from these registries and cohort, it is difficult to clearly distinguish between diabetic nephropathy and primary kidney disease in a general clinical setting. This is a limitation of these studies. Moreover, the JRBR and JKDR had no follow-up data. In contrast to these two cross-sectional registries, the JDNCS is a prospective cohort study to evaluate cardiovascular events and progression of kidney dysfunction. Future analysis of data from these two registries and one cohort will provide valuable clinical and pathological information of type 2 diabetes in Japan.

In conclusion, there are few national registries of diabetic nephropathy to evaluate prognosis in Japan. Future analysis of prospective cohort studies, such as the JDNCS, will provide clinical information on epidemiology, and renal and cardiovascular outcomes of type 2 diabetic patients in Japan.

Acknowledgments This study was supported in part by a Grant-in-Aid for Progressive Renal Diseases Research, Research on Rare and Intractable Disease, and a Grant-in-Aid for Diabetic Nephropathy and Nephrosclerosis Research, from the Ministry of Health, Labour and Welfare of Japan.

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

References

1. Wada T, Shimizu M, Toyama T, Hara A, Kaneko S, Furuichi K. Clinical impact of albuminuria in diabetic nephropathy. Clin Exp Nephrol. 2012;16:96–101.
2. Wada J, Makino H. Historical chronology of basic and clinical research in diabetic nephropathy and contributions of Japanese scientists. Clin Exp Nephrol. 2009;13:405–14.
3. Sugiyama H, Yokoyama H, Sato H, Saito T, Kohda Y, Nishi S, Tsuruya K, Kiyomoto H, Iida H, Sasaki T, Higuchi M, Hattori M, Oka K, Kagami S, Nagata M, Kawamura T, Honda M, Fukasawa Y, Fukatsu A, Morozumi K, Yoshikawa N, Yuzawa Y, Matsuo S, Kiyohara Y, Joh K, Taguchi T, Makino H. Japan Renal Biopsy Registry: the first nationwide, web-based, and prospective registry system of renal biopsies in Japan. Clin Exp Nephrol. 2011;15:493–503.
4. Matsuo S, Imai E, Horio M, Yasuda Y, Tomita K, Nitta K, Yamagata K, Tomino Y, Yokoyama H, Hishida A. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis. 2009;53:982–92.
5. Ninomiya T, Perkovic V, de Galan BE, Zoungas S, Pillai A, Jardine M, Patel A, Cass A, Neal B, Poulter N, Mogensen CE, Cooper M, Marre M, Williams B, Hamet P, Mancia G, Woodward M, Macmahon S, Chalmers J. Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. J Am Soc Nephrol. 2009;20:1813–21.
6. Bruno G, Merletti F, Bargero G, Novelli G, Melis D, Soddu A, Perotto M, Pagano G, Cavallo-Perin P. Estimated glomerular filtration rate, albuminuria and mortality in type 2 diabetes: the Casale Monferrato study. Diabetologia. 2007;50:941–8.
7. So WY, Kong AP, Ma RC, Ozaki R, Szeto CC, Chan NN, Ng V, Ho CS, Lam CW, Chow CC, Cockram CS, Chan JC, Tong PC. Glomerular filtration rate, cardiorenal end points, and all-cause mortality in type 2 diabetic patients. Diabetes Care. 2006;29:2046–52.
8. Nakai S, Masakane I, Shigematsu T, Hamano T, Yamagata K, Watanabe Y, Itami N, Ogata S, Kimata N, Shinoda T, Syouji T, Suzuki K, Taniguchi M, Tsuchida K, Nakamoto H, Nishi S, Nishi H, Hashimoto S, Hasegawa T, Hanafusa N, Fujii N, Marubayashi S, Morita O, Wakai K, Wada A, Iseki K, Tsubakihara Y. An overview of regular dialysis treatment in Japan (as of 31 December 2007). Ther Apher Dial. 2009;13:457–504.
9. Yokoyama H, Sone H, Oishi M, Kawai K, Fukumoto Y, Kobayashi M. 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;24:1212–9.
10. Caramori ML, Fioretto P, Mauer M. Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. Diabetes. 2003;52:1036–40.

Long-term Outcomes of Japanese Type 2 Diabetic Patients With Biopsy-Proven Diabetic Nephropathy

MIHO SHIMIZU, MD, PHD[1,2]
KENGO FURUICHI, MD, PHD[1,2]
TADASHI TOYAMA, MD[1,2]
SHINJI KITAJIMA, MD[1,2]
AKINORI HARA, MD, PHD[1,2]
KIYOKI KITAGAWA, MD, PHD[1,2]
YASUNORI IWATA, MD, PHD[1,2]
NORIHIKO SAKAI, MD, PHD[1,2]
TOSHINARI TAKAMURA, MD, PHD[2]
MITSUHIRO YOSHIMURA, MD, PHD[3]
HITOSHI YOKOYAMA, MD, PHD[4]
SHUICHI KANEKO, MD, PHD[2]
TAKASHI WADA, MD, PHD[1,5]
AND THE KANAZAWA STUDY GROUP FOR RENAL DISEASES AND HYPERTENSION

OBJECTIVE—We evaluated the structural-functional relationships and the prognostic factors for renal events, cardiovascular events, and all-cause mortality in type 2 diabetic patients with biopsy-proven diabetic nephropathy.

RESEARCH DESIGN AND METHODS—Japanese type 2 diabetic patients with biopsy-proven diabetic nephropathy (n = 260) were enrolled. Patients were stratified by albuminuria (proteinuria) and estimated glomerular filtration rate (eGFR) at the time of renal biopsy. The outcomes were the first occurrence of renal events (requirement of dialysis or a 50% decline in eGFR from baseline), cardiovascular events (cardiovascular death, nonfatal myocardial infarction, coronary interventions, or nonfatal stroke), and all-cause mortality.

RESULTS—The factors associated with albuminuria (proteinuria) regardless of eGFR were hematuria, diabetic retinopathy, low hemoglobin, and glomerular lesions. The factors associated with low eGFR regardless of albuminuria (proteinuria) were age and diffuse, nodular, tubulointerstitial, and vascular lesions. The glomerular, tubulointerstitial, and vascular lesions in patients with normoalbuminuria (normal proteinuria) and low eGFR were more advanced compared to those in patients with normoalbuminuria (normal proteinuria) and maintained eGFR. In addition, compared to patients with micro-/macroalbuminuria (mild/severe proteinuria) and low eGFR, their tubulointerstitial and vascular lesions were similar or more advanced in contrast to glomerular lesions. The mean follow-up period was 8.1 years. There were 118 renal events, 62 cardiovascular events, and 45 deaths. The pathological determinants were glomerular lesions, interstitial fibrosis and tubular atrophy (IFTA), and arteriosclerosis for renal events, arteriosclerosis for cardiovascular events, and IFTA for all-cause mortality. The major clinical determinant for renal events and all-cause mortality was macroalbuminuria (severe proteinuria).

CONCLUSIONS—Our study suggests that the characteristic pathological lesions as well as macroalbuminuria (severe proteinuria) were closely related to the long-term outcomes of biopsy-proven diabetic nephropathy in type 2 diabetes.

Diabetic nephropathy occurs in 20–40% of patients with diabetes (1). The prevalence of diabetic nephropathy is increasing in proportion to the increase in prevalence of diabetes, and it has been predicted to continue to increase in future (2). Diabetes is a risk factor of cardiovascular disease and death, and diabetic nephropathy further increases these risks (3). In addition, diabetic nephropathy is the leading cause of end-stage renal disease requiring dialysis or transplantation in developed countries (4–6).

In recent years, many clinical studies have suggested strict glycemic control and blood pressure management by use of appropriate medication to suppress the onset and progression of diabetic nephropathy. Thus, it is important to identify patients at risk in the early stages to improve prognosis in patients with diabetic nephropathy (1). Albuminuria and glomerular filtration rate (GFR) are recommended for use as clinical markers of diabetic nephropathy (1,7–9). On the other hand, selection of pathological markers is complicated because a variety of renal lesions can be found in diabetic nephropathy in addition to factors such as obesity, hypertension, dyslipidemia, and aging, which are frequently complicated in type 2 diabetes, causing a wide variety of pathological changes (10).

We previously reported on the clinical factors related to the development and progression of renal lesions in diabetic nephropathy by the evaluation of serial renal biopsies or autopsy (11). In this report, we demonstrated a significant relationship between the progression of diabetic glomerulosclerosis and clinical factors such as the control of blood glucose, type of diabetes, age at onset, type of treatment, and degree of obesity.

After this study, we conducted a long-term retrospective study to evaluate the structural-functional relationships and the predictive impacts of clinicopathological parameters for renal events, cardiovascular events, and all-cause mortality among Japanese patients with biopsy-proven diabetic nephropathy in type 2 diabetes.

RESEARCH DESIGN AND METHODS

—A total of 260 patients who were diagnosed with diabetic nephropathy in type 2 diabetes at Kanazawa University Hospital or Kanazawa Medical Center between 1985 and 2010 were

From the [1]Division of Nephrology, Kanazawa University Hospital, Kanazawa, Japan; and [2]Department of Disease Control and Homeostasis, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan; the [3]Department of Nephrology and Rheumatology, National Hospital Organization Kanazawa Medical Center, Kanazawa, Japan; the [4]Division of Nephrology, Kanazawa Medical University, Uchinada, Japan; and the [5]Department of Laboratory Medicine, Kanazawa University Graduate School of Medical Sciences, Kanazawa, Japan.
Corresponding author: Takashi Wada, twada@m-kanazawa.jp.
Received 5 February 2013 and accepted 28 May 2013.
DOI: 10.2337/dc13-0298
This article contains Supplementary Data online at http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc13-0298/-/DC1.
© 2013 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.

Diabetes Care Publish Ahead of Print, published online October 2, 2013

included in this study. The diagnosis of diabetes was based on the criteria of the Japanese Diabetic Society (12). The diagnosis of diabetic nephropathy was confirmed by histological characteristics, such as glomerular hypertrophy, thickened capillary basement membranes, diffuse mesangial expansion (sclerosis), nodular mesangial sclerosis, exudative lesions such as capsular drop or fibrin cap, mesangiolysis, capillary microaneurysm, or hyalinosis of afferent and efferent arterioles, using appropriate standards for renal biopsy including light microscopy, electron microscopy, and immunofluorescence examination. Patients with other glomerular diseases concomitant with diabetic nephropathy were excluded from this study. Renal biopsy was performed for precise diagnosis of renal lesions with the consent of each patient. The study protocol was approved by the medical ethics committee of Kanazawa University and Kanazawa Medical Center.

Clinical examinations

Age, sex, 24-h urinary albumin excretion, 24-h urinary protein excretion, urine dipstick test results (proteinuria and hematuria), serum creatinine, estimated GFR (eGFR), duration of diabetes, presence of diabetic retinopathy, HbA_{1c}, BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, and hemoglobin were used as baseline clinical parameters at the time of renal biopsy. eGFR for Japanese patients was calculated using the following equation: eGFR (mL/min/1.73 m^2) = 194 × serum creatinine$^{-1.094}$ × age$^{-0.287}$ (if female, ×0.739) (13). HbA_{1c} levels were presented as National Glycohemoglobin Standardization Program values according to the recommendations of the Japanese Diabetic Society (12) and International Federation of Clinical Chemistry values.

Based on the new classification of chronic kidney disease, albuminuria at baseline was categorized as normoalbuminuria (<30 mg/day [category A1]), microalbuminuria (≥30 and <300 mg/day [category A2]), and macroalbuminuria (≥ 300 mg/day [category A3]) (7,8). We classified proteinuria among patients for whom albuminuria was not evaluated as normal proteinuria (<0.15 g/day or urine dipstick negative or trace [category A1]), mild proteinuria (≥0.15 and <0.5 g/day or urine dipstick + [category A2]), and severe proteinuria (≥0.5 g/day or urine dipstick ≥2+ [category A3]) (7,8). When results were inconsistent, we gave priority to 24-h urinary albumin excretion, 24-h urinary protein excretion, and urine dipstick test results—in that order. In addition, eGFR at baseline was categorized as ≥60 mL/min/1.73 m^2 (categories G1–2) and <60 mL/min/1.73 m^2 (categories G3a-5) for categorical analyses comparing risks.

Outcomes

The outcomes for this study were the first occurrence of renal events (requirement of dialysis or a 50% decline in eGFR from baseline), cardiovascular events (cardiovascular death, nonfatal myocardial infarction, coronary interventions, or nonfatal stroke), and all-cause mortality. The patients were followed up until the end of 2011 or death.

Pathological examinations

For light microscopic examination, renal biopsy specimens were fixed in 10% phosphate-buffered formalin (pH 7.2), embedded in paraffin, and sliced into sections 4 μm thick. These specimens were stained with periodic acid Schiff (PAS) reagent, periodic acid silver methenamine, hematoxylin-eosin, and Mallory-Azan and examined by light microscopy. The severity of diffuse lesions of glomeruli was graded on a scale of 0 to 4 according to the description by Gellman et al. (14) as follows: grade 0, all glomeruli appear normal; grade 1, local lesions present within each glomerulus and focal lesions present within the kidney; grade 2, mesangial thickening is diffuse within the glomerulus and generalized throughout the kidney; grade 3, capillary lumina are narrowed and obliterated only locally; and grade 4, the lumen is generally narrowed and the entire glomerulus is ischemic and appears to be hyalinized (14–17) (Supplementary Fig. 1*A–D*). Nodular lesions, exudative lesions, and mesangiolysis were simply shown as their presence or absence in each specimen (15–17) (Supplementary Fig. 1*E–G*). The severity of interstitial fibrosis and tubular atrophy (IFTA) and interstitial inflammation was scored according to the description by Tervaert et al. (18). The severity of IFTA was evaluated and graded on a scale from 0 to 3: grade 0, no IFTA; grade 1, <25%; grade 2, 25–50%; and grade 3, >50% (18). The severity of interstitial inflammation was evaluated and graded on a scale from 0 to 2: grade 0, absent; grade 1, infiltration only in relation to IFTA; and grade 2, infiltration in areas without IFTA (18). The severity of arteriolar hyalinosis was evaluated and graded on a scale from 0 to 3 according to the description by Takazakura et al. (11) as follows: grade 0, normal appearance without PAS-positive deposit; grade 1, a light PAS-positive thickening is observed but at less than half the circumference of the arteriole in many arterioles; grade 2, most vessel walls are moderately thickened with PAS-positive deposition without apparent luminal narrowing; and grade 3, a heavy thickening of the majority of the vessel walls is seen with luminal narrowing or obliteration (Supplementary Fig. 1*H–J*). The severity of arteriosclerosis was evaluated and graded on a scale from 0 to 2 according to the description by Tervaert et al. (18) as follows: grade 0, no intimal thickening; grade 1, intimal thickening less than thickness of media; and grade 2, intimal thickening greater than thickness of media (Supplementary Fig. 1*K* and *L*). Renal tissue specimens were examined by four nephrologists.

Statistical analysis

Data are expressed as means ± SD. Comparisons of continuous variables among groups were performed using the Mann-Whitney *U* test for nonparametric data. Comparisons of categorical variables among groups were performed using χ^2 test. The survival curves were obtained using the Kaplan-Meier method and compared by log-rank test. The influence of different categories of albuminuria (proteinuria) and eGFR on each outcome was evaluated with the use of the Cox proportional hazards model after adjustment for age and sex. The results are presented as hazard ratios (HRs) and 95% CI. Patients with normoalbuminuria (normal proteinuria) and eGFR ≥60 mL/min/1.73 m^2 were served as the reference group in the analyses. A multivariate Cox proportional hazard regression model was used to select factors that significantly affected the incidence of each outcome and to estimate the risks. The following variables were incorporated as covariates: age, sex, microalbuminuria (mild proteinuria), macroalbuminuria (severe proteinuria), eGFR, duration of diabetes, presence of diabetic retinopathy, HbA_{1c}, BMI, systolic blood pressure, total cholesterol, and hemoglobin as clinical covariates or diffuse lesions, nodular lesions, exudative lesions, mesangiolysis, IFTA, interstitial inflammation, arteriolar hyalinosis, and arteriosclerosis as pathological covariates. All analyses were carried out using SPSS, version 19 (SPSS, Tokyo, Japan). Two-sided $P < 0.05$

was considered indicative of statistical significance.

RESULTS

Baseline characteristics

The baseline characteristics of 260 patients are shown in Table 1. In the clinical parameters, the mean age was 58.2 years, and 63.1% of the patients were male. Among the 95 patients for whom daily urinary albumin excretion measurements were available, 10 (10.5%) showed normoalbuminuria (A1), 31 (32.6%) showed microalbuminuria (A2), and 54 (56.8%) showed macroalbuminuria (A3). Among the 231 patients for whom daily urinary protein excretion measurements were available, 31 (13.4%) showed normal proteinuria (A1), 44 (19.0%) showed mild proteinuria (A2), and 156 (67.5%) showed severe proteinuria (A3). Among the 256 patients for whom urinary dipstick protein test results were available, 53 (20.7%) showed negative (A1), 19 (7.4%) showed trace (A1), 42 (16.4%) showed + (A2), 63 (26.4%) showed 2+ (A3), and 79 (30.9%) showed ≥3+ (A3). The mean serum creatinine was 1.4 mg/dL, and the mean eGFR was 58.0 mL/min/1.73 m^2. The proportions with eGFR ≥90 (G1), 60–89 (G2), 45–59 (G3a), 30–44 (G3b), 15–29 (G4), and <15 (G5) mL/min/1.73 m^2 were 15.0%, 25.8%, 21.9%, 18.5%, 12.7%, and 6.2%, respectively.

The proportions of patients stratified by albuminuria (proteinuria) and eGFR categories are demonstrated in Supplementary Table 1. The proportions of patients with normoalbuminuria (normal proteinuria), microalbuminuria (mild proteinuria), and macroalbuminuria (severe proteinuria) were 16.5% (43 of 260), 21.2% (55 of 260), and 62.3% (162 of 260), respectively. The proportions of patients with eGFR ≥60 and <60 mL/min/1.73 m^2 were 40.8% (106 of 260) and 59.2% (154 of 260), respectively. The proportions of patients with normoalbuminuria (normal proteinuria), microalbuminuria (mild proteinuria), and macroalbuminuria (severe proteinuria) among those with eGFR ≥60 mL/min/1.73 m^2 were 26.4% (28 of 106), 29.2% (31 of 106), and 44.3% (47 of 106), respectively. The proportions of patients with normoalbuminuria (normal proteinuria), microalbuminuria (mild proteinuria), and macroalbuminuria (severe proteinuria) among those with eGFR <60 mL/min/1.73 m^2 were 9.7% (15 of 154), 15.6% (24 of 154), and 74.7% (115 of 154), respectively.

Table 1—*Clinical characteristics of patients at the time of renal biopsy* (n = 260)

Age (years)	58.2 ± 11.4
Male	164 (63.1)
Kidney-related parameters	
Urine albumin category (mg/day), *n* = 95	
Normoalbuminuria (<30)	10 (10.5)
Microalbuminuria (30–299)	31 (32.6)
Macroalbuminuria (≥300)	54 (56.8)
Urine protein category (g/day), *n* = 231	
Normal proteinuria (<0.15)	31 (13.4)
Mild proteinuria (0.15–0.49)	44 (19.0)
Severe proteinuria (≥0.5)	156 (67.5)
Dipstick test results, *n* = 256	
–	53 (20.7)
±	19 (7.4)
+	42 (16.4)
2+	63 (24.6)
≥3+	79 (30.9)
Serum creatinine (mg/dL)	1.4 ± 1.3
eGFR (mL/min/1.73 m^2)	58.0 ± 31.7
≥90	39 (15.0)
60–89	67 (25.8)
45–59	57 (21.9)
30–44	48 (18.5)
<30	49 (18.8)
Hematuria (%)	39.1
Diabetes parameters	
Diabetes duration (years)	11.2 ± 8.1
Diabetic retinopathy (%)	79.5
HbA_{1c} (%)	8.2 ± 2.3
HbA_{1c} (mmol/mol)	61.6 ± 24.7
Other major risk factors	
BMI (kg/m^2)	23.2 ± 3.7
Systolic blood pressure (mmHg)	142.1 ± 21.4
Diastolic blood pressure (mmHg)	76.8 ± 12.0
Total cholesterol (mg/dL)	218.9 ± 83.2
Hemoglobin (g/dL)	12.1 ± 2.4

Data are means ± SD or *n* (%).

Clinical and pathological features associated with albuminuria (proteinuria) and low eGFR

The baseline clinical and pathological features were compared among subgroups stratified by albuminuria (proteinuria) and eGFR categories (Table 2). Clinical and pathological factors associated with micro-/macroalbuminuria (mild/severe proteinuria) regardless of eGFR categories were hematuria, diabetic retinopathy, low hemoglobin, and glomerular lesions. On the other hand, clinical and pathological factors associated with low eGFR regardless of albuminuria (proteinuria) categories were age, diffuse lesions, nodular lesions, tubulointerstitial lesions, and vascular lesions. Glomerular lesions in patients with normoalbuminuria (normal proteinuria) were less advanced for both eGFR ≥60 and eGFR <60 mL/min/1.73 m^2 categories. On the other hand, as to tubulointerstitial and vascular lesions in patients with normoalbuminuria (normal proteinuria), there were different trends between eGFR ≥60 and eGFR <60 mL/min/1.73 m^2 categories. In the eGFR ≥60 ml/min/1.73 m^2 category, tubulointerstitial and vascular lesions in patients with normoalbuminuria (normal proteinuria) were less advanced compared with those in patients with micro-/macroalbuminuria (mild/severe proteinuria). However, in the eGFR <60 ml/min/1.73 m^2 category, tubulointerstitial and vascular lesions in patients with normoalbuminuria (normal proteinuria) were similar or more advanced compared with those in patients with micro-/macroalbuminuria (mild/severe

Table 2—*Baseline clinical and pathological features of patients stratified by albuminuria (proteinuria) and eGFR categories*

	Normoalbuminuria (normal proteinuria)		Micro-/macroalbuminuria (mild/severe proteinuria)		*P* for normo (normal) vs. micro (mild)/macro (severe)	
	eGFR ≥60 mL/min/1.73 m^2	eGFR <60 mL/min/1.73 m^2	eGFR ≥60 mL/min/1.73 m^2	eGFR <60 mL/min/1.73 m^2	eGFR ≥60 mL/min/1.73 m^2	eGFR <60 mL/min/1.73 m^2
n	28	15	78	139		
Clinical parameters						
Age (years)	48.8 ± 13.8	62.5 ± 6.2**	53.8 ± 10.8	62.1 ± 9.6††	0.10	0.74
Male	53.6	46.7	65.4	65.5	0.27	0.15
Serum creatinine (mg/dL)	0.7 ± 0.1	1.2 ± 0.4**	0.7 ± 0.2	1.9 ± 1.5††	0.95	<0.01
eGFR (mL/min/1.73 m^2)	86.0 ± 16.4	46.0 ± 10.6**	89.0 ± 26.4	36.4 ± 15.5††	0.81	<0.05
Hematuria	4.2	7.7	32.8	51.9†	<0.01	<0.01
Diabetes duration (years)	8.0 ± 7.4	7.4 ± 6.4	9.6 ± 6.6	13.1 ± 8.8††	0.21	<0.05
Diabetic retinopathy	41.7	50.0	81.7	87.9	<0.01	<0.01
HbA_{1c} (%)	8.5 ± 2.4	8.3 ± 2.2	8.4 ± 2.5	7.4 ± 2.0††	0.53	0.35
HbA_{1c} (mmol/mol)	65.0 ± 26.2	64.1 ± 25.1	68.8 ± 27.1	57.1 ± 22.3††	0.53	0.35
BMI (kg/m^2)	23.5 ± 2.2	22.2 ± 2.2	22.9 ± 4.8	23.5 ± 3.4†	0.07	0.34
Systolic blood pressure (mmHg)	132.0 ± 17.1	129.3 ± 14.3	138.8 ± 22.1	146.9 ± 21.0††	0.31	<0.01
Diastolic blood pressure (mmHg)	75.0 ± 11.2	75.6 ± 10.0	77.6 ± 12.0	76.8 ± 12.4	0.26	0.91
Total cholesterol (mg/dL)	192.7 ± 37.4	196.6 ± 51.5	215.0 ± 54.4	227.6 ± 100.6	0.07	0.31
Hemoglobin (g/dL)	14.2 ± 1.6	13.0 ± 1.8	13.2 ± 2.1	11.1 ± 2.2††	<0.05	<0.01
Pathological parameters						
Diffuse lesion (0–4)	0.9 ± 0.6	1.5 ± 0.9*	2.0 ± 0.9	2.4 ± 0.8†	<0.01	<0.01
Nodular lesion	0.0	20.0*	44.7	65.1††	<0.01	<0.01
Exudative lesion	0.0	6.7	25.0	44.1††	<0.01	<0.01
Mesangiolysis	0.0	0.0	29.6	30.2	<0.01	<0.05
IFTA (0–3)	1.1 ± 1.0	2.3 ± 0.7**	1.6 ± 0.9	2.1 ± 0.9††	<0.05	0.41
Interstitial inflammation (0–2)	0.9 ± 0.8	1.5 ± 0.5**	0.9 ± 0.5	1.2 ± 0.5††	0.61	<0.05
Arteriolar hyalinosis (0–3)	1.4 ± 1.1	2.4 ± 0.8**	1.8 ± 1.0	2.2 ± 0.8††	0.07	0.33
Arteriosclerosis (0–2)	0.7 ± 0.6	1.9 ± 0.4**	1.2 ± 0.7	1.5 ± 0.5††	<0.01	<0.05

Data are means ± SD or % unless otherwise indicated. Differences among albuminuria (proteinuria) and eGFR categories are compared by Mann-Whitney *U* test for continuous variables and χ^2 test for categorical variables. micro, microalbuminuria; macro, macroalbuminuria; normo, normoalbuminuria. **$P < 0.01$ vs. normoalbuminuria (normal proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group. ††$P < 0.01$ vs. micro-/macroalbuminuria (mild/severe proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group. *$P < 0.05$ vs. normoalbuminuria (normal proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group. †$P < 0.05$ vs. micro-/macroalbuminuria (mild/severe proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group.

proteinuria) in contrast to glomerular lesions (Supplementary Fig. 2*A–C*).

Prognosis of renal events, cardiovascular events, and all-cause mortality

Follow-up data were available for renal events in 229 patients and for cardiovascular events and all-cause mortality in 233 patients. The mean duration of follow-up was 8.1 years (range 5–9,739 days) during 1985–2011. There were a total of 118 renal events, 62 cardiovascular events, and 45 deaths (Supplementary Table 2). Event-free rate of renal events in patients with macroalbuminuria (severe proteinuria) was significantly lower than in those with normoalbuminuria (normal proteinuria) or microalbuminuria (mild proteinuria) for both eGFR ≥60 and <60 mL/min/1.73 m^2 categories (vs. normoalbuminuria [normal proteinuria] and eGFR ≥60 mL/min/1.73 m^2, $P < 0.01$; vs. microalbuminuria [mild proteinuria] and eGFR ≥60 mL/min/1.73 m^2, $P < 0.01$; vs. normoalbuminuria [normal proteinuria] and eGFR <60 mL/min/1.73 m^2, $P < 0.01$; and vs. microalbuminuria [mild proteinuria] and eGFR <60 mL/min/1.73 m^2, $P < 0.01$) (Fig. 1*A* and *B*). Event-free rate of cardiovascular events showed no significant differences between albuminuria (proteinuria) categories for both eGFR ≥60 and <60 mL/min/1.73 m^2 categories. Event-free rate of all-cause mortality in patients with macroalbuminuria (severe proteinuria) was significantly lower than in those with normoalbuminuria (normal proteinuria) or microalbuminuria (mild proteinuria) in the eGFR <60 mL/min/1.73 m^2 category (vs. normoalbuminuria [normal proteinuria] and eGFR <60 mL/min/1.73 m^2, $P < 0.05$; vs. microalbuminuria [mild proteinuria] and eGFR <60 mL/min/1.73 m^2, $P < 0.01$) (Fig. 1*C* and *D*). Event-free rates of renal events, cardiovascular events, and all-cause mortality in patients with eGFR <60 mL/min/1.73 m^2 were significantly lower than in those with eGFR ≥60 mL/min/1.73 m^2 only among patients with macroalbuminuria (severe proteinuria) (renal events $P < 0.01$, cardiovascular events $P < 0.05$, all-cause mortality $P < 0.01$).

Risks of renal events, cardiovascular events, and all-cause mortality stratified by albuminuria (proteinuria) and eGFR categories

HRs of renal events, cardiovascular events, and all-cause mortality were calculated in subgroups of patients stratified

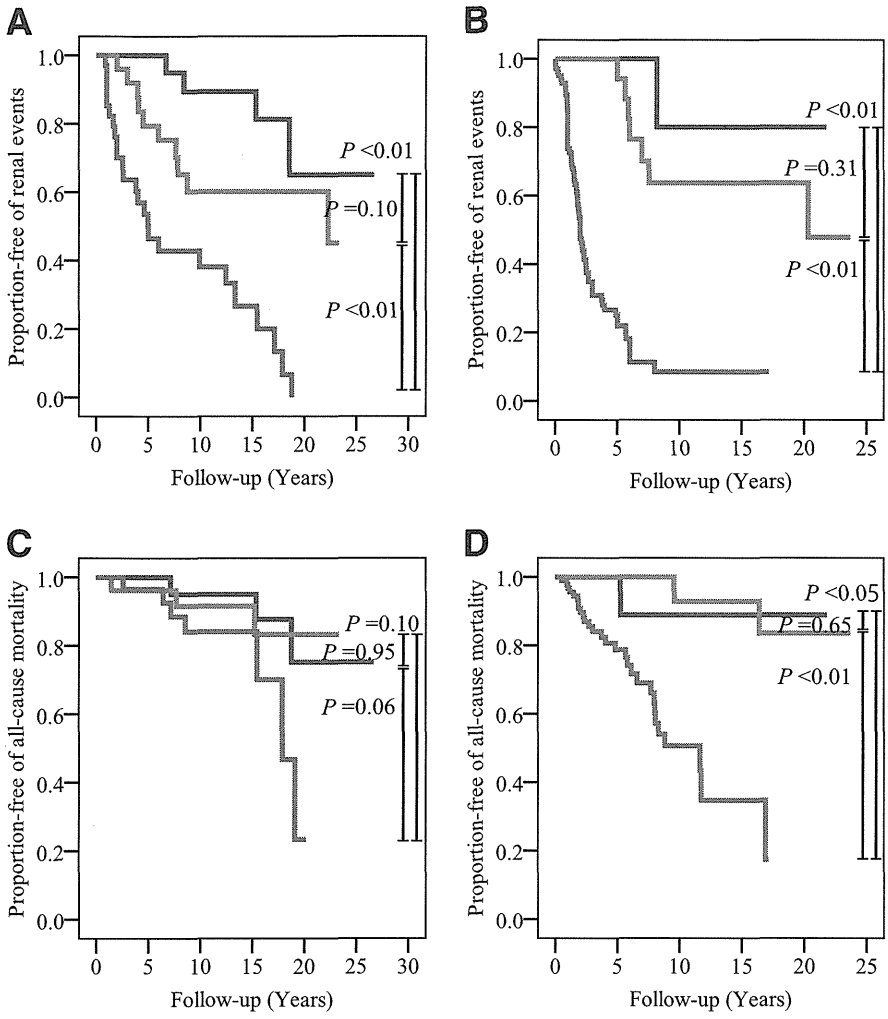

Figure 1—*Event-free rate stratified by albuminuria (proteinuria) and eGFR categories.* A: *Event-free rate of renal events stratified by albuminuria (proteinuria) in the eGFR ≥60 mL/min/1.73 m^2 category according to the Kaplan-Meier method. Blue line, normoalbuminuria (normal proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group* (n = 24)*; green line, microalbuminuria (mild proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group* (n = 27)*; red line, macroalbuminuria (severe proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group* (n = 37)*. Differences between groups were compared by a log-rank test.* B: *Event-free rate of renal events stratified by albuminuria (proteinuria) in the eGFR <60 mL/min/1.73 m^2 category according to the Kaplan-Meier method. Blue line, normoalbuminuria (normal proteinuria) and eGFR <60 mL/min/1.73 m^2 group* (n = 14)*; green line, microalbuminuria (mild proteinuria) and eGFR <60 mL/min/1.73 m^2 group* (n = 21)*; red line, macroalbuminuria (severe proteinuria) and eGFR <60 mL/min/1.73 m^2 group* (n = 106)*. Differences between groups were compared by a log-rank test.* C: *Event-free rate of all-cause mortality stratified by albuminuria (proteinuria) in the eGFR ≥60 mL/min/1.73 m^2 category according to the Kaplan-Meier method. Blue line, normoalbuminuria (normal proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group* (n = 25)*; green line, microalbuminuria (mild proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group* (n = 27)*; red line, macroalbuminuria (severe proteinuria) and eGFR ≥60 mL/min/1.73 m^2 group* (n = 38)*. Differences between groups were compared by a log-rank test.* D: *Event-free rate of all-cause mortality stratified by albuminuria (proteinuria) in the eGFR <60 mL/min/1.73 m^2 category according to Kaplan-Meier method. Blue line, normoalbuminuria (normal proteinuria) and eGFR <60 mL/min/1.73 m^2 group* (n = 14)*; green line, microalbuminuria (mild proteinuria) and eGFR <60 mL/min/1.73 m^2 group* (n = 22)*; red line, macroalbuminuria (severe proteinuria) and eGFR <60 mL/min/1.73 m^2 group* (n = 107)*. Differences between groups were compared by a log-rank test.*

by albuminuria (proteinuria) and eGFR categories after adjustment for age and sex (Supplementary Table 2). The group of patients with normoalbuminuria (normal proteinuria) and eGFR ≥60 mL/min/1.73 m^2 served as a reference group. HRs of renal events were 8.99-fold higher risk (95% CI 3.07–26.37) in patients with macroalbuminuria (severe proteinuria) and eGFR ≥60 mL/min/1.73 m^2 and 20.82-fold higher risk (95% CI 7.12–60.85) in patients with macroalbuminuria (severe proteinuria) and eGFR <60 mL/min/1.73 m^2. HRs of cardiovascular events was 3.11-fold higher risk (95% CI 1.15–8.39) in patients with macroalbuminuria (severe proteinuria) and eGFR <60 mL/min/1.73 m^2. HRs of all-cause mortality was 5.87-fold higher risk (95% CI 1.62–21.25) in patients with macroalbuminuria (severe proteinuria) and eGFR <60 mL/min/1.73 m^2. Reduced eGFR was not predictive of renal events, cardiovascular events, and all-cause mortality except in patients with macroalbuminuria (severe proteinuria).

Clinical and pathological parameters associated with renal events, cardiovascular events, and all-cause mortality

The results of multivariate Cox proportional hazard regression analysis are shown in Table 3. Young age, macroalbuminuria (severe proteinuria), low eGFR, presence of diabetic retinopathy, high systolic blood pressure, low hemoglobin, advanced diffuse lesions, presence of nodular lesions, presence of exudative lesions, presence of mesangiolysis, advanced IFTA, and advanced arteriosclerosis were the independent risk factors for renal events. High systolic blood pressure and advanced arteriosclerosis were the independent risk factors for cardiovascular events. High age, macroalbuminuria (severe proteinuria), high systolic blood pressure, and advanced IFTA were the independent risk factors for all-cause mortality.

CONCLUSIONS—The present retrospective study is the first report to describe the pathological features with accompanying long-term clinical outcomes among the patients with normoalbuminuria (normal proteinuria) and low eGFR in type 2 diabetes. The glomerular, tubulointerstitial, and vascular lesions in patients with normoalbuminuria (normal proteinuria) and low eGFR were more advanced compared to those in patients with normoalbuminuria (normal proteinuria) and maintained eGFR. In addition, compared to patients with micro-/macroalbuminuria (mild/severe proteinuria) and low eGFR, their tubulointerstitial and vascular lesions were similar or more advanced in contrast to glomerular lesions.

Furthermore, we showed that the evaluation of renal pathology provides

Table 3—*Parameters identified by multivariate Cox proportional hazard regression analysis associated with renal events, cardiovascular events, and all-cause mortality*

Parameters	HR	95% CI	*P*
Renal events			
Age (−10 years)	1.37	(1.01–1.05)	<0.01
Macroalbuminuria (severe proteinuria)	3.89	(2.15–7.05)	<0.01
eGFR (−10 mL/min/1.73 m^2)	1.11	(1.00–1.02)	<0.05
Retinopathy	2.49	(1.23–5.04)	<0.01
Systolic blood pressure (+10 mmHg)	1.12	(1.00–1.02)	<0.05
Hemoglobin (−1 g/dL)	1.42	(1.26–1.59)	<0.01
Diffuse lesion (score +1)	1.39	(1.01–1.91)	<0.05
Presence of nodular lesion	1.82	(1.02–3.25)	<0.01
Presence of exudative lesion	1.89	(1.15–3.11)	<0.05
Presence of mesangiolysis	1.55	(0.95–2.53)	<0.05
IFTA (score +1)	1.49	(1.13–1.97)	<0.01
Arteriosclerosis (score +1)	1.70	(1.18–2.43)	<0.01
Cardiovascular events			
Systolic blood pressure (+10 mmHg)	1.17	(1.00–1.03)	<0.05
Arteriosclerosis (score +1)	1.90	(1.17–3.08)	<0.05
All-cause mortality			
Age (+10 years)	2.55	(1.05–1.15)	<0.01
Macroalbuminuria (severe proteinuria)	4.21	(1.77–10.01)	<0.01
Systolic blood pressure (+10 mmHg)	1.22	(1.00–1.04)	<0.05
IFTA (score +1)	1.92	(1.20–3.09)	<0.01

HRs are adjusted for clinical covariates(age, sex, microalbuminuria [mild proteinuria], macroalbuminuria [severe proteinuria], eGFR, duration of diabetes, presence of diabetic retinopathy, HbA_{1c}, BMI, systolic blood pressure, total cholesterol, and hemoglobin) or pathological covariates (diffuse lesions, nodular lesions, exudative lesions, mesangiolysis, IFTA, interstitial inflammation, arteriolar hyalinosis, and arteriosclerosis).

practical information concerning overall management including renal events and cardiovascular events of diabetic nephropathy in type 2 diabetes. Glomerular lesions, IFTA, and arteriosclerosis were identified as the pathological determinants for renal events. In addition, arteriosclerosis was identified as the pathological determinant for cardiovascular events, and IFTA was identified as the pathological determinant for all-cause mortality.

Clinically, we revealed that macroalbuminuria (severe proteinuria) has a higher impact for renal events and all-cause mortality than low eGFR, whereas the impact of low eGFR on clinical outcomes was observed only in patients with macroalbuminuria (severe proteinuria).

First, we evaluated the structural-functional relationships of diabetic nephropathy in type 2 diabetes. As to the renal lesions related to albuminuria (proteinuria), our results showed that hematuria, diabetic retinopathy, low hemoglobin, and glomerular lesions were increased and more advanced with progression of albuminuria (proteinuria) categories regardless of eGFR. Previous studies in type 1 and type 2 diabetes have shown that the major renal pathological changes of diabetic nephropathy associated with increasing urinary albumin (protein) excretion are mesangial expansion and glomerular basement membrane thickening (19). Further, previous reports in type 2 diabetes have found that nodular lesions and mesangiolysis are correlated with urinary albumin (protein) excretion consistently with our results (15,20–23). Although the presence of hematuria has been considered one of the atypical features indicating the presence of nondiabetic renal disease, several studies have suggested a positive association between the severity of albuminuria and the development of hematuria in patients with diabetic nephropathy, in accordance with our results (24). Our results suggest that the presence of hematuria is associated with more advanced histological alterations in diabetic nephropathy. However, previous studies of biopsy-proven diabetic nephropathy did not correlate the pathological changes with the presence of hematuria (24).

As to the renal lesions related to low eGFR with micro-/macroalbuminuria (mild/severe proteinuria), our results showed that more advanced diffuse, nodular, exudative, tubulointerstitial, and vascular lesions compared to those related to maintained eGFR with micro-/macroalbuminuria (mild/severe proteinuria). In type 1 diabetes, previous studies evaluating structural-functional relationships in diabetic nephropathy among patients ranging from normoalbuminuria to proteinuria demonstrated that the main lesions that determine low GFR shift from glomerular lesions to interstitial lesions (19,25). Our results demonstrate similarities to those in patients with type 1 diabetes and more severe vascular lesions, perhaps reflecting older age and hypertension.

Remarkably, we confirmed the pathological features related to low eGFR without albuminuria (proteinuria). In our study, 9.7% of patients with low eGFR (<60 mL/min/1.73 m^2) were not associated with albuminuria (proteinuria). The frequencies of normoalbuminuria observed in patients with low GFR have been reported to be 22–24% in type 1 diabetes and 32–71% in type 2 diabetes (25,26). These results suggest that normoalbuminuric renal insufficiency is not uncommon among diabetic patients, especially in type 2 diabetes. This study revealed that the glomerular, tubulointerstitial, and vascular lesions in patients with normoalbuminuria (normal proteinuria) and low eGFR were more advanced compared to those in patients with normoalbuminuria (normal proteinuria) and maintained eGFR. In addition, compared to patients with micro-/macroalbuminuria (mild/severe proteinuria) and low eGFR, the tubulointerstitial and vascular lesions in patients with normoalbuminuria (normal proteinuria) and low eGFR in type 2 diabetes were similar or more advanced in contrast to glomerular lesions. Our results suggest that tubulointerstitial lesions observed among patients with normoalbuminuria (normal proteinuria) and low eGFR are strongly affected by vascular lesions rather than by glomerular lesions. A previous study in type 1 diabetes showed that the pathological features among the patients with normoalbuminuria and low GFR included more advanced diabetic glomerular lesions compared to those among the patients with normoalbuminuria and maintained GFR (25). An animal model of type 2 diabetes, the Cohen diabetic rat, which shows progressive depression of renal function without proteinuria, was also reported to show typical diabetic glomerulosclerosis (27). Therefore, further examinations are required to determine the pathophysiological

conditions of patients with normoalbuminuria and low GFR in type 2 diabetes.

Next, we evaluated the pathological impact of glomeruli, tubulointerstitium, and vessels on renal events, cardiovascular events, and all-cause mortality. As to glomerular lesions related to renal events, diffuse lesions, nodular lesions, exudative lesions, and mesangiolysis were identified as the pathological determinants in this study. Previous reports have found that diffuse lesions, nodular lesions, and mesangiolysis are associated with renal outcome in accordance with our results (15,21–23).

In addition to glomerular lesions, IFTA was identified as the pathological determinant for renal events and all-cause mortality in this study. There are numerous studies suggesting that tubulointerstitial damage, as well as glomerular damage, contributes to a decline in renal function (21,28). However, this is the first report identifying IFTA as the predictor of all-cause mortality in diabetic nephropathy. In IgA nephropathy, a Japanese scoring system consisting of clinical findings and histological grades has been reported to predict 10-year risk of end-stage renal disease as well as all-cause mortality risk (29). Even though the histological evaluation is not commonly applied in patients with diabetic nephropathy and we are unable to assess sufficiently how confounding factors influenced our results, the evaluation of renal lesions in addition to clinical findings may improve mortality risk prediction in diabetic nephropathy.

Furthermore, arteriosclerosis in renal biopsy specimens was identified as the pathological determinant for renal events and cardiovascular events in this study. Arteriosclerosis included in the evaluation proposed by the Renal Pathology Society in the U.S. has been shown to worsen glomerular lesions in diabetic nephropathy (18,28). In addition, several autopsy-based studies have shown that intimal thickness of small renal arteries and renal arteriolar hyalinization are strongly associated with atherosclerotic lesions in the coronary arteries, aorta, and major cerebral vessels (30–32). These data support our results.

Considering these findings, various pathological lesions in glomeruli, tubulointerstitium, and vessels were orchestrated to promote and escalate diabetic kidney injuries, resulting in renal failure. It is important to determine whether pathological information from renal biopsy improves the predictive power when added to albuminuria and renal dysfunction. Based on our results, it is reasonable to predict renal prognosis of diabetic nephropathy by combination of clinical and pathological parameters. Prospective studies to develop a prognostic model by research biopsy may be useful for addressing this issue. Furthermore, we speculate that the evaluation of renal pathology provides a key for overall management including renal events and cardiovascular events in patients with diabetic nephropathy.

Finally, our study highlighted the impact of albuminuria (proteinuria) on clinical outcomes of patients with biopsy-proven diabetic nephropathy in type 2 diabetes. Patients with macroalbuminuria (severe proteinuria) had higher incidence of renal events and all-cause mortality than patients with normoalbuminuria (normal proteinuria) or microalbuminuria (mild proteinuria). In addition, macroalbuminuria (severe proteinuria) was a major clinical determinant of renal events and all-cause mortality in this study. Supporting our notion, previous studies have found that the renal outcome of patients with normoalbuminuria and low GFR is better than that of patients with albuminuria, even with maintained GFR (33–35). These results suggest that albuminuria has a greater impact than low GFR on predicting the development and progression of diabetic nephropathy. However, recent studies showed that the higher levels of urinary albumin excretion within the normal range predict faster decline in GFR and higher incidence of cardiovascular disease in type 2 diabetic patients (36,37). In addition, our study shows histological alterations even in the normoalbuminuria (normal proteinuria) category, although it is possible that including negative proteinuria as well as trace proteinuria in the normoalbuminuria (normal proteinuria) category affected the results. Further, some previous studies have found that albuminuria and renal function independently predict renal events, cardiovascular events, and death in diabetic patients (38–40). Therefore, further studies on clinical impacts of low GFR with or without albuminuria and new biomarkers for early and definitive diagnosis of diabetic nephropathy are required.

There are some limitations in this study. First, this study had a retrospective design that was dependent on collectable data. Second, there was likely an influence of bias through limitation of subjects to patients with renal biopsy. Thirdly, there was the lack of quantitative structural measurements. Fourth, the data for proteinuria including dipstick test results were used when data for albuminuria were not available. Finally, treatment contents were not evaluated. These limitations may have placed significant constraints on the interpretation of the results, particularly related to differences in renal and cardiovascular outcomes. However, clinical examination by long-term observation in 260 patients with biopsy-proven diabetic nephropathy is of importance for understanding the pathophysiology of diabetic kidney lesions and clinical outcomes.

In conclusion, the current study of Japanese type 2 diabetic patients with biopsy-proven diabetic nephropathy suggest that the characteristic pathological lesions and macroalbuminuria (severe proteinuria) are closely related to the long-term outcomes of diabetic nephropathy in type 2 diabetes.

Acknowledgments—This study was supported in part by a grant-in-aid for Diabetic Nephropathy Research and for Diabetic Nephropathy and Nephrosclerosis Research from the Ministry of Health, Labor and Welfare of Japan.

No potential conflicts of interest relevant to this article were reported.

M.S. and T.W. designed the study protocol, researched data, contributed to discussion, wrote the manuscript, and reviewed and edited the manuscript. K.F. and T.To designed the study protocol, researched data, contributed to discussion, and reviewed and edited the manuscript. S.Ki., A.H., K.K., Y.I., and N.S. researched data, and contributed to discussion. T.Ta., M.Y., H.Y., and S.Ka. researched data, contributed to discussion, and reviewed and edited the manuscript. All authors approved the final version. T.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Parts of this study were presented in abstract form at the 72nd Scientific Sessions of the American Diabetes Association, Philadelphia, Pennsylvania, 8–12 June 2012.

The authors thank Dr. Hiroshi Kida (National Hospital Organization Kanazawa Medical Center) for his helpful comments on this study.

References

1. American Diabetes Association. Standards of medical care in diabetes—2013. Diabetes Care 2013;36(Suppl. 1):S11–S66
2. de Boer IH, Rue TC, Hall YN, Heagerty PJ, Weiss NS, Himmelfarb J. Temporal trends in the prevalence of diabetic kidney

disease in the United States. JAMA 2011; 305:2532–2539
3. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, Holman RR; UKPDS GROUP. Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney Int 2003;63:225–232
4. Nakai S, Iseki K, Itami N, et al. An overview of regular dialysis treatment in Japan (as of 31 December 2010). Ther Apher Dial 2012;16:483–521
5. United States Renal Data System. USRDS 2012 Annual Data Report. Am J Kidney Dis 2013;61(Suppl. 1):e165–e192
6. Steenkamp R, Castledine C, Feest T, Fogarty D. UK Renal Registry 13th Annual Report (December 2010): Chapter 2: UK RRT prevalence in 2009: national and centre-specific analyses. Nephron Clin Pract 2011;119(Suppl. 2):c27–c52
7. Levey AS, de Jong PE, Coresh J, et al. The definition, classification, and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney Int 2011;80:17–28
8. Japanese Society of Nephrology. In *Clinical Practice Guidebook for Diagnosis and Treatment of Chronic Kidney Disease 2012*. Tokyo, Japan, Tokyo-Igakusya, 2012, p. 1–4
9. Wada T, Shimizu M, Toyama T, Hara A, Kaneko S, Furuichi K. Clinical impact of albuminuria in diabetic nephropathy. Clin Exp Nephrol 2012;16:96–101
10. Fioretto P, Mauer M, Brocco E, et al. Patterns of renal injury in NIDDM patients with microalbuminuria. Diabetologia 1996; 39:1569–1576
11. Takazakura E, Nakamoto Y, Hayakawa H, Kawai K, Muramoto S. Onset and progression of diabetic glomerulosclerosis; a prospective study based on serial renal biopsies. Diabetes 1975;24:1–9
12. Committee of the Japan Diabetes Society on the Diagnostic Criteria of Diabetes Mellitus. Report of the Committee on the Classification and Diagnostic Criteria of Diabetes Mellitus. J Diabetes Invest 2010; 1:212–228
13. Matsuo S, Imai E, Horio M, et al.; Collaborators developing the Japanese equation for estimated GFR. Revised equations for estimated GFR from serum creatinine in Japan. Am J Kidney Dis 2009;53:982–992
14. Gellman DD, Pirani CL, Soothill JF, Muehrcke RC, Kark RM. Diabetic nephropathy: a clinical and pathologic study based on renal biopsies. Medicine (Baltimore) 1959;38:321–367
15. Saito Y, Kida H, Takeda S, et al. Mesangiolysis in diabetic glomeruli: its role in the formation of nodular lesions. Kidney Int 1988;34:389–396
16. Wada T, Furuichi K, Sakai N, et al. Up-regulation of monocyte chemoattractant protein-1 in tubulointerstitial lesions of human diabetic nephropathy. Kidney Int 2000;58:1492–1499
17. Sakai N, Wada T, Furuichi K, et al. Involvement of extracellular signal-regulated kinase and p38 in human diabetic nephropathy. Am J Kidney Dis 2005;45: 54–65
18. Tervaert TW, Mooyaart AL, Amann K, et al.; Renal Pathology Society. Pathologic classification of diabetic nephropathy. J Am Soc Nephrol 2010;21:556–563
19. Mauer SM, Steffes MW, Ellis EN, Sutherland DE, Brown DM, Goetz FC. Structural-functional relationships in diabetic nephropathy. J Clin Invest 1984; 74:1143–1155
20. Stout LC, Kumar S, Whorton EB. Focal mesangiolysis and the pathogenesis of the Kimmelstiel-Wilson nodule. Hum Pathol 1993;24:77–89
21. Oh SW, Kim S, Na KY, et al. Clinical implications of pathologic diagnosis and classification for diabetic nephropathy. Diabetes Res Clin Pract 2012;97:418–424
22. Furuichi K, Hisada Y, Shimizu M, et al. Matrix metalloproteinase-2 (MMP-2) and membrane-type 1 MMP (MT1-MMP) affect the remodeling of glomerulosclerosis in diabetic OLETF rats. Nephrol Dial Transplant 2011;26:3124–3131
23. Wada T, Shimizu M, Yokoyama H, et al. Nodular lesions and mesangiolysis in diabetic nephropathy. Clin Exp Nephrol 2013;17:3–9
24. Akimoto T, Ito C, Saito O, et al. Microscopic hematuria and diabetic glomerulosclerosis—clinicopathological analysis of type 2 diabetic patients associated with overt proteinuria. Nephron Clin Pract 2008;109:c119–c126
25. Caramori ML, Fioretto P, Mauer M. Low glomerular filtration rate in normoalbuminuric type 1 diabetic patients: an indicator of more advanced glomerular lesions. Diabetes 2003;52:1036–1040
26. Macisaac RJ, Jerums G. Diabetic kidney disease with and without albuminuria. Curr Opin Nephrol Hypertens 2011;20:246–257
27. Yagil C, Barak A, Ben-Dor D, et al. Non-proteinuric diabetes-associated nephropathy in the Cohen rat model of type 2 diabetes. Diabetes 2005;54:1487–1496
28. Bohle A, Wehrmann M, Bogenschütz O, Batz C, Müller CA, Müller GA. The pathogenesis of chronic renal failure in diabetic nephropathy. Investigation of 488 cases of diabetic glomerulosclerosis. Pathol Res Pract 1991;187:251–259
29. Bjørneklett R, Vikse BE, Bostad L, Leivestad T, Iversen BM. Long-term risk of ESRD in IgAN; validation of Japanese prognostic model in a Norwegian cohort. Nephrol Dial Transplant 2012;27:1485–1491
30. Tracy RE, MacLean CJ, Reed DM, Hayashi T, Gandia M, Strong JP. Blood pressure, nephrosclerosis, and age autopsy findings from the Honolulu Heart Program. Mod Pathol 1988;1:420–427
31. McGill HC Jr, Strong JP, Tracy RE, McMahan CA, Oalmann MC; The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. Relation of a postmortem renal index of hypertension to atherosclerosis in youth. Arterioscler Thromb Vasc Biol 1995;15: 2222–2228
32. Burchfiel CM, Tracy RE, Chyou PH, Strong JP. Cardiovascular risk factors and hyalinization of renal arterioles at autopsy. The Honolulu Heart Program. Arterioscler Thromb Vasc Biol 1997;17: 760–768
33. Kramer CK, Leitão CB, Pinto LC, Silveiro SP, Gross JL, Canani LH. Clinical and laboratory profile of patients with type 2 diabetes with low glomerular filtration rate and normoalbuminuria. Diabetes Care 2007;30:1998–2000
34. Hoefield RA, Kalra PA, Baker PG, et al. The use of eGFR and ACR to predict decline in renal function in people with diabetes. Nephrol Dial Transplant 2011;26:887–892
35. Bruno G, Merletti F, Bargero G, et al. Estimated glomerular filtration rate, albuminuria and mortality in type 2 diabetes: the Casale Monferrato study. Diabetologia 2007;50:941–948
36. Babazono T, Nyumura I, Toya K, et al. Higher levels of urinary albumin excretion within the normal range predict faster decline in glomerular filtration rate in diabetic patients. Diabetes Care 2009;32: 1518–1520
37. Ruggenenti P, Porrini E, Motterlini N, et al.; BENEDICT Study Investigators. Measurable urinary albumin predicts cardiovascular risk among normoalbuminuric patients with type 2 diabetes. J Am Soc Nephrol 2012;23:1717–1724
38. Yokoyama H, Oishi M, Kawai K, Sone H; Japan Diabetes Clinical Data Management Study Group. Reduced GFR and microalbuminuria are independently associated with prevalent cardiovascular disease in Type 2 diabetes: JDDM study 16. Diabet Med 2008;25:1426–1432
39. Ninomiya T, Perkovic V, de Galan BE, et al.; ADVANCE Collaborative Group. Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. J Am Soc Nephrol 2009;20:1813–1821
40. Drury PL, Ting R, Zannino D, et al. Estimated glomerular filtration rate and albuminuria are independent predictors of cardiovascular events and death in type 2 diabetes mellitus: the Fenofibrate Intervention and Event Lowering in Diabetes (FIELD) study. Diabetologia 2011;54: 32–43

PLOS ONE

The Impacts of Albuminuria and Low eGFR on the Risk of Cardiovascular Death, All-Cause Mortality, and Renal Events in Diabetic Patients: Meta-Analysis

Tadashi Toyama[1], Kengo Furuichi[1], Toshiharu Ninomiya[2], Miho Shimizu[1], Akinori Hara[1], Yasunori Iwata[1], Shuichi Kaneko[3], Takashi Wada[1,4]*

1 Division of Nephrology, Kanazawa University Hospital, Kanazawa, Japan, **2** Department of Medicine and Clinical Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan, **3** Department of Disease Control and Homeostasis, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan, **4** Department of Laboratory Medicine, Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kanazawa, Japan

Abstract

Background: Precise effects of albuminuria and low estimated glomerular filtration rate (eGFR) on cardiovascular mortality, all-cause mortality, and renal events in diabetic patients are uncertain.

Materials and Methods: A systematic review was conducted of the literature through MEDLINE, EMBASE, and CINHAL from 1950 to December 2010. Cohort studies of diabetic patients providing adjusted relative risk (RR) of albuminuria and eGFR for risks of cardiovascular mortality, all-cause mortality, and renal events were selected. Two reviewers screened abstracts and full papers of each study using standardized protocol.

Results: We identified 31 studies fulfilling the criteria from 6546 abstracts. With regard to the risk of cardiovascular mortality, microalbuminuria (RR 1.76, 95%CI 1.38–2.25) and macroalbuminuria (RR 2.96 95%CI 2.44–3.60) were significant risk factors compared to normoalbuminuria. The same trends were seen in microalbuminuria (RR 1.60, 95%CI 1.42–1.81), and macroalbuminuria (RR 2.64, 95%CI 2.13–3.27) for the risk of all-cause mortality, and also in microalbuminuria (RR 3.21, 95%CI 2.05–5.02) and macroalbuminuria (RR 11.63, 95%CI 5.68–23.83) for the risk of renal events. The magnitudes of relative risks associated with low eGFR along with albuminuria were almost equal to multiplying each risk rate of low eGFR and albuminuria. No significant factors were found by investigating potential sources of heterogeneity using subgroup analysis.

Conclusions: High albuminuria and low eGFR are relevant risk factors in diabetic patients. Albuminuria and low eGFR may be independent of each other. To evaluate the effects of low eGFR, intervention, or race, appropriately designed studies are needed.

Citation: Toyama T, Furuichi K, Ninomiya T, Shimizu M, Hara A, et al. (2013) The Impacts of Albuminuria and Low eGFR on the Risk of Cardiovascular Death, All-Cause Mortality, and Renal Events in Diabetic Patients: Meta-Analysis. PLoS ONE 8(8): e71810. doi:10.1371/journal.pone.0071810

Editor: Yan Gong, College of Pharmacy, University of Florida, United States of America

Received March 11, 2013; **Accepted** July 3, 2013; **Published** August 30, 2013

Copyright: © 2013 Toyama et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Funding: This study was supported in part by a Grant-in-Aid for Diabetic Nephropathy Research, from the Ministry of Health, Labor and Welfare of Japan. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. No additional external funding received for this study.

Competing Interests: The authors have declared that no competing interests exist.

* E-mail: twada@m-kanazawa.jp

Introduction

The prevalence of diabetes is increasing globally, and management of diabetic complications is particularly important. [1,2,3] Diabetic nephropathy, resulting in end-stage renal events requiring renal replacement therapy, is one of the most common complications. Furthermore, in the course of diabetic nephropathy, patients have higher rates of mortality from cardiovascular disease. [4] Albuminuria is an early marker of diabetic nephropathy, and previous reports described the association between albuminuria and risks of adverse cardiovascular and kidney events. [5,6] Albuminuria is often used as a surrogate marker for the risk of fatal and non-fatal events in clinical trials of antihyperglycemic medications or in antihypertensive therapy. [7,8,9] Similarly, low eGFR, which is a common manifestation of progressed diabetic nephropathy, has also been demonstrated to be an independent risk factor for cardiovascular events and death. [10,11] Recent evidence suggests that both high albuminuria and low eGFR are independent risk factors for progressive kidney failure and cardiovascular disease. [10] In addition, the magnitudes of risk for progressive kidney failure, cardiovascular disease, and all-cause mortality were different between studies, and the unevenness may have been due to differences in study design or characteristics of participants. It is important to clarify these problems to apply this evidence to individuals.

To manage diabetic nephropathy, it is necessary to clarify the precise magnitude of the risks for cardiovascular mortality, all-cause mortality, and renal events according to the status of the patient. These observations may be useful for the screening of high-risk patients or considering interventions. Therefore, we conducted a systematic review and meta-analysis of published

studies on diabetic nephropathy to provide an accurate estimation of the influence of albuminuria and low eGFR.

Methods

Data Sources and Searches

We conducted a systematic review of disease prognosis. A systematic review of the available literature according to MOOSE (meta-analysis of observational studies on epidemiology) guidelines was conducted. MEDLINE (http://ovidsp.ovid.com/), EMBASE (http://www.embase.com/), and CINHAL (http://www.ebscohost.com/cinahl/) from 1950 until December 2010 were searched, and the related literature were identified. Search strategies consisted of medical subject headings and text words, including all spellings of proteinuria, albuminuria, microalbuminuria, macroalbuminuria, and glomerular filtration rate combined with cardiovascular diseases, mortality, renal events (Table 1), and limited to cohort studies of diabetic patients. References from identified studies were also screened manually.

Study Selection

Studies were included if they were cohort studies on diabetic patients that estimated the relative risk (RR) and 95% confidence intervals (CIs) of albuminuria or low eGFR on cardiovascular mortality, all-cause mortality, or renal events, and the estimates were derived from Cox proportional hazard models. The definitions of albuminuria were pre-specified (Table 2). Studies were included if they met the definitions of albuminuria in Table 2. Cardiovascular mortality was defined as death from coronary events and/or stroke, which may be on the basis of International Classification of Diseases codes. Renal events were defined as renal replacement therapy, renal transplantation, or loss of renal function. Loss of renal function is defined as sustained eGFR or creatinine clearance below 60 ml/min/1.73 m^2 or less, halving of eGFR, or doubling of serum creatinine.

Data Extraction and Quality Assessment

The literature search and screening were performed by two of the authors (TT and MS). Authors independently judged the contents of abstracts and full papers in duplicate using standardized data collection form. Additional data were not collected from authors of literature. To eliminate the potential influences of specific disease, studies were excluded if their cohorts included patients with specific complications. Studies were also excluded if they reported estimates of influences without any information about standard error, and if they did not yield an estimate that was not adjusted at least by age.

Data Synthesis and Analysis

Random-effects model were used to obtain summary estimates of RR and 95% CI. Summary estimates were obtained separately according to the level of albuminuria (microalbuminuria, macroalbuminuria, any level of albuminuria). If only subgroups of the estimate were reported (e.g., by gender), these were pooled by fixed-effects model as a within-study summary estimate. We also investigated studies providing RR associated with low eGFR according to the level of albuminuria. If the study population was representative of a particular level of eGFR (e.g., eGFR >60), it was handled as stratified. To evaluate the influences of albuminuria and low eGFR, compare the relative risks pooled by fixed-effects model according to stratified category of albuminuria (micro- and macroalbuminuria), low eGFR (< 60 mL/min/

Table 1. Search Strategies.

1: diabetes mellitus AND (proteinuria OR albuminuria OR microalbuminuria OR macroalbuminuria)
2: (diabetic nephropathy)
3: (kidney failure, chronic) OR (glomerular filtration rate)
4: (cardiovascular diseases) OR (cerebrovascular disorders)
5: mortality OR death
6: (cohort studies) OR (case-control studies)
(1 or 2) and (3 or 4 or 5) and 6

terms associated with Medical Subject Headings.
doi:10.1371/journal.pone.0071810.t001

Table 2. Definitions of Albuminuria.

Measurement Method	Microalbuminuria	Macroalbuminuria	Any level of albuminuria
24 hour urine collection	30–300 mg/day or 20–200 μg/min	>300 mg/day or >200 μg/min	>30 mg/day or >20 μg/min
(proteinuria)	N/A	>0.3–0.5 g/day	N/A
Spot urine albumin creatinine ratio	30–300 mg/g or 3.4–34 mg/mmol	>300 mg/g or >34 mg/mmol	>30 mg/g or >3.4 mg/mmol
(proteinuria)	N/A	>0.3–0.5 g/g	N/A
Spot urine albumin concentration	3–30 mg/dl	>30 mg/dl	>3 mg/dl
(proteinuria)	N/A	>0.3–0.5 g/l	N/A
Spot urine dipstick	Specific microalbuminuria dipstick positive	N/A	N/A

Abbreviation: N/A, not available.
Based on Sarnak et al. [12].
doi:10.1371/journal.pone.0071810.t002

1.73 m^2) and normal eGFR (≥ 60 mL/min/1.73 m^2) regardless of the reference category of eGFR. Heterogeneity between studies was assessed using Cochran Q test and I^2 value. Potential sources of heterogeneity were examined by subgroup analysis comparing summary estimates from subset of studies categorized by characters of participants or study design. Univariate meta-regression was used to compare the subgroups. Begg's test [13] and Egger's test [14] were used to evaluate possible publication bias (where $P<0.05$ was taken to indicate statistical significance). To evaluate an influence of a single study, sensitivity analysis is performed to examine the exclusion of any single study altered the magnitude of relative risk or test for heterogeneity. All analyses were performed using Stata (release 11.2; Stata Corporation, College Station, TX). For all tests, a two-sided p-value below 0.05 was considered significant.

Results

Literature Search and Characteristics of Studies

The systematic database search yielded 6546 studies, of which 326 papers were reviewed in full (Figure 1). Finally, 31 studies that fulfilled the criteria were included in the analysis, including information for 148350 participants. The crude incidence rates were 19.1 deaths from cardiovascular disease, 35.7 deaths, and 11.7 renal events (per 1000 person-years, respectively). The process of study identification is shown in the flow chart, and the study characteristics are listed in Table 3 and Table 4. Studies consisted of four studies of type 1 diabetic patients, 23 studies of type 2 diabetic patients, one study of type 1 and type 2 diabetic patients, and 3 studies of unknown type of diabetic patients. The study size was in the range of 146 to 94934, and the average follow-up period was in the range of 3 to 19 years. Regarding cardiovascular mortality, Asian population study was not included according to the criteria. We pooled the risk of two studies [15,16] reporting only subgroups of the estimate.

Micro- and macroalbuminuria were defined as risk factors in 25 studies. Any level of albuminuria (i.e., micro- or macroalbuminuria) was defined as a risk factor in 7 studies. In these studies, various means of expression of albuminuria were adopted. The magnitude of microalbuminuria was expressed as urinary albumin excretion rate (n?12), urinary albumin-creatinine ratio on spot urine samples (n?10), spot urinary albumin concentration (n?6), qualitative test of albuminuria (n?2), or urinary protein excretion rate (n?1). Almost all of the estimates were adjusted for multiple risk factors including age. In one study [17], the estimate was not adjusted for age because age was not a statistically significant risk.

Association of Albuminuria with Risk of Cardiovascular Mortality

Microalbuminuria was associated with 1.76 (95% confidence interval [CI] 1.38–2.25) times greater risk of cardiovascular mortality as compared with normoalbuminuria (Figure 2), with strong heterogeneity among studies ($I^2 = 66\%$, $p = 0.003$ for heterogeneity). We found no significant evidence of publication bias. Subgroup analysis did not determine the suspected source of heterogeneity (Figure S1). Age stratified analysis showed no trends neither micro- nor macroalbuminuria (Figure S2). Macroalbuminuria was associated with about 2.96 (95%CI 2.44–3.60) times greater risk of cardiovascular mortality compared with normoal-

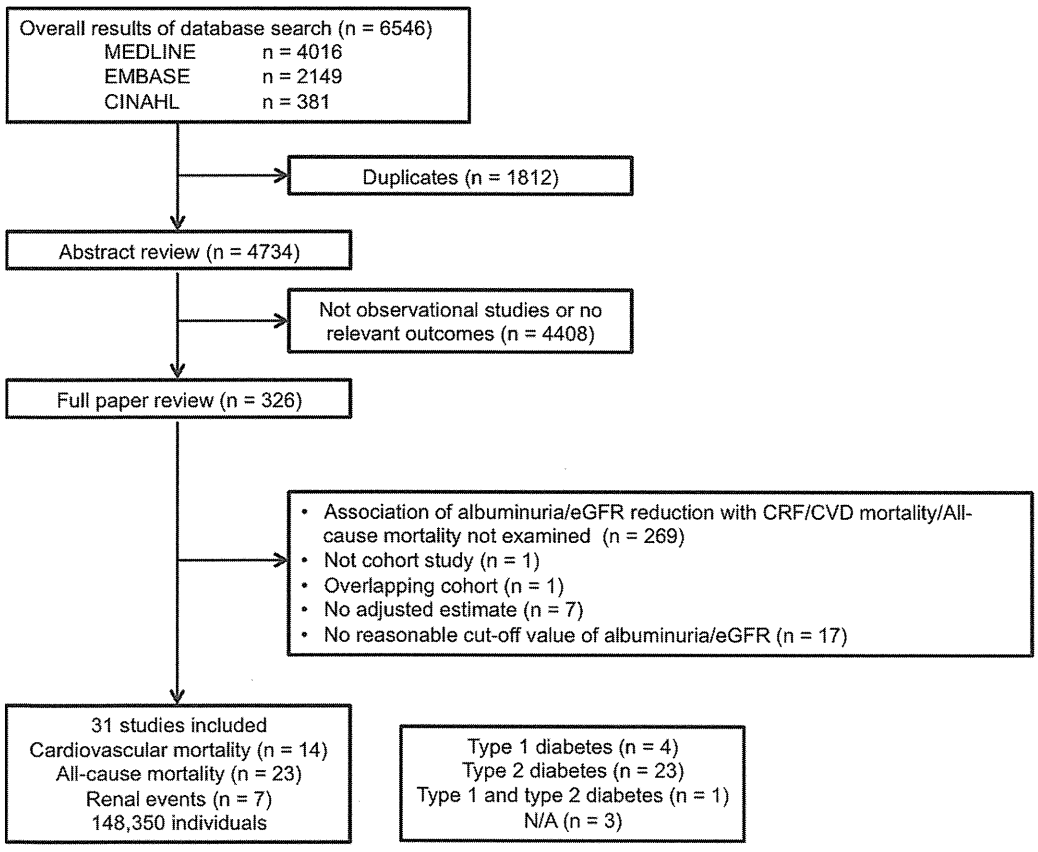

Figure 1. Process for identification of eligible studies Abbreviation: N/A, not available.
doi:10.1371/journal.pone.0071810.g001

Table 3. Characteristic of Studies Reporting on the Association between Albuminuria or low eGFR and Subsequent Risk of Adverse Outcomes.

Author	Year	Country	Study size	%male	%white	Endpoints[a]		No. of CV mortality	No. of all-cause mortality	No. of renal events
Jager[15]	2010	Netherlands	173	48.0	100.0	CV mortality		16		
O'Hare[16]	2010	US	94,934	98.0	87.0	All-cause mortality			25481	
Grauslund[17]	2010	Denmark	389	55.0	N/A	CV mortality	All-cause mortality	N/A	117	
Molitch[18]	2010	US	1,439	52.5	N/A		Renal events			89
Ninomiya[10]	2009	Multicountries	10,640	57.0	N/A	CV mortality	All-cause mortality Renal events	432	817	107
Groop[19]	2009	Finland	4,201	51.8	N/A		All-cause mortality		291	
de Boer[20]	2009	US	691	42.1	80.6	CV mortality	All-cause mortality	169	378	
Vlek[6]	2008	Netherlands	759	76.5	N/A	CV mortality	All-cause mortality	49	82	
Luk[21]	2008	China	5,829	49.8	N/A		Renal events			741
Tong[22]	2007	China	4,416	42.9	N/A		All-cause mortality Renal events		110	221
Bruno[23]	2007	Italy	1,538	43.4	N/A	CV mortality	All-cause mortality	331	670	
Roy[24]	2006	US	725	41.7	0.0		All-cause mortality		131	
So[25]	2006	Hong Kong	4,421	43.2	N/A		Renal events			212
Retnakaran[26]	2006	UK	5,032	59.0	81.0		Renal events			584
Xu[27]	2005	USA	1,953	37.6	N/A[e]	CV mortality	All-cause mortality	223	627	
Yuyun[28]	2003	UK	427	62.1	N/A		All-cause mortality		56	
Bruno[29]	2003	Italy	1,408	43.6	N/A		Renal events			82
Jude[30]	2002	UK	340	66.5	66.8	CV mortality	All-cause mortality	44	63	
Ostgren[31]	2002	Sweden	400	50.5	N/A		All-cause mortality		131	
Stehouwer[32]	2002	Netherlands	328	61.6	N/A		All-cause mortality		113	
Gerstein[33]	2001	North and South America and Europe	3,498	62.9	N/A		All-cause mortality		431	
de Grauw[34]	2001	Netherlands	262	39.0	N/A		All-cause mortality		57	
Florkowski[35]	2001	New Zealand	447	46.5	N/A		All-cause mortality		187	
Casiglia[36]	2000	Italy	683	50.2	N/A	CV mortality		68		
Valmadrid[37]	2000	US	840	45.0	N/A	CV mortality	All-cause mortality	364	529	
Hänninen[38]	1999	Finland	252	53.2	N/A		All-cause mortality		21	
Mattock[39]	1998	U.K.	146	56.2	100.0	CV mortality	All-cause mortality	20	36	
Beilin[40]	1996	Australia	666	47.1	N/A	CV mortality	All-cause mortality	80	167	

Table 3. Cont.

Author	Year	Country	Study size	%male	%white	Endpoints[a]	No. of CV mortality	No. of all-cause mortality	No. of renal events
Rossing[41]	1996	Denmark	939	52.5	N/A	CV mortality All-cause mortality	74	207	
Gall[42]	1995	Denmark	328	61.5	N/A	CV mortality	29		
Neil[43]	1993	U.K.	246	50.8	N/A	All-cause mortality		93	

Follow-Up (years)	Mean age (years)	Type of DM[b]	Duration of DM (years)	mean eGFR (ml/min/1.73m^2)	sBP (mmHg)	dBP (mmHg)	Study type[c]	Use of RASS inhibitors (%)	Adjustment of BP or HT	Stratification of eGFR (ml/min/1.73m^2)	Level of Adjustment[d]
	64	T2DM	N/A	67.8	139	83	Obs	N/A	NO	N/A	Age, sex, obesity, HT, TCHOL, TG, HDL, preexistent IHD, current smoking
6.4	66.6	N/A	N/A	75.3	139	74	Obs	60.7	YES	≥90, 89-60, 59-45, 44-30, 29-15	Age, sex, BMI, sBP, dBP, race, eGFR, comorbidity, medication use
13	45.8	T1DM	30.0	68.0	N/A	N/A	Obs	N/A	NO	N/A	Age, sex, DM duration
19.3	27.1	T1DM	5.8	112.6	114	72	Trial	51.0	NO	N/A	Mean arterial pressure, ACE inhibitor use
4.3	66	T2DM	7	80.6	145	81	Trial	50.0	YES	eGFR ≥90/60-89/<60	Age, sex, DM duration, HbA1c, BMI, sBP, history of currently treated HT, logTG, HDL, LDL, ECG abnormalities, current smoking, current drinking, history of macrovascular dis.
7	33.0	T1DM	23.2	75.9	134	80	Obs	28.9	NO	N/A	Age, duration of DM, HbA1c, eGFR, macrovascular disease
10	77.7	N/A	N/A	76.0	138	69	Obs	25.3	YES	60 ≧, <60	Age, sex, BMI, HT, DM duration, TCHO, hypoglycemic medications, race, smoking, lipid-lowering medications, prevalent cardiovascular disease, and prevalent congestive heart failure.
4	59.7	N/A	N/A	113.3	142	82	Obs	22.4	YES	>60, <60	Age, sex, sBP, dBP, HDL, LDL, vascular history, smoking
4.6	54.1	T2DM	6.2	91.0	133	76	Obs	N/A	YES	N/A	Age, sex, BMI, HT, DM duration, HbA1c, Retinopathy, central obesity, hypertriglyceridemia
3.4	57.6	T2DM	5.3	110.3	135	77	Obs	9.3	YES	N/A	Renal failure: Age, waist circumference, sBP; All-cause mortality: Age, sex, BMI
11	68.6	T2DM	10.8	N/A	155	78	Obs	N/A	YES	>60, <60	Age, sex, HT, HbA1c, apoB/apoA1, smoking, fibrinogen
3	29	T1DM	8.0	N/A	N/A	N/A	Obs	N/A	YES	N/A	Age, BMI, dBP, diabetic retinopathy severity level, socio economic status, macroangiopathy, heavy alcohol consumption
3.3	57.6	T2DM	6.9	91.0	134	77	Obs	37.8	YES	>90/60-89/30-39/15-29	Age, sex, DM duration, HbA1c, BMI, sBP, retinopathy, TG, HDL, LDL, smoking, RAAS inhibition
15	52.4	T2DM	0	82.3	135	83	Trial	N/A	YES	N/A	Age, sex, ethnicity, Cr, smoking, waist, height, sBP, retinopathy
8.8	57.1	T2DM	9.7	65.7	136	N/A	Obs	N/A	YES	N/A	Age, sex, BMI, HT, DM duration, TG, HDL, LDL, study center, percent of American Indian heritage, current alcohol drinking, smoking, preexisting CVD
5	53.4	T1+T2DM	14.3	N/A	N/A	N/A	Obs	N/A	YES	N/A	Age, sex, BMI, mean BP, duration of DM, TCHO, smoking, type of DM, baseline cardiovascular history, rate of change of albuminuria over 1 year
6.7	68.1	T2DM	10.7	66.0	154	88	Obs	N/A	NO	N/A	Age, sex, attained time of follow-up

Table 3. Cont.

Follow-Up (years)	Mean age (years)	Type of DM[b]	Duration of DM (years)	mean eGFR (ml/min/ 1.73m^2)	sBP (mmHg)	dBP (mmHg)	Study type[c]	Use of RASS inhibitors (%)	Adjustment of BP or HT	Stratification of eGFR (ml/min/1.73m^2)	Level of Adjustment[d]
5.3	60.8	T2DM	5.1	N/A	161	88	Obs	N/A	YES		Age, sex, HT, DM duration, HbA1c, TCHO, sCr, race, pre-existing IHD
5.9	69.6	T2DM	8.9	89.0	160	84	Obs	N/A	NO	N/A	Age and sex
9.0	53.8	T2DM	6.4	67.6	151	86	Obs	N/A	YES	N/A	Age, sex, BMI, sBP, DM duration, HbA1c, TCHOL, prior cardiovascular disease
4.5	65.4	T2DM	11.4	73.5	142	80	Trial	50.5	YES	N/A	Age, sex, abdominal obesity, HT, DM duration,HbA1c, dyslipidemia, diabetes status, smoking status, and sCr, use of oral agents or insulin
6	66	T2DM	5	N/A	155	82	Obs	N/A	NO		Age, sex, duration of DM
10	62.2	T2DM	9.6	N/A	N/A	N/A	Obs	N/A	YES	N/A	Age, sex, DM duration, BMI, HbA1c, HT, PVD, smoking, glucose, TCHOL, HDL, TG, cerebrovascular disease, peripheral neuropathy, coronary artery disease, metformin, sulphonylurea, combined oral, insulin
6	63.2	T2DM	N/A	N/A	156	90	Obs	N/A	YES	mean -2S.D. >60	Age, coronary artery disease, sustained arterial hypertension
12	67.9	T2DM	15.1	N/A	143	76	Obs	N/A	YES	N/A	Age, sex, glycemic control, insulin use, alcohol intake, physical activity, history of CVD, intake of antihyptertensive agents, and the presence and severity of diabetic retinopathy
5	58	T2DM	6.0	N/A	145	90	Obs	N/A	NO	N/A	Age, sex, coronary heart disease
7	59	T2DM	5	N/A	144	84	Obs	N/A	NO	N/A	Age, sex, HbA1c, TCHO, preexistent coronary heart disease
4.8	63	T2DM	13	N/A	154	82	Obs	N/A	YES	N/A	Age, sex, DM duration, HbA1c, BMI, sBP, dBP, retiinopathy, TCHO, HDL, TG, age at diagnosis, smoking, fasting glucose, urea, loss of pinprick sensation, leg claudication, number of abscent foot pulses, CHD, carebrovascular disease
9.2	39.6	T2DM	18.6	N/A	136	82	Obs	N/A	YES	N/A	Age, sex, HT, smoking, HbA1c, smoking, height, sCr, social class, overt nephropthy
5.3	55.9	T2DM	6.3	N/A	151	86	Obs	N/A	YES	N/A	Age, HbA1c, sBP, coronary heart disease
6.1	66.2	T2DM	9.1	N/A	160	89	Obs	N/A	NO	N/A	Age, DM duration, retinopathy, lens opacity, intermittent claudication

[a]Endpoints: CV mortality, cardiovascular mortality.
[b]Type of DM: N/A, type of DM is not documented; T1DM, population with type 1 DM; T2DM, population with type 2 DM.
[c]Study type: Obs, based on the cohort of observational study; Trial, based on the cohort of clinical trial.
[d]Level of Adjustment: ACE, angiotensin converting enzyme; Apo, apolipoprotein; BMI, body mass index; CVD, cardiovascular disease; dBP, diastolic blood pressure; DM, diabetes mellitus; ECG, electrocardiogram; HbA1c, glycosylated hemoglobin A1c; HDL, high-density lipoproteins; HT, hypertension; IHD, ischemic heart disease; LDL, low-density lipoproteins; PVD, peripheral vascular disease; RAAS, Renin-Angiotensin-Aldosterone System; sBP, systolic blood pressure; sCr, serum creatinine TCHO, total cholesterol; TG, triglycerides;
[e]Cohort of American Indians.
Other abbreviations: N/A, not available; CV mortality, cardiovascular mortality; sBP, systolic blood pressure; dBP, diastolic blood pressure.
doi:10.1371/journal.pone.0071142.t003

Table 4. Definitions of Albuminuria, eGFR categories and Outcomes.

Author	Urine measurement method[a]	Definition of microalbuminuria	Definition of macroalbuminuria	Definition of any level of albuminuria	eGFR categories	Criteria of renal failure	Criteria of CV mortality	Definition of CV disease[b]
Jager [15]	ACR			>2.0 mg/mmol			ICD code 390–459	Heart/Brain
O'Hare [16]	ACR	30–299 mg/gCr	≥300 mg/gCr					
Grauslund [17]	spot	30–299 mg/L	≥300 mg/L				ICD-9 codes 430.0–438.9 ICD-10 codes I20.0–I25.9, I60.0–I60.9	Heart/Brain
Molitch [18]	AER	30–300 mg/24 h	>300 mg/24 h			sustained eGFR<60		
Ninomiya [10]	ACR	30–300 mg/gCr	>300 mg/gCr		>90, 60–89, <60	death as a result of kidney disease, requirement for dialysis or transplantation, or doubling of serum creatinine to >200 μmol/L	death as a result of coronary heart disease or cerebrovascular disease	Heart/Brain
Groop [19]	AER	20–200 μg/min	>200 μg/min					
de Boer [20]	ACR			≥30 mg/gCr	≥60, <60		death from coronary heart disease, myocardial infarction, sudden cardiac death, or stroke	Heart/Brain
Vlek [6]	ACR			>3 mg/mmol	>60, ≤60		Vascular death, Stroke, Myocardial infarction	Heart/Brain
Luk [21]	ACR	2.5–30 mg/mmol (women) 3.5–30 mg/mmol (men)	>30 mg/mmol			ICD-9 code 250.4, 585, 586 ICD-9 procedure code 39.95 (hemodialysis), 54.98 (peritoneal dialysis)		
Tong [22]	ACR	3.5–25 mg/mmol	≥25 mg/mmol			eGFR halving, eGFR <15 ml/min/1.73 m^2, death as a result of renal causes or need for dialysis		
Bruno [23]	AER	20–200 μg/min	>200 μg/min		≥60, <60		ICD code 390–459	Heart/Brain
Roy [24]	AER	20–200 μg/min	>200 μg/min					
So [25]	ACR	3.5–25 mg/mmol	≥25 mg/mmol	>3.5 mg/mmol	>90, 60–89, 30–59, 15–29	Reduction in eGFR by 50% or progression to eGFR 15 ml/min/1.73 m^2 (stage 5) or renal dialysis or death secondary to renal causes		
Retnakaran [26]	spot	50–299 mg/L	≥300 mg/L			Creatinine clearance ≤60 ml/min per 1.73 m^2		
Xu [27]	ACR	≥30, <300 mg/gCr	≥300 mg/gCr				definite fatal MI, definite sudden death due to CHD, definite or possible fatal CHD, definite or possible fatal stroke, definite or possible fatal CHF, and other fatal CVD	Heart/Brain
Yuyun [28]	AER	30–300 mg/24 h	>300 mg/24 h					
Bruno [29]	AER	20–200 ug/min	>200 ug/min			ESRD (need for dialysis) or chronic renal failure		
Jude [30]	PER		Urine protein ≥0.5 g/24 h				from death certificates	Heart/Brain

Table 4. Cont.

Author	Urine measurement method[a]	Definition of microalbuminuria	Definition of macroalbuminuria	Definition of any level of albuminuria	eGFR categories	Criteria of renal failure	Criteria of CV mortality	Definition of CV disease[b]
Ostgren [31]	qualitative	Specific microalbumiuria dipstick positive						
Stehouwer [32]	AER	30–299 mg/24 h	≥300 mg/24 h					
Gerstein [33]	ACR			>2.0 mg/mmol exclude dipstick–positive proteinuria				
de Grauw [34]	spot	20–200 mg/L	>200 mg/L					
Florkowski [35]	spot			≥50 mg/l				
Casiglia [36]	AER	30–300 mg/24 h	>300 mg/24 h		>60, ≤60		from the hospital of physicians'files	Heart/Brain
Valmadrid [37]	qualitative	Agglutination inhibition assay positive,and reagent strip negative	Urine protein ≥0.3 g/L				ICD9 codes 402, 404, 410–414, 428, 430–438	Heart/Brain
Hänninen [38]	AER			≥20 μg/min				
Mattock [39]	AER	20–200 μg/min	UAER >200 μg/min				from death certificates	Heart
Beilin [40]	spot	30–300 mg/L	≥300 mg/L				ICD9 codes 390 to 458, 410 to 414	Heart/Brain
Rossing [41]	AER	31–299 mg/24 h	≥300 mg/24 h				from death certificate	Heart/Brain
Gall [42]	AER	30–299 mg/24 h	AER ≥300 mg/24 h				from death certificates	Heart/Brain
Neil [43]	spot	40–200 mg/L	UAC >200 mg/L					

[a]Urine measurement method: ACR, albumin creatinine ratio; AER, albumin excretion rate; PER, protein excretion rate; spot, spot urinary albumin concentration; qualitative, qualitative detection of albumin in urine.
[b]*Definition of CV disease: Heart, ischemic heart disease; Brain, cerebrovascular disease.*
doi:10.1371/journal.pone.0071810.t004

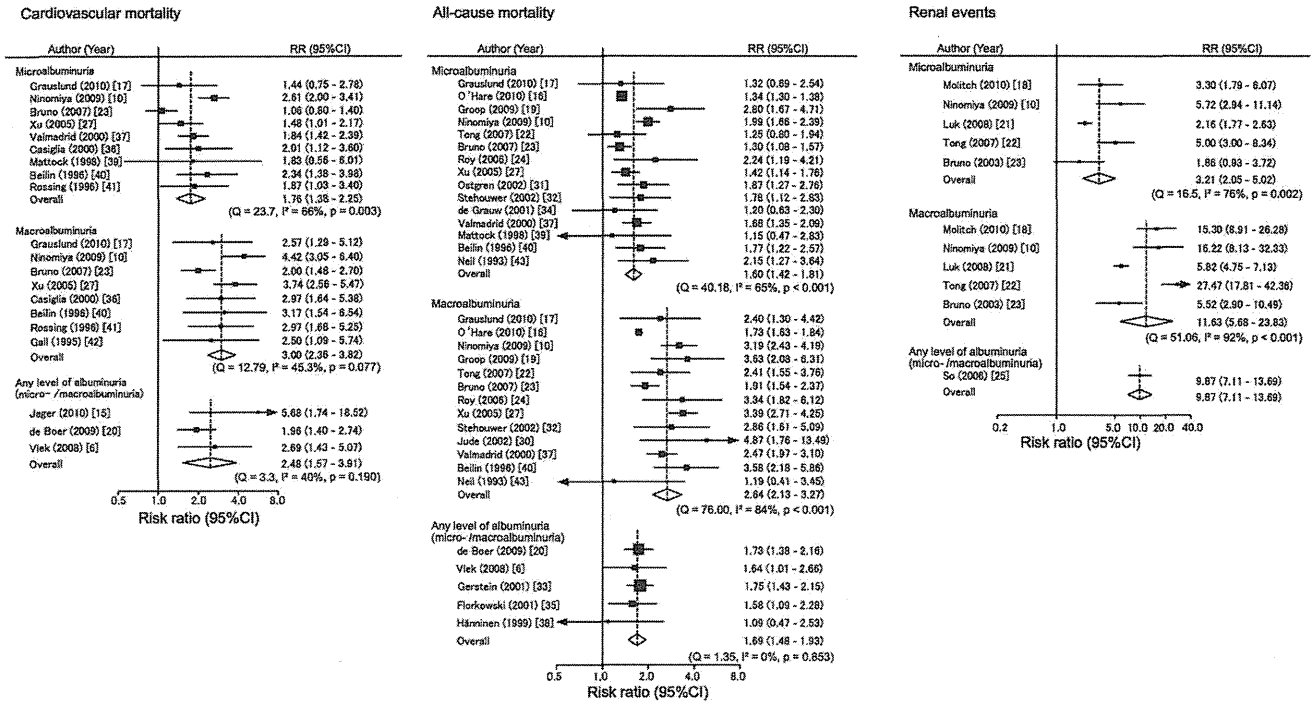

Figure 2. Risk ratio for the association between albuminuria and cardiovascular mortality, all-cause mortality, and renal events compared with normoalbuminuria. Abbreviations: CI, confidence interval; RR, risk ratio.
doi:10.1371/journal.pone.0071810.g002

buminuria, and there was no significant evidence of heterogeneity among studies. These findings suggest that there is a dose-dependent association between albuminuria and the risk of cardiovascular mortality: the influence of macroalbuminuria was significantly higher than that of microalbuminuria ($p = 0.026$). In the three studies for which information was available, any level of albuminuria was associated with about 2.48 times (95%CI 1.57–3.91) greater risk of cardiovascular mortality compared with normoalbuminuria, without any evidence of heterogeneity in the association.

Association of Albuminuria with Risk of All-cause Mortality

Summary estimates of the influences of microalbuminuria and macroalbuminuria on all-cause mortality were 1.60 (95%CI 1.42–1.81) and 2.64 (95%CI 2.13–3.27), respectively (Figure 2): the associations were heterogeneous among studies for both ($I^2 = 65\%$ and 84%, both $p<0.001$ for heterogeneity). There was some evidence of publication bias in microalbuminuria and macroalbuminuria (Egger's test *P*?0.014 and *P*?0.015, respectively), which may have overestimated the strength of the association. Subgroup analysis did not determine the suspected source of heterogeneity. As to the racial difference, relative risks were not significantly different between Asians and non-Asians. A study in veterans

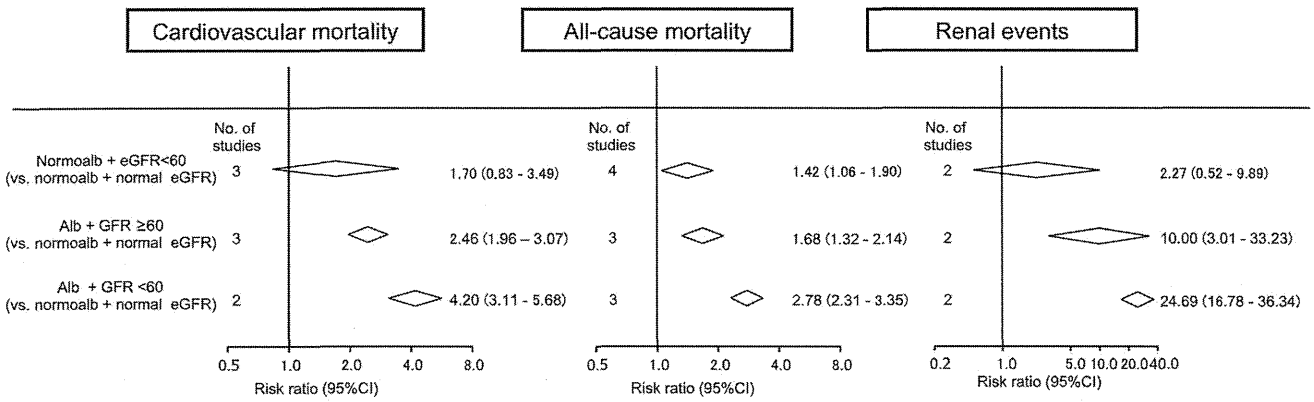

Figure 3. Risk ratio for the association of low eGFR with the risk of each outcome according to the presence of albuminuria, compared with normal eGFR and normoalbuminuria. Albuminuria was defined as any level of albuminuria or pooled estimate of microalbuminuria and macroalbuminuria. Abbreviations: normoalb, normoalbuminuria; alb, albuminuria.
doi:10.1371/journal.pone.0071810.g003

(O'Hare et al.) [18] yielded a lower risk of all-cause mortality (HR 1.34 [95%CI 1.30–1.38] for microalbuminuria, HR 1.73 [95%CI 1.63–1.84] for macroalbuminuria), but the source of heterogeneity was not apparent (Figure S1). In age-stratified analysis, there was no significant difference between younger and older age (Figure S2). Sensitivity analysis excluding this study [18], with the highest weight in this meta-analysis, showed a similar relative risk in microalbuminuria (HR 1.65 [95% CI 1.46 – 1.87]) and macroalbuminuria (HR 2.77 [95% CI 2.34 – 3.27]); the test for heterogeneity was insignificant in microalbuminuria (I^2?41.0%, *P*?0.06), and was still significant for macroalbuminuria (I^2?51.1%, *P*?0.02). The summary estimate of the influence of any level of albuminuria for the risk of all-cause mortality was 1.69 (95%CI 1.48–1.93).

Association of Albuminuria with Risk of Renal Events

Summary estimates of the influences of microalbuminuria and macroalbuminuria on renal events were 3.21 (95%CI 2.05–5.02) and 11.63 (95%CI 5.68–23.83), respectively (Figure 2): the risk estimates of micro- and macroalbuminuria were diverse across studies ($I^2 = 76\%$ and 92%, $p = 0.02$ and $p<0.001$ for heterogeneity). We found no significant evidence of publication bias. Subgroup analysis did not show any significant differences between characteristics of participants or study design (Figure S1). Asians have almost the same risk for renal events as non-Asians in both micro- and macroalbuminuria. Age stratified analysis showed no trends in microalbuminuric or macroalbuminuric patients (Figure S2). One study evaluating the influences of any level of albuminuria showed the same trend.

Combined Impacts of Low eGFR on Albuminuria

A few studies [6,10,19,20] evaluated the combined influence of low eGFR on albuminuria in terms of the risk for the outcomes. As compared to those with normoalbuminuria, the risk of cardiovascular mortality tended to increase by 1.70-fold (95%CI 0.83–3.49) in subjects with normoalbuminuria and eGFR of <60 mL/min/1.73 m^2 (Figure 3). Similarly, the presence of albuminuria was significantly associated with 2.46-fold (95%CI 1.96–3.07) increased risk of cardiovascular mortality. Furthermore, subjects with both albuminuria and eGFR <60 mL/min/1.73 m^2 were at 4.20 times (95%CI 3.11–5.68) higher risk of cardiovascular mortality compared to those with neither of these risk factors.

Discussion

This study explored the influences of albuminuria and low eGFR on cardiovascular mortality, all-cause mortality, and renal events in diabetic patients using meta-analysis methods with 148350 cases. Microalbuminuria and macroalbuminuria are significant risk factors for each outcome. Similar to the influences of albuminuria, low eGFR also increased the risk of each adverse outcome.

This meta-analysis suggested that low eGFR and albuminuria may be independent risk factors for cardiovascular mortality, all-cause mortality, and renal events. Recent published new CKD staging from Kidney Disease: Improving Global Outcomes (KDIGO) was defined by these two factors, eGFR and albuminuria. [44,45] However, conventional staging of diabetic nephropathy was classified only by degree of albuminuria. [46] Many reports and meta-analysis indicated albuminuria as one of the main risk factors for cardiovascular mortality and all-cause mortality in diabetic patients. [47] Although the number of reports was limited, some indicated the influences of low eGFR on the risk of each outcome in diabetic nephropathy. [10,19,20] However, other reports concluded that low eGFR was not always a significant risk factor for these outcomes. [6,25] Thus, the influences of albuminuria and low eGFR are not consistent among studies adjusted for each other. Further large prospective studies are needed to clarify the independent influences of albuminuria and low eGFR on the three outcomes in diabetic nephropathy.

The interaction between eGFR and albuminuria may be important in considering the possibility of albuminuria and low eGFR as independent risk factors for the three outcomes. Previous meta-analyses of general and high-risk cohorts indicated no interaction between eGFR and albuminuria on the risks of cardiovascular mortality, all-cause mortality, and renal events. [48,49] Similarly, in our results of diabetic nephropathy consisting of 4 data or less, stratified analysis demonstrated that the magnitudes of relative risks of these events with low eGFR and albuminuria were almost equivalent to those obtained by multiplying each risk rate of low eGFR and albuminuria. These results suggested that there is no interaction between eGFR and albuminuria in each adverse outcome. In our meta-analysis, only two studies evaluated the interaction between eGFR and albuminuria. [10,25] One of these studies that included stratified analysis indicated that increasing risk of cardiovascular mortality and all-cause mortality in low eGFR were significantly higher in patients with macroalbuminuria but not those with normoalbuminuria. [25] Moreover, in a previous meta-analysis, one of eight general and high-risk cohorts showed significant interaction between eGFR and albuminuria for the risk of ESRD. [49] Based on these studies, the significance of the interaction between eGFR and albuminuria is still variable. Detailed analysis of cohort studies, including an unusual case of diabetic nephropathy, such as low eGFR with normoalbuminuria and high GFR with macroalbuminuria, are needed to resolve the precise interaction of them.

There was heterogeneity among studies for cardiovascular mortality, all-cause mortality, and renal events in the presence of microalbuminuria or macroalbuminuria. There are some possible causes of the heterogeneity in this study. One of the possible reasons is a large cohort with different results from the others. Another possible reason is the diversity of study design. A large study with an exceptional setting [18] may lead to heterogeneity of the outcome. The report by O'Hare et al. had the highest weight in this meta-analysis, and its relative risk was even lower than the pooled risk of all-cause mortality. [18] Therefore, this large cohort study of veterans should have some different setting from other studies. The multiplicity of study design is an unavoidable limitation of meta-analyses, which is another possible reason of heterogeneity. The entry criteria, treatment, or adjustment for confounders were different between studies, and the different settings may affect results to uneven extents. Although some other factors, such as blood pressure control or use of ACE inhibitors for renal events, are possible factors for heterogeneity, these factors were not fully evaluated in the studies included in this analysis. [50,51] Based on these results, standardization of study design is needed, including treatment strategy or adjustment of confounders.

As diabetes is a common disease with high risk of macrovascular and microvascular complications, we focused on diabetic patients. In this sense, we excluded patients without diabetes from this study. Due to this restriction of subjects, our study precisely compared the outcomes of the studies of diabetic cohorts. On the other hand, out study was not able to describe the risk of patients with diabetes compared to those without diabetes.

The strength of this study is the listing of all studies allowing readers to see the inconsistency across cohorts. The limitations of this study should also be noted. First, the numbers of studies

regarding the associations between low eGFR and cardiovascular mortality, all-cause mortality, and renal events were small. Although low eGFR was considered as a risk factor for cardiovascular events according to the guidelines developed by KDIGO in 2002, there were few studies from this viewpoint prior to this time. [44] Second, each study had its own definition of normal eGFR as the reference category for multivariate analysis. Some studies [10,19] defined normal eGFR as >90 mL/min/1.73 m^2, while others [6,20] used a definition of >60 mL/min/1.73 m^2. The difference in definition may have affected the magnitude of pooled risk ratio for each outcome. Third, there were differences in measurement and expression of albuminuria, such as daily excretion of albumin, or the ratio of urinary albumin to creatinine. Moreover, measurement of urinary albumin was still not standardized. [52,53,54] A standardized method for measurement of albuminuria is essential for comparing data across studies. Furthermore, collection of urine was also not standardized. Spot urine sample collection in the morning or daily collection of urine would lead to different magnitudes of risk ratio.' [55] With regard to expression of urinary albumin, some guidelines [56,57,58] use albumin/creatinine ratio. However, other expressions were also used in different studies, such as 24-h excretion or concentration of urinary albumin. Fourth, there may be problems associated with reporting bias, especially for renal events. Some studies measuring serum creatinine at baseline did not report renal outcome. The outcome reporting bias may have increased the influence of renal outcome, which is a very large risk ratio compared with cardiovascular or all-cause mortality. Fifth, the numbers of studies reporting the influence of low eGFR were small. Our search strategy limited objects as "diabetes with albuminuria/proteinuria" or "diabetic nephropathy." Therefore, studies of diabetic patients with low eGFR may not have been included in our systematic review due to our search strategy. Sixth, making the best use of information about study design or baseline characteristics, the threshold of study size was not used as a limitation in study selection. These selection criteria resulted in more than half of the selected studies consisted of less than 1000 participants.

With regard to the effects of albuminuria and eGFR in diabetic patients, the Chronic Kidney Disease Prognosis Consortium (CKDPC) reported a precise estimate of risk [59]. In addition, our study provided further information showing the inconsistency of study design or subgroup analysis, and presented pooled risk ratio by category of albuminuria and low eGFR for use in clinical care. Moreover, information about intervention or race (except Caucasian) is limited in both the report of CKDPC and this systematic review.

In summary, we conducted a systematic review and meta-analysis, including 148350 cases, and described the impacts of albuminuria and low eGFR on the risks of cardiovascular mortality, all-cause mortality, and renal events. Micro- and macroalbuminuria were significant risk factors for all three outcomes, and low eGFR and albuminuria may be independent risk factors. There was less evidence exploring the influences of low eGFR as independent risk factor on the outcomes. To evaluate the effects of low eGFR, intervention, or race, including Asian subjects, individual patient data meta-analysis or long-term prospective studies based on individual patient data are needed.

Supporting Information

Figure S1 Subgroup analysis for examination of potential sources of heterogeneity in the association between micro- or macroalbuminuria and cardiovascular mortality, all-cause mortality or renal events.
(TIFF)

Figure S2 Age stratified analysis for the association between albuminuria and cardiovascular mortality, all-cause mortality, and renal events compared with normoalbuminuria.
(TIFF)

Author Contributions

Conceived and designed the experiments: TT KF TN MS AH YI SK TW. Performed the experiments: TT TN MS. Analyzed the data: TT TN TW. Contributed reagents/materials/analysis tools: TT TN TW. Wrote the paper: TT KF TN SK TW.

References

1. Danaei G, Finucane MM, Lu Y, Singh GM, Cowan MJ, et al. (2011) National, regional, and global trends in fasting plasma glucose and diabetes prevalence since 1980: systematic analysis of health examination surveys and epidemiological studies with 370 country-years and 2·7 million participants. Lancet 378: 31–40.
2. Yang SH, Dou KF, Song WJ (2010) Prevalence of diabetes among men and women in China. The New England journal of medicine 362: 2425–6; author reply 2426.
3. Soriguer F, Goday A, Bosch-Comas A, Bordiú E, Calle-Pascual A, et al. (2012) Prevalence of diabetes mellitus and impaired glucose regulation in Spain: the Di@bet.es Study. Diabetologia 55: 88–93.
4. Adler AI, Stevens RJ, Manley SE, Bilous RW, Cull CA, et al. (2003) Development and progression of nephropathy in type 2 diabetes: the United Kingdom Prospective Diabetes Study (UKPDS 64). Kidney international 63: 225–232.
5. Wada T, Shimizu M, Toyama T, Hara A, Kaneko S, et al. (2012) Clinical impact of albuminuria in diabetic nephropathy. Clinical and experimental nephrology 16: 96–101.
6. Vlek ALM, Van der Graaf Y, Spiering W, Algra A, Visseren FLJ (2008) Cardiovascular events and all-cause mortality by albuminuria and decreased glomerular filtration rate in patients with vascular disease. Journal of internal medicine 264: 351–360.
7. The Diabetes Control and Complications Trial Research Group (1993) The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The New England journal of medicine 329: 977–986.
8. Ohkubo Y, Kishikawa H, Araki E, Miyata T, Isami S, et al. (1995) Intensive insulin therapy prevents the progression of diabetic microvascular complications in Japanese patients with non-insulin-dependent diabetes mellitus: a randomized prospective 6-year study. Diabetes research and clinical practice 28: 103–117.
9. Haller H, Ito S, Izzo JL, Januszewicz A, Katayama S, et al. (2011) Olmesartan for the delay or prevention of microalbuminuria in type 2 diabetes. The New England journal of medicine 364: 907–917.
10. Ninomiya T, Perkovic V, De Galan BE, Zoungas S, Pillai A, et al. (2009) Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. Journal of the American Society of Nephrology?: JASN 20: 1813–1821.
11. Yokoyama H, Oishi M, Kawai K, Sone H (2008) Reduced GFR and microalbuminuria are independently associated with prevalent cardiovascular disease in Type 2 diabetes: JDDM study 16. Diabetic medicine: a journal of the British Diabetic Association 25: 1426–1432.
12. Sarnak MJ, Levey AS, Schoolwerth AC, Coresh J, Culleton B, et al. (2003) Kidney disease as a risk factor for development of cardiovascular disease: a statement from the American Heart Association Councils on Kidney in Cardiovascular Disease, High Blood Pressure Research, Clinical Cardiology, and Epidemiology and Prevention. Circulation 108: 2154–2169.
13. Begg CB, Mazumdar M (1994) Operating characteristics of a rank correlation test for publication bias. Biometrics 50: 1088–1101.
14. Egger M, Davey Smith G, Schneider M, Minder C (1997) Bias in meta-analysis detected by a simple, graphical test. BMJ (Clinical research ed) 315: 629–634.
15. Florkowski CM, Scott RS, Coope PA, Moir CL (2001) Predictors of mortality from type 2 diabetes mellitus in Canterbury, New Zealand; a ten-year cohort study. Diabetes research and clinical practice 53: 113–120.
16. Tong PCY, Kong AP, So WY, Yang X, Ng MCY, et al. (2007) Interactive effect of retinopathy and macroalbuminuria on all-cause mortality, cardiovascular and renal end points in Chinese patients with Type 2 diabetes mellitus. Diabetic medicine?: a journal of the British Diabetic Association 24: 741–746.