Renal Failure

ISSN: 0886-022X (Print) 1525-6049 (Online) Journal homepage: https://www.tandfonline.com/loi/irnf20

Association of plasma growth arrest-specific protein 6 (Gas6) concentrations with albuminuria in patients with type 2 diabetes

Aybala Erek-Toprak, Ozlem Bingol-Ozakpinar, Zeynep Karaca, Mehmet Ali Cikrikcioglu, Mehmet Hursitoglu, Ahmet Riza Uras, Khosrow Adeli & Fikriye Uras

To cite this article: Aybala Erek-Toprak, Ozlem Bingol-Ozakpinar, Zeynep Karaca, Mehmet Ali Cikrikcioglu, Mehmet Hursitoglu, Ahmet Riza Uras, Khosrow Adeli & Fikriye Uras (2014) Association of plasma growth arrest-specific protein 6 (Gas6) concentrations with albuminuria in patients with type 2 diabetes, Renal Failure, 36:5, 737-742, DOI: 10.3109/0886022X.2014.883997

To link to this article: https://doi.org/10.3109/0886022X.2014.883997

RENAL FAILURE

http://informahealthcare.com/rnf
ISSN: 0886-022X (print), 1525-6049 (electronic)

Ren Fail, 2014; 36(5): 737–742
 DOI: 10.3109/0886022X.2014.883997

informa
healthcare

CLINICAL STUDY

Association of plasma growth arrest-specific protein 6 (Gas6) concentrations with albuminuria in patients with type 2 diabetes*

Aybala Erek-Toprak[1], Ozlem Bingol-Ozakpinar[2], Zeynep Karaca[2], Mehmet Ali Cikrikcioglu[3], Mehmet Hursitoglu[4], Ahmet Riza Uras[5], Khosrow Adeli[6], and Fikriye Uras[2]

[1]Department of Biochemistry Istanbul, Istanbul Medeniyet University School of Medicine, Istanbul, Turkey, [2]Department of Biochemistry, School of Pharmacy, Marmara University, Istanbul, Turkey, [3]Department of Internal Medicine, Bezmialem Vakif University School of Medicine, Istanbul, Turkey, [4]Department of Internal Medicine, Kanuni Sultan Suleyman Training & Research Hospital, Istanbul, Turkey, [5]Haydarpaşa Numune Training and Research Hospital, Clinical Chemistry Laboratory, Istanbul, Turkey, and [6]Clinical Biochemistry, Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, University of Toronto, Toronto, ON, Canada

Abstract

Aims: New biomarkers are required to detect diabetic nephropathy earlier in persons with type 2 diabetes mellitus. Recent experimental studies indicate that growth arrest-specific protein 6 (Gas6) may have a role in pathogenesis of complications associated with diabetes. The objective of the current study is to examine whether plasma Gas6 concentrations are associated with albuminuria in persons with type 2 diabetes mellitus. *Methods*: About 32 patients with diabetes which have micro or macroalbuminuria, 37 patients with diabetes and normoalbuminuria, and 30 healthy volunteers were recruited. Plasma Gas6 levels were measured by ELISA. Hemoglobin A_{1c} (HbA_{1c}), serum C reactive protein, fibrinogen and 24-h urine samples for microalbuminuria were analyzed by Primus PDQ, Beckman Coulter Immage 800, STA Compact and Roche Cobas Integra 800 analyzer, respectively. Statistical analysis was performed using SPSS (Statistical Package for Social Sciences) for Windows 11.5. *Results*: There was a noteworthy difference among the three groups for Gas6 according to the Kruskal–Wallis test ($p < 0.01$). Plasma Gas6 concentrations were higher in patients with micro or macroalbuminuria [20.9 ng/mL (16.7–27.0); median (25–75% percentile)] compared to patients with normoalbuminuria [16.5 ng/mL (13.1–22.9)], and healthy controls [15.3 ng/mL (8.3–33.6)]. *Conclusions*: In conclusion, this is the first study indicating that plasma Gas6 levels are associated with albuminuria in patients with type 2 diabetes. This study could be considered a starting point to focus on the association between Gas6 and diabetic nephropathy.

Keywords

Albuminuria, biomarkers, diabetic nephropathy, Gas6, growth arrest-specific protein

History

Received 7 October 2013
Revised 24 December 2013
Accepted 28 December 2013
Published online 11 February 2014

Introduction

More than 371 million people worldwide suffer from diabetes mellitus and by 2030 this number is expected to rise to more than 550 million.[1] Diabetes mellitus is the principal factor in chronic kidney disease, responsible for up to 45% of end-stage renal disease.[2] In people with diabetes, nephropathy is defined as the presence of proteinuria. It is characterized by structural abnormalities and is reversible in the early stage.[3] The pathophysiological characteristics of diabetic nephropathy are still in debate. Many attempts have been made to find out new biomarkers for early stage detection of diabetic nephropathy.[4–6]

It has been shown that mesangial cell proliferation is increased in response to a number of growth factors and cytokines: growth arrest-specific 6 (Gas6),[4] platelet-derived growth factor,[7] basic fibroblast growth factor,[8] angiotensin II[6] and interleukin-6.[9] Gas6, a newly discovered vitamin K-dependent protein, is a ligand for TYRO-3, AXL and MER (TAM receptors). Gas6/TAM signaling is involved in cell migration, survival, and adhesion.[10–12]

Initially, one gene that was highly expressed in serum starved cultured cells of NIH 3T3 was Gas6.[13] More recently, it has been classified as a vitamin K-dependent protein, with a similar structure to protein S.[14] Gas6 has an N-terminal γ-carboxylated glutamyl domain containing 11–12 γ-carboxylated glutamyl residues. Vitamin K-dependent carboxylase enzyme modifies glutamyl residues to γ-carboxylated glutamyl residues post-translationally.[15] Carboxylation helps binding of the vitamin K-dependent clotting factors to membrane phospholipids on platelets which lead to the triggering of the coagulation pathway.[16–18] In the absence of carboxylation, the

*This study was presented in part at the AACC Annual Meeting, 24–28 July 2011, Atlanta, USA and at the 36th FEBS Congress, Biochemistry for Tomorrow's Medicine, June 25–30, 2011, Lingotto Conference Centre, Torino, Italy.

Address correspondence to Aybala Erek Toprak, MD, Clinical Chemistry Lab, Göztepe Training and Research Hospital, İstanbul Medeniyet University, Dr. Erkin Cad., 34730 Kadıköy, Istanbul, Turkey. Tel.: +90 0216 5664000/9023; E-mail: aybalaerek@yahoo.com

coagulation process becomes defective.[19,20] It has not been shown that Gas6 has a direct effect on the coagulation cascade.

Gas6 is expressed in the lung, intestine and terminally differentiated cells including endothelial cells within the capillaries and as well as in vascular smooth muscle cells.[21,22] Both Gas6 and its receptor AXL are expressed in the kidney principally in intraglomerular mesangial cells.[23,24] Mesangial cell proliferation, a hallmark of glomerular sclerosis, was stimulated by Gas6/AXL.[4,5,25] Putdown of mesangial cell proliferation with either warfarin, an inhibitor of gamma-carboxylation, or an extracellular portion of AXL suggested that Gas6 plays a role in glomerular disease.[26]

Some experimental studies indicate that Gas6-AXL may be involved in cardiovascular and renal complications of diabetes. It was reported that Gas6 signaling increased survival of vascular smooth muscle cells in a low glucose environment, while Gas6 signaling increased cell migration in high glucose.[27] Animal models have suggested potential roles for Gas6 and TAM receptors in the development of diabetic nephropathy and allograft rejection.[4,23,28] Diabetic nephropathy was less severe in Gas6 knockout mice or when Gas6 activity was inhibited by warfarin treatment.[5]

Despite these advances in our understanding of Gas6, the clinical importance of the Gas6/TAM pathway in the molecular mechanism of complications associated with diabetes mellitus is unknown. Hung et al.[29] showed that plasma Gas6 concentrations were decreased in patients with type 2 diabetes and Gas6 levels were also associated with some markers of endothelial malfunctioning and inflammation.

Albuminuria is not always a reliable indicator for proliferation of the mesangial cell matrix in the kidney.[30,31] Approximately half of diabetes with microalbuminuria progress to obvious nephropathy.[32] Microalbuminuria is often associated with heart failure or arterial hypertension in type 2 diabetes. Therefore, novel biomarkers are required for earlier detection of diabetic nephropathy. This study hypothesizes that there is a link between plasma Gas6 concentrations and albuminuria in patients with type 2 diabetes. The objective of this study is to examine whether plasma Gas6 levels are associated with albuminuria in patients with type 2 diabetes.

Materials and methods

Study participants

This study included three groups of subjects. Besides the control group ($n = 30$, healthy volunteers), there were two groups of type 2 diabetes: one group with normoalbuminuria (DM-NA) ($n = 37$) whose urinary albumin excretions (UAE) were <30 mg/24 h and the other group with albuminuria (DM-AL) ($n = 32$) whose urinary albumin excretions were >30 mg/24 h. The DM-AL group consisted of 14 patients with microalbuminuria (UAE: 30–300 mg/24 h) and 18 with macroalbuminuria (UAE >300 mg/24 h). All the participants gave written informed consent, and the study was overseen by the local ethics committee in the light of the principles of the (revised) Declaration of Helsinki.

None of the study participants were on anticoagulation therapy or vitamin K supplementation. Other exclusion criteria were: lactation, pregnancy, hypothyroidism, peripheral disease of the arteries, lower extremity varicoses, chronic venous insufficiency, ischemic heart disease, inflammatory diseases, chronic kidney disease, alcoholism, general malignancies, use of antidepressants or neuroleptics, high levels of hyperlipidemia, chronic hepatitis, cirrhosis of the liver, bronchial asthma, chronic obstructive pulmonary disease, collagen diseases, local or systemic infections and persistent transaminase elevation.

Samples

All samples were collected between 8:00 a.m. and 11:00 a.m. after an overnight fast (10–12 h). The following parameters were measured: HbA_{1c} (EDTA whole blood); C-reactive protein (CRP) (serum); fibrinogen, and Gas6 (3.2% citrated plasma). Urine (24 h) samples were used for the measurement of albumin. The plasma was obtained after centrifugation of the blood samples at $3000 \times g$ for 15 min and was kept frozen at $-80\,^{\circ}C$ for no longer than 2 months.

Laboratory measurements

A boronate affinity assay was performed for hemoglobin A_{1c} (HbA_{1c}) (Primus PDQ, Primus Diagnostics, Kansas City, MO). The IMMAGE 800 nephelometric analyzer (Beckman Coulter, Fullerton, CA) was used to measure CRP concentrations. Fibrinogen was analyzed on STA Compact (Diagnostica Stago, Asnieres sur Seine, France). Urinary albumin excretion was measured in urine samples (24 h) by a Cobas Integra 800 Analyzer (Roche Diagnostics, Mannheim, Germany).

Human Gas6 sandwich ELISA development kit (DuoSet Economy Pack, 45 Plate) provided by R&D Systems (Minneapolis, MN) was used for the determination of Gas6 in human plasma. Since this kit is designed for the analysis of cell culture supernates, it has been optimized and validated to assay Gas6 in plasma in our laboratory. After optimization, it was used to assay plasma Gas6 levels in the study population. Briefly, microtiter plates (96 wells) were coated with 100 μL primary antibody and incubated overnight at $4\,^{\circ}C$. The following day the wells were washed five times with a washing buffer [phosphate buffered saline containing 0.05% Tween-20] and then blocked with 5% bovine serum albumin in phosphate buffered saline for 2 h at room temperature. After five washes with a washing buffer, 100 μL of diluted standard solutions or samples were added to the wells and incubated for 1 h at $37\,^{\circ}C$. Then the manufacturer's recommendations were followed. The absorbance was measured at 450 nm in a microplate reader.

Statistical analysis

''Mann–Whitney *U*'' and a nonparametric test, ''Kruskal–Wallis analysis of variance'' (ANOVA), were chosen to compare the groups. A $p < 0.05$ value was accepted as significant level. Any significant results with Kruskal–Wallis ANOVAs were then followed up by ''Bonferroni-adjusted Mann–Whitney *U*'' tests to compare the two groups and the actual p values were reported. Spearman's correlation coefficients were calculated for correlation analysis.

Results

The general characteristics of patients with and without albuminuria are shown in Table 1. There were two patient groups; one with normoalbuminuria (DM-NA) ($n = 37$) whose urinary albumin excretions (UAE) were <30 mg/24 h and the other group with albuminuria (DM-AL) ($n = 32$) whose urinary albumin excretions were >30 mg/24 h. The DM-AL group consisted of 14 patients with microalbuminuria (UAE: 30–300 mg/24 h) and 18 with macroalbuminuria (UAE >300 mg/24 h). The plasma level of Gas6 was 20.9 ng/mL (16.7–27.0); median (25–75% percentile) in patients with albuminuria (macro- or microalbuminuria) (DM-AL); 16.5 ng/mL (13.1–22.9) in patients with normoalbuminuria (DM-NA) and 15.3 ng/mL (8.3–21.8) in healthy controls (Figure 1A). Kruskal–Wallis test revealed a significant difference among the three groups with respect to albuminuria concentration ($p < 0.01$) (Figure 1B).

In the patients with type 2 diabetes, plasma Gas6 levels were significantly higher in patients with albuminuria than those of the patients with normoalbuminuria when Bonferroni Adjusted Mann–Whitney *U* test was used to compare the two groups ($p < 0.0167$). Plasma Gas6 levels in type 2 diabetes with normoalbuminuria were not significant at $p = 0.140$ compared to the healthy controls, since the Bonferroni correction requires a p value to be <0.0167. There were no significant gender and age differences in plasma concentration of Gas6 in the diabetic or nondiabetic group, or the duration of diabetes. The Gas6 level in the group of type 2 diabetes with microalbuminuria appeared lower than the level in type 2 diabetes with macroalbuminuria, but did not reach statistical significance using the Mann–Whitney *U* test ($p > 0.05$) (Figure 2).

We also examined the relationship between Gas6 levels and albuminuria using Spearman's correlation. There was a significant correlation between Gas6 levels and albuminuria (rho = 0.356, $p < 0.01$), as well as between Gas6 and HbA_{1c} (rho = 0.331, $p < 0.01$) whereas Gas6 had no correlation with CRP, and fibrinogen either. Moreover significant correlation was observed between fibrinogen and either CRP (rho = 0.000) or albuminuria (rho = 0.456, $p < 0.01$).

A statistically significant difference for HbA_{1c} among the groups was noted by the Kruskal–Wallis ANOVA ($p < 0.001$)

Table 1. Main demographic and clinical characteristics of subjects.

	DM-AL[a]	DM-NA[b]	Healthy controls
n	32 (14(microal) + 18(macroalb))	37	30
Male	11	16	15
Female	21	21	15
Age	59 (6.7)	53.2 (12.1)	39.4 (4.6)
Duration of diabetes (years)	11.1 (6.1)	6.7 (6.2)	–
HbA_{1c}			
%	8.6 (7.3–9.6)	7.2 (5.9–8.6)	5 (4.6–5.6)
mmol/mol	71 (56–81)	55 (41–71)	31 (27–38)
Albuminuria (mg/24 h)	485 (105–1545)	4 (1.8–9.4)	2 (1–3)
Microalbuminuria (mg/24 h)	125	–	–
Macroalbuminuria (mg/24 h)	1959	–	–
Gas6 (ng/ml)	20.9 (16.7–27.0)	16.5 (13.1–22.9)	15.3 (8.3–21.8)
CRP (mg/l)	6 (3–11)	5 (3–7)	3 (2–4)
Fibrinogen (μmol/l)	15.1 (11.7–16.3)	11.6 (10.1–12.9)	7.9 (6.9–8.9)

[a]DM-AL: Diabetes type 2 with albuminuria (micro and macro).
[b]DM-NA: Diabetes type 2 with normoalbuminuria.

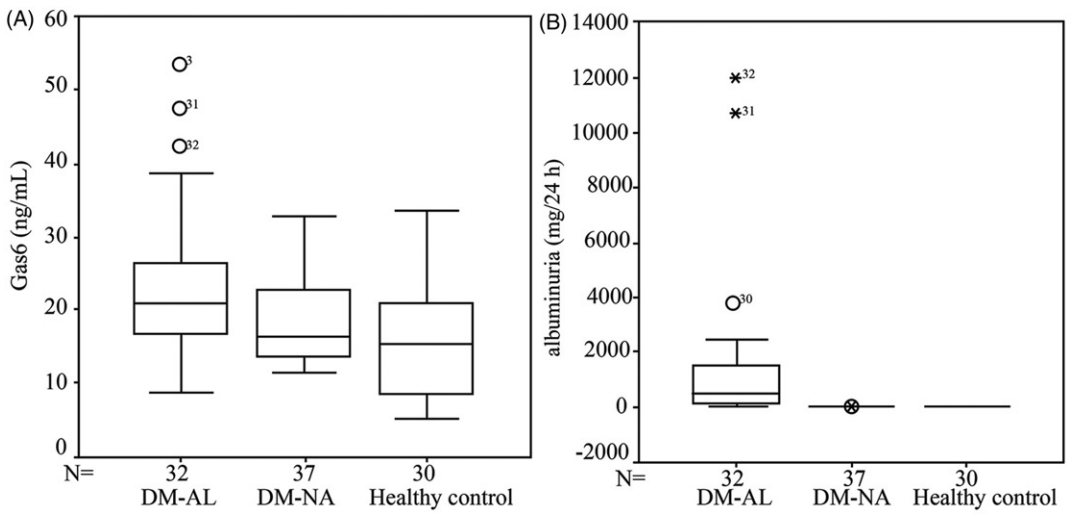

Figure 1. Comparison of the study groups: type 2 diabetic patients with albuminuria (DM-AL) or with normoalbuminuria (DM-NA), and healthy control. (A) Plasma Gas6 concentrations and (B) albuminuria.

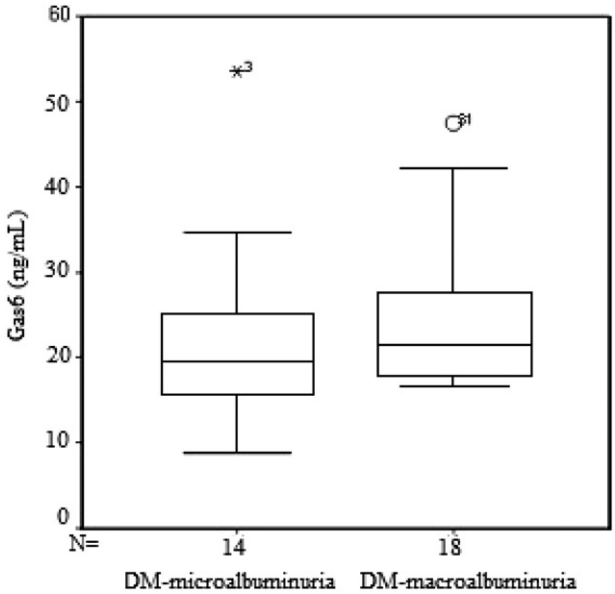

Figure 2. Comparison of the plasma Gas6 concentrations in type 2 diabetic patients with micro- or macro-albuminuria.

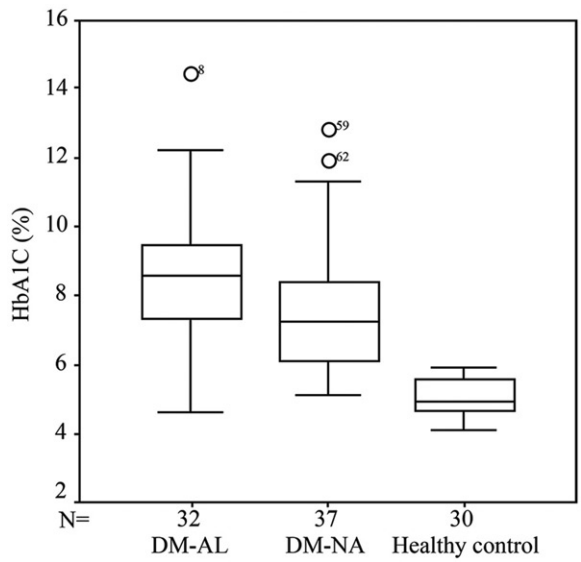

Figure 3. HbA_{1c} in the study groups: Type 2 diabetic patients with albuminuria (DM-AL) or with normoalbuminuria (DM-NA), and healthy control[a].

(Figure 3). Bonferroni Adjusted Mann–Whitney *U* test revealed that HbA_{1c} concentration was not significantly different between the type 2 diabetes subgroups of with albuminuria (DM-AL) and with normoalbuminuria (DM-NA) ($p > 0.0167$). However, HbA_{1c} levels of DM-AL ($p < 0.0167$) and DM-NA ($p < 0.0167$) were higher compared to the healthy control group.

Discussion

Because of the global increase in diabetes, diabetic nephropathy is becoming a major issue. Diagnosis and treatment in the early stages of nephropathy may avert this major complication and new biomarkers are needed. In this study, we examined whether plasma Gas 6 levels are associated with albuminuria in patients with type 2 diabetes and found a positive correlation between plasma Gas6 levels and albuminuria. Microalbuminuria concentrations are used to indicate diabetic nephropathy, but it is not always found to correspond to matrix expansion of mesangial cells during the early stage.[2] In the present study, for the patients with type 2 diabetes it was found that plasma Gas6 levels were significantly higher in patients with albuminuria than those in patients with normoalbuminuria. The plasma Gas6 levels between subgroups of type 2 diabetes mellitus were checked and it was found that it was lower in the group with microalbuminuria than the group with macroalbuminuria but was not statistically different. There was a significant correlation between albuminuria and plasma Gas6 levels and as well as between Gas6 and HbA_{1c} whereas Gas6 had no correlation with CRP, or fibrinogen. Moreover, significant correlation was observed between fibrinogen and either CRP or albuminuria.

Although currently microalbuminuria is used for a diagnostic marker to predict future overt nephropathy, neither the sensitivity nor the specificity of microalbuminuria is high enough to detect it. Another difficulty with microalbuminuria is the false positive results. Reasons for false positives are: congestive heart failure, infection strenuous exercise within 24 h, fever, marked hyperglycemia, marked hypertension, pregnancy, hematuria, and urinary tract infection.[2] A new indicator in the early phase of kidney injury would be helpful to predict diabetic nephropathy, which is reversible at this phase.

Some studies suggested a role for inflammation as a cause of pathogenesis of diabetic complications. Lengyel et al.[33] showed that protein S activity and fibrinogen levels were elevated during the onset stages of diabetic nephropathy. Tessari et al.[34] reported that the expression of both fibrinogen and albumin is increased in patients with type 2 diabetes who have elevated levels of urinary albumin excretion. Takebayashi et al.[35] measured fibrinogen and hsCRP in patients with diabetes and reported that fibrinogen and hsCRP, considered independently, might be closely related to diabetic microangiopathy. Increasingly, evidence suggests that inflammation may have a role in the development of diabetic nephropathy. This may initiate the activation of some pathways in the kidney. This new point of view explains diabetic nephropathy together with inflammatory markers, as an inflammatory disease.[36,37] In agreement with those studies, a significant correlation between fibrinogen and CRP, as well as between fibrinogen and albuminuria was found in the current study.

Cavet et al.[38] reported significant effects of variable glucose concentrations on the receptor of Gas6, AXL signaling, in vascular smooth muscle cells. Glucose levels affected Gas6-AXL signaling, with increased activation of Akt and mTOR at low glucose levels, and increased activation of ERK1/2 at high glucose levels. In the study, at high glucose levels, Gas6-AXL signaling induced migration; at low glucose levels, inhibition of apoptosis by Gas6-AXL signaling was greater. Their study suggests that altered Gas6-AXL signaling contributes to vascular dysfunction in diabetes.

There is a potential role for Gas6 in the development of complications in diabetes. Transgenic mice ectopically expressing AXL in myeloid cells, were observed to develop phenotypes similar to noninsulin-dependent diabetes mellitus.[39] These animals displayed hyperglycemia, severe insulin resistance, hyperinsulinemia, hepatic lipids and pancreatic islet dysplasia, progressive obesity, but did not exhibit hyperphagia.

According to the results of our study, it appears likely that there is an association between plasma Gas6 levels and albuminuria in patients with type 2 diabetes. There are some limitations of our study: (1) the number of patients in each group was small and (2) although the changes may have been statistically significant, they were small and it is questionable if they are useful as clinical discriminators of risk. This study may serve as a reasonable starting point to explain the association between plasma Gas6 levels with diabetic nephropathy.

In conclusion, this is the first study indicating that plasma Gas6 concentrations are associated with albuminuria in patients with type 2 diabetes. Further well designed, appropriately sized and long-term follow-up clinical studies are needed to elucidate the actual role of Gas6 in diabetic nephropathy.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

The authors are grateful to W. Greg Miller, Department of Pathology, Virginia Commonwealth University, for critical review of the manuscript. All authors contributed to the conception and design, acquisition of data or analysis and interpretation of data, drafting the article or revising it critically, and approved the final version of the article for publication.

References

1. International Diabetes Federation Diabetes Atlas, 5th ed. Available at: http://www.idf.org/diabetesatlas/5e/Update2012. Accessed May 2013.
2. KDOQI: KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. *Am J Kidney Dis.* 2007;49(2 Suppl 2):12–154.
3. Kanwar YS, Wada J, Sun L, et al. Diabetic nephropathy: mechanisms of renal disease progression. *Exp Biol Med.* 2008; 233:4–11.
4. Yanagita M. The role of the vitamin K dependent growth factor Gas6 in glomerular pathophysiology. *Curr Opin Nephrol Hypertens.* 2004;13:465–470.
5. Nagai K, Arai H, Yanagita M, et al. Growth arrest-specific gene 6 is involved in glomerular hypertrophy in the early stage of diabetic nephropathy. *J Biol Chem.* 2003;278:18229–18234.
6. Mima A, Arai H, Matsubara T, et al. Urinary Smad1 is a novel marker to predict later onset of mesangial matrix expansion in diabetic nephropathy. *Diabetes.* 2008;57:1712–1722.
7. Johnson RJ, Floege J, Couser WG, Alpers CE. Role of platelet derived growth factor in glomerular disease. *J Am Soc Nephrol.* 1993;4:119–128.
8. Floege J, Eng E, Lindner V, et al. Rat glomerular mesangial cells synthesize basic fibroblast growth factor. Release, upregulated synthesis, and mitogenicity in mesangial proliferative glomerulonephritis. *J Clin Invest.* 1992;90:362–369.
9. Ryffel B, Car BD, Gunn H, Roman D, Hiestand P, Mihatsch MJ. Interleukin-6 exacerbates glomerulonephritis in (NZBxNZW)F1 mice. *Am J Pathol.* 1994;144:927–937.
10. Goruppi S, Ruaro E, Schneider C. Gas6, the ligand of Axl tyrosine kinase receptor, has mitogenic and survival activities for serum starved NIH3T3 fibroblasts. *Oncogene.* 1996;12:471–480.
11. McCloskey P, Fridell YW, Attar E, et al. Gas6 mediates adhesion of cells expressing the receptor tyrosine kinase Axl. *J Biol Chem.* 1997;272:23285–23291.
12. Fridell YW, Villa Jr J, Attar EC, Liu ET. Gas6 induces Axl-mediated chemotaxis of vascular smooth muscle cells. *J Biol Chem.* 1998;273:7123–7126.
13. Schneider C, King RM, Philipson L. Genes specifically expressed at growth arrest of mammalian cells. *Cell.* 1998;54:787–793.
14. Manfioletti G, Brancolini C, Avanzi G, Schneider C. The protein encoded by a growth arrest-specific gene (Gas6) is a new member of the vitamin K-dependent proteins related to protein S, a negative coregulator in the blood coagulation cascade. *Mol Cell Biol.* 1993; 13:4976–4985.
15. Esmon CT, Sadowski JA, Suttie JW. A new carboxylation reaction. The vitamin K-dependent incorporation of H14CO3− into prothrombin. *J Biol Chem.* 1975;250:4744–4748.
16. Tetik S, Uras F, Eksioglu-Demiralp E, Turay Yardimci K. Low-density lipoprotein specifically binds glycoprotein IIb/IIIa. A flow cytometric method for ligand-receptor interaction. *Clin Appl Thromb Hemost.* 2008;14:210–219.
17. Ozsavci D, Yardimci T, Demirel GY, Demiralp E, Uras F, Onder E. Flow cytometric assay of platelet glycoprotein receptor numbers in hypercholesterolemia. *Platelets.* 2002;13:223–229.
18. Ozsavci D, Yardimci T, Demirel GY, Uras F, Hekim N, Ulutin ON. Apo-A1 binding to platelets detected by flow cytometry. *Thromb Res.* 2001;103:117–122.
19. Uras F, Uras AR, Yardımcı T, Sardana MK. Determination of the N-terminal amino acid sequence of the purified prothrombin from a patient with liver cirrhosis. *Thromb Res.* 2000;99:277–283.
20. Koldas M, Uras F. Avidin-biotin ELISA for measurement of prothrombin in human plasma. *Thromb Res.* 2001;102:221–227.
21. Avanzi GC, Callicchio M, Bottarel F, et al. Gas 6 inhibits granulocyte adhesion to endothelial cells. *Blood.* 1998;91: 2334–2340.
22. Nakano T, Higashino K, Kikuchi N, et al. Vascular smooth muscle cell-derived, Gla-containing growth potentiating factor for Ca (2+)-mobilizing growth factors. *J Biol Chem.* 1995;270:5702–5705.
23. Fiebeler A, Park JK, Muller DN, et al. Growth arrest specific protein 6/Axl signaling in human inflammatory renal diseases. *Am J Kidney Dis.* 2004;43:286–295.
24. Arai H, Nagai K, Doi T. Role of growth arrest-specific gene 6 in diabetic nephropathy. *Vitam Horm.* 2008;78:375–392.
25. Nagai K, Matsubara T, Mima A, et al. Gas6 induces Akt/mTOR-mediated mesangial hypertrophy in diabetic nephropathy. *Kidney Int.* 2005;68:552–561.
26. Yanagita M, Ishii K, Ozaki H, et al. Mechanism of inhibitory effect of warfarin on mesangial cell proliferation. *J Am Soc Nephrol.* 1999;10:2503–2509.
27. Suzuki LA, Poot M, Gerrity RG, Bornfeldt KE. Diabetes accelerates smooth muscle accumulation in lesions of atherosclerosis: lack of direct growth-promoting effects of high glucose levels. *Diabetes.* 2001;50:851–860.
28. Yin, JL, Pilmore HL, Yan YQ, et al. Expression of growth arrest-specific gene 6 and its receptors in a rat model of chronic renal transplant rejection. *Transplantation.* 2002;73:657–660.
29. Hung YJ, Lee CH, Chu NF, Shieh YS. Plasma protein growth arrest-specific 6 levels are associated with altered glucose tolerance, inflammation, and endothelial dysfunction. *Diabetes Care.* 2010;33:1840–1844.
30. Chavers BM, Bilous RW, Ellis EN, Steffes MW, Mauer SM. Glomerular lesions and urinary albumin excretion in type I diabetes without overt proteinuria. *N Engl J Med.* 1989;320:966–970.
31. Fioretto P, Steffes MW, Mauer M. Glomerular structure in nonproteinuric IDDM patients with various levels of albuminuria. *Diabetes.* 1994;43:1358–1364.
32. Dihazi H, Müller GA, Lindner S, et al. Characterization of diabetic nephropathy by urinary proteomic analysis: identification of a processed ubiquitin form as a differentially excreted protein in diabetic nephropathy patients. *Clin Chem.* 2007;53: 1636–1645.
33. Lengyel Z, Voros P, Toth LK, et al. Urinary albumin excretion is correlated to fibrinogen levels and protein S activity in patients

with type 1 diabetes mellitus without overt diabetic nephropathy. *Wien Klin Wochenschr.* 2004;116:240–245.
34. Tessari P, Kiwanuka E, Barazzoni R, Vettore M, Zanetti M. Diabetic nephropathy is associated with increased albumin and fibrinogen production in patients with type 2 diabetes. *Diabetologia.* 2006;49:1955–1961.
35. Takebayashi K, Suetsugu M, Matsutomo R, Wakabayashi S, Aso Y, Inukai T. Correlation of high-sensitivity C-reactive protein and plasma fibrinogen with individual complications in patients with type 2 diabetes. *South Med J.* 2006;99:23–27.
36. Navarro-Gonzalez JF, Mora-Fernandez C, de Fuentes MM, Garcia-Perez J. Inflammatory molecules and pathways in the pathogenesis of diabetic nephropathy. *Nat Rev Nephrol.* 2011; 7: 327–340.
37. Lu J, Randell E, Han Y, Adeli K, Krahn J, Meng QH. Increased plasma methylglyoxal level, inflammation, and vascular endothelial dysfunction in diabetic nephropathy. *Clin Biochem.* 2011;44: 307–311.
38. Cavet ME, Smolock EM, Ozturk OH, et al. Gas6-axl receptor signaling is regulated by glucose in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol.* 2008;28:886–891.
39. Augustine KA, Rossi R, Van G, et al. Noninsulin-dependent diabetes mellitus occurs in mice ectopically expressing the human Axl tyrosine kinase receptor. *J Cell Physiol.* 1999;181:433–447.