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

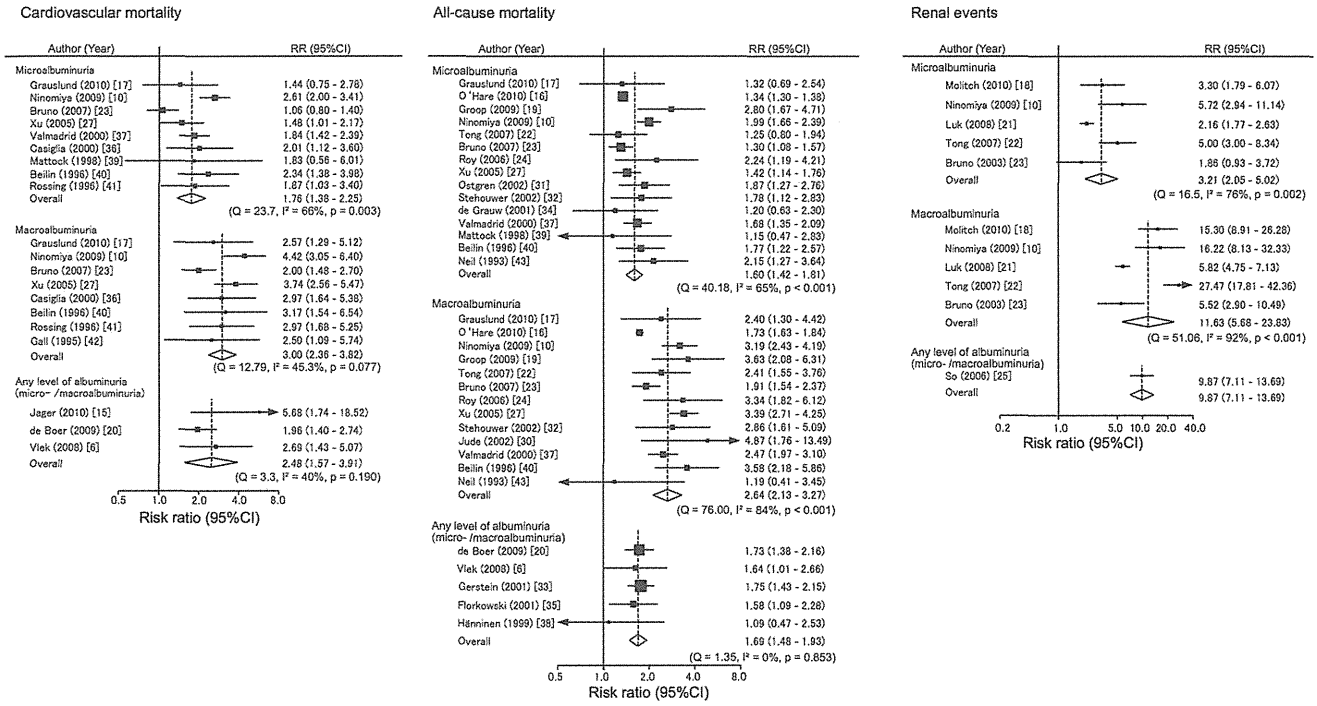

Figure 2. Risk ratio for the association between albuminuria and cardiovascular mortality, all-cause mortality, and renal events compared with normoalbuminuria. Abbreviations: CI, confidence interval; RR, risk ratio.
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buminuria, and there was no significant 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

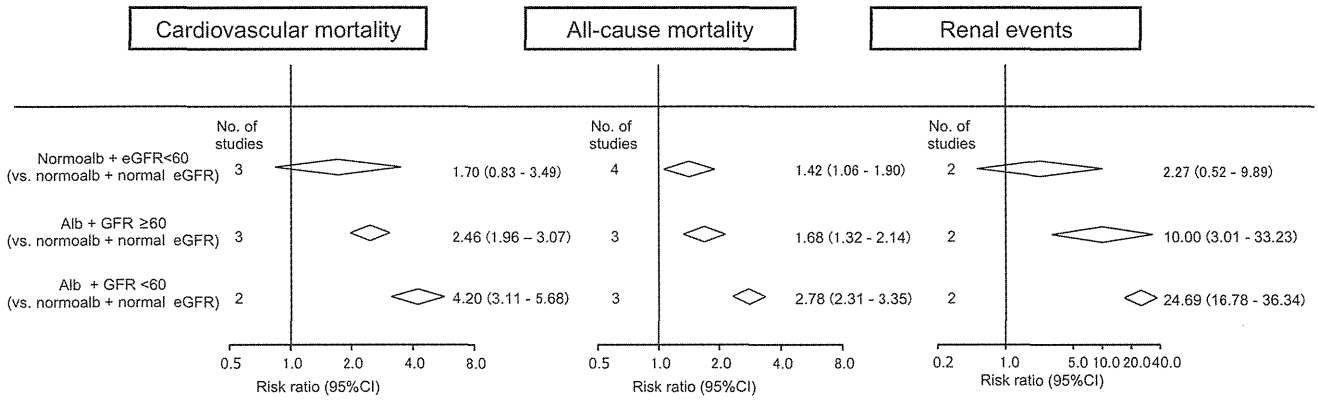

Figure 3. Risk ratio for the association of low eGFR with the risk of each outcome according to the presence of albuminuria, compared with normal eGFR and normoalbuminuria. Albuminuria was defined as any level of albuminuria or pooled estimate of microalbuminuria and macroalbuminuria. Abbreviations: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

17. Molitch ME, Steffes M, Sun W, Rutledge B, Cleary P, et al. (2010) Development and progression of renal insufficiency with and without albuminuria in adults with type 1 diabetes in the diabetes control and complications trial and the epidemiology of diabetes interventions and complications study. Diabetes care 33: 1536–1543.
18. O'Hare AM, Hailpern SM, Pavkov ME, Rios-Burrows N, Gupta I, et al. (2010) Prognostic implications of the urinary albumin to creatinine ratio in veterans of different ages with diabetes. Archives of internal medicine 170: 930–936.
19. So WY, Kong APS, Ma RCW, Ozaki R, Szeto CC, et al. (2006) Glomerular filtration rate, cardiorenal end points, and all-cause mortality in type 2 diabetic patients. Diabetes care 29: 2046–2052.
20. De Boer IH, Katz R, Cao JJ, Fried LF, Kestenbaum B, et al. (2009) Cystatin C, albuminuria, and mortality among older adults with diabetes. Diabetes care 32: 1833–1838.
21. Jager A, Kostense PJ, Ruhé HG, Heine RJ, Nijpels G, et al. (1999) Microalbuminuria and peripheral arterial disease are independent predictors of cardiovascular and all-cause mortality, especially among hypertensive subjects: five-year follow-up of the Hoorn Study. Arteriosclerosis, thrombosis, and vascular biology 19: 617–624.
22. Grauslund J, Jørgensen TMM, Nybo M, Green A, Rasmussen LM, et al. (2010) Risk factors for mortality and ischemic heart disease in patients with long-term type 1 diabetes. Journal of diabetes and its complications 24: 223–228.
23. Groop P, Thomas MC, Moran JL, Wadèn J, Thorn LM, et al. (2009) The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. Diabetes 58: 1651–1658.
24. Luk AOY, So W-Y, Ma RCW, Kong APS, Ozaki R, et al. (2008) Metabolic syndrome predicts new onset of chronic kidney disease in 5,829 patients with type 2 diabetes: a 5-year prospective analysis of the Hong Kong Diabetes Registry. Diabetes care 31: 2357–2361.
25. Bruno G, Merletti F, Bargero G, Novelli G, Melis D, et al. (2007) Estimated glomerular filtration rate, albuminuria and mortality in type 2 diabetes: the Casale Monferrato study. Diabetologia 50: 941–948.
26. Roy M, Rendas-Baum R, Skurnick J (2006) Mortality in African-Americans with Type 1 diabetes: The New Jersey 725. Diabetic medicine: a journal of the British Diabetic Association 23: 698–706.
27. Retnakaran R, Cull CA, Thorne KI, Adler AI, Holman RR (2006) Risk factors for renal dysfunction in type 2 diabetes: U.K. Prospective Diabetes Study 74. Diabetes 55: 1832–1839.
28. Xu J, Lee ET, Best LG, Begum M, Knowler WC, et al. (2005) Association of albuminuria with all-cause and cardiovascular disease mortality in diabetes: the Strong Heart Study. The British Journal of Diabetes & Vascular Disease 5: 334–340.
29. Yuyun MF, Dinneen SF, Edwards OM, Wood E, Wareham NJ (2003) Absolute level and rate of change of albuminuria over 1 year independently predict mortality and cardiovascular events in patients with diabetic nephropathy. Diabetic medicine?: a journal of the British Diabetic Association 20: 277–282.
30. Bruno G, Biggeri A, Merletti F, Bargero G, Ferrero S, et al. (2003) Low incidence of end-stage renal disease and chronic renal failure in type 2 diabetes: a 10-year prospective study. Diabetes care 26: 2353–2358.
31. Jude EB, Anderson SG, Cruickshank JK, Srivatsa A, Tentolouris N, et al. (2002) Natural history and prognostic factors of diabetic nephropathy in type 2 diabetes. QJM: monthly journal of the Association of Physicians 95: 371–377.
32. Ostgren CJ, Lindblad U, Melander A, Råstam L (2002) Survival in patients with type 2 diabetes in a Swedish community: skaraborg hypertension and diabetes project. Diabetes care 25: 1297–1302.
33. Stehouwer CDA, Gall M, Twisk JWR, Knudsen E, Emeis JJ, et al. (2002) Increased urinary albumin excretion, endothelial dysfunction, and chronic low-grade inflammation in type 2 diabetes: progressive, interrelated, and independently associated with risk of death. Diabetes 51: 1157–1165.
34. Gerstein HC, Mann JF, Yi Q, Zinman B, Dinneen SF, et al. (2001) Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. JAMA: the journal of the American Medical Association 286: 421–426.
35. De Grauw WJ, Van de Lisdonk EH, Van Gerwen WH, Verstappen M, Van den Hoogen HJ, et al. (2001) Microalbuminuria in patients with Type 2 diabetes mellitus from general practice: course and predictive value. Diabetic medicine?: a journal of the British Diabetic Association 18: 139–143.
36. Casiglia E, Zanette G, Mazza a, Donadon V, Donada C, et al. (2000) Cardiovascular mortality in non-insulin-dependent diabetes mellitus. A controlled study among 683 diabetics and 683 age- and sex-matched normal subjects. European journal of epidemiology 16: 677–684.
37. Valmadrid CT, Klein R, Moss SE, Klein BE (2000) The risk of cardiovascular disease mortality associated with microalbuminuria and gross proteinuria in persons with older-onset diabetes mellitus. Archives of internal medicine 160: 1093–1100.
38. Hänninen J, Takala J, Keinänen-Kiukaanniemi S (1999) Albuminuria and other risk factors for mortality in patients with non-insulin-dependent diabetes mellitus aged under 65 years: a population-based prospective 5-year study. Diabetes research and clinical practice 43: 121–126.
39. Mattock MB, Barnes DJ, Viberti G, Keen H, Burt D, et al. (1998) Microalbuminuria and coronary heart disease in NIDDM: an incidence study. Diabetes 47: 1786–1792.
40. Beilin J, Stanton KG, McCann VJ, Knuiman MW, Divitini ML (1996) Microalbuminuria in type 2 diabetes: an independent predictor of cardiovascular mortality. Australian and New Zealand journal of medicine 26: 519–525.
41. Rossing P, Hougaard P, Borch-Johnsen K, Parving HH (1996) Predictors of mortality in insulin dependent diabetes: 10 year observational follow up study. BMJ (Clinical research ed) 313: 779–784.
42. Gall MA, Borch-Johnsen K, Hougaard P, Nielsen FS, Parving HH (1995) Albuminuria and poor glycemic control predict mortality in NIDDM. Diabetes 44: 1303–1309.
43. Neil A, Hawkins M, Potok M, Thorogood M, Cohen D, et al. (1993) A prospective population-based study of microalbuminuria as a predictor of mortality in NIDDM. Diabetes care 16: 996–1003.
44. Inventory BD, Index CD (2002) K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. American journal of kidney diseases: the official journal of the National Kidney Foundation 39: S1–266.
45. Levey AS, De Jong PE, Coresh J, Nahas M El, Astor BC, et al. (2010) The definition, classification and prognosis of chronic kidney disease: a KDIGO Controversies Conference report. Kidney international 80: 17–28.
46. Gross JL, De Azevedo MJ, Silveiro SP, Canani LH, Caramori ML, et al. (2005) Diabetic nephropathy: diagnosis, prevention, and treatment. Diabetes care 28: 164–176.
47. Dinneen SF, Gerstein HC (1997) The association of microalbuminuria and mortality in non-insulin-dependent diabetes mellitus. A systematic overview of the literature. Archives of internal medicine 157: 1413–1418.
48. Van der Velde M, Matsushita K, Coresh J, Astor BC, Woodward M, et al. (2011) Lower estimated glomerular filtration rate and higher albuminuria are associated with all-cause and cardiovascular mortality. A collaborative meta-analysis of high-risk population cohorts. Kidney international 79: 1341–1352.
49. Gansevoort RT, Matsushita K, Van der Velde M, Astor BC, Woodward M, et al. (2011) Lower estimated GFR and higher albuminuria are associated with adverse kidney outcomes in both general and high-risk populations. A collaborative meta-analysis of general and high-risk population cohorts. Kidney International 80: 1–12.
50. Pohl MA, Blumenthal S, Cordonnier DJ, De Alvaro F, Deferrari G, et al. (2005) Independent and additive impact of blood pressure control and angiotensin II receptor blockade on renal outcomes in the irbesartan diabetic nephropathy trial: clinical implications and limitations. Journal of the American Society of Nephrology?: JASN 16: 3027–3037.
51. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD (1993) The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. The Collaborative Study Group. The New England journal of medicine 329: 1456–1462.
52. Miller WG, Bruns DE, Hortin GL, Sandberg S, Aakre KM, et al. (2009) Current issues in measurement and reporting of urinary albumin excretion. Clinical chemistry 55: 24–38.
53. Sviridov D, Meilinger B, Drake SK, Hoehn GT, Hortin GL (2006) Coelution of other proteins with albumin during size-exclusion HPLC: Implications for analysis of urinary albumin. Clinical chemistry 52: 389–397.
54. Brinkman JW, Bakker SJL, Gansevoort RT, Hillege HL, Kema IP, et al. (2004) Which method for quantifying urinary albumin excretion gives what outcome? A comparison of immunonephelometry with HPLC. Kidney international Supplement: S69–75.
55. Witte EC, Lambers Heerspink HJ, De Zeeuw D, Bakker SJL, De Jong PE, et al. (2009) First morning voids are more reliable than spot urine samples to assess microalbuminuria. Journal of the American Society of Nephrology: JASN 20: 436–443.
56. Diabetes VII, In C (2011) Standards of medical care in diabetes–2011. Diabetes care 34 Suppl 1: S11–61.
57. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. (2007). American journal of kidney diseases?: the official journal of the National Kidney Foundation 49: S12–154.
58. Levey AS, Eckardt K-U, Tsukamoto Y, Levin A, Coresh J, et al. (2005) Definition and classification of chronic kidney disease: a position statement from Kidney Disease: Improving Global Outcomes (KDIGO). Kidney international 67: 2089–2100.
59. Fox CS, Matsushita K, Woodward M, Bilo HJG, Chalmers J, et al. (2012) Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. Lancet 380: 1662–1673.

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REVIEW ARTICLE

Nodular lesions and mesangiolysis in diabetic nephropathy

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Abstract Diabetic nephropathy is a leading cause of end-stage renal failure all over the world. Advanced human diabetic nephropathy is characterized by the presence of specific lesions including nodular lesions, doughnut lesions, and exudative lesions. Thus far, animal models precisely mimicking advanced human diabetic nephropathy, especially nodular lesions, remain to be fully established. Animal models with spontaneous diabetic kidney diseases or with inducible kidney lesions may be useful for investigating the pathogenesis of diabetic nephropathy. Based on pathological features, we previously reported that diabetic glomerular nodular-like lesions were formed during the reconstruction process of mesangiolysis. Recently, we established nodular-like lesions resembling those seen in advanced human diabetic nephropathy through vascular endothelial injury and mesangiolysis by administration of monocrotaline. Here, in this review, we discuss diabetic nodular lesions and its animal models resembling human diabetic kidney lesions, with our hypothesis that endothelial cell injury and mesangiolysis might be required for nodular lesions.

Keywords Diabetic nephropathy · Nodular lesion · Mesangiolysis · Glomerulosclerosis · Extracellular matrix · Matrix metalloproteinase

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Introduction

The number of patients with chronic kidney disease (CKD) on dialysis due to diabetic nephropathy is increasing worldwide. In Japan, the annual incidence of starting dialysis due to diabetic nephropathy has been in first place since 1998, indicating diabetic nephropathy as one of the most important causes of end-stage kidney disease. In clinical settings, insights for functional–pathological relationships in diabetic nephropathy are required for better prognosis. Generally speaking, it appears that in patients with type 2 diabetes, kidney structural changes are more heterogenous and diabetic glomerulopathy lesions are less severe than in type 1 diabetes patients with similar urine albumin levels [1]. The presence of persistent albuminuria is the hallmark of clinical diagnosis of diabetic nephropathy [1, 2]. However, some type 2 diabetic patients with microalbuminuria and proteinuria have lesions showing strikingly normal glomerular appearance or less advanced glomerular diseases than those observed in type 1 diabetes with similar urine albumin excretion [3, 4].

Characteristic pathologic changes of diabetic nephropathy are accumulation of extracellular matrix (ECM) in

glomerular and tubulointerstitial tissues, and exudative lesions [5]. Among these diabetic lesions, specific glomerular lesions in advanced human diabetic nephropathy include nodular lesions, arteriolar lesions (both efferent and afferent arterioles), and doughnut lesions. Nodular lesions are of importance for advanced diabetic nephropathy, which correlates with massive proteinuria in clinical settings. Nodular lesions were first described by Kimmelstiel and Wilson in 1936 [6]. In contrast, nodular lesions are hardly observed in experimental diabetic models showing high glucose levels without other insults. Insults in addition to glucose levels, such as endothelial cell nitric oxide synthase (eNOS) deficiency [7], integrin $\alpha 1$ deficiency [8] and the presence of Thy-1.1 nephritis [9], may be required for the formation of diabetic nodular lesions. Therefore, deeper insights into the pathogenesis of diabetic advanced lesions, including nodular lesions, are required. However, pathogenesis of these specific lesions in advanced diabetic nephropathy remains to be fully investigated. Further studies would be required to determine the pathogenesis of nodular lesions, as well as other lesions characteristic to advanced diabetic nephropathy, such as exudative lesions.

Here in this manuscript, we focus on pathogenesis and lessons from animal models of nodular lesions characteristic to advanced human glomerular lesions, both in type 1 and 2 diabetic patients.

Nodular lesions in advanced human diabetic nephropathy

Nodular lesions in human diabetic nephropathy have been reported in 10–50 % of biopsy specimens in both type 1 and type 2 diabetes [5]. Nodular lesions contain extracellular matrix components, including type IV collagen, which exists in normal glomerular structure. Electron microscopic studies revealed that nodular lesions consisted of accumulation of mesangial matrix with visible collagen fibrils, fibrils of unknown origin, small lipid particles, and cell debris [10–12]. Nodular lesions appear to develop from the central area of a stalk of a glomerular tuft, and encroach upon the surrounding capillary lumens [12]. In contrast, we previously reported that mesangiolysis might play a role in the formation of nodular lesions, with the deposition of type VI collagen in glomeruli (Fig. 1) [13, 14]. Nodular lesions possibly associated with mesangiolysis sometimes contain laminated structure. Surrounding the strongly Periodic Acid Methenamine silver (PAM)-positive materials, weakly PAM-positive materials with circumferential lamination as nodular lesions were observed (Fig. 1). Type IV collagen was mainly detected in the strongly PAM-positive area, whereas type VI collagen was detected in the weakly PAM-positive area. In this study, we examined 355 kidney specimens obtained from 327 patients with primary diabetes mellitus [13]. This report hypothesizes that: (1) the mesangiolysis is the initial lesion occurring in glomeruli in the process of diabetic nodule formation, and disturbed blood flow into glomeruli, caused by diabetic arteriolosclerosis, may be implicated in the development of the mesangiolysis; and (2) concentric compression of the lysed mesangial matrix by recanalized capillaries forms layered structures and ultimately completed diabetic nodules [13]. Hong et al. reported that diabetic patients with biopsy-proven nodular lesions had longer diabetic durations, more severe renal damage, and exhibited a poorer kidney prognosis. Furthermore, patients with nodular lesions more frequently revealed evidence of diabetic retinopathy [15]. Supporting this notion, Schwartz et al. [16] described that patients with Kimmelstiel–Wilson nodules had elevated serum creatinines and more severe overall retinopathy than those without Kimmelstiel–Wilson nodules. In contrast, kidney biopsy specimens from patients at presentation without overt clinical manifestations of glucose intolerance revealed typical nodular glomerulosclerosis, a negative deposition based on an immunofluorescence study, and neither any significant electron dense deposits nor fibrils on electron microscopy [17]. In this study, Sanai et al. concluded that diabetic nodular glomerulosclerosis occurred in patients without overt diabetes, suggesting the role of factors additional to prolonged hyperglycemia in the pathogenesis of this disorder. Interestingly, supporting this notion, nodular lesions have been also observed in membranoproliferative glomerulonephritis, amyloidosis, and light-chain deposition disease without diabetes [5]. In addition, nodular lesions are also observed in Goodpasture's syndrome [18]. Among these diseases, the strongly PAM-positive materials increased in glomeruli. However, mesangiolysis is not always observed in patients with nodular lesions with or without diabetic conditions. We previously reported nodular lesions in diabetes coexisting with mesaigiolysis or without mesangiolysis [13], although underlining mechanisms remain to be investigated. Further studies to determine mechanisms involved in the pathogenesis of nodular lesions with or without diabetes would be required.

Nodular-like lesions and mesangiolysis in an experimental model

To address the hypothesis that vascular endothelial injury and mesangiolysis are closely related to the formation of nodular lesions in diabetes, we attempted to induce nodular-like lesions resembling those seen in human patients by administration of monocrotaline [19]. In this study, marked mesangial expansion was observed in Otsuka Long-Evans

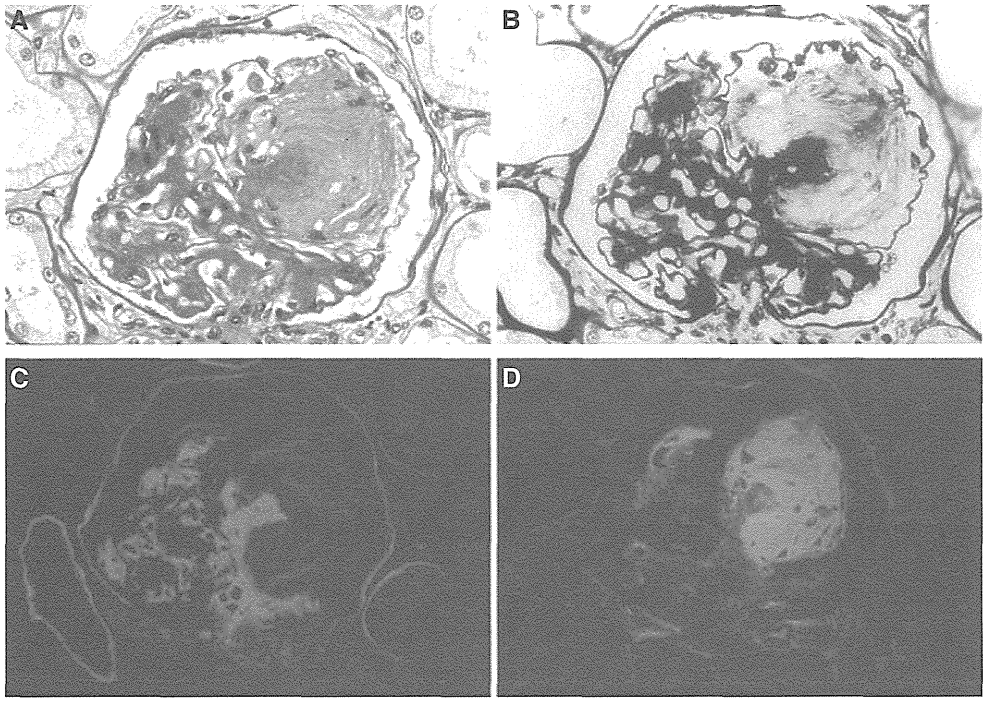

Fig. 1 Human diabetic nodular lesions by light microscopy, using serial sections. Surrounding the strongly Periodic Acid Methenamine silver (PAM)-positive materials, weakly PAM-positive materials with circumferential lamination as nodular lesions were observed (**a** Periodic Acid-Schiff (PAS) stain, ×250, **b** PAM stain, ×250). Type IV collagen was mainly detected in the strongly PAM-positive area (**c** ×250), and type VI collagen in the weakly PAM-positive area (**d** ×250). (From Ref. [14], reproduced with permission from Japanese Society of Nephrology)

Tokushima Fatty (OLETF) rats, in contrast to Long-Evans Tokushima Otsuka (LETO) rats, used as controls, after monocrotaline injection. Moreover, some glomeruli showed typical mesangiolytic lesions and nodular-like lesions in monocrotaline-treated OLETF rats at 50 weeks, concomitant with glomerular capillary endothelial cell swelling and nodular-like lesions and mesangiolysis gradually increased in monocrotaline-treated OLETF rats (Fig. 2) [19]. Matrix metalloproteinase (MMP)-2 and membrane-type 1 (MT1)-MMP proteins increased in the expanded mesangial lesions in OLETF rats as compared to LET rats. Gelatin zymography revealed an increase in 62-kDa activated MMP-2 in the culture supernatants of isolated glomeruli from OLETF rats. In situ enzymatic activity of MMP in the mesangial areas was also detected in 50-week-old monocrotaline-injected OLETF rats. The most compelling part of our study was proceeding to mesangiolysis under hyperglycemic conditions, possibly followed by the formation of nodular-like lesions. These results suggest that diabetic conditions and some toxic events associated with monocrotaline injection during the process of reconstruction from mesangiolysis may be required for the formation of nodular-like lesions resembling human ones in this model, although the lamination of nodules in this model was incomplete.

Nodular-like lesions in experimental models

The Animal Models of Diabetic Complications Consortium (AMDCC), created in 2001, describes research criteria for validating a progressive mouse model of diabetic nephropathy [20]. Especially, pathology of kidneys defines criteria consisting of four issues: (1) advanced mesangial matrix expansion ± nodular sclerosis and mesangiolysis; (2) any degree of arteriolar hyalinosis; (3) glomerular basement membrane thickening by > 50 % over baseline; and (4) tubulointerstitial fibrosis. AMDCC investigators conclude that no current model meets them all. Therefore, the criteria should be viewed as goals, rather than requirements [20].

A few reports have described experimental diabetic models resembling human nodular lesions. Inagi et al. [21] utilized megsin overexpression in a receptor for advanced glycation end products (RAGE) and inducible nitric oxide synthase (iNOS) transgenic mice. In this particular model, the triple transgenic mice overexpressing megsin, RAGE, and iNOS developed, at an early age (16 weeks), severe albuminuria and kidney damage, characterized by development of mesangial expansion, and tubulointerstitial damage, with all of the characteristics of human diabetic nephropathy. In addition, 30–40 %

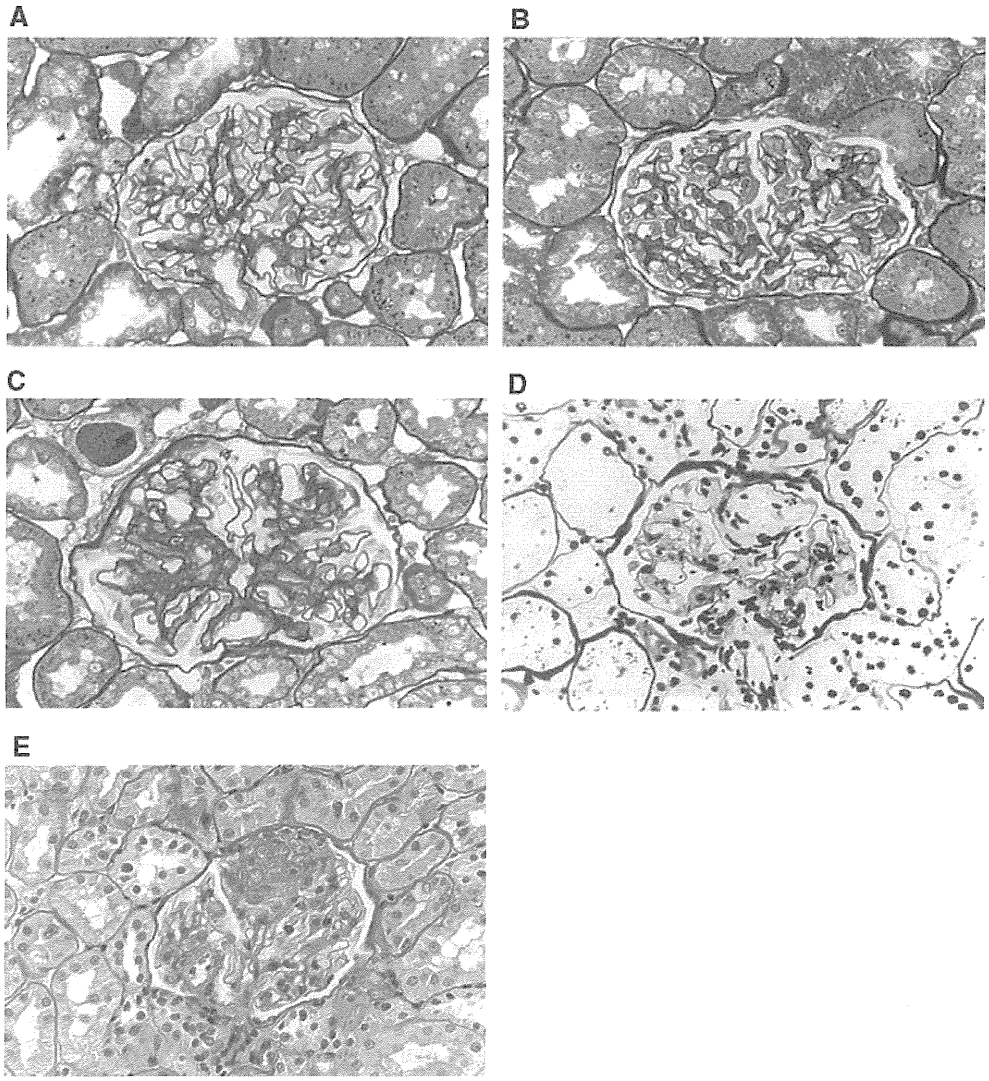

Fig. 2 Mesangiolysis and diffuse glomerulosclerosis detected in monocrotaline-injected Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Representative kidney tissues obtained from a saline-injected Long-Evans Tokushima Otsuka (LETO) rat (50 weeks **a**) and a saline-injected OLETF (50 weeks **c**). A monocrotaline-injected LETO (50 weeks **b**), and a monocrotaline-injected OLETF (50 weeks **d**, **e**) showing mesangiolysis and nodular-like lesions. **a**–**d** PAM stain, ×400; **e** PAS stain, ×400. (From Ref. [19], reproduced with permission from The European Renal Association, European Dialysis and Transplant Association)

of glomeruli exhibit nodule-like lesions with an increase in local oxidative stress, which may be relevant to previous reports describing the possible involvement of oxidative stress [22]. Further, endothelial nitric oxide synthase (eNOS) (−/−) mice, backcrossed to C57BLKS/J db/db mice, exhibited dramatic albuminuria, arteriolar hyalinosis, increased glomerular basement membrane thickness, mesangial expansion, mesangiolysis, and focal segmental and early nodular glomerulosclerosis by 26 weeks, although the severity of hyperglycemia was similar to C57BLKS/J db/db mice [7]. Remarkably, eNOS(−/−) C57BLKS db/db exhibited decreases in GFR to levels < 50 % of that in eNOS(+/+) C57BLKS db/db. Similarly, The $eNOS^{-/-}/lepr^{db/db}$ double-knockout mice showed significant glomerular injury, including mesangiolysis, microaneurysms, increased mesangial matrix expansion with nodular lesions (nodular glomerulosclerosis) and globally sclerotic glomeruli [23]. Further, in streptozotocin-induced eNOS knockout mice, deficiency of eNOS-derived NO causes glomerular endothelial injury in the setting of diabetes and results in overt albuminuria and glomerular mesangiolysis and nodular-like lesions in nephropathy-resistant inbred C57BL6 mice [24]. Supportingly, the level of eNO may be one of the critical determinants for whether patients with diabetes are at risk for developing nephropathy, including nodular-like lesions [25].