original article

Gender-specific association of adiponectin as a predictor of progression of chronic kidney disease: The Mild to Moderate Kidney Disease Study

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Progressive renal vascular sclerosis is a key feature of chronic kidney disease (CKD). Adiponectin, an adipokine with potent anti-inflammatory and antiatherosclerotic properties, is associated with insulin resistance, type II diabetes and cardiovascular disease. In this study, we evaluated the predictive value of adiponectin for the progression of CKD in patients enrolled in the Mild to Moderate Kidney Disease Study. The primary end point was defined as a doubling of the baseline serum creatinine and/or terminal renal failure in 177 patients who completed a prospective follow-up of 7 years. Patients who reached a progression endpoint ($n = 65$) were significantly older, had higher baseline serum creatinine, proteinuria and adiponectin concentrations and more components of the metabolic syndrome. A gender-stratified Cox model revealed adiponectin in men as a significant predictor of progression after adjustment for age, glomerular filtration rate, and proteinuria. Male patients with adiponectin levels above their ROC analysis-derived optimal cutoff of 4 μg/ml had a significantly faster progression than patients below this point. This prospective long-term study in patients with CKD indicates high adiponectin as a novel independent predictor of disease progression in men but not in women. Our observation may be relevant for other conditions of progressive vascular sclerosis and diabetic nephropathy.

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The kidney contains a vast amount of vessels of different size and function with an enormous endothelial surface. As a consequence, pathophysiological conditions involving the vascular bed are not only related to atherosclerotic changes of major vessels of the heart and the brain, but also to vascular changes within the kidney. It has been even proposed that glomerulosclerosis and atherosclerosis share common patho-physiological pathways.[1] However, little is known about the exact mechanisms,[2] and factors related to atherosclerosis as well as glomerular–endothelial injury might be interesting candidates to be involved in the progression of kidney disease.

One of these putative candidates is adiponectin, the major adipocyte-secretory protein. It has been demonstrated to improve insulin sensitivity and to possess anti-inflammatory and antiatherosclerotic properties.[3] Hypoadiponectinaemia has been found to be associated with insulin resistance,[4,5] obesity, and other features of the metabolic syndrome[6] as well as type II diabetes mellitus and cardiovascular disease.[7–9] Recent data suggested that the presence of a metabolic syndrome might be a causal factor for chronic kidney disease (CKD).[10,11] In addition, we observed that insulin resistance is present already in patients with mild degrees of renal impairment and even in patients with primary CKD and normal glomerular filtration rate (GFR).[12] Because of the relationship between adiponectin and the metabolic syndrome, we decided to investigate the predictive value of adiponectin for progression in a prospective 7-year follow-up study in a cohort of non-diabetic patients with mostly mild to moderate primary CKD.

RESULTS

Baseline characteristics

A total of 177 patients (78%) from the baseline cohort could be assessed during the follow-up. The remaining 50 patients lost to follow-up had moved away and/or did not return for another visit in the outpatients departments. They had a significantly better kidney function than those who came to follow-up examinations: GFR 91 vs 64 ml/min/1.73 m^2,

serum creatinine 1.55 vs 2.15 mg/dl, proteinuria 0.6 vs 1.0 g/24 h. Only 12 of those 50 patients (24%) were in the GFR groups 1–3 (GFR below 60 ml/min/1.73 m^2) compared with 94 of 177 patients with follow-up (53%). They had also lower adiponectin levels (5.3 vs 6.3 µg/ml) and higher hemoglobin levels (14.4 vs 13.6). They tended to have a lower number of metabolic syndrome components (2.24 vs 2.43) but both groups did not differ significantly with respect to age and sex.

Baseline clinical characteristics and laboratory data of the patients with follow-up are reported in the first column of Table 1. Table 2 shows the correlations of adiponectin, age, GFR, proteinuria with the various components of the metabolic syndrome. During follow-up, 65 patients progressed to a renal end point, which was terminal renal failure requiring renal replacement therapy in 29 patients and doubling of baseline serum creatinine without reaching end-stage renal disease in 36 patients. Table 1 further presents data of patients with and without disease progression. Patients who had reached a progression end point were significantly older, had higher baseline serum creatinine, and adiponectin levels and protein excretion rates as well as lower GFR. In addition, more components of the metabolic syndrome were present in these patients ($P < 0.005$). There

were no significant differences between men and women either in the time of observation until the primary study end point or the end of the observation period was reached (48.9 ± 19.7 vs 44.5 ± 19.1 months, $P = 0.21$) nor the frequency of progression (37 vs 36%, $P = 0.83$).

Adiponectin as a predictor of kidney disease progression

Age- and sex-adjusted Cox regression analysis revealed high adiponectin levels as a significant predictor of disease progression (Table 3). A metabolic syndrome was not a significant predictor for disease progression ($P = 0.32$). However, when the singular components of the metabolic syndrome were tested in the Cox regression analysis, we found triglycerides and high-density lipoprotein cholesterol levels to be related to the progression of disease. An interaction term between adiponectin concentrations and sex was highly significant ($P = 0.001$). Therefore, all further analyses were stratified for men and women.

In women, adiponectin concentrations were not a significant predictor in either model. On the contrary, in men adiponectin was a significant predictor of disease progression in all models adjusting for age alone or additionally adjusting for GFR, proteinuria, metabolic

Table 1 | Baseline clinical and laboratory data of 177 patients who completed follow-up with further stratification into those without and with progression of kidney disease during the follow-up period

	All patients (*n*=177)	Non-progressors (*n*=112)	Progressors (*n*=65)
Sex (male/female), *n* (%)	118/59 (67/33%)	74/38 (66/34%)	44/21 (68/32%)
Age (years)	46.4 ± 12.2	44.8 ± 12.6	49.1 ± 11.1*
Follow-up time (years)[a]	47 ± 20	55 ± 16	34 ± 18****
	(32; 53; 63)	(52; 59; 66)	(18; 34; 46)
BMI (kg/m^2)	25.2 ± 3.7	24.8 ± 3.5	25.7 ± 3.9
Current smokers, *n* (%)	34 (19%)	18 (16%)	16 (25%)
Systolic blood pressure (mm Hg)	137 ± 20	136 ± 22	137 ± 17
Diastolic blood pressure (mm Hg)	87 ± 13	86 ± 14	88 ± 12
Blood pressure medication, *n* (%)	143 (81%)	82 (73%)	61 (94%)***
Serum creatinine (mg/dl)	2.15 ± 1.22	1.54 ± 0.61	3.21 ± 1.31****
Glomerular filtration rate (ml/min/1.73 m^2)	64 ± 39	79 ± 38	38 ± 25****
	(35; 54; 89)	(50; 74; 99)	(20; 33; 46)
Proteinuria (g/24 h/1.73 m^2)	1.01 ± 0.92	0.87 ± 0.95	1.25 ± 0.83****
	(0.20; 0.70; 1.63)	(0.14; 0.46; 1.25)	(0.61; 1.09; 1.78)
Serum albumin (g/dl)	4.56 ± 0.40	4.57 ± 0.43	4.53 ± 0.36
High-sensitivity C-reactive protein (mg/l)	0.28 ± 0.31	0.28 ± 0.32	0.29 ± 0.31
Adiponectin (µg/ml)	6.31 ± 4.43	5.89 ± 4.27	7.05 ± 4.63*
	(3.43; 5.04; 7.73)	(2.93; 4.60; 7.34)	(4.10; 5.49; 8.61)
Metabolic syndrome, *n* (%)[b]	85 (48%)	47 (42%)	38 (59%)*
Metabolic factors, *n*[c]	2.43 ± 1.17	2.25 ± 1.21	2.74 ± 1.02***
Insulin (mU/l)	13.50 ± 9.70	13.72 ± 11.14	13.11 ± 6.61
Triglycerides (mg/dl)	172 ± 95	159 ± 93	194 ± 96***
	(104; 144; 223)	(97; 131; 201)	(121; 181; 244)
HDL cholesterol (mg/dl)	44 ± 15	46 ± 15	40 ± 13**
Glucose (mg/dl)	98 ± 15	99 ± 16	97 ± 14
Use of fibrates, *n* (%)	9 (5%)	6 (5%)	3 (5%)

BMI, body mass index; HDL, high-density lipoprotein.
Data are provided as mean ± s.d. (25th, 50th, 75th percentile where appropriate) or *n* (%).
*$P<0.05$; **$P<0.01$; ***$P<0.005$; ****$P<0.001$ – comparison between progressors and non-progressors.
[a]Follow-up time was calculated as the time from enrollment until the primary study end point or the end of the observation period was reached.
[b]Definition according to the scientific statement from the American Heart Association and the National Heart, Lung, and Blood Institute. Three of the following five parameters had to be present: elevated triglycerides ⩾150 mg/dl (1.7 mmol/l) or on drug treatment for elevated triglycerides, reduced HDL-cholesterol <40 mg/dl (1.03 mmol/l) in men, <50 mg/dl (1.3 mmol/l) in women or on drug treatment for reduced HDL-cholesterol, hypertension: ⩾130 mm Hg systolic blood pressure or ⩾85 mm Hg diastolic blood pressure or on antihypertensive drug treatment in a patient with a history of hypertension, elevated fasting glucose: ⩾100 mg/dl or on drug treatment for elevated glucose; as waist circumference was not available in our cohort we used BMI>30 kg/m^2.
[c]Metabolic factors: average number of factors considered in the definition of metabolic syndrome (see footnote above).

Table 2 | Spearman correlation coefficients of adiponectin, age, GFR, proteinuria, and the various components of the metabolic syndrome stratified for men (left lower triangle, *n*=118) and women (right upper triangle, *n*=59)

	Age	Adiponectin	GFR	Proteinuria	BMI	Triglycerides	HDL cholesterol	Glucose	Hypertension
Age	—	0.17	−0.53****	−0.21	0.38***	0.20	−0.09	0.21	0.31*
Adiponectin	0.10	—	−0.09	0.05	0.17	0.02	0.20	0.02	0.04
GFR	−0.24*	−0.26**	—	−0.17	−0.31*	−0.36**	0.11	−0.15	−0.33*
Proteinuria	0.06	0.14	−0.20*	—	0.05	−0.14	0.03	0.01	0.11
BMI	0.40****	−0.10	−0.12	−0.01	—	0.25	−0.15	0.32*	0.37***
Triglycerides	0.16	−0.09	−0.25**	0.08	0.23*	—	−0.36**	0.08	0.16
HDL cholesterol	0.02	0.26**	0.18*	0.10	−0.14	−0.48****	—	0.02	0.06
Glucose	0.17	−0.13	0.12	−0.04	0.32***	0.19*	−0.05	—	0.21
Hypertension	0.32****	0.05	−0.24**	0.11	0.19*	0.16	−0.03	0.07	—

Correlations of the right upper triangle are those of women (*n*=59) and those of the left lower triangle are those of men (*n*=118).
BMI, body mass index; GFR, glomerular filtration rate; HDL, high-density lipoprotein.
*$P<0.05$, **$P<0.01$, ***$P<0.005$, ****$P<0.001$.

Table 3 | Association of different variables with progression of kidney disease during the observation period using age- and sex-adjusted Cox proportional hazards regression models

	Entire group	
Variable (increment)	HR per unit increment (95% CI) adjusted for age and sex	*P*
GFR (10 ml/min/1.73 m^2)	0.67 (0.59–0.76)	<0.0001
Proteinuria (1 g/24 h/1.73 m^2)	1.30 (1.02–1.65)	0.032
Adiponectin (1 μg/ml)	1.08 (1.02–1.15)	0.005
Adiponectin ⩾4 μg/ml	2.53 (1.32–4.85)	0.01
Metabolic syndrome (1=yes, 0=no)	1.32 (0.77–2.27)	0.32
Single components of metabolic syndrome		
Hypertension (1=yes, 0=no)[a]	4.94 (0.66–36.99)	0.12
BMI (1 kg/m^2)	1.01 (0.94–1.09)	0.73
BMI >30 kg/m^2 (1=yes, 0=no)[a]	0.83 (0.35–1.95)	0.67
Triglycerides (1 mg/dl)	1.002 (0.999–1.004)	0.21
Triglycerides ⩾150 mg/dl (1=yes, 0=no)[a]	1.86 (1.09–3.18)	0.02
HDL cholesterol (1 mg/dl)	0.977 (0.956–0.998)	0.03
HDL cholesterol <40/<50 mg/dl for men and women, respectively (1=yes, 0=no)[a]	1.41 (0.83–2.38)	0.21
Glucose (1 mg/dl)	0.99 (0.97–1.01)	0.16
Glucose ⩾100 mg/dl (1=yes, 0=no)[a]	0.75 (0.43–1.29)	0.29

BMI, body mass index; CI, confidence interval; GFR, glomerular filtration rate; HDL, high-density lipoprotein; HR, hazards ratio.
[a]Dichotomization of these parameters included in the definition of the metabolic syndrome was performed according to the scientific statement from the American Heart Association and the National Heart, Lung, and Blood Institute.

syndrome, or singular components of the metabolic syndrome ($P<0.0001$) (Table 4). The estimates for adiponectin were very stable and independent for the component of the metabolic syndrome adjusted (Table 4). Adiponectin levels were still significantly associated with disease progression in men even when adjusted for asymmetric dimethylarginine or apolipoprotein A-IV, which we recently showed to be significant predictors of progression.[13,14] The same holds true for the type of renal disease as well as the use or kind of antihypertensive medications when adjusted for baseline GFR (data not shown). We further observed that male patients in the lowest compared with the highest tertile of body mass index (BMI) showed a clear trend to higher adiponectin levels (6.55±4.30 vs 5.31±4.52 μg/ml, $P=0.098$) and a higher probability of disease progression (hazard ratio (HR) (95% CI) 2.46 (1.03–5.89), $P=0.043$).

Separately for men and women, we constructed Kaplan–Meier curves of the progression-free period comparing patients with high versus low serum adiponectin concentrations using the sex-specific optimal cutoff. This optimal cutoff was derived from receiver operating characteristics analysis and refers to the value, which optimizes sensitivity and false positive rates for the study outcome and was 4 μg/ml for both sexes. Male patients with adiponectin levels above this threshold had a worse prognosis and significantly faster progression to the end point compared with those with adiponectin levels below this threshold (log-rank test, $P=0.0005$) (Figure 1): the mean follow-up time to progression was 54.9 (95% CI: 47.8–62.0) months compared with 73.2 (95% CI 67.8–78.6) months, respectively. In women, there was no evidence for a difference in progression-free period in relation to the adiponectin level.

Additional analyses

When Cox regression analysis was performed for both end points separately, adiponectin was a highly significant

Table 4 | Association of adiponectin with progression of kidney disease during the observation period using various adjusted Cox proportional hazards regression models stratified by sex

Stratified by sex	Women		Men	
Adjusted for	HR (95% CI) of disease progression per 1 µg/ml increment of adiponectin	P	HR (95% CI) of disease progression per 1 µg/ml increment of adiponectin	P
Age, GFR	0.98 (0.83–1.16)	0.83	1.16 (1.09–1.24)	<0.0001
Age, GFR, proteinuria	0.96 (0.81–1.13)	0.61	1.16 (1.08–1.23)	<0.0001
Age, GFR, proteinuria, HDL-C	0.96 (0.82–1.13)	0.61	1.16 (1.09–1.24)	<0.0001
Age, GFR, proteinuria, BMI	0.96 (0.81–1.13)	0.61	1.16 (1.08–1.24)	<0.0001
Age, GFR, proteinuria, triglycerides	0.93 (0.78–1.11)	0.45	1.16 (1.09–1.24)	<0.0001
Age, GFR, proteinuria, hypertension	0.96 (0.81–1.13)	0.60	1.15 (1.08–1.23)	<0.0001
Age, GFR, proteinuria, HOMA-IR	0.93 (0.79–1.11)	0.44	1.15 (1.08–1.22)	<0.0001
Age, GFR, proteinuria, metabolic syndrome	0.96 (0.81–1.13)	0.62	1.16 (1.08–1.23)	<0.0001
Adjusted for	HR (95% CI) of disease progression for adiponectin ≥4 µg/ml	P	HR (95% CI) of disease progression for adiponectin ≥4 µg/ml	P
Age, GFR	0.71 (0.18–2.80)	0.63	2.27 (1.07–4.80)	0.03
Age, GFR, proteinuria	0.57 (0.14–2.36)	0.44	2.28 (1.08–4.80)	0.03

CI, confidence interval; GFR, glomerular filtration rate; HDL, high-density lipoprotein; HR, hazards ratio; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance.

predictor for reaching terminal renal failure (HR 1.21, 95% CI 1.11–1.32, $P < 0.001$) and a tendency towards an increased HR for doubling of serum creatinine (HR 1.09, 95% CI 0.98–1.22, $P = 0.11$) in men. Adiponectin was not predictive for either of the end points in women.

Regarding sex differences in the baseline variables it should be noted that adiponectin levels were significantly different between men and women (5.64 ± 4.22 vs 7.66 ± 4.58 µg/ml, $P = 0.004$). Men suffered more often from a metabolic syndrome (56 vs 32%, $P = 0.003$) and had significantly more components of the metabolic syndrome compared with women (2.67 ± 1.14 vs 1.95 ± 1.07, $P < 0.001$) (Figure 2). These included a significantly higher prevalence of low high-density lipoprotein cholesterol (64 vs 39%, $P = 0.002$), high triglyceride (56 vs 39%, $P = 0.034$), and high glucose levels (45 vs 19%, $P = 0.001$) in men compared with women.

DISCUSSION

Recent cross-sectional studies showed impaired kidney function to be associated with increased adiponectin levels.[5,12,15] Potential mechanistic explanations are changes in the ligand/receptor reactivity as shown for other hormone/receptor systems in renal failure,[16] reduced adiponectin clearance by the kidney,[17] or a counter-regulatory response to metabolic derangements in renal failure.[18] Therefore, kidney function seems to be an important determinant of adiponectin levels. However, prospective studies investigating whether adiponectin levels serve as a predictor of CKD progression are missing. Our prospective study in patients with non-diabetic primary CKD identified high adiponectin levels as a novel predictor of CKD progression in men, but not in women, independent of other predictors of disease progression.

Controversy in the literature: is the association to be reshaped?

Considering the bulk of the literature, the finding of high adiponectin levels as predictor of CKD progression, at least in men, was unanticipated considering that low rather than high adiponectin levels were reported to be associated with obesity, type II diabetes mellitus, and cardiovascular disease in the general population. In contrast to these cross-sectional findings some,[19–24] but not all,[7–9,24] prospective studies failed to document an association between low adiponectin levels and cardiovascular events. Studies in selected groups of patients with various diseases at baseline showed even associations of high adiponectin levels with various outcomes. An investigation in type I diabetes patients found increased concentrations of adiponectin and a positive association with soluble vascular cell adhesion molecule-1 that is a marker of generalized vascular dysfunction.[25] The Ludwigshafen Risk and Cardiovascular Health Study investigated almost 2500 patients with coronary artery disease at baseline and followed them for more than 5 years. Increased adiponectin levels were strongly associated with all-cause and cardiovascular mortality.[26] Similar results were reported by the Modification of Diet in Renal Disease study in patients with a mean GFR of 33 ml/min/1.73 m^2.[27] Recently, in the study of Kistorp *et al.*,[28] high adiponectin levels were predictive for mortality in patients with chronic heart failure (CHF), independent of CHF severity. It has recently been suggested that adiponectin increases energy expenditure and induces weight loss through a direct effect on the brain.[29] It has been proposed that in the context of increased energy expenditure high plasma adiponectin levels might not be beneficial in CHF. And the same is potentially true in renal patients. This would be in line with our observation that male patients in the lowest tertile of BMI had higher
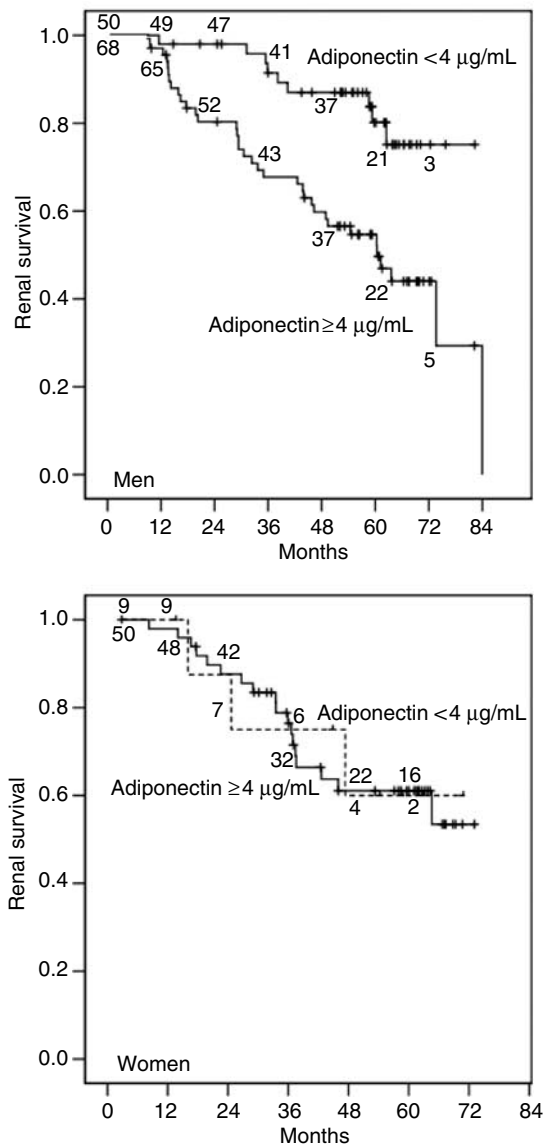
Figure 1 | **Kaplan–Meier curves of renal end points in male (upper panel) and female (lower panel) patients with plasma adiponectin concentrations above and below the sex-specific cutoff of 4 μg/ml.** This most useful cutoff value of adiponectin for predicting disease progression was derived from receiver operating characteristics analysis. At this value, the greatest sum of sensitivity and specificity was obtained. Male patients with adiponectin levels above the cut-point showed a significantly faster progression than those below this value (log-rank test, $P = 0.0005$). No significant difference was observed in women ($P = 0.92$). Each small tick line at the survival curves indicates censoring of a patient. Numbers near the survival curves represent the number of patients at risk with plasma adiponectin levels below and above the cut-off at the times 0, 12, 24, 36, 48, 60, and 72 months. Consider that a disease progression in one patient close to the end of the observation period when only few patients are still at risk has a dramatic impact on the decline of the curve, which has to be interpreted with caution.

adiponectin levels and were more likely to progress. In this respect, CHF and CKD patients may have much in common and many traditional risk factors such as hypercholestrole-
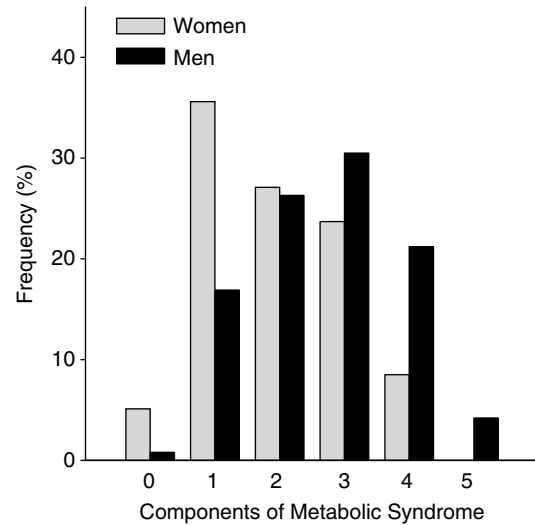

Figure 2 | **Frequency of patients according to the concomitant number of components of the metabolic syndrome stratified by sex.** In male patients, significantly more components of the metabolic syndrome were present as compared with women (Mantel–Haenszel test: $\chi^2 = 15.0$, df $= 1$, $P = 0.0001$). The factors considered in this analysis are listed in the footnote of Table 1.

mia, hypertension, or high BMI may provide beneficial outcomes, a well known constellation which is called 'reverse epidemiology'.[30] Taken together, from studies in patients with type I diabetes mellitus, pre-existing coronary artery disease, CHF as well as in patients with CKD, it might be concluded that the well known association of low adiponectin levels with adverse outcome in the general population is not necessarily valid in patients with severe and chronic disease and probably even turns into the opposite direction. It is conceivable that the upregulation of adiponectin is a compensatory attempt to attenuate endothelial and vascular damage as recently suggested.[25]

Adiponectin resistance as an alternative explanation

An alternative explanation for the association of high adiponectin levels with progression of CKD in men could be adiponectin resistance[31,32] caused by a dysfunction of adiponectin itself or a dysfunction or downregulation of adiponectin receptors with consecutive counter-regulatory increased adiponectin secretion. Such adiponectin resistance might be analogous to the finding of the virtually absent uptake of adiponectin across the coronary bed found in diabetic compared with non-diabetic patients.[32] It is also in accordance with major post-translational modifications of adiponectin caused by glycosylation as observed recently.[33] As advanced glycation products as well as the frequency of components of the metabolic syndrome are increased in kidney disease, resulting alterations of the adiponectin molecule might be an intriguing hypothesis which could explain the paradox phenomenon of high adiponectin levels with CKD progression.

Kidney functional considerations

The observed association of adiponectin and CKD progression, however, could also reflect a functional impairment of the kidney, which is less related to diminished glomerular filtration (and which we considered already by measuring and adjusting for GFR) than to other non-filtration-related consequences of kidney dysfunction; or in other words, a residual confounding of kidney function not related to GFR.

Sex differences

Adiponectin levels were not predictive for CKD progression in women although women have significantly higher adiponectin levels compared with men. An explanation for this finding might again be the presence of adiponectin resistance, which could be more pronounced in men than in women. This assumption is in line with the observation that in our cohort of women a metabolic syndrome was less frequent and the number of components of the metabolic syndrome was lower compared with men, which was mainly the case for the lipid and glucose components. This finding is different from the general population in which the metabolic syndrome tends to be more frequent in women.[34] We speculate that the ligand–receptor interaction might be less disturbed in women compared with men, which could be due to less functionally impaired adiponectin molecules in women as discussed above. It is of interest in this context that in a recent study in 839 children aged 8 years, adiponectin levels were associated with body weight, BMI, waist circumference, and fasting and 30-min insulin levels, but the associations were opposite in boys, with positive associations, and girls, with inverse associations.[35] Whether a complex age- and sex-related regulation of adiponectin metabolism with possible functional consequences plays a role in adults has to be investigated in upcoming studies. Data in mice showing that females have not only higher levels of adiponectin but also increased proportions of the high-molecular weight complexes.[36] Therefore, different adiponectin bioactivity could be an explanation for the sex-specific findings.

Limitations of the study

One study limitation is the fact that we did not assess waist circumference according to the American Heart Association (AHA) and National Heart, Lung, and Blood Institute (NHLBI) criteria for clinical diagnosis of the metabolic syndrome. Instead, we used obesity defined by $BMI > 30 \, kg/m^2$ to consider the obesity component of the metabolic syndrome. However, as recent discussions doubted the common pathogenetic roots of this syndrome[37] we have put more emphasis on adiponectin and the singular components of the metabolic syndrome.

Another limitation is that we have no clinical data on CHF. Further, the applied exclusion criteria in our study (e.g., diabetic nephropathy or age > 65 years) resulted in a selected group of patients and our findings may therefore not be applicable to other types of CKD such as diabetic nephro-

pathy or to other ethnicities. However, a recent case–control study in type I diabetic patients found a significant association of the A-allele of the adiponectin-promoter single nucleotide polymorphism rs17300539 with diabetic nephropathy.[38] A systematic investigation of the adiponectin gene found this single nucleotide polymorphism to be strongly associated with adiponectin concentrations with about 20% higher adiponectin levels in carriers of the A allele.[39] Our observation of high adiponectin levels with progression of primary CKD is in line and extends the association of the A allele and its higher adiponectin levels with diabetic nephropathy.

To test the confounding effect of relevant risk factors on the predictive power of adiponectin on CKD progression, the multiple Cox regression analysis might be underpowered in women. However, even models that included only GFR and adiponectin with and without age were far away from any significance.

Finally, it might be considered a limitation that we do not have follow-up data on 50 (22%) patients of the baseline cohort. However, as these patients had a far better kidney function at baseline than the patients under prospective observation, we assume that most of them remained stable without symptoms over years and therefore no more visited the outpatient ward. A survival bias might be excluded considering this far better kidney function and the fact that even in the patients under prospective observation only three out of 177 patients died, which might be explained by the relatively low average age of 46 years at study entry and the regular care by nephrologists. Nevertheless, we additionally performed two simulation analyses adding each of these 50 patients to the followed 177 patients once as progressor and once as non-progressor. Both simulations resulted still in significant associations for adiponectin in men, which were more pronounced in the second simulation (data not shown).

In summary, in our prospective study with 7 years of follow-up in patients with non-diabetic CKD, we identified adiponectin as a novel predictor for CKD progression in men but not in women. This observation may be of relevance for other conditions of progressive vascular sclerosis as well as for patients with diabetic nephropathy.

MATERIALS AND METHODS
Patients and baseline investigations

We examined 227 Caucasian patients aged between 18 and 65 years with non-diabetic CKD and various degrees of renal impairment. They were recruited from eight nephrology departments in Germany, Austria, and South Tyrol by one physician who visited all participating centers as described earlier.[14,40] The study was approved by the institutional ethic committees and subjects gave written informed consent. Patients had stable renal function for at least 3 months before entry into the study. Exclusion criteria were treatment with immunosuppressive agents, fish oil or erythropoietin, serum creatinine above 6 mg/dl, diabetes mellitus of any type, malignancy, liver, thyroid or infectious disease, nephrotic syndrome (defined as proteinuria $> 3.5 \, g/1.73 \, m^2/day$), organ transplantation, allergy to ionic contrast media, and pregnancy. Patient history,

including smoking habits and antihypertensive treatment, was recorded by interview and confirmed by checking patient records. This was complemented by clinical examination including assessment of BMI, blood glucose, and blood pressure. Diagnosis of diabetes mellitus of any kind was based on several measurements of fasting blood glucose.

The primary cause of CKD was glomerulonephritis in 97 (biopsy-confirmed in 90) patients, adult polycystic kidney disease in 37 patients, interstitial nephritis in 24 patients, other types of kidney disease in 43 patients, and unknown in 26 patients.

Prospective follow-up and definition of renal end points

After the baseline investigation, patients were followed prospectively until the primary study end point or the end of the observation period was reached. The primary end point was defined as doubling of baseline serum creatinine and/or terminal renal failure necessitating renal replacement therapy.

Laboratory measurements

Blood samples for the measurement of adiponectin and other parameters were taken after an overnight fast of at least 12 h. Adiponectin plasma concentrations were measured with an enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN, USA). GFR was assessed in all patients using the iohexol clearance technique as described in detail elsewhere.[41] Criteria for clinical diagnosis of metabolic syndrome were defined according to the scientific statement from the AHA and the NHLBI.[42] We have also quantified insulin sensitivity in our patients using the Homeostasis Model Assessment of Insulin Resistance: plasma insulin (mU/l) × plasma glucose (mg/dl)−405.

Statistical analysis

Comparisons of variables between various groups were performed using unpaired *t*-tests, nonparametric Wilcoxon rank sum tests and Pearson's χ^2 test. The HRs for CKD progression were estimated using a Cox proportional hazards regression model adjusted for age and other risk factors of disease progression. To consider the possibility of a nonlinear association between continuous variables of metabolic syndrome components (BMI, triglycerides, high-density lipoprotein cholesterol) with progression-free period, the analysis was also performed using categorical variables using the cutoff, which defines each of those components (defined in the footnote to Table 1). Finally, we tested for multicollinearity and fulfillment of proportional hazard assumptions.

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