Reduced GFR and albuminuria in Chinese type 2 diabetes mellitus patients are both independently associated with activation of the TNF- α system

D. P. K. Ng · M. Fukushima · B. C. Tai · D. Koh ·
H. Leong · H. Imura · X. L. Lim

Received: 2 April 2008 / Accepted: 5 August 2008 / Published online: 7 October 2008
© Springer-Verlag 2008

Abstract

Aims/hypothesis The involvement of chronic inflammation in albuminuria and renal function was investigated in a cross-sectional study of 320 type 2 diabetic Chinese patients from the Singapore Diabetes Cohort Study.

Methods Plasma levels of TNF- α and its two cellular receptors and of IL-6 and C-reactive protein (CRP) were measured. A composite TNF- α score was extracted using principal component analysis. Multiple linear regression analysis was implemented to evaluate the relationship between $\log_e$ (ln) albumin:creatinine ratio (ACR) and estimated GFR (eGFR) with the inflammatory variables and other clinical covariates. A Bonferroni correction was applied based on the total number of variables entered into regression analyses.

Results ln ACR was significantly associated with TNF- α score independently of eGFR even after a Bonferroni correction. TNF- α score was also significantly associated with eGFR independently of ln ACR even after correction for

multiple testing. These findings were similar when the individual molecules of the TNF- α system were analysed separately instead of using the composite TNF- α score. No association was observed for IL-6 and CRP with either renal trait. Diabetes duration was a significant predictor for ln ACR but not eGFR. Conversely, age was significantly associated with eGFR but not ln ACR.

Conclusions/interpretation Activation of the TNF- α system may potentially exert independent effects on ln ACR and eGFR in type 2 diabetes. Because of the study design, one may also consider the possibility that changes in these renal traits may conversely be responsible for such an inflammatory response.

Keywords Albuminuria · Bonferroni correction · CRP · Glomerular filtration rate · IL-6 · Inflammation · Modification of diet in renal disease · TNF- α

Electronic supplementary material The online version of this article (doi:10.1007/s00125-008-1162-1) contains supplementary material, which is available to authorised users.

D. P. K. Ng (✉) · B. C. Tai · D. Koh · X. L. Lim
Department of Community, Occupational and Family Medicine,
Yong Loo Lin School of Medicine,
National University of Singapore,
16 Medical Drive MD3,
Singapore 117597, Singapore
e-mail: cofnpkd@nus.edu.sg

M. Fukushima · H. Imura
Health Informatics Research Group,
Foundation for Biomedical Research and Innovation,
Kobe, Japan

H. Leong
Clinical Services, National Healthcare Group Polyclinics,
Singapore, Singapore

Abbreviations

ACR	albumin:creatinine ratio
CRP	C-reactive protein
eGFR	estimated GFR
HPFS	Health Professionals Follow-up Study
MAP	mean arterial pressure
p_c	Bonferroni-corrected p value
SDCS	Singapore Diabetes Cohort Study
sTNFR1	soluble receptor 1 for TNF- α
sTNFR2	soluble receptor 2 for TNF- α

Introduction

Chronic low-grade inflammation has been postulated to be a pathogenetic mechanism underlying the development of diabetic nephropathy [1]. Although inflammation has

traditionally been conceived as a process that stems from macrophage infiltration, more recent evidence suggests that even cells distant from the blood stream, such as podocytes, can produce inflammatory cytokines [2]. Indeed, preliminary observations of a systemic inflammation have been reported in diabetic patients with diabetic nephropathy. Specifically, albuminuria in both type 1 and type 2 diabetic patients has been associated with serum levels of TNF- α , IL-6 and C-reactive protein (CRP) [1, 3].

While albuminuria has been the primary endpoint of interest in these studies, it is much less clear whether this pro-inflammatory response is also implicated in the modulation of GFR, a measure of renal function. This aspect is particularly of interest with newer insights from genetic studies that provocatively suggest that variation in albuminuria and GFR are possibly two different underlying phenotypes in diabetic nephropathy [4, 5]. Extending this hypothesis, various risk factors may play a role in causing either phenotype, or even both [6]. With a mind to the above, we have here set out to clarify whether systemic inflammation is associated with variation in both albuminuria and GFR in type 2 diabetes in a sample of 320 type 2 diabetic Chinese patients residing in Singapore.

Methods

Patients Type 2 diabetic Chinese patients included in this study were from among patients recruited into the Singapore Diabetes Cohort Study (SDCS). The research protocol was approved by both the National University of Singapore Institutional Review Board (NUS-012) and the National Healthcare Group Domain-Specific Review Board (C/05/118) and patients participating in this study gave informed consent. Briefly, since 2004 all patients previously diagnosed with type 2 diabetes and who were being treated at primary care facilities of the National Healthcare Group Polyclinics in Singapore were invited to join the SDCS. Of the patients approached, 91% agreed to participate in the study and formed a part of our SDCS patient group. Consenting patients completed a questionnaire to elicit information on demographics, lifestyle factors and medical family history and also had their physical measurements taken. Blood and spot urine specimens were obtained for laboratory analyses and medical records were reviewed to obtain information on their metabolic control and the presence of comorbidities and complications. A sample of 320 patients with fasting blood specimens was available at the time of this study. Only Chinese patients were included in this present study.

Laboratory measurements CRP was measured by an ultra-sensitive competitive immunoassay, as described previously [7]. IL-6 was measured by an ELISA (Quantikine IL-6;

R&D Systems, Oxford, UK) as described previously [8]. TNF- α was measured by enzyme immunoassay (Quantikine HS Human TNF- α immunoassay; R&D systems, Minneapolis, MN, USA) as previously described, and the soluble forms of its two cellular receptors, sTNFR1 and sTNFR2, were measured by ELISAs (Quantikine Human sTNFR1 and sTNFR2 immunoassays; R&D Systems, Minneapolis) [9, 10].

Statistical analyses Renal function was estimated by the simplified Modification of Diet in Renal Disease equation where the estimated GFR (eGFR) = $186.3 \times (\text{plasma creatinine in } \mu\text{mol/l} \times 0.011)^{-1.154} \times (\text{age in years})^{-0.203} \times 0.742$ for women $\times 1.21$ if the individual is black [11]. Mean arterial pressure (MAP) was calculated as $(2 \times \text{diastolic blood pressure (mmHg)} + [\text{systolic blood pressure (mmHg)}]) / 3$.

All statistical analyses were performed using STATA (version 10). Student's *t* test was used to compare differences in means between two groups of interest. In the case of skewed data (e.g. albumin:creatinine ratio [ACR], triacylglycerol level, CRP and diabetes duration), natural logarithmic ($\log_e$ [ln]) transformations were carried out to normalise the data before the *t* test was implemented. Further, the Pearson's correlation coefficient was estimated to evaluate the strength of relationship between continuous outcomes of interest. A composite score of TNF- α , sTNFR1 and sTNFR2 was extracted using principal component analysis with varimax rotation. Since the three molecules of the TNF- α system were highly correlated, the composite score was subsequently used in the multiple linear regression analysis when assessing the relationship of ln ACR and eGFR with inflammatory cytokines (namely, IL-6, CRP and composite TNF- α score). In our reasoning, a composite TNF- α score may potentially represent a useful way of summarising the information provided by all three molecules of the TNF- α system. At the same time, using the TNF- α score bypassed the problem of multi-collinearity if TNF- α , sTNFR1 and sTNFR2 were included simultaneously in regression analyses. TNF- α score correlated most strongly with sTNFR2, followed by sTNFR1 and last, TNF- α (Electronic Supplementary Material [ESM] Fig. 1). As supplementary analyses, regression was repeated considering only one molecule of the TNF- α system at a time while excluding the others. As assessed by the fit of the regression models (R^2), the TNF- α score appeared to perform better than TNF- α alone, and was comparable to the individual receptors (ESM Tables 1, 2, 3, 4, 5, 6).

Multiple linear regression analysis was implemented to evaluate the relationship between ln ACR and eGFR with potential risk factors. Besides the inflammatory variables (IL-6, CRP and TNF- α score), the covariates that were initially considered in the regression analyses were age at recruitment, sex, diabetes duration, BMI, WHR, HbA_{1c}, ln triacylglycerol, MAP, total cholesterol, LDL-cholesterol,

HDL-cholesterol and diabetes treatment modality. For the variable selection, nested models were compared using the *F* statistic. Bonferroni corrections were applied based on the total number of inflammatory variables and covariates tested. A Bonferroni-corrected *p* value (*p_c*) <0.05 was considered statistically significant.

Results

Patient characteristics

Selected characteristics of the 320 study patients are shown in Table 1. Both sexes were similar in all the various measurements except that females had smaller WHRs (*p_c*<0.05) and higher HDL-cholesterol levels (*p_c*<0.05). Serum CRP levels were also higher among females compared with males (*p_c*<0.05). The two renal measurements (i.e. ln ACR and eGFR) were only modestly correlated (Pearson's correlation coefficient *r* value −0.204, uncorrected *p*<0.001) (Fig. 1).

Inflammatory molecules Plasma levels of five inflammatory molecules of interest (IL-6, CRP, TNF-α, sTNFR1 and sTNFR2) were measured. The presence of these molecules were correlated with each other with *r* values ranging from

a modest 0.203 (ln CRP vs TNF-α), to a higher 0.835 (sTNFR1 vs sTNFR2) (Fig. 1). As anticipated, the three molecules of the TNF-α system (which included TNF-α and its two receptors) were also highly correlated with *r*>0.649 (Fig. 1).

Association of plasma inflammatory markers and covariates with albuminuria (ACR) In the multivariate analyses, ln ACR was significantly associated with TNF-α score, with a unit change in TNF-α score resulting in a 0.20 unit change in ln ACR (ACR in g/kg) (95% CI 0.13–0.27, uncorrected *p*<0.001). This association between ln ACR and TNF-α score persisted regardless of whether eGFR was excluded (Model 1) or deliberately retained (Model 2) in the model (Table 2). This association remained significant even after correction for multiple testing (*p_c*<0.05) (Table 2). Multivariate analyses considering the individual molecules of the TNF-α system alone instead of the composite TNF-α score yielded similar results (ESM Tables 1, 2, 3). Neither CRP nor IL-6 levels were associated with ln ACR (uncorrected *p*>0.05) (Table 2). This lack of association with IL-6 and CRP was evident even in regression analyses that excluded TNF-α score (data not shown). Among the covariates, only diabetes duration showed a clear association with ln ACR after taking into

Table 1 Characteristics of the Chinese type 2 diabetes patients

Characteristic	All (<i>n</i> =320)	Male (<i>n</i> =153)	Female (<i>n</i> =167)	Uncorrected <i>p</i> value
Age at recruitment (years)	64 (10)	63 (10)	65 (9)	0.158
Diabetes duration (years)	9 (9)	9 (9)	9 (9)	0.997
BMI (kg/m ²)	25.7 (3.7)	25.5 (3.3)	25.9 (4.0)	0.334
WHR	0.90 (0.06)	0.94 (0.05)	0.86 (0.06)	<0.001 ^a
HbA _{1c} (%)	7.4 (1.0)	7.4 (1.0)	7.4 (1.0)	0.748
MAP (mmHg)	97 (8)	97 (9)	96 (8)	0.165
Triacylglycerol (mmol/l)	1.32 (0.34–4.60)	1.30 (0.34–4.60)	1.33 (0.38–4.07)	0.250
Total cholesterol (mmol/l)	4.73 (0.80)	4.63 (0.74)	4.82 (0.85)	0.041
LDL-cholesterol (mmol/l)	2.80 (0.70)	2.82 (0.67)	2.78 (0.73)	0.597
HDL-cholesterol (mmol/l)	1.26 (0.31)	1.17 (0.30)	1.34 (0.31)	<0.001 ^a
Diabetes treatment modality, <i>n</i> (%)				
Diet and exercise	68 (21.7)	37 (24.7)	31 (18.9)	0.534
Oral hypoglycaemic agents only	211 (67.2)	95 (63.3)	116 (70.7)	
Oral hypoglycaemic agents and insulin	28 (8.9)	14 (9.3)	14 (8.5)	
Insulin alone	7 (2.2)	4 (2.7)	3 (1.8)	
ACR (g/kg) ^b	9.6 (1.4–3,905.7)	9.2 (1.4–1,492.7)	9.9 (1.4–3,905.7)	0.249
eGFR (ml min ^{−1} 1.73 m ^{−2})	78.9 (24.6)	76.9 (21.72)	80.7 (26.85)	0.166
IL-6 (ng/l)	3.35 (2.29)	2.96 (1.62)	3.52 (2.74)	0.023
TNF-α (ng/l)	1.81 (0.74)	1.80 (0.72)	1.83 (0.76)	0.709
sTNFR1 (ng/l)	1,544.7 (621.9)	1,541.1 (518.2)	1,547.9 (704.2)	0.922
sTNFR2 (ng/l)	2,472.3 (859.6)	2,481.6 (751.9)	2,464.0 (948.8)	0.854
CRP (μg/l)	1,237 (70–9,761)	924 (70–9,761)	1,605 (96–8,476)	<0.001 ^a

Data are presented as means (SD), medians (range) or *n* (%)

^a *p_c*<0.05

^b To convert ACR in g/kg to μg/μmol, divide by 8.57

Fig. 1 Pearson's correlation matrix for eGFR, ln ACR and inflammatory molecules. ** $p<0.01$; *** $p<0.001$. eGFR and ln ACR are expressed in $\text{ml min}^{-1} 1.73 \text{ m}^{-2}$ and g/kg , respectively (to convert ACR in g/kg to $\mu\text{g}/\mu\text{mol}$, divide by 8.57). Inflammatory molecules are in ng/l except for CRP ($\mu\text{g/l}$)

account multiple testing (uncorrected $p<0.001$, $p_c<0.05$) (Table 2).

Association of plasma inflammatory markers with eGFR
We next hypothesised that the inflammatory markers studied might be associated with eGFR independently of ln ACR. To test this possibility, we repeated the regression analyses with eGFR as the dependent variable (Table 3). The TNF-α score was associated with eGFR whether in the absence (Model 1) or with deliberate retention of ln ACR as a covariate (Model 2) (Table 3); this association remained significant even after correction for multiple testing ($p_c<0.05$). On average, a unit increase in TNF-α score is associated

with a reduction in eGFR of 12 units (95% CI 9–14). Multivariate analyses considering the individual molecules of the TNF-α system alone instead of the composite TNF-α score yielded similar results (ESM Tables 4, 5, 6). Neither IL-6 nor CRP showed any significant association with eGFR (uncorrected $p>0.05$), even with the exclusion of TNF-α score from the regression analyses (data not shown). In contrast to our analyses with ln ACR as the outcome variable, age was the only covariate that showed significant evidence for association with eGFR (uncorrected $p<0.001$; $p_c<0.05$) (Table 3). Although the type of diabetes treatment modality was associated with the various TNF variables (namely, TNF-α score, TNF-α, sTNFR1 and sTNFR2)

Table 2 Association between ln ACR and inflammatory variables in multivariate analyses^a

Model	Estimate (95% CI)	Uncorrected p value	R^2
Model 1 (without eGFR)			
Inflammatory variable TNF-α score	0.20 (0.13, 0.27)	<0.001 ^b	25.1
Covariate			
ln triacylglycerol	0.15 (0.01, 0.30)	0.041	
MAP	0.01 (0.002, 0.02)	0.011	
Diabetes duration	0.02 (0.01, 0.02)	<0.001 ^b	
Total cholesterol	0.12 (0.04, 0.21)	0.006	
Model 2 (including eGFR)			
Inflammatory variables TNF-α score	0.23 (0.14, 0.31)	<0.001 ^b	25.2
Covariate			
ln triacylglycerol	0.16 (0.02, 0.31)	0.030	
MAP	0.01 (0.002, 0.02)	0.011	
Diabetes duration	0.02 (0.01, 0.02)	<0.001 ^b	
Total cholesterol	0.12 (0.03, 0.21)	0.006	
eGFR	0.002 (−0.001, 0.005)	0.257	

^a Inflammatory variables and covariates that were initially entered into multivariate analyses are listed in Table 1

^b $p_c<0.05$

^c Only inflammatory variables and covariates showing an uncorrected $p<0.05$ are shown unless otherwise indicated

Table 3 Association between eGFR and inflammatory variables in multivariate analyses^a

^a Inflammatory variables and covariates that were initially entered into multivariate analyses are listed in Table 1

^b $p < 0.05$

^c Only inflammatory variables and covariates showing an uncorrected $p < 0.05$ are shown unless otherwise indicated

Model	Estimate (95% CI)	Uncorrected p value	R^2
Model 1 (without ln ACR)			41.8
Inflammatory variable TNF- α score	-11.58 (-13.92, -9.25)	<0.001 ^b	
Covariate			
ln triacylglycerol	-4.59 (-9.03, -0.15)	0.043	
Age	-0.75 (-0.98, -0.51)	<0.001 ^b	
Model 2 (including ln ACR)			41.7
Inflammatory variable TNF- α score	-11.96 (-14.44, -9.48)	<0.001 ^b	
Covariate			
ln triacylglycerol	-4.91 (-9.41, -0.41)	0.032	
Age	-0.74 (-0.98, -0.51)	<0.001 ^b	
ln ACR	1.61 (-1.98, 5.21)	0.377	

(ESM Table 7), it did not emerge as significant predictor of either ln ACR or eGFR in regression analyses.

Discussion

In this present study, we sought to investigate the potential role of inflammation in modulating albuminuria and eGFR in Chinese patients with type 2 diabetes. Our most salient finding was that activation of the TNF- α system was strongly implicated in both renal traits (ln ACR and eGFR). Moreover, this pro-inflammatory effect on one trait was clearly independent of its effect on the other (Tables 2, 3). Given the correlation between ln ACR and eGFR (Fig. 1), this intriguing finding would suggest that although there may be evidence of a systemic inflammation mediated by the TNF- α system in type 2 diabetic patients, the phenotypic manifestation of this underlying process may be distinct. Specifically, some of these patients may manifest albuminuria, while in others there may be an early decline in renal function instead. This observation may suggest that environmental or even genetic factors could potentially influence the association between the TNF- α system and these renal traits. The growing realisation that genetic modulation of albuminuria and impaired renal function may be attributed to different chromosomal susceptibility loci would at least seem to be consistent with this notion [4, 5].

In human studies, the involvement of TNF- α in diabetic nephropathy has primarily been confined to its association with albuminuria [2]. Specifically, serum TNF- α was associated with albuminuria in a pilot study of 65 type 2 diabetic patients [12]. This finding has since been confirmed in a subsequent larger study of 160 diabetic patients [3]. Such a positive association has similarly been reported among type 2 diabetic Japanese patients [13] as well as in Europids with type 1 diabetes [14]. In experimental models of diabetes, urinary albumin excretion has been correlated with urinary levels of TNF- α and with renal TNF- α (also known as *TNF*) gene expression [15, 16]. Inhibition of

TNF- α using two different modalities (the monoclonal antibody infliximab and the p38 mitogen-activated protein kinase inhibitor FR167653) ameliorated urinary albumin excretion with a concomitant reduction in TNF- α excretion in a streptozotocin-induced rat model of diabetes [17]. Taken together, these previous results from both human and animal studies corroborate our present positive findings that the activation of the TNF- α system is very likely implicated in diabetes-induced albuminuria.

In contrast to the literature on albuminuria, much less is known about the possible effect of the TNF- α system on eGFR in diabetes. Indeed, in our literature review, we came across only one report so far and this was based on a cross-sectional analysis of the Health Professionals Follow-Up Study (HPFS). Although that study did find an association between serum levels of sTNFR2 and eGFR in type 2 diabetes [18], there are a couple of important caveats. First, because of the study design, that association was only restricted to men. Second, the potential confounding effect of albuminuria on the association between sTNFR2 levels and eGFR was not evaluated. Also, the statistical significance of the association was not corrected for multiple hypothesis testing. These deficiencies have now been addressed in our present study.

The potential involvement of the TNF- α system in the impairment of renal function may be glimpsed from other studies that have not primarily focused on diabetes [19, 20]. Higher TNFR2 levels have been associated with reduced renal function among predominantly non-diabetic individuals with a history of myocardial infarction [19]. In rodent models, neutralisation of TNF- α by administration of its soluble receptor (TNFRp55) ameliorated the loss in GFR brought on by i.p. injection of bacterial lipopolysaccharide [20]. This detrimental effect of TNF- α on renal dysfunction may be potentially mediated by the effect of this cytokine on the transcriptional regulation of the potent vasoconstrictor endothelin-1 in endothelial and mesangial cells of the glomerulus [21], possibly in concert with a profound alteration in gene expression of various renal glucose transporters

including sodium-dependent co-transporters *SGLT1*, -2 and -3 (also known as *SLC5A1*, -2 and -3, respectively), as well as the glucose transporters *GLUT1* (also known as *SLC2A1*) and *GLUT2* (also known as *SLC2A1*) [22].

Our findings with regard to other inflammatory molecules, IL-6 and CRP, were less convincing in comparison to the TNF- α system. We did not observe any association of IL-6 with albuminuria. This result differed from the earlier findings among type 1 diabetic Europids by Saraheimo et al. [1] This discordance may be due to differences in ethnicity and type of diabetes. It is also unclear if the reported association with IL-6 would have remained statistically significant after correction for multiple hypotheses testing. Of relevance, another previous study had failed to find any significant association of serum IL-6 with raised albumin excretion rates in a study of 72 type 1 diabetic Europids [23].

It has been proposed that CRP may be associated with albuminuria in a study of 194 type 1 diabetic Europids, but since that finding was only borderline significant (uncorrected $p=0.016$) [1], it may still be compatible with our current negative results. With respect to eGFR, our CRP findings were concordant with the reported findings from the cross-sectional analysis of 732 type 2 diabetic men in the HPFS [18]. It was noted in our study that plasma CRP levels were higher in women compared with men, an observation that was consistent with previous reports on diabetic patients [24, 25]. The reason for this sex difference has not been clearly established, although it may be related to the use of oral contraceptive pills [25]. Since information on the use of oral contraceptives was not captured in our study population, this association cannot be confirmed at the moment.

The primary focus of our study has been to examine the possible role of inflammation in diabetic nephropathy. Nevertheless, our concomitant covariate analyses did also highlight several notable findings. Diabetes duration was strikingly associated with ln ACR but not eGFR. On the other hand, age was significantly associated with eGFR, but not ln ACR.

Our study is not devoid of shortcomings. By virtue of its cross-sectional design, conclusions about temporal relationships and the underlying patho-physiological mechanisms are necessarily limited. As such, an alternative interpretation of our findings may be that albuminuria and reduced eGFR could have caused or propagated the observed inflammatory response. This cause-and-effect relationship can only be established using large cohort studies. In the absence of such studies, our study has nevertheless deepened our insight into the possible aetiological factors. Another limitation is that renal function was estimated from creatinine-based equations since direct radioisotope GFR measurements on more than 300 patients included in this

study would have represented a logistical challenge. Albuminuria was also determined on a single random urine sample and this may have reduced the power of our study. Although error in the determination of albuminuria and in the estimation of the true GFR would certainly have blunted any potential association, our positive findings, especially with regards to the TNF- α system, were in our opinion quite convincing.

Limitations aside, our study has several merits. This study was performed on a well-characterised group of patients and its substantial sample size afforded small p values that we then corrected using the conservative Bonferroni procedure. The latter measure gave us confidence that our positive findings were robust and therefore likely to represent real associations. In addition, we have improved upon previous studies by evaluating all three components of the TNF- α system (i.e. TNF- α , sTNFR1 and sTNFR2) instead of focusing only on one [3, 18]. Finally, we have also performed the appropriate statistical analyses with both albuminuria and eGFR as dependent variables, while at the same time, assessing the potential confounding effect of one renal trait on the other.

In conclusion, our study has furnished evidence that the activation of the TNF- α system is likely to exert independent effects on albuminuria and renal function in type 2 diabetes. While the relevance of our findings to diabetes care will require further investigation, our study suggests that exploration of newer therapies targeted at diminishing TNF- α -mediated chronic inflammation may prove beneficial in the prevention and treatment of diabetic nephropathy.

Acknowledgements This research was supported by grant NMRC/1018/2005 from the National Medical Research Council, Singapore, the Leading Project of Biostimulation, the Kobe Translational Research Cluster, and the Knowledge Cluster Initiative from the Ministry of Education, Culture, Sports, Science and Technology, Japan. We thank the doctors and staff of the National Healthcare Group Polyclinics and the National University of Singapore for contributing to the recruitment of patients into the Singapore Diabetes Cohort Study.

Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

References

1. Saraheimo M, Teppo AM, Forsblom C, Fagerudd J, Groop PH (2003) Diabetic nephropathy is associated with low-grade inflammation in Type 1 diabetic patients. *Diabetologia* 46:1402–1407
2. Fornoni A, Ijaz A, Tejada T, Lenz O (2008) Role of inflammation in diabetic nephropathy. *Curr Diabetes Rev* 4:10–17
3. Navarro JF, Mora C, Muros M, García J (2006) Urinary tumour necrosis factor- α excretion independently correlates with clinical markers of glomerular and tubulointerstitial injury in type 2 diabetic patients. *Nephrol Dial Transplant* 21:3428–3434

4. Krolewski AS, Poznik GD, Placha G et al (2006) A genome-wide linkage scan for genes controlling variation in urinary albumin excretion in type II diabetes. *Kidney Int* 69:129–136
5. Placha G, Poznik GD, Dunn J et al (2006) A genome-wide linkage scan for genes controlling variation in renal function estimated by serum cystatin C levels in extended families with type 2 diabetes. *Diabetes* 55:3358–3365
6. Placha G, Canani LH, Warram JH, Krolewski AS (2005) Evidence for different susceptibility genes for proteinuria and ESRD in type 2 diabetes. *Adv Chronic Kidney Dis* 12:155–169
7. Taniguchi A, Nagasaka S, Fukushima M et al (2002) C-reactive protein and insulin resistance in non-obese Japanese type 2 diabetic patients. *Metabolism* 51:1578–1581
8. Taniguchi A, Fukushima M, Ohya M et al (2006) Interleukin 6, adiponectin, leptin, and insulin resistance in nonobese Japanese type 2 diabetic patients. *Metabolism* 55:258–262
9. Taniguchi A, Fukushima M, Nakai Y et al (2006) Soluble tumor necrosis factor receptor 1 is strongly and independently associated with serum homocysteine in nonobese Japanese type 2 diabetic patients. *Diabetes Care* 29:949–950 (letter)
10. Ohya M, Taniguchi A, Fukushima M et al (2005) Three measures of tumor necrosis factor alpha activity and insulin resistance in nonobese Japanese type 2 diabetic patients. *Metabolism* 54:1297–1301
11. National Kidney Foundation (2002) K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 39(Suppl 1):S1–266
12. Navarro JF, Mora C, Maca M, Garca J (2003) Inflammatory parameters are independently associated with urinary albumin in type 2 diabetes mellitus. *Am J Kidney Dis* 42:53–61
13. Moriwaki Y, Yamamoto T, Shibutani Y et al (2003) Elevated levels of interleukin-18 and tumor necrosis factor- α in serum of patients with type 2 diabetes: relationship with diabetic nephropathy. *Metabolism* 52:605–608
14. Schram MT, Chaturvedi N, Schalkwijk CG, Fuller JH, Stehouwer CDA, EURODIAB Prospective Complications Study Group (2005) Markers of inflammation are cross-sectionally associated with microvascular complications and cardiovascular disease in type 1 diabetes—the EURODIAB Prospective Complications Study. *Diabetologia* 48:370–378
15. Kalantarina K, Awad AS, Siragy HM (2003) Urinary and renal interstitial concentrations of TNF- α increase prior to raise in albuminuria in diabetic rats. *Kidney Int* 64:1208–1213
16. Navarro JF, Milena FJ, Mora C et al (2005) Tumor necrosis factor- α gene expression in diabetic nephropathy: relationship with urinary albumin excretion and effect of angiotensin-converting enzyme inhibition. *Kidney Int* 99(Suppl):S98–S102
17. Moriwaki Y, Inokuchi T, Yamamoto A et al (2007) Effect of TNF- α inhibition on urinary albumin excretion in experimental diabetic rats. *Acta Diabetol* 44:215–218
18. Lin J, Hu FB, Rimm EB, Rifai N, Curhan GC (2006) The association of serum lipids and inflammatory biomarkers with renal function in men with type II diabetes mellitus. *Kidney Int* 69:336–342
19. Tonelli M, Sacks F, Pfeffer M, Jhangri GS, Curhan G (2005) The Cholesterol and Recurrent Events (CARE) Trial Investigators: biomarkers of inflammation and progression of chronic kidney disease. *Kidney Int* 68:237–245
20. Knotek M, Rogachev B, Wang W et al (2001) Endotoxemic renal failure in mice: role of tumor necrosis factor independent of inducible nitric oxide synthase. *Kidney Int* 59:2243–2249
21. Marsden PA, Brenner BM (1992) Transcriptional regulation of the endothelin-1 gene by TNF- α . *Am J Physiol Cell Physiol* 262: C854–C861
22. Schmidt C, Hoehrl K, Bucher M (2007) Regulation of renal glucose transporters during severe inflammation. *Am J Physiol Renal Physiol* 292:F804–F811
23. Myrup B, de Maat M, Rossing P, Gram J, Kluft C, Jespersen J (1996) Elevated fibrinogen and the relation to acute phase response in diabetic nephropathy. *Thromb Res* 81:485–490
24. Mackness B, Hine D, McElduff P, Mackness M (2006) High C-reactive protein and low paraoxonase 1 in diabetes as risk factors for coronary heart disease. *Atherosclerosis* 186:396–401
25. Jenkins AJ, Rothen M, Klein RL et al (2008) Cross-sectional associations of C-reactive protein with vascular risk factors and vascular complications in the DCCT/EDICT cohort. *J Diabetes its Complicat* 22:153–163