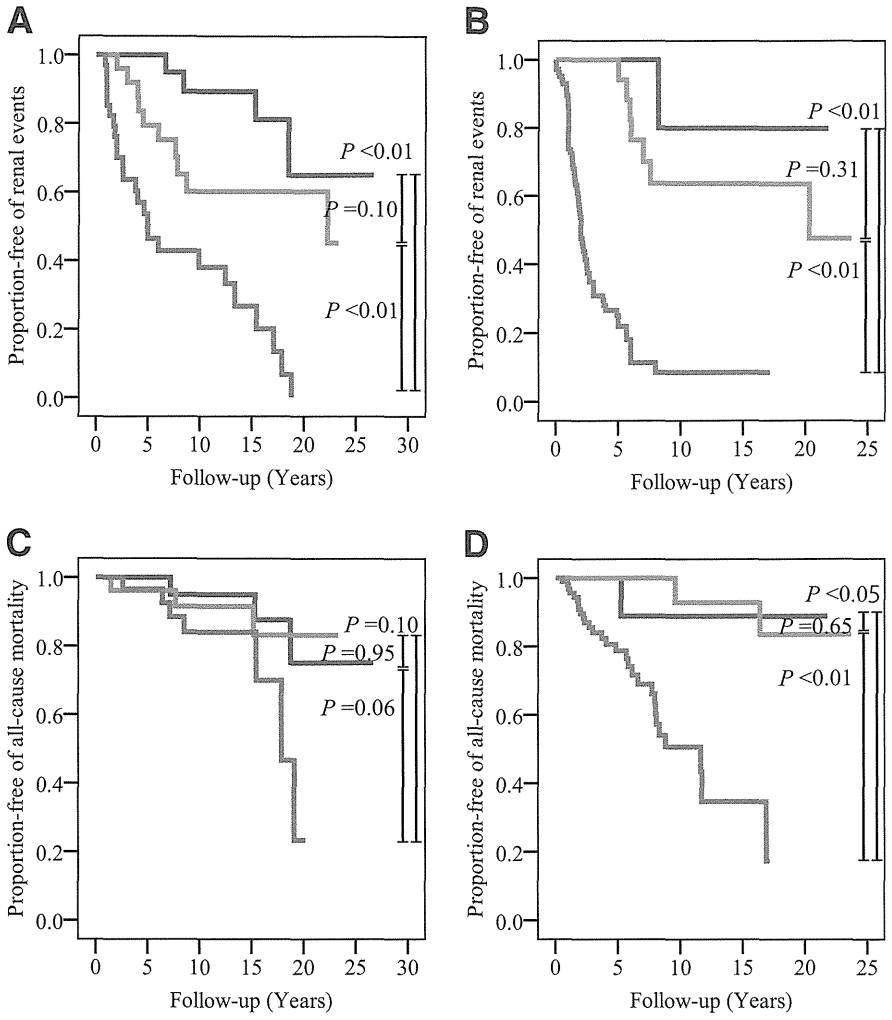

Figure 1—*Event-free rate stratified by albuminuria (proteinuria) and eGFR categories.* A: *Event-free rate of renal events stratified by albuminuria (proteinuria) in the eGFR ≥60 mL/min/1.73 m^2 category according to the Kaplan-Meier method. Blue line, normoalbuminuria (normal proteinuria) and eGFR ≥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.

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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.

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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

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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

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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.

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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.
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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-

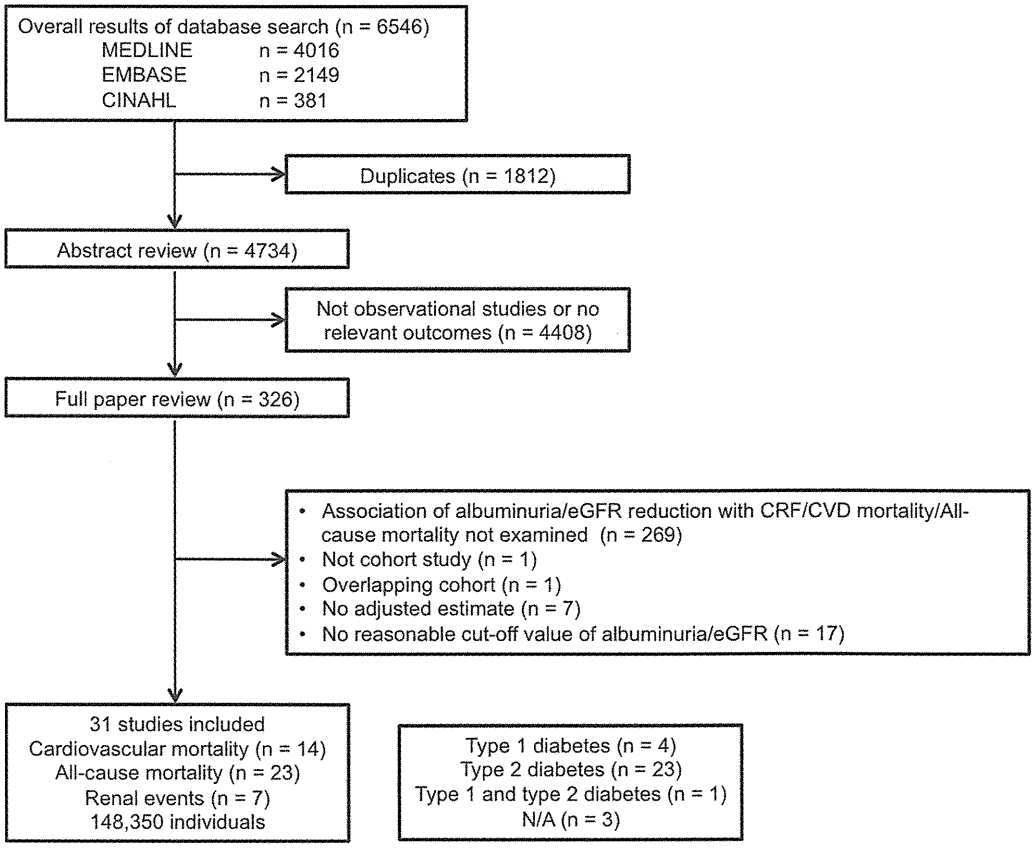

Figure 1. Process for identification of eligible studies Abbreviation: N/A, not available.
doi:10.1371/journal.pone.0071810.g001

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.
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