Original Article

Risk Factors for Renal Dysfunction in Type 2 Diabetes

U.K. Prospective Diabetes Study 74

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Not all patients with type 2 diabetes develop renal dysfunction. Identifying those at risk is problematic because even microalbuminuria, often used clinically as an indicator of future renal dysfunction, does not always precede worsening renal function. We sought to identify clinical risk factors at diagnosis of type 2 diabetes associated with later development of renal dysfunction. Of 5,102 U.K. Prospective Diabetes Study (UKPDS) participants, prospective analyses were undertaken in those without albuminuria (n = 4,031) or with normal plasma creatinine (n = 5,032) at diagnosis. Stepwise proportional hazards multivariate regression was used to assess association of putative baseline risk factors with subsequent development of albuminuria (microalbuminuria or macroalbuminuria) or renal impairment (Cockcroft-Gault estimated creatinine clearance <60 ml/min or doubling of plasma creatinine). Over a median of 15 years of follow-up 1,544 (38%) of 4,031 patients developed albuminuria and 1,449 (29%) of 5,032 developed renal impairment. Of 4,006 patients with the requisite data for both outcomes, 1,534 (38%) developed albuminuria and 1,132 (28%) developed renal impairment. Of the latter, 575 (51%) did not have preceding albuminuria. Development of albuminuria or renal impairment was independently associated with increased baseline systolic blood pressure, urinary albumin, plasma creatinine, and Indian-Asian ethnicity. Additional independent risk factors for albuminuria were male sex, increased waist circumference, plasma triglycerides, LDL cholesterol, HbA_{1c} (A1C), increased white cell count, ever having smoked, and previous retinopathy. Additional independent risk factors for renal impairment were female sex, decreased waist circumference, age, increased insulin sensitivity, and previous sensory neuropathy. Over a median of 15 years from diagnosis of type 2 diabetes, nearly 40% of UKPDS patients developed albuminuria and nearly 30% developed renal impairment. Distinct sets of risk factors are associated with the development of these two outcomes, consistent with the concept that they are not linked inexorably in type 2 diabetes. *Diabetes* 55:1832–1839, 2006

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CVD, cardiovascular disease; ESRD, end-stage renal disease; FPG, fasting plasma glucose; HOMA, homeostasis model assessment; UKPDS, U.K. Prospective Diabetes Study; WESDR, Wisconsin Epidemiologic Study of Diabetic Retinopathy.

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Type 2 diabetes is the leading cause of end-stage renal disease (ESRD) in the Western world (1,2). Many, but not all, patients with type 2 diabetes develop renal dysfunction during their lifetime. In the U.K. Prospective Diabetes Study (UKPDS), 24.9% of patients developed microalbuminuria within 10 years of diagnosis of type 2 diabetes, but only 0.8% developed ESRD, as assessed by elevated plasma creatinine (>250 μmol/l) or the need for renal replacement therapy (3). Annual rates of transition between successive stages within the classical paradigm of normoalbuminuria to microalbuminuria to macroalbuminuria to ESRD were in the range of 2–3% per year, suggesting that many individuals will not necessarily progress to worsening renal outcomes, even after developing microalbuminuria (3). The ability to identify those patients most likely to progress to poor renal outcomes would allow the institution of appropriate interventions in a timely manner. Our objective in the current report was to identify clinically evident risk factors at the time of diagnosis of type 2 diabetes that are associated with the subsequent development of renal dysfunction in patients recruited to the UKPDS.

RESEARCH DESIGN AND METHODS

The UKPDS was a clinical trial designed to evaluate the effects of improved blood glucose control and/or blood pressure control on the incidence of complications in patients with type 2 diabetes. The UKPDS, whose recruitment and selection process has been reported previously (4), received ethical committee approval in each of the 23 participating clinical centers and conformed to the guidelines of the Declaration of Helsinki. All participants provided informed consent. Briefly, 5,102 of 7,616 patients with newly diagnosed type 2 diabetes had fasting plasma glucose (FPG) levels ≥6.0 mmol/l on two successive occasions after referral and entered the study between 1977 and 1991. Exclusion criteria included myocardial infarction or stroke within the preceding year, severe vascular disease, uncontrolled hypertension, proliferative or preproliferative retinopathy, plasma creatinine ≥175 μmol/l, treatment with systemic steroids, and severe previous illness that would limit life or require extensive systemic treatment. Those recruited were aged 25–65 years, with 81% self-reported white Caucasian, 10% Indian Asian, and 9% Afro-Caribbean. After a 3-month dietary run-in period, therapies for glycemic control were allocated randomly according to the UKPDS protocol (4). From 1987, a subset of hypertensive participants (n = 1,148) was also allocated randomly to therapies for blood pressure control (4). All patients were followed quarterly in UKPDS clinics until the trial ended in 1997. For the current analyses, baseline data were taken as those recorded at the end of the dietary run-in period.

Biochemical and clinical measurements. Biochemical analyses were performed by the UKPDS central laboratory (5,6) apart from annual plasma creatinine measurements and triennial white blood cell counts, which were undertaken by local National Health Service clinical chemistry laboratories. Annual morning urine samples were collected in thiomersal tubes and

transported to the central laboratory at 4°C. From 1988 to the end of the study, urine samples were stored at 4°C and albumin levels measured by an immunoturbidimetric method (5,7). Before 1988, urinary albumin was measured by radioimmunoassay in samples stored at −20°C (8). Urinary albumin concentrations were standardized over time to account for the different assay and storage methodologies (9). HbA_{1c} (A1C) was measured by high-performance liquid chromatography (Diamat automated glycosylated hemoglobin analyzer; Biorad, Hemel Hempstead, U.K.), with a normal range of 4.5–6.2% certified by the NGSP (National Glycohemoglobin Standardization Programme) as comparable to the DCCT (Diabetes Control and Complications Trial) (5). FPG, fasting plasma insulin, triglycerides, and cholesterol (total, HDL, and LDL) were measured as described previously (5).

Anthropometric measurements, including weight, height, waist and hip measurements, and blood pressure assessment were performed as described previously (5,6). BMI was calculated as weight (kg) divided by the square of the height (m). The Homeostasis Model Assessment (version 2.0) (10) was used to derive β-cell function (%B) and insulin sensitivity (%S), using the homeostasis model assessment (HOMA) Calculator (available online from http://www.dtu.ox.ac.uk). Creatinine clearance was estimated using the Cockcroft-Gault formulas (11) for males: (140 − age [in years]) × weight (in kg)/72 × plasma creatinine (in μg/dl) and for females (140 − age [in years]) × weight (in kg) × 0.85/72 × plasma creatinine (in μg/dl).

Previous retinopathy at baseline was defined as a modified Early Treatment Diabetic Retinopathy Study "191" score ≥20/20, a history of retinal photocoagulation, or vitreous hemorrhage (12). Previous sensory neuropathy was defined as bilateral absence of ankle or patellar reflexes or a vibration perception threshold ≥25 V for both great toes. Where information was missing from a limb because of a diabetes-related amputation, worst-case values were imputed. Where information was missing from a limb because of a non–diabetes-related amputation, the values available for the other limb were substituted. Previous cardiovascular disease (CVD) was defined as Minnesota-coded electrocardiogram evidence (13) of a myocardial infarction or a history of myocardial infarction, angina, or a transient ischemic attack. Hypertension was defined as a systolic blood pressure ≥140 mmHg and/or diastolic blood pressure ≥90 mmHg and/or use of antihypertensive therapy.

Renal outcomes. The renal outcomes assessed were two measures of albuminuria (micro- and macroalbuminuria) and two measures of renal impairment (reduced creatinine clearance and doubling of baseline plasma creatinine). Microalbuminuria was defined as a urinary albumin concentration 50–299 mg/l inclusive, to account for the effects of storage at −20°C (5), and macroalbuminuria as a urinary albumin concentration ≥300 mg/l. A reduced glomerular filtration rate was defined as an estimated creatinine clearance ≤60 ml/min per 1.73 m^2. The time of occurrence of these outcomes was taken as the 1st year at which the event occurred, provided that the subsequent assessment confirmed the same result or worse.

Statistical analysis. Statistical analyses were performed using SAS 8.2 and 9.1.3 (SAS Institute, Cary, NC). Data are the means ± SD, median (interquartile range), or geometric mean (1-SD interval). Proportional hazards regression with discrete time intervals was used to evaluate associations between potential risk factors and renal outcomes. Hazard ratios (HRs) are presented with 95% CIs. Univariate analyses were limited for albuminuria models to participants without albuminuria at baseline (n = 4,031) and for renal impairment models to participants with estimated creatinine clearance ≥60 ml/min per 1.73 m^2 and normal plasma creatinine (females <120 μmol/l, males <130 μmol/l) at baseline (n = 5,032). Multivariate models were limited to 2,392 subjects with data for all relevant covariates. Variables were considered for inclusion in stepwise multivariate models if significant at $P < 0.01$ in univariate analyses for any of the four renal outcomes or if they were known to be clinically relevant. Patients were censored if deceased or if they remained event free at the end of follow-up. Putative baseline risk factors examined were age at diagnosis; sex; ethnicity; smoking status; weight; waist circumference; systolic blood pressure; diastolic blood pressure; history of hypertension; FPG; A1C; HOMA %B; HOMA %S; total, LDL, and HDL cholesterol; triglycerides; white cell count; urine albumin; plasma creatinine; and previous retinopathy, sensory neuropathy, or CVD. Plasma triglycerides were log transformed. Age at diagnosis, sex, and ethnicity were included in all multivariate analyses, whereas A1C and LDL cholesterol were tested in preference to FPG and total cholesterol, respectively. Systolic blood pressure was tested in preference to diastolic blood pressure or history of hypertension.

RESULTS

Table 1 shows baseline demographic and clinical characteristics for the 4,031 and 5,032 patients with the requisite data available for the albuminuria and renal impairment models, respectively. Median A1C for both cohorts was 6.9% with mean blood pressure 135/83 mmHg. Figure 1 shows the cumulative incidence of new-onset albuminuria and renal impairment over time. Altogether, 1,544 of 4,031 (38%) patients developed albuminuria and 1,449 of 5,032 (29%) developed renal impairment.

Of 4,006 patients with the requisite data for both outcomes, 1,534 (38%) developed albuminuria, 1,132 (28%) developed renal impairment, and 557 (14%) developed both conditions. Of the 1,534 patients who developed albuminuria, 977 (64%) did not develop renal impairment during the study, 372 (24%) developed renal impairment subsequent to developing albuminuria, and 12% developed renal impairment before developing albuminuria. Of the 1,132 patients who developed renal impairment, 575 (51%) did not develop albuminuria during the study, 185 (16%) developed albuminuria subsequent to developing renal impairment, and 33% developed albuminuria before developing renal impairment.

Microalbuminuria. In univariate models (Table 2), Indian-Asian ethnicity (HR 1.60), smoking history (1.28), urinary albumin (1.009), waist circumference (1.017), systolic blood pressure (1.17), diastolic blood pressure (1.24), history of hypertension (1.49), FPG (1.07), A1C (1.08), plasma triglycerides (1.13), white cell count (1.07), and previous CVD (1.58) were associated with increased risk of development of microalbuminuria ($P < 0.01$), whereas HOMA %S (0.97) was associated with a decreased risk. In multivariate models (Table 3), Indian-Asian ethnicity (HR 2.02) systolic blood pressure (1.15), plasma triglycerides (1.09), urinary albumin (1.004), white cell count (1.06), A1C (1.08) waist circumference (1.01), previous retinopathy (1.25), previous CVD (1.46), smoking history (1.20), and male sex (1.18) were independently associated with development of microalbuminuria.

Macroalbuminuria. In univariate models (Table 2), urinary albumin (HR 1.010), plasma creatinine (1.010), waist circumference (1.021), systolic blood pressure (1.18), history of hypertension (1.74), FPG (1.08), total cholesterol (1.20), plasma triglycerides (1.19), white cell count (1.07), and previous CVD (1.64) were associated with development of macroalbuminuria ($P < 0.01$). In multivariate models (Table 3), urinary albumin (HR 1.009), plasma creatinine (1.087), waist circumference (1.016), systolic blood pressure (1.15), A1C (1.10), LDL cholesterol (1.17), and plasma triglycerides (1.15) were independently associated, whereas white cell count, smoking history, and previous retinopathy were no longer significant.

Reduced creatinine clearance. In univariate models (Table 2), age at diagnosis (HR 2.07), height (1.07), urinary albumin (1.003), plasma creatinine (1.18), systolic blood pressure (1.20), diastolic blood pressure (1.07), history of hypertension (1.90), FPG (1.02), A1C (1.05), HOMA %S (1.02), total cholesterol (1.20), LDL cholesterol (1.22), HDL cholesterol (1.85), previous retinopathy (1.38), previous neuropathy (1.36), and previous CVD (1.71) were associated with an increased risk of development of reduced creatinine clearance ($P < 0.01$). Male sex (HR 0.53), Indian-Asian ethnicity (0.76), waist circumference (0.96), weight (0.76), and HOMA %B (0.97) were independently associated with reduced risk of development of reduced creatinine clearance. In multivariate models (Table 3), baseline plasma creatinine (HR 1.34), systolic blood pressure (1.11), age at diagnosis (2.15), height (1.05), Indian-Asian ethnicity (1.93), ever smoking (1.25), previous retinopathy (1.26), and urinary albumin (1.009) were independently associated with increased risk of development

TABLE 1
Baseline demographic and clinical characteristics of subjects included in the albuminuria and renal insufficiency models

Variable	Albuminuria models	Renal insufficiency models
n	4,031	5,032
Age at diagnosis (years)	52.6 ± 8.7	52.4 ± 8.8
Male	2,433 (60)	2,969 (59)
Ethnicity		
White Caucasian	3,320 (82)	4,155 (82)
Afro-Caribbean	307 (7.6)	385 (7.6)
Indian Asian	404 (10.0)	492 (9.7)
Smoking status		
Current smoker	1,235 (30)	1,556 (31)
Ex-smoker	1,389 (34)	1,694 (33)
Never smoked	1,405 (35)	1,780 (35)
Urinary albumin (mg/l)*	9 (4–20)	9 (4–20)
Plasma creatinine (μmol/l)*	82 (71–92)	81 (71–92)
BMI (kg/m^2)	27.7 ± 5.3	27.5 ± 5.4
Waist (cm)	96 ± 13	95 ± 13
Systolic blood pressure (mmHg)	135 ± 20	135 ± 20
Diastolic blood pressure (mmHg)	83 ± 10	83 ± 11
Hypertensive	1,820 (45)	2,255 (44)
On antihypertensive therapy	800 (20)	966 (19)
FPG (mmol/l)*	8.0 (6.6–10.1)	8.2 (6.8–11.0)
A1C (%)*	6.9 (5.9–8.1)	6.9 (5.9–8.1)
HOMA %B*	55.9 (34.5–84.0)	55.5 (34.3–83.3)
HOMA %S*	52.1 (36.3–75.1)	51.7 (36.1–74.9)
Total cholesterol (mmol/l)	5.38 ± 1.13	5.39 ± 1.12
LDL cholesterol (mmol/l)	3.50 ± 1.03	3.51 ± 1.03
HDL cholesterol (mmol/l)	1.07 ± 0.24	1.07 ± 0.24
Plasma triglycerides (mmol/l)†	1.54 (0.92–2.56)	1.55 (0.93–2.59)
White blood cell count ($\times 10^9$/l)	6.6 (5.5–8.1)	6.6 (5.5–8.1)
Previous retinopathy	543 (19)	681 (20)
Previous sensory neuropathy	847 (22)	1094 (22)
Previous CVD	761 (19)	974 (19)

Data are the means ± SD or *n* (%), unless otherwise indicated. *Median (interquartile range); †geometric mean (1-SD interval).

of reduced creatinine clearance (Table 3). Male sex (HR 0.55) and waist circumference (0.95) were independently associated with reduced risk of development of reduced creatinine clearance.

Doubling of plasma creatinine. In univariate models (Table 2), urinary albumin (HR 1.008), systolic blood pressure (1.36), diastolic blood pressure (1.80), and history of hypertension (3.09) were associated with an increased risk of doubling of plasma creatinine ($P < 0.01$). The only factors independently associated with doubling of plasma creatinine were systolic blood pressure (HR 1.38) and previous sensory neuropathy (1.79), with HDL cholesterol (2.78) significant at $P < 0.05$.

DISCUSSION

This report shows that over a median of 15 years after diagnosis of type 2 diabetes, 38% of UKPDS participants developed albuminuria and 29% developed renal impairment. Importantly, a substantial proportion of patients

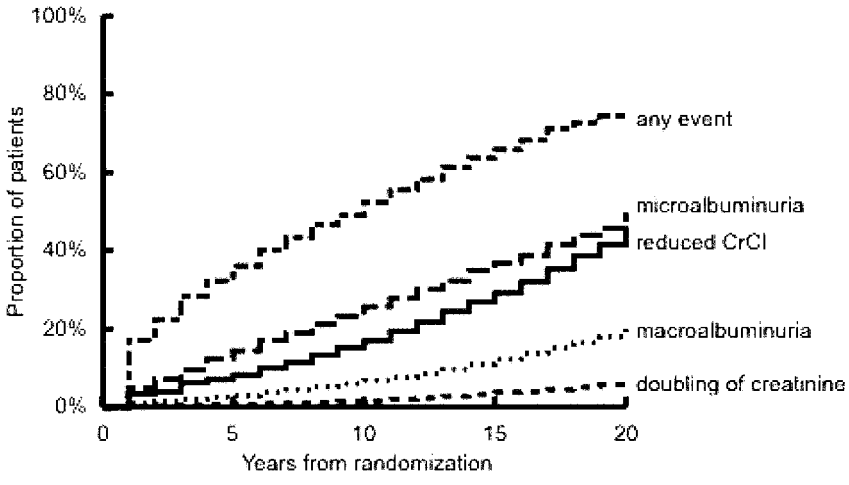

FIG. 1. Kaplan-Meier plots of proportion of patients with microalbuminuria, macroalbuminuria, reduced creatinine clearance (CrCl), doubling of plasma creatinine, or any one of these, after diagnosis of diabetes.

TABLE 2
Univariate proportional hazards analyses for albuminuria and renal insufficiency outcomes

	Albuminuria (n = 4,031)				Renal insufficiency (n = 5,032)			
	Microalbuminuria (N events = 1,430)		Macroalbuminuria (N events = 420)		Creatinine clearance ≤60 ml/min per 1.73 m^2 (N events = 1,428)		Doubling of plasma creatinine (N events = 169)	
Variable	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
Age at diagnosis (per 5 years)	1.04 (1.00–1.09)	0.08	1.06 (0.97–1.16)	0.18	2.07 (1.98–2.17)	<0.0001	1.06 (0.91–1.23)	0.48
Sex (male)	1.18 (1.00–1.39)	0.045	1.42 (1.05–1.93)	0.023	0.53 (0.48–0.59)	<0.0001	0.73 (0.43–1.22)	0.22
Ethnicity								
White Caucasian	1		1		1		1	
Afro-Caribbean	0.98 (0.72–1.35)	0.92	0.94 (0.52–1.70)	0.84	1.17 (0.98–1.41)	0.085	0.69 (0.21–2.21)	0.53
Indian Asian	1.60 (1.26–2.05)	0.00015	1.72 (1.13–2.62)	0.011	0.76 (0.62–0.93)	0.0065	0.97 (0.39–2.44)	0.95
Smoking status								
Never	1		1		1		1	
Ever	1.28 (1.08–1.51)	0.0046	1.31 (0.96–1.79)	0.088	0.93 (0.83–1.04)	0.19	1.31 (0.75–2.28)	0.35
Urinary albumin (per 20 mg/l)	1.009 (1.06–1.011)	<0.0001	1.010 (1.007–1.013)	<0.0001	1.003 (0.999–1.008)	0.14	1.008 (1.003–1.014)	0.0019
Plasma creatinine (per 10 μmol/l)	1.21 (0.99–1.05)	0.20	1.010 (1.038–1.157)	0.00093	1.18 (1.14–1.21)	<0.0001	0.90 (0.82–0.99)	0.032
Weight (per 5 kg)	1.05 (1.03–1.08)	<0.0001	1.05 (1.01–1.10)	0.014	0.762 (0.745–0.779)	<0.0001	1.03 (0.95–1.12)	0.45
Waist (cm)	1.017 (1.013–1.021)	<0.0001	1.021 (1.014–1.029)	<0.0001	0.960 (0.955–0.965)	<0.0001	1.005 (0.993–1.017)	0.41
Height (cm)	1.00 (0.99–1.01)	0.75	1.00 (0.99–1.01)	0.75	1.07 (1.05–1.08)	<0.0001	0.98 (0.95–1.00)	0.052
Systolic blood pressure (per 10 mmHg)	1.17 (1.13–1.22)	<0.0001	1.18 (1.10–1.26)	<0.0001	1.20 (1.17–1.23)	<0.0001	1.36 (1.22–1.52)	<0.0001
Diastolic blood pressure (per 10 mmHg)	1.24 (1.14–1.34)	<0.0001	1.16 (1.00–1.34)	0.047	1.07 (1.02–1.13)	0.0062	1.80 (1.41–2.28)	<0.0001
Hypertensive	1.49 (1.28–1.75)	<0.0001	1.74 (1.31–2.33)	0.00016	1.90 (1.71–2.11)	<0.0001	3.09 (1.77–5.39)	<0.0001
On antihypertensive therapy	1.28 (1.07–1.55)	0.0087	1.52 (1.10–2.10)	0.012	1.74 (1.55–1.97)	<0.0001	1.54 (0.86–2.74)	0.14
FPG (mmol/l)	1.07 (1.05–1.10)	<0.0001	1.08 (1.03–1.13)	0.00081	1.021 (1.007–1.035)	0.0036	1.09 (1.01–1.18)	0.019
A1C (%)	1.08 (1.03–1.12)	0.0004	1.10 (1.02–1.18)	0.015	1.05 (1.02–1.08)	0.0016	1.06 (0.93–1.22)	0.37
HOMA %B (per 10%)	0.99 (0.97–1.01)	0.16	0.97 (0.94–1.01)	0.18	0.97 (0.95–0.98)	0.000034	0.96 (0.89–1.03)	0.26
HOMA %S (per 10%)	0.97 (0.95–0.99)	0.0044	0.95 (0.91–0.99)	0.019	1.02 (1.01–1.03)	0.000012	0.97 (0.90–1.03)	0.32
Total cholesterol (mmol/l)	1.08 (1.00–1.16)	0.042	1.20 (1.06–1.36)	0.0037	1.20 (1.15–1.26)	<0.0001	1.04 (0.82–1.32)	0.75
LDL cholesterol (mmol/l)	1.07 (0.99–1.15)	0.088	1.17 (1.02–1.34)	0.026	1.22 (1.15–1.28)	<0.0001	1.07 (0.83–1.38)	0.59
HDL cholesterol (mmol/l)	0.69 (0.49–0.96)	0.026	0.53 (0.28–0.99)	0.048	1.85 (1.50–2.28)	<0.0001	1.67 (0.60–4.64)	0.33
Plasma triglycerides mmol/l)*	1.13 (1.07–1.19)	<0.0001	1.19 (1.11–1.27)	<0.0001	0.96 (0.86–1.07)	0.44	0.86 (0.63–1.16)	0.32
White cell count (10^9/l)	1.07 (1.03–1.11)	0.00037	1.07 (1.00–1.14)	0.056	0.98 (0.95–1.00)	0.093	0.99 (0.87–1.13)	0.86
Previous retinopathy	1.36 (1.12–1.64)	0.0017	1.46 (1.05–2.05)	0.027	1.38 (1.18–1.61)	0.000043	1.78 (1.00–3.16)	0.051
Previous sensory neuropathy	1.16 (0.96–1.40)	0.13	1.40 (1.01–1.96)	0.045	1.36 (1.21–1.54)	<0.0001	1.45 (0.81–2.61)	0.22
Previous CVD	1.58 (1.31–1.90)	<0.0001	1.64 (1.18–2.28)	0.003	1.71 (1.51–1.93)	<0.0001	1.22 (0.65–2.31)	0.54

*Log_{10} transformed.

TABLE 3
HRs derived from a stepwise proportional hazards regression models for albuminuria outcomes and renal insufficiency outcomes (n = 2,167)

	Albuminuria						Renal insufficiency					
	Microalbuminuria (N events = 756)			Macroalbuminuria (N events = 219)			Creatinine clearance ≤60 ml/min per 1.73 m^2 (N events = 584)			Doubling of plasma creatinine (N events = 58)		
Variable	Order entered	HR (95% CI)	P	Order entered	HR (95% CI)	P	Order entered	HR (95% CI)	P	Order entered	HR (95% CI)	P
Age at diagnosis (per 5 years)	—	1.01 (0.97–1.06)	0.58	—	1.02 (0.94–1.12)	0.59	—	2.15 (1.98–2.34)	<0.0001	—	0.91 (0.77–1.07)	0.25
Sex (male)	—	1.18 (1.01–1.39)	0.041	—	1.47 (1.06–2.02)	0.020	—	0.550 (0.424–0.715)	<0.0001	—	0.87 (0.51–1.48)	0.61
Ethnicity	—	—	—	—	—	—	—	—	—	—	—	—
White Caucasian	—	1	—	—	1	—	—	1	—	—	1	—
Afro-Caribbean	—	1.21 (0.89–1.65)	0.22	—	1.05 (0.59–1.86)	0.87	—	1.26 (0.91–1.76)	0.17	—	0.40 (0.10–1.68)	0.21
Indian Asian	—	2.02 (1.59–2.60)	<0.0001	—	2.07 (1.36–3.15)	0.00066	—	1.93 (1.38–2.72)	0.00015	—	1.51 (0.59–3.90)	0.39
Urinary albumin (per 20 mg/l)	3	1.004 (1.002–1.007)	0.00066	1	1.009 (1.005–1.012)	<0.0001	5	1.009 (1.002–1.015)	0.0075	—	—	—
Plasma creatinine (per 10 μmol/l)	—	—	—	8	1.087 (1.005–1.175)	0.038	2	1.34 (1.28–1.40)	<0.0001	—	—	—
Smoking status (ever)	9	1.20 (1.01–1.42)	0.036				7	1.25 (1.03–1.52)	0.022	—	—	—
Waist (cm)	7	1.010 (1.004–1.016)	0.00042	4	1.016 (1.006–1.026)	0.0019	1	0.95 (0.94–0.96)	<0.0001	—	—	—
Height (cm)	—	—					3	1.05 (1.036–1.072)	<0.0001	—	—	—
Systolic blood pressure (per 10 mmHg)	1	1.15 (1.11–1.20)	<0.0001	3	1.15 (1.07–1.24)	0.00019	4	1.107 (1.06–1.16)	0.000012	1	1.39 (1.23–1.57)	<0.0001
A1C (%)	6	1.08 (1.03–1.12)	0.00031	6	1.10 (1.02–1.18)	0.011	—	—	—	—	—	—
LDL cholesterol (mmol/l)	—	—	—	7	1.17 (1.02–1.33)	0.022	—	—	—	—	—	—
HDL cholesterol (mmol/l)	—	—	—	—	—	—	—	—	—	3	2.78 (1.01–7.68)	0.049
Plasma triglycerides (mmol/l)*	2	1.09 (1.04–1.14)	<0.0001	2	1.15 (1.09–1.21)	<0.0001	—	—	—	—	—	—
White cell count (10^9/l)	5	1.06 (1.02–1.10)	0.0012	—	—	—	—	—	—	—	—	—
Previous retinopathy	8	1.25 (1.05–1.49)	0.012	—	—	—	7	1.255 (1.020–1.544)	0.032	—	—	—
Previous sensory neuropathy	—	—	—	—	—	—	—	—	—	2	1.84 (1.03–3.30)	0.039
Previous CVD	4	1.46 (1.23–1.73)	<0.0001	5	1.58 (1.16–2.15)	0.0041	—	—	—	—	—	—

*Log_{10} transformed.

developed one outcome but not the other. Whereas systolic blood pressure, Indian-Asian ethnicity, urinary albumin excretion, and plasma creatinine were risk factors for both albuminuria and renal impairment, other risk factors for these two outcomes were distinct. These findings are consistent with the concept that albuminuria and renal impairment may not necessarily reflect the same underlying pathology in type 2 diabetes.

The finding that nearly 40% of UKPDS patients developed albuminuria during >20 years after the diagnosis of type 2 diabetes is comparable to previous data from the Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR) (14). In the WESDR, by 15 years after the diagnosis of diabetes, 45.2% of participants had developed albuminuria. Although confirming the high risk of albuminuria in patients with type 2 diabetes, this analysis also demonstrates a high incidence (29%) of renal impairment in this patient population. A significant degree of discordance between development of albuminuria and renal impairment is apparent. Of those patients who developed renal impairment, 61% did not have albuminuria beforehand and 39% never developed albuminuria during the study. Of the patients that developed albuminuria, only 24% subsequently developed renal impairment during the study. These data thus do not support the classical paradigm of albuminuria always preceding renal impairment in the progression of diabetic kidney disease.

Albuminuria is used clinically as a marker of nephropathic risk in type 2 diabetes (2). However, it has recently been recognized that the probability of progression to macroalbuminuria in microalbuminuric subjects is not as high as once believed (2), and both stabilization of microalbuminuria without progression and regression of albuminuria are observed (2,15,16). Moreover, underlying renal structural lesions spanning a broad range of severity have been documented in both normoalbuminuric and microalbuminuric patients (2,17). In addition, in a cross-sectional analysis from the Third National Heath and Nutrition Examination Survey, 30% of adults with type 2 diabetes and chronic renal insufficiency exhibited neither albuminuria nor retinopathy, features presumed to be indicative of classic diabetic glomerulosclerosis (18). These findings suggest that microalbuminuria alone may not provide optimal identification of patients with type 2 diabetes at higher risk of renal impairment, and thus identification of other risk factors is needed. Furthermore, distinction needs to be drawn between risk factors for albuminuria and those for renal impairment.

The most highly associated risk factors for incident albuminuria were systolic blood pressure, plasma triglycerides, urinary albumin, and Indian-Asian ethnicity. The central importance of blood pressure as a risk factor for both albuminuria and renal impairment in type 2 diabetes has been well documented in previous observational studies (19,20). Furthermore, antihypertensive therapy has been shown to reduce the incidence of albuminuria and preserve renal function in clinical trials, including the UKPDS (21–23). In contrast to blood pressure, plasma triglycerides have exhibited inconsistent associations with incident proteinuria and renal dysfunction in both population and diabetic cohorts (24–29). In prospective studies in type 2 diabetes, an elevated triglyceride-to-HDL ratio has been independently associated with the progression of microalbuminuria (30), whereas hypertriglyceridemia has predicted the need for future renal replacement therapy (31). On the other hand, however, some investigators have found no independent association between triglycerides and renal outcomes, with univariate relationships abolished on multivariate adjustment (19,32). Compared with earlier studies in type 2 diabetes, it is important to note that the current analysis involves a larger patient population with longer follow-up and extensive clinical and metabolic characterization, allowing for more complete multivariate adjustment. Furthermore, fasting plasma triglyceride concentration consistently emerged as a strong independent determinant of both microalbuminuria and macroalbuminuria. The current findings support a role for hypertriglyceridemia in the early pathophysiology of albuminuria in type 2 diabetes. Consistent with this concept, fenofibrate decreased the progression to microalbuminuria in patients with type 2 diabetes in both the Diabetes Atherosclerosis Intervention Study (33) and the FIELD (Fenofibrate Intervention and Event Lowering in Diabetes) study (34).

Other studies have identified baseline urinary albumin as an independent risk factor for the development of albuminuria (35,36) and renal impairment (37,38). In contrast, Indian-Asian ethnicity has not been previously implicated in this context. An increased incidence of renal failure has been estimated previously in U.K. Indian-Asian patients with type 2 diabetes (39). In addition, cross-sectional studies have shown a higher prevalence of microalbuminuria in Indian Asians compared with Caucasians in both type 2 diabetes and population cohorts (40,41). The current report confirms these earlier observations and demonstrates that Indian-Asian ethnicity is independently associated with the development of both albuminuria and renal insufficiency in type 2 diabetes.

Previous prospective studies have identified several other risk factors for incident albuminuria in type 2 diabetes, including age, male sex, duration of diabetes, smoking, obesity, and the presence of retinopathy (16,19,20,35,42,43). Although confirming the multifactorial nature of the risk profile underlying albuminuria, the current analysis has also identified white cell count as an independent risk factor for microalbuminuria, though not for macroalbuminuria. This finding raises the possibility that subclinical inflammation may contribute to incident albuminuria. Indeed, both type 2 diabetes and atherosclerotic vascular disease have been characterized as states of chronic low-grade inflammation, as manifested by increased serum concentrations of inflammatory biomarkers, including leukocytes (44,45). Whereas white cell count has been prospectively associated with incident CVD (46), its relevance to microvascular disease in type 2 diabetes has received limited attention to date. Three studies have reported a cross-sectional association between peripheral leukocyte count and urinary albumin excretion in type 2 diabetes (47–49). The current report demonstrates additionally an independent prospective relationship between baseline white cell count and incident microalbuminuria. Although the biological mechanisms underlying this association remain to be elucidated, it should be noted that activated leukocytes secrete a variety of potentially nephrotoxic cytokines and can promote oxidative stress (47, 48).

The most highly associated risk factors for creatinine clearance ≤60 ml/min per 1.73 m^2 were plasma creatinine, systolic blood pressure, age, female sex, height, and decreased waist circumference. Both serum creatinine and blood pressure have been associated independently with the development of renal impairment in previous

studies (32,39). Although both were risk factors for albuminuria and renal impairment in the current analysis, as were Indian-Asian ethnicity and urinary albumin, the associations with sex and waist circumference highlight the substantial differences between the respective risk factor profiles for albuminuria and renal insufficiency. Indeed, sex and waist circumference exhibited paradoxical associations with these outcomes, with male sex and increased central obesity linked to albuminuria and with female sex and decreased waist circumference associated with renal impairment. Although the basis for these differences is not clear, the discordance between their respective risk factor profiles may reflect pathophysiological differences between albuminuria and renal impairment. In genetic analysis of UKPDS participants, a single polymorphism at the paraoxonase-2 gene locus is paradoxically associated with increased risk of albuminuria but decreased likelihood of renal insufficiency (50). These findings also support the notion of discordance between these two outcomes.

Strengths of this study include the prospective design, which ensured that measurement of risk factors preceded the development of albuminuria and renal impairment; the recruitment of patients at diagnosis of type 2 diabetes, such that the risk factors identified may reflect early events in the pathophysiology of renal disease; and the large study population and long follow-up, which provided sufficient power to evaluate numerous risk factors. In addition, the requirement of two consecutive abnormal tests when defining outcomes helped improve the specificity. Similarly, the evaluation of two albuminuria outcomes and two renal impairment outcomes of differing severity supports the robustness of the identification of the risk factors because those factors implicated in both relevant analyses have been identified. There were, however, relatively few macroalbuminuria or doubling of creatinine events and insufficient power to examine possible effects of randomized therapy.

A potential limitation of the current study is the lack of ability to account for possible regression of albuminuria over time. A second limitation is that the use of ACE inhibitors was not addressed specifically in this analysis. ACE inhibition has been associated with a decreased incidence of microalbuminuria in patients with type 2 diabetes and hypertension (51). However, in the UKPDS blood pressure control study, there was no difference in the efficacy of captopril and atenolol in reducing the incidence of diabetes complications, including albuminuria (52).

In conclusion, by a median of 15 years from diagnosis of type 2 diabetes, nearly 40% of UKPDS patients developed albuminuria and nearly 30% developed renal impairment. Many patients developed one of these outcomes but not the other. Whereas systolic blood pressure, Indian-Asian ethnicity, urinary albumin excretion, and plasma creatinine were risk factors for both albuminuria and renal impairment, other risk factors for these two outcomes were distinct. These findings are consistent with the concept that albuminuria and renal impairment are not inexorably linked in type 2 diabetes.

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REFERENCES

1. Ritz E, Orth S: Nephropathy in patients with type 2 diabetes mellitus. *N Engl J Med* 341:1127–1133, 1999
2. Caramori ML, Fioretto P, Mauer M: The need for early predictors of diabetic nephropathy risk: is albumin excretion rate sufficient? *Diabetes* 49:1399–1408, 2000
3. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR: Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). *Kidney Int* 63:225–232, 2003
4. UK Prospective Diabetes Study Group: UK Prospective Diabetes Study VIII: study design, progress and performance. *Diabetologia* 34:877–890, 1991
5. UK Prospective Diabetes Study Group: UK Prospective Diabetes Study. XI. Biochemical risk factors in type 2 diabetic patients at diagnosis compared with age-matched normal subjects. *Diabet Med* 11:534–544, 1994
6. UKPDS Group: Intensive blood glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837–853, 1998
7. UKPDS Group: UK Prospective Diabetes Study. IX. Relationships of urinary albumin and N-acetylglucosaminidase to glycaemia and hypertension at diagnosis of type 2 (non-insulin-dependent) diabetes mellitus and after 3 months diet therapy. *Diabetologia* 36:835–842, 1992
8. Manley SE, Burton ME, Fisher KE, Cull CA, Turner RC: Decreases in albumin/creatinine and N-acetylglucosaminidase/creatinine ratios in urine samples stored at -20°C. *Clin Chem* 38:2294–2299, 1992
9. Cull CA, Manley SE, Stratton IM, Holman RR, Turner RC, Matthews DR: Approach to maintaining comparability of biochemical data during long-term clinical trials. *Clin Chem* 43:1913–1918, 1997
10. Levy J, Matthews D, Hermans M: Correct homeostasis model assessment (HOMA) evaluation uses the computer program. *Diabetes Care* 21:2191–2192, 1998
11. Cockroft D, Gault M: Prediction of creatinine clearance from serum creatinine. *Nephron* 16:31–41, 1976
12. Kohner EM, Aldington SJ, Stratton IM, Manley SE, Holman RR, Matthews DR, Turner RC: United Kingdom Prospective Diabetes Study, 30: diabetic retinopathy at diagnosis of non-insulin-dependent diabetes mellitus and associated risk factors. *Arch Ophthalmol* 116:297–303, 1998
13. Rose GA, Blackburn H, Gillum RF, Prineas RJ: *Cardiovascular Survey Methods*. Geneva, World Health Organization, 1982
14. Valmadrid CT, Klein R, Moss SE, Klein BE, Cruickshanks KJ: Alcohol intake and the risk of coronary heart disease mortality in persons with older-onset diabetes mellitus. *JAMA* 282:239–246, 1999
15. Nosadini R, Velussi M, Brocco E, Bruseghin M, Abaterusso C, Saller A, Dalla Vestra M, Carraro A, Bortoloso E, Sambataro M, Barzon I, Frigato F, Muollo B, Chiesura-Corona M, Pacini G, Baggio B, Piarulli F, Sfriso A, Fioretto P: Course of renal function in type 2 diabetic patients with abnormalities of albumin excretion rate. *Diabetes* 49:476–484, 2000
16. Tabaei B, Al-Kassab A, Ilag L, Zawacki C, Herman W: Does microalbuminuria predict nephropathy? *Diabetes Care* 24:1560–1576, 2001
17. Fioretto P, Mauer M, Brocco E, Velussi M, Frigato F, Muollo B, Sambataro M, Abaterusso C, Baggio B, Crepaldi G, Nosadini R: Patterns of renal injury

in NIDDM patients with microalbuminuria. *Diabetologia* 39:1569–1576, 1996
18. Kramer H, Nguyen Q, Curham G, Hsu C: Renal insufficiency in the absence of albuminuria and retinopathy among adults with type 2 diabetes mellitus. *JAMA* 289:3273–3277, 2003
19. 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* 159:998–1004, 1998
20. Park JY, Kim HK, Chung YE, Kim SW, Hong SK, Lee KU: Incidence and determinants of microalbuminuria in Koreans with type 2 diabetes. *Diabetes Care* 21:530–534, 1998
21. Bakris G, Williams M, Dworkin L, Elliott W, Epstein M, Toto R, Tuttle K, Douglas J, Hsueh W, Sowers J: Preserving renal function in adults with hypertension and diabetes: a consensus approach: National Kidney Foundation Hypertension and Diabetes Executive Committees Working Group. *Am J Kidney Dis* 36:530–534, 2000
22. Schrier RW, Estacio RO, Esler A, Mehler P: Effects of aggressive blood pressure control in normotensive type 2 diabetic patients on albuminuria, retinopathy and strokes. *Kidney Int* 61:1086–1097, 2002
23. UK Prospective Diabetes Study Group: Tight blood pressure control and risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 38. *BMJ* 317:703–713, 1998
24. Chen H, Guh J, Chang J, Hsieh M, Shin S, Lai Y: Role of lipid control in diabetic nephropathy. *Kidney Int* 67:S60–S62, 2005
25. Tozawa M, Iseki K, Iseki C, Oshiro S, Ikemiya Y, Takishita S: Triglyceride, but not total cholesterol or low-density lipoprotein cholesterol levels, predict development of proteinuria. *Kidney Int* 62:1743–1749, 2002
26. Muntner P, Coresh J, Smith J, Eckfeldt J, Klag M: Plasma lipids and risk of developing renal dysfunction: the Atherosclecrosis Risk in Communities Study. *Kidney Int* 58:293–301, 2000
27. Fox C, Larson M, Leip E, Culleton B, Wilson P, Levy D: Predictors of new-onset kidney disease in a community-based population. *J Am Med Assoc* 291:844–850, 2004
28. Hovind P, Tarnow L, Rossing P, Jensen B, Graae M, Torp I, Binder C, Parving H: Predictors for the development of microalbuminuria and macroalbuminuria in patients with type 1 diabetes: inception cohort study. *BMJ* 328:1105–1109, 2004
29. Coonrod B, Ellis D, Becker D, Bunker C, Kelsey S, Lloyd C, Drash A, Kuller L, Orchard T: Predictors of microalbuminuria in individuals with IDDM: Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes Care* 16:1376–1383, 1993
30. Smulders Y, Rakic M, Stehouwer C, Weijers R, Slaats E, Silberbusch J: Determinants of progression of microalbuminuria in patients with NIDDM: a prospective study. *Diabetes Care* 20:999–1005, 1997
31. Cusick M, Chew EY, Hoogwerf B, Agron E, Wu L, Lindley A, Ferris FL 3rd, the Early Treatment Diabetic Retinopathy Study Research Group: Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), ETDRS26. *Kidney Int* 66:1173–1179, 2004
32. Bruno G, Biggeri A, Merletti F: Low incidence of end-stage renal disease and chronic renal failure in type 2 diabetes: a 10-year prospective study. *Diabetes Care* 26:2353–2358, 2003
33. Ansquer JC, Foucher C, Rattier S, Taskinen MR, Steiner G, DAIS Investigators: Fenofibrate reduces progression to microalbuminuria over 3 years in a placebo-controlled study in type 2 diabetes: results from the Diabetes Atherosclerosis and risk factors Study (DAIS). *Am J Kidney Dis* 45:485–493, 2005
34. Keech A, Simes RJ, Barter P, Best J, Scott R, Taskinen MR, Forder P, Pillai A, Davis T, Glasziou P, Drury P, Kesaniemi YA, Sullivan D, Hunt D, Colman P, d'Emden M, Whiting M, Ehnholm C, Laakso M, FIELD Study Investigators: Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. *Lancet* 366:1849–1861, 2005
35. Gall M, Hougaard P, Borch-Johnsen K, Parving H: Risk factors for development of incipient and overt diabetic nephropathy in patients with non-insulin dependent diabetes mellitus: prospective, observational study. *BMJ* 314:783–788, 1997
36. Murussi M, Baglio P, Gross JL, Silveiro SP: Risk factors for microalbuminuria and macroalbuminuria in type 2 diabetic patients: a 9-year follow-up study. *Diabetes Care* 25:1101–1103, 2002
37. Rossing K, Christensen PK, Hovind P, Tarnow L, Rossing P, Parving HH: Progression of nephropathy in type 2 diabetic patients. *Kidney Int* 66:1596–1605, 2004
38. Keane WF, Brenner BM, de Zeeuw D, Grunfeld JP, McGill J, Mitch WE, Ribeiro AB, Shahinfar S, Simpson RL, Snapinn SM, Toto R, the RENAAL Study Investigators: The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: the RENAAL Study. *Kidney Int* 63:1499–1507, 2003
39. Burden AC, McNally PG, Feehally J, Walls J: Increased incidence of end-stage renal failure secondary to diabetes mellitus in Asian ethnic groups in the United Kingdom. *Diabet Med* 9:641–645, 1992
40. Mather HM, Chaturvedi N, Kehely AM: Comparison of prevalence and risk factors for microalbuminuria in South Asians and Europeans with type 2 diabetes mellitus. *Diabet Med* 15:672–677, 1998
41. Fischbacher C, Bhopal R, Rutter M, Unwin N, Marshall S, White M, Alberti K: Microalbuminuria is more frequent in South Asian than in European origin populations: a comparative study in Newcastle, UK. *Diabet Med* 20:31–36, 2003
42. Forsblom C, Sane T, Groop P-H, Saloranta C, Ekstrand A, Groop L, Totterman K: Predictors of progression from normoalbuminuria to microalbuminuria in NIDDM. *Diabetes Care* 21:1932–1938, 1998
43. 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* 67:258–266, 2005
44. Pickup J, Crook M: Is type 2 DM a disease of the innate immune system? *Diabetologia* 41:1241–1248, 1998
45. Libby P, Ridker P, Marseri A: Inflammation and atherosclerosis. *Circulation* 105:1135–1143, 2002
46. Brown D, Giles W, Croft J: White blood cell count: an independent predictor of coronary heart disease mortality among a national cohort. *J Clin Epidemiol* 54:316–322, 2001
47. Tong P, Lee K, So W: White blood cell count is associated with macro- and microvascular complications in Chinese patients with type 2 diabetes. *Diabetes Care* 27:216–222, 2004
48. Chung F, Tsai J, Chang D, Shin S, Lee Y: Peripheral total and differential leukocyte count in diabetic nephropathy: the relationship of plasma leptin to leukocytosis. *Diabetes Care* 28:1710–1717, 2005
49. Cavalot F, Massucco P, Perna P, Traversa M, Anfossi G, Trovati M: White blood cell count is positively correlated with albumin excretion rate in subjects with type 2 diabetes. *Diabetes Care* 12:2354–2355, 2002
50. Calle R, McCarthy M, Banerjee P, Zeggini E, Cull C, Thorne K, Wiltshire S, Terra S, Meyer D, Richmond J, Mancuso J, Milos P, Fryburg D, Holman R: Effect of paraoxonase 2 (PON2) polymorphisms on development and progression of diabetic nephropathy in UKPDS patients [article online], 2005. Available from http://scientificsessions.diabetes.org/Abstracts/
51. Ruggenenti P, Fassi A, Ilieva AP, Bruno S, Iliev IP, Brusegan V, Rubis N, Gherardi G, Arnoldi F, Ganeva M, Ene-Iordache B, Gaspari F, Perna A, Bossi A, Trevisan R, Dodesini AR, Remuzzi G; Bergamo Nephrologic Diabetes Complications Trial (BENEDICT) Investigators: Preventing microalbuminuria in type 2 diabetes. *N Engl J Med* 351:1941–1951, 2004
52. UK Prospective Diabetes Study Group: Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 39. *BMJ* 317:713–720, 1998