (micro)albuminuria [1,2]. In 2002, the Kidney Disease Outcome Quality Initiative of the National Kidney Foundation proposed guidelines for the diagnosis and classification of chronic kidney disease, including diabetic kidney disease [3], in which chronic kidney disease was defined and staged mainly according to estimated GFR (eGFR). Recently, the classification of chronic kidney disease has been modified to include both eGFR and albuminuria stages, creating the 'heat map' that describes the relative risks for renal and cardiovascular outcomes [4].

It is generally believed that microalbuminuria is an early clinical manifestation of diabetic kidney disease, and that decreased GFR occurs secondarily, mainly in people with longstanding diabetes. This clinical course appears to be typical for those with Type 1 diabetes [5]. In contrast, the natural history of chronic kidney disease in Type 2 diabetes appears to be heterogeneous. We [6] and others [7,8] identified a subgroup of people with Type 2 diabetes who had decreased GFR despite normoalbuminuria. These cross-

What's new?

- This was a cohort study showing differences in risk factors for the onset of albuminuria and decreased estimated GFR (< 60 ml/min/1.73 m^2) in people with Type 2 diabetes mellitus without albuminuria or renal impairment.

- Risk factors common to both conditions were diabetic retinopathy, high HbA$_{1c}$ level and urinary albumin excretion.

- Additional risk factors for albuminuria were male gender and elevated uric acid levels. Additional risk factors for decreased estimated GFR were older age, high systolic blood pressure, low estimated GFR and low HDL cholesterol levels.

- This study supports the hypothesis that both common and distinct pathophysiological mechanisms are involved in the development of chronic kidney disease in diabetes.

sectional observations suggest that risk factors for albuminuria and those for decreased GFR may differ; however, previous longitudinal studies in people with Type 2 diabetes have focused exclusively on risk factors for albuminuria [9–13]. We conducted the present observational cohort study to investigate, therefore, whether onset of albuminuria and decreased eGFR share common risk factors or have unique sets of risk factors in people with Type 2 diabetes mellitus.

Subjects and methods

Study population

This was a single-centre longitudinal observational historical cohort study in Japanese people with Type 2 diabetes, conducted using a hospital database. Data were retrieved from hospital records of adults with Type 2 diabetes, aged ≥ 20 years, who consecutively visited the Diabetes Centre, Tokyo Women's Medical University Hospital in Tokyo, Japan between 1 November 2003 and 31 March 2005. Cross-sectional and longitudinal studies involving these subjects have been reported previously [6,14]. For the present study, we selected people with Type 2 diabetes with normoalbuminuria and eGFR ≥ 60 ml/min/1.73 m^2 at baseline, both of which were determined within 90 days. Pregnant women and people with infectious and malignant diseases were excluded.

The study protocol was approved by the Ethics Committee of Tokyo Women's Medical University School of Medicine, who provided a waiver of informed consent.

Baseline data

Information on baseline physical and laboratory examinations were collected directly from electronic medical records maintained by the hospital. Diabetic retinopathy status was assessed through funduscopic examination by experienced ophthalmologists, and classified simply as absent or present. History of cardiovascular disease was defined as a previous episode of stroke, transient ischaemic attack or coronary artery disease.

Laboratory examinations included HbA$_{1c}$, serum lipid, haemoglobin, uric acid and creatinine levels and urinary albumin-to-creatinine ratio in a first morning urine specimen. HbA$_{1c}$ was measured by high-performance liquid chromatography using a set of calibrators assigned by the Japan Diabetes Society. To standardize HbA$_{1c}$ values to the National Glycohaemoglobin Standardization Program (NGSP) units and International Federation of Clinical Chemistry (IFCC), the following formulae were used: HbA$_{1c}$ (NGSP; %) = 1.02 × measured HbA$_{1c}$ (Japan Diabetes Society; %) + 0.25 [15], HbA$_{1c}$ (IFCC; mmol/mol) = 10.78 × measured HbA$_{1c}$ (Japan Diabetes Society; %) – 18.59 [16]. HDL cholesterol was determined enzymatically. LDL cholesterol was determined directly by a homogeneous enzymatic assay or calculated using the Friedewald equation when the serum triglyceride level was < 400 mg/dl [17]. Non-HDL cholesterol was calculated as total cholesterol minus HDL cholesterol. Urinary albumin was measured by an immunoturbidimetric method using an automated analyser (Hitachi, Tokyo, Japan) and normalized to urinary creatinine levels.

Normo-, micro- and macroalbuminuria were defined as an albumin-to-creatinine ratio < 30 mg/g, 30–299 mg/g and ≥ 300 mg/g, respectively. GFR was estimated using the following three-variable equation, as proposed by the Japanese Society for Nephrology: eGFR (ml/min/1.73 m^2) = 194 × serum creatinine (mg/dl) -1.094 × age (years) - 0.287 × ([if female] × 0.739) [18]. The definition and laboratory assay methods for these variables were unchanged during the study period.

Follow-up and outcomes

Subjects were seen periodically at the outpatient clinic of the hospital until 31 March 2013 by diabetes specialists who had been instructed to measure urinary albumin-to-creatinine ratio and serum creatinine at least once a year as part of routine outpatient examinations. Individuals who died during follow-up or were lost to follow-up were included until the time of death or the last observation.

In the present study, two independent outcomes were specified. The first outcome was onset of albuminuria (urinary albumin-to-creatinine ratio ≥ 30 mg/g), confirmed on at least two consecutive urinary albumin-to-creatinine ratio measurements (albuminuria cohort). The second outcome was decreased eGFR defined as eGFR < 60 ml/min/1.73 m^2 observed on at least two consecutive eGFR measurements (GFR cohort).

Statistical analysis

Data were expressed as percentages, arithmetic means ± SD, or geometric means with 95% CIs, as appropriate according to the data distribution. Serum triglyceride levels and urinary albumin-to-creatinine ratios were logarithmically transformed to correct for the skewed distribution. Student's *t*-test and Fisher's exact probability test were used to compare continuous and categorical data, respectively.

The cumulative incidence of each outcome was estimated using Kaplan–Meier analysis, and the statistical differences between groups were examined using the log-rank test. Hazard ratios and the corresponding 95% CIs for each outcome were calculated using univariate and multivariate Cox regression analysis. In the multivariate Cox model, the following variables were considered as putative covariates: age, sex, duration of diabetes, presence of diabetic retinopathy, history of cardiovascular disease, BMI, systolic and diastolic blood pressure, use of renin-angiotensin system blockers (angiotensin-converting enzyme inhibitors and angiotensin receptor blockers), calcium channel blockers, insulin, oral hypoglycaemic drugs, HbA$_{1c}$, triglycerides, HDL cholesterol, non-HDL cholesterol, uric acid, haemoglobin, eGFR and urinary albumin-to-creatinine ratio at baseline. These variables were then selected using a stepwise procedure, specifying the significant levels for entering an additional explanatory variable into the models as 0.25 and for removing an explanatory variable from the model as 0.15.

In the survival analysis assessing one of the two outcomes (for example, onset of albuminuria), whether or not the other outcome (in this case, decrease in eGFR) occurred during follow-up was not considered. These two outcomes may be interdependent, however, so we conducted a sensitivity analysis. In the sensitivity analysis assessing one of the two outcomes, people who reached the other outcome earlier were censored at the time when the other outcome occurred.

All statistical analysis was performed using SAS version 9.3 (SAS Institute, Cary, NC, USA). *P* values < 0.05 were taken to indicate statistical significance.

Results

Among 1802 Japanese people with Type 2 diabetes assessed for eligibility between 1 November 2003 and 31 March 2005, 1655 and 1777 people had sufficient baseline and follow-up data to qualify for inclusion in the albuminuria and eGFR cohorts, respectively. Table 1 shows baseline characteristics for people in the albuminuria and eGFR cohorts. As 1630 individuals overlapped (98.5% of the albuminuria and 91.7% of the eGFR cohort), the demographic and clinical characteristics of the two cohorts were almost identical.

In the albuminuria cohort, 181 of 1655 people (10.9%) progressed to micro- or macroalbuminuria during the median (interquartile range) follow-up period of 6.9 (3.5–8.1) years, with a 5-year cumulative incidence of 8.3% (Fig. 1). In the eGFR cohort, 316 of 1777 people (17.8%) had a decreased eGFR (< 60 ml/min/1.73 m^2) during the median (interquartile range) follow-up period of 8.0 (3.8–8.5) years. The 5-year cumulative incidence of decreased eGFR was 10.4% (Fig. 1). Of 1630 people with the requisite

Table 1 Demographic and laboratory data at baseline in the albuminuria and estimated GFR cohorts

	Albuminuria cohort (*n* = 1655)	eGFR cohort (*n* = 1777)
Men, %	59.2	58.7
Age, years	57 ± 12	58 ± 12
Duration of diabetes, years	13 ± 9	13 ± 9
History of cardiovascular disease, %	10.0	10.4
Body mass index, kg/m^2	23.8 ± 3.6	23.8 ± 3.6
Diabetic retinopathy, %	32.5	32.1
Medication for diabetes: oral hypoglycaemic agent /insulin, %	52.0/27.9	51.8/27.5
Systolic blood pressure, mmHg	132 ± 19	133 ± 19
Diastolic blood pressure, mmHg	76 ± 11	76 ± 11
Use of renin-angiotensin system blockers, %	23.1	21.4
Use of calcium channel blockers, %	19.0	19.2
Laboratory data		
HbA$_{1c}$, mmol/mol	65.0 ± 16.6	65.0 ± 16.6
HbA$_{1c}$, %	8.1 ± 1.5	8.1 ± 1.5
HDL cholesterol, mmol/l	1.40 ± 0.38	1.40 ± 0.39
Non-HDL cholesterol, mmol/l	3.69 ± 0.87	3.68 ± 0.87
Creatinine, μmol/l	62.9 ± 12.3	62.7 ± 12.3
eGFR, ml/min/1.73 m^2	81.4 ± 15.5	81.5 ± 15.5
Uric acid, μmol/l	294.5 ± 73.4	293.7 ± 73.1
Haemoglobin, g/l	141 ± 14	141 ± 14
Urinary albumin-to-creatinine ratio, mg/g	10.1 (9.8–10.3)	10.1 (9.9–10.4)

eGFR, estimated GFR.
Data are expressed as percentage, mean ± sd or geometric mean (95% CI).
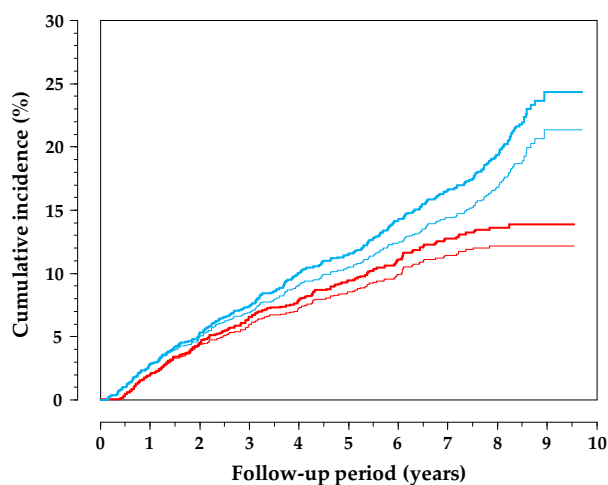
FIGURE 1 Cumulative incidence of reaching albuminuria outcome (red lines) and eGFR outcome (blue lines) in 1655 and 1777 Japanese people with Type 2 diabetes, respectively. Bold lines are cumulative incidence calculated in the original analysis. Thin lines are cumulative incidence calculated in the sensitivity analysis in which individuals who reached the other outcome earlier were censored at the time when the outcome occurred.

data for both outcomes, 118 had onset of albuminuria alone, 236 had decreased GFR alone, and 61 had both onset of albuminuria and decreased eGFR.

In the univariate Cox regression analysis, male gender, higher BMI, presence of diabetic retinopathy, use of renin-angiotensin system blockers, calcium blockers and insulin, non-use of oral hypoglycaemic drugs, higher HbA$_{1c}$ levels, urinary albumin-to-creatinine ratio and serum uric acid levels and lower HDL cholesterol levels were all associated with an increased risk of development albuminuria (Table 2). In the multivariate models with the stepwise selection procedure, male gender, presence of diabetic retinopathy and higher HbA$_{1c}$ level, urinary albumin-to-creatinine ratio and serum uric acid remained as significantly predictive of the development of albuminuria (Table 2). Lower non-HDL cholesterol level, which was not significantly associated with the onset of albuminuria in the univariate model, was selected in the multivariate model.

In the univariate Cox regression analysis of predictors for decreased eGFR, the variables significantly associated with increased risk of development of renal impairment are listed in Table 3. In the multivariate model, older age, presence of diabetic retinopathy, higher HbA$_{1c}$ level, urinary albumin-to-creatinine ratio, systolic blood pressure, and lower eGFR and HDL cholesterol were independently associated with decreased eGFR (Table 3).

In the sensitivity analysis for the onset of albuminuria, in which individuals were censored when decreased eGFR occurred before the onset of albuminuria or the final observational period, the cumulative incidence of reaching the albuminuria endpoint was lower than that of the original analysis (Fig 1.). In the Cox regression analysis, the same

variables were selected as significantly associated with albuminuria onset in the primary multivariate analysis (Table 2). In the sensitivity analysis for decreased eGFR, the cumulative incidence of reaching the eGFR endpoint was lower. HDL cholesterol and urinary albumin-to-creatinine ratio were no longer significantly associated with the outcome (Table 3).

Discussion

In this single-centre observational cohort study in Japanese people with Type 2 diabetes without evidence of chronic kidney disease, we observed a higher cumulative incidence of decreased eGFR (eGFR < 60 ml/min/1.73 m^2) than of albuminuria. The presence of diabetic retinopathy and higher HbA$_{1c}$ levels and urinary albumin-to-creatinine ratio at baseline were associated with an increased risk of both onset of albuminuria and decreased eGFR. Additional independent predictors were associated separately with the two outcomes. In sensitivity analysis for the outcome of decreased eGFR in which individuals who developed albuminuria was censored at the onset of albuminuria, the significant association of urinary albumin-to-creatinine ratio disappeared.

The observation that decreased eGFR antedated the onset of albuminuria in many participants in this study is not surprising. In our previous cross-sectional study, 15.5% of patients with Type 2 diabetes with normoalbuminuria presented with eGFR < 60 ml/min/1.73 m^2 [6]. In a cross-sectional study from the Third National Health and Nutrition Examination Survey, 30% of adults with Type 2 diabetes without albuminuria had renal insufficiency [19]. These results are also consistent with a previous longitudinal study in the UK, showing that half of the people with Type 2 diabetes progressed to renal impairment without preceding albuminuria [20]; therefore, the natural history of kidney disease in people with Type 2 diabetes may differ from that of people with Type 1 diabetes, in whom decreased eGFR is generally found to occur after the onset of macroalbuminuria. Alternatively, the natural history of diabetic kidney disease may have been changed after the introduction of renin-angiotensin system blockers for the treatment of hypertension in people with diabetes [21]. Nevertheless, our results indicate differences in the risk factors for the pathogenesis of albuminuria and decreased eGFR in people with Type 2 diabetes. Whether such is the case with Type 1 diabetes remains unknown.

Previous observational studies have suggested several predictors of onset of albuminuria; however, few studies have assessed predictors of decreased eGFR in people with Type 2 diabetes [19,22–25]. In the present study, both the renal outcomes shared several baseline predictors, including the presence of diabetic retinopathy and elevated HbA$_{1c}$ levels and urinary albumin-to-creatinine ratio. The association of retinopathy and onset of albuminuria are consistent with the results of the UK Prospective Diabetes Study [20];

Table 2 Univariate and multivariate Cox proportional hazards regression analysis with stepwise selection procedure to determine predictors of onset of albuminuria

Variable	Univariate			Multivariate			Multivariate (sensitivity analysis)		
	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Age (years)	1.01	0.99–1.02	0.263	-			-		
Men (vs women)	1.48	1.09–2.03	0.014	1.69	1.21–2.35	0.002	1.78	1.25–2.53	0.002
Duration of diabetes (years)	1.01	0.99–1.03	0.283	-			-		
BMI (kg/m^2)	1.04	1.00–1.08	0.043	-			-		
Retinopathy (yes vs no)	2.07	1.55–2.77	< 0.001	1.57	1.17–2.12	0.003	1.48	1.07–2.05	0.018
Medication for diabetes, oral hypoglycaemic agent (vs none)	0.73	0.55–0.98	0.036	-			-		
Medication for diabetes, insulin (vs none)	1.58	1.17–2.13	0.003	-					
Systolic blood pressure (mmHg)	1.01	0.99–1.01	0.096	-			-		
Diastolic blood pressure (mmHg)	1.00	0.99–1.02	0.652	-			-		
Use of renin-angiotensin system blockers (yes vs no)	1.85	1.36–2.50	< 0.001	-			-		
Use of calcium channel blockers (yes vs no)	1.80	1.31–2.48	< 0.001	-			-		
History of cardiovascular disease (yes vs no)	1.08	0.67–1.76	0.748	-			-		
Laboratory data									
HbA$_{1c}$ (mmol/mol)	1.03	1.02–1.03	< 0.001	1.02	1.01–1.03	< 0.001	1.02	1.01–1.03	< 0.001
HDL cholesterol (mmol/l)	0.50	0.33–0.76	0.001	-			-		
Non-HDL cholesterol (mmol/l)	0.87	0.73–1.03	0.113	0.80	0.67–0.96	0.014	0.83	0.69–0.99	0.045
eGFR (ml/min/1.73 m^2)	1.00	0.99–1.01	0.397	-			-		
Log [urinary albumin-to-creatinine ratio (mg/g)]	239.58	108.42–529.42	< 0.001	219.45	95.75–502.93	< 0.001	202.60	85.15–482.05	< 0.001
Uric acid (μmol/l)	1.00	1.00–1.01	< 0.001	1.00	1.00–1.01	< 0.001	1.00	1.00–1.01	0.002
Haemoglobin (g/l)	1.00	0.99–1.01	0.752	-			-		

eGFR, estimated GFR; HR, hazard ratio.

however, a higher HbA$_{1c}$ level was not predictive of decreased eGFR, while male gender and higher systolic blood pressure were predictive in the UK prospective study, suggesting some dissociations between studies.

When assessing both of these renal outcomes in the same study, one precedent outcome may affect another outcome. For example, if people with normoalbuminuria progressed to micro- or macroalbuminuria during follow-up, these people were likely to have a reduction in GFR thereafter to a greater extent than those who still had normoalbuminuria during follow-up. We conducted a sensitivity analysis, therefore, to exclude these 'antecedent effects'. In this analysis, baseline urinary albumin-to-creatinine ratio and HDL cholesterol were excluded as predictors of decreased eGFR (Table 3), suggesting that an increase in urinary albumin-to-creatinine ratio during follow-up as well as a higher baseline albumin-to-creatinine ratio, even within the normal range, is associated with future GFR decline. By contrast, sensitivity analysis for onset of albuminuria yielded the same predictors as in the original analysis, suggesting that antecedent decreased eGFR may have little or no effect on the development of albuminuria.

Predictors that were unique for the onset of albuminuria were male gender, lower non-HDL cholesterol levels and higher uric acid levels. Unique predictors for decreased GFR were older age, higher systolic blood pressure and lower eGFR and HDL cholesterol levels. These associations appear to be generally consistent with previous studies, with some discrepancies, possibly attributable to differences in the inclusion criteria or ethnicity, as well as possible differences in definitions used for outcomes. In the Action in Diabetes and Vascular Disease: Preterax and Diamicron MR Controlled Evaluation (ADVANCE) study, for example, participants were selected based on high risk of vascular events, and major kidney-related outcomes were defined as a composite of doubling of serum creatinine, renal replacement therapy or death as a result of kidney disease [23]. The Verona Diabetes Study included Italian people with Type 2 diabetes with baseline eGFR ≥ 60 ml/min/1.73 m^2 but with a broad range of albuminuria stages; the GFR outcome was defined as the annual rate of decrease in eGFR [25]. An observational study from the Steno Diabetes Centre identified determinants of renal function loss in Danish people

Table 3 Univariate and multivariate Cox proportional hazards regression analysis with stepwise selection procedure to determine predictors of decreased estimated GFR

Variable	Univariate			Multivariate			Multivariate (sensitivity analysis)		
	HR	95% CI	P	HR	95% CI	P	HR	95% CI	P
Age (years)	1.07	1.06–1.09	< 0.001	1.04	1.02–1.05	< 0.001	1.04	1.02–1.05	< 0.001
Men (vs women)	1.14	0.90–1.43	0.271	-			-		
Duration of diabetes (years)	1.03	1.02–1.04	< 0.001	-			-		
BMI (kg/m^2)	0.99	0.96–1.02	0.535	-			-		
Retinopathy (yes vs no)	1.68	1.34–2.10	< 0.001	1.36	1.08–1.71	0.008	1.40	1.09–1.79	0.007
Medication for diabetes, oral hypoglycaemic agent (vs none)	0.94	0.76–1.18	0.605	-			-		
Medication for diabetes, insulin (vs none)	1.18	0.93–1.50	0.176	-			-		
Systolic blood pressure (mmHg)	1.01	1.01–1.02	< 0.001	1.01	1.00–1.01	0.031	1.01	1.00–1.01	0.022
Diastolic blood pressure (mmHg)	0.99	0.98–1.00	0.172	-			-		
Use of renin-angiotensin system blockers (yes vs no)	1.46	1.15–1.86	0.002	-			-		
Use of calcium channel blockers (yes vs no)	1.95	1.54–2.48	< 0.001	-			-		
History of cardiovascular disease (yes vs no)	2.17	1.63–2.91	< 0.001	-			-		
Laboratory data									
HbA$_{1c}$ (mmol/mol)	1.01	1.00-1.02	0.002	1.02	1.01–1.03	< 0.001	1.02	1.01–1.03	< 0.001
HDL cholesterol (mmol/l)	0.72	0.53–0.97	0.031	0.71	0.52–0.98	0.035	-		
Non-HDL cholesterol (mmol/l)	0.98	0.86-1.11	0.723	-			-		
eGFR (ml/min/1.73 m^2)	0.88	0.87–0.90	< 0.001	0.89	0.87–0.90	< 0.001	0.87	0.86–0.89	< 0.001
Log [urinary albumin-to-creatinine ratio (mg/g)]	3.53	2.14-5.82	< 0.001	2.74	1.64–4.57	< 0.001	-		
Uric acid (μmol/l)	1.00	1.00-1.00	< 0.001	-			-		
Haemoglobin (g/l)	0.99	0.98-0.99	0.008	-			-		

eGFR, estimated GFR; HR, hazard ratio.

with Type 2 diabetes with macroalbuminuria, and also used the annual rate of decrease in eGFR as the outcome measure [22]. In contrast to these studies, we studied people with Type 2 diabetes without albuminuria and renal impairment; predictors of renal outcomes in people with more advanced stages of chronic kidney disease could be determined in future studies.

In the present study, we focused on the two kidney disease outcomes, onset of albuminuria and reduced eGFR, in diabetes in association with several pathological entities. Chronic kidney disease in people with diabetes may include nephrosclerosis and ischaemic nephropathy as well as traditional diabetic nephropathy. These two renal manifestations may reflect different pathological mechanisms, and may describe how urinary albumin-to-creatinine ratio and GFR are not ideal biomarkers for kidney disease; additional biomarkers of kidney disease are under investigation [26] and may be useful for future studies to address pathological mechanisms of disease.

The present study has numerous limitations. First, we examined an ethnically and socially homogeneous population visiting an outpatient clinic in an urban university hospital in Japan; therefore, generalization of our findings may be limited. Second, the number of observed outcomes was still small. Third, we addressed the association of renal outcomes with baseline values of albumin-to-creatinine ratio and eGFR values without assessing intraindividual changes

in these variables during follow-up. In addition, covariates were also analysed using only subjects' baseline data and not average data during follow-up or time-dependent variables. Fourth, regarding the manifestation of reduced eGFR, we assessed a reduction in eGFR to < 60 ml/min/1.73 m^2. Other outcomes, including reduction of eGFR by half, which is almost equivalent to doubling of serum creatinine level, or by > 30% from baseline, should be addressed in future studies. Other study limitations include: 1) the estimation of GFR rather than a direct measure, 2) possible selection bias resulting from exclusion of people without requisite data for both outcomes (a common limitation for such historical cohort studies), and 3) lack of information on the use of renin-angiotensin system blockers during the follow-up period, which may affect the renal outcomes. Nevertheless, the study's large sample size, long duration of follow-up, improved specificity of outcomes because of the requirement of two consecutive measurements of urinary albumin-to-creatinine ratio and eGFR, and consistent use of first-morning specimens, strengthen its potential relevance to clinical practice.

In conclusion, the presence of diabetic retinopathy, higher HbA$_{1c}$ levels and greater urinary albumin excretion were associated with both onset of albuminuria and decreased eGFR in Japanese people with Type 2 diabetes. Additional independent risk factors for albuminuria were male gender and elevated uric acid levels. Additional risk factors for

decreased eGFR were older age, higher systolic blood pressure, lower eGFR and lower HDL cholesterol levels. The identification of both common and distinct predictive factors for the onset of albuminuria and decreased eGFR support the hypothesis that both common and distinct pathophysiological mechanisms are involved in the development of chronic kidney disease in people with diabetes. Ultimately, whether reduction of these risk factors improves renal hard endpoints, progression to end-stage renal disease or renal death, in people with Type 2 diabetes will require elucidation in interventional studies.

References

1 Mongensen CE. Microalbuminuria predicts clinical proteinuria and early mortality in maturity-onset diabetes. *N Engl J Med* 1984; **310**: 356–360.

2 Viberti GC, Hill RD, Jarrett RJ, Argyropoulos A, Mahmud U, Keen H. Microalbuminuria as a predictor of clinical nephropathy in insulin-dependent diabetes mellitus. *Lancet* 1982; **1**: 1430–1432.

3 National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**(Suppl. 1): S1–S266.

4 Levey AS, de Jong PE, Coresh J, El Nahas M, Astor BC, Matsushita K *et al*. The definition, classification, and prognosis of chronic kidney disease: a KDIGO controversies conference report. *Kidney Int* 2011; **80**: 17–28.

5 Mongensen CE, Christensen CK, Vittinghus E. The stages in diabetic renal disease. With emphasis on the stage of incipient diabetic nephropathy. *Diabetes* 1983; **32**(Suppl. 2): 64–78.

6 Ohta M, Babazono T, Uchigata Y, Iwamoto Y. Comparison of the prevalence of chronic kidney disease in Japanese patients with type 1 and type 2 diabetes. *Diabet Med* 2010; **27**: 1017–1023.

7 Yokoyama H, Sone H, Oishi M, Kawai K, Fukumoto Y, Kobayashi M *et al*. Prevalence of albuminuria and renal insufficiency and associated clinical factors in type 2 diabetes: the Japan Diabetes Clinical Data Management study (JDDM15). *Nephrol Dial Transplant* 2009; **24**: 1212–1219.

8 Thomas MC, MacIsaac RJ, Jerums G, Weekes A, Moran J, Shaw JE *et al*. Nonalbuminuric renal impairment in type 2 diabetic patients and in the general population (National Evalution of the Frequency of Renal Impairment cO-existing with NIDDM [NEFRON] 11). *Diabetes Care* 2009; **32**: 1497–1502.

9 Tabaei B, Al-Kassab A, Ilag L, Zawacki C, Herman W. Does microalbuminuria predict nephropathy? *Diabetes Care* 2001; **24**: 1560–1576.

10 Ravid M, Brosh D, Ravid-Safran D, Levy Z, Rachmani R. Main risk factors for nephropathy in type 2 diabetes mellitus are plasma cholesterol levels, mean blood pressure, and hyperglycemia. *Arch Intern Med* 1998; **159**: 998–1004.

11 Park JY, Kim HK, Chung YE, Kim SW, Hong SK, Lee KU. Incidence and determinants of microalbuminuria with type 2 diabetes. *Diabetes Care* 1998; **21**: 530–534.

12 Forsblom C, Groop PH, Ekstrand A, Totterman KJ, Sane T, Saloranta C *et al*. Predictors of progression from normoalbuminuria to microalbuminuria in NIDDM. *Diabetes Care* 1998; **21**: 1932–1938.

13 Cederholm J, Eliasson B, Nilsson PM, Weiss L, Gudbjornsdottir S, the Steering Committee of the Swedish National Diabetes Register. Microalbuminuria and risk factors in type 1 and type 2 diabetic patients. *Diabetes Res Clin Pract* 2005; **67**: 258–266.

14 Toya K, Babazono T, Hanai K, Uchigata Y. Association of serum bilirubin levels with development and progression of albuminuria, and decline in estimated glomerular filtration rate in patients with type 2 diabetes mellitus. *J Diabetes Invest* 2014; **5**: 228–235.

15 Kashiwagi A, Kasuga M, Araki E, Oka Y, Hanafusa T, Ito H *et al*. Committee on the standardization of diabetes mellitus-related laboratory testing of Japan diabetes society. International clinical harmonization of glycated hemoglobin in Japan: from Japan diabetes society to national glycohemoglobin standardization program values. *J Diabetes Investig* 2012; **3**: 39–40.

16 Hoelzel W, Weykamp C, Jeppsson JO, Miedema K, Barr JR, Goodall I *et al*. IFCC reference system for measurement of hemoglobin A1c in human blood and the national standardization schemes in the United States, Japan, and Sweden: a method-comparison study. *Clin Chem* 2004; **50**: 166–174.

17 Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; **18**: 499–502.

18 Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K *et al*. Revised equations for estimated GFR from serum creatinine in Japan. *Am J Kidney Dis* 2009; **53**: 982–992.

19 Kramer HJ, Nguyen QD, Curhan G, Hsu CY. Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. *JAMA* 2003; **289**: 3273–3277.

20 Retnakaran R, Cull CA, Thorne KI, Adler AI, Holman RR. Risk factors for renal dysfunction in type 2 diabetes: U.K. Prospective Diabetes Study 74. *Diabetes* 2006; **55**: 1832–1838.

21 Araki S, Haneda M, Sugimoto T, Isono M, Isshiki K, Kashiwagi A *et al*. Factors associated with frequent remission of microalbuminuria in patients with type 2 diabetes. *Diabetes* 2005; **54**: 2983–2987.

22 Rossing K, Christensen PK, Hovind P, Tarnow L, Rossing P, Parving HH. Progression of nephropathy in type 2 diabetic patients. *Kidney Int* 2004; **66**: 1596–1605.

23 Jardine MJ, Hata J, Woodward M, Perkovic V, Ninomiya T, Arima H *et al*. ADVANCE Collaborative Group. Prediction of kidney-related outcomes in patients with type 2 diabetes. *Am J Kidney Dis* 2012; **60**: 770–778.

24 Afghahi H, Cederholm J, Eliasson B, Gudbjornsdottir S, Hadimeri H, Svensson MK. Risk factors for the development of albuminuria and renal impairment in type 2 diabetes – the Swedish National Diabetes Register (NDR). *Nephrol Dial Transplant* 2011; **26**: 1236–1243.

25 Zoppini G, Targher G, Chonchol M, Ortalda V, Negri C, Stoico V *et al*. Predictors of estimated GFR decline in patients with type 2 diabetes and preserved kidney function. *Clin J Am Soc Nephrol* 2012; **7**: 401–408.

26 Macisaac RJ, Ekinci EI, Jerums G. Markers of and risk factors for the development and progression of diabetic kidney disease. *Am J Kidney Dis* 2014; **63**(2 Suppl. 2): S39–562.