View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by Elsevier - Publisher Connector

© 2008 International Society of Nephrology

see commentary on page 549

Serum adiponectin predicts all-cause mortality and end stage renal disease in patients with type I diabetes and diabetic nephropathy

Anders Jorsal[1], Lise Tarnow[1], Jan Frystyk[2], Maria Lajer[1], Allan Flyvbjerg[2], Hans-Henrik Parving[3,4], Nathalie Vionnet[5] and Peter Rossing[1]

[1]Steno Diabetes Center, Gentofte, Denmark; [2]Medical Department M (Diabetes and Endocrinology), University of Aarhus, Aarhus, Denmark; [3]Department of Medical Endocrinology, Rigshospitalet, University Hospital of Copenhagen, Copenhagen, Denmark; [4]Faculty of Health Science, University of Aarhus, Aarhus, Denmark and [5]L'Institut National de la Santé et de la Recherche Médicale (INSERM), U525, Université Pierre et Marie Curie-Paris 6, Paris, France

Adiponectin levels are increased in patients with type I diabetes especially in the presence of microangiopathy. Here we determined the predictive value of serum adiponectin levels and 8 adiponectin gene polymorphisms for mortality, cardiovascular events and end-stage renal disease in type I diabetic patients. This prospective, observational follow-up study of type I diabetics consisted of 438 patients with overt diabetic nephropathy that were compared to 440 type I patients with normal albumin excretion. These two groups were followed an average of 8 years and generally matched for gender, age and duration of diabetes. Cox regression analysis of 373 patients showed a covariate-adjusted hazard ratio for all-cause mortality of 1.46 for a change of one standard deviation in log10 of serum adiponectin. There was no association with cardiovascular events; however, serum adiponectin levels predicted end stage renal disease in a covariate-adjusted analysis. Two of eight gene polymorphisms, found in the 878 patients, were associated with increased serum adiponectin levels but none of the polymorphisms were associated with a renal or cardiovascular outcome. These studies show that high serum adiponectin levels predict mortality and progression to end stage renal disease in type I diabetic patients.

Kidney International (2008) **74,** 649–654; doi:10.1038/ki.2008.201; published online 21 May 2008

KEYWORDS: adiponectin; end stage renal disease; nephropathy; type I diabetes

Correspondence: *Anders Jorsal, Steno Diabetes Center, Niels Steensens Vej 2, Gentofte 2820, Denmark. E-mail: ajrs@steno.dk*

Received 31 October 2007; revised 28 February 2008; accepted 11 March 2008; published online 21 May 2008

Proteinuric patients with type I diabetes have an increased mortality compared to the general population, mainly explained by end-stage renal disease (ESRD) and cardiovascular disease (CVD).[1]

The adipocyte secretes a number of peptides labeled adipocytokines or adipokines. The most abundantly secreted adipokine is adiponectin, which is induced during adipocyte differentiation. In experimental studies and in type II diabetic patients, adiponectin has been shown to possess anti-inflammatory, antiatherogenic, and cardioprotective properties.[2] Furthermore, adiponectin has been suggested to enhance insulin action.[3,4]

Recently, two cell surface receptors for adiponectin have been identified. The two receptors are expressed in most tissues, but liver and muscle showed by far the most prominent expression.[5] Receptor activation has been shown to stimulate AMP-activated protein kinase and peroxisome proliferator-activated receptor-γ, fatty-acid oxidation, and glucose uptake.[5,6] These actions suggest a role of adiponectin as an endogenous insulin sensitizer.[3,4]

Low concentrations of adiponectin have been associated with obesity,[7,8] type II diabetes,[7,9] and coronary artery disease.[9–12] In addition, with respect to kidney disease in type I diabetes, serum adiponectin levels are increased and associated with microangiopathy.[11,13–15] The levels of adiponectin are influenced by genetic variations in the *adiponectin* (*ADIPOQ*) gene.[16] However, the relationship between concentrations of adiponectin and microangiopathy is not fully understood, but we have previously shown that genetic variations in the *ADIPOQ* gene are associated with the risk of diabetic nephropathy.[17] This prompted us to investigate whether serum levels of adiponectin and eight polymorphisms in the *ADIPOQ* gene predict all-cause mortality, CVD events, and ESRD in a well-characterized population of type I diabetic patients with or without diabetic nephropathy. Furthermore, we wanted to investigate whether associations with genetic variants are driven through increased adiponectin levels.

RESULTS

Baseline characteristics

This was a prospective observational follow-up study with a median follow-up time until end point or last visit of 8.1 (0.0–12.8) years. From Denmark, 952 patients were originally included in the EURAGEDIC case–control study.[18] In 52 patients no follow-up was possible and 22 individuals had at least one unreported genotype due to technical reasons. The remaining study population included two groups: 438 cases with type I diabetes and diabetic nephropathy and 440 controls with type I diabetes for more than 15 years and persistent normoalbuminuria. Baseline clinical and laboratory characteristics of the 878 patients are shown in Table 1. Patients with nephropathy were younger, received more antihypertensive treatment, had higher HbA_{1c}, blood pressure, serum creatinine and total cholesterol, but lower concentrations of high-density lipoprotein cholesterol than patients with normoalbuminuria ($P<0.05$). There was a correlation between adiponectin and the following parameters: Age ($r=0.17$), serum levels of creatinine ($r=0.41$), weight ($r=-0.28$), urinary albumin excretion rate ($r=0.29$), and high-density lipoprotein cholesterol ($r=0.27$), and all P-values were <0.001. On average, glomerular filtration rate (GFR) was well preserved in patients with diabetic nephropathy, but nevertheless, in type I diabetic patients serum adiponectin was significantly higher in individuals with diabetic nephropathy, as previously published.[13] Furthermore, after adjustment for the presence of nephropathy, patients carrying the minor allele in −11387 and the non-A-allele in +2033 had significant elevated adiponectin concentrations, $P=0.031$ and 0.040. However, associations disappeared after Bonferroni correction for multiple testing. Allele and genotype frequencies for the eight variants in the *ADIPOQ* gene were compatible with the Hardy–Weinberg equilibrium. Allele frequencies for all polymorphisms are shown in Table 2. Interestingly, the two polymorphisms associated with increased adiponectin levels were also nominally associated with diabetic nephropathy in the case–control study: The A-allele in −11387 was associated with diabetic nephropathy (A-allele frequency: 9.4 versus 6.6% in cases and controls, respectively, $P=0.014$), as well as +2033 (the non-A-allele frequency: 37.0 versus 41.3% in cases and controls, respectively, $P=0.050$).

Follow-up data

In 373 patients, circulating levels of adiponectin were measured. Data were evaluated with adiponectin as a continuous variable. During follow-up, 19 (10.9%) patients with normoalbuminuria and 79 (39.9%) patients with macroalbuminuria died. Cox regression analysis revealed an increased risk for all-cause mortality with a hazard ratio (HR) of 1.75 (1.47–2.10, $P<0.001$) for a change of one s.d. (0.21) in $\log_{10}$ of serum adiponectin. Adiponectin remained an independent predictor of all-cause mortality in the Cox regression model (covariate-adjusted (sex, age, ± nephropathy, systolic blood pressure, HbA_{1c}, serum creatinine, serum cholesterol, and antihypertensive treatment) HR 1.46 (1.07–2.00, $P=0.018$)). This association was linear and the interaction term between case–control group and adiponectin level was significant ($P=0.037$). However, no associations were seen when analyzing cases and controls separately in a multivariate Cox regression model ($P=0.428$ and 0.093). In addition, one s.d. increase of $\log_{10}$ of serum adiponectin was associated with an increased risk of the combined end point

Table 1 | Baseline clinical and laboratory characteristics of the 878 patients divided according to nephropathy status

	Nephropathy (*N*=438)	Normoalbuminuria (*N*=440)	*P*-value
Sex (men/women)	267/171	233/207	0.017
Age (years)	42.3 ± 10.4	45.4 ± 11.5	<0.001
Duration of diabetes (years)	28.4 ± 8.8	27.7 ± 10.1	0.29
BMI (kg/m^2)	24.2 ± 3.3	24.2 ± 3.1	0.80
HbA_{1c} (%)	9.4 ± 1.5	8.4 ± 1.1	<0.001
Antihypertensive treatment (%)	77.3	16.6	<0.001
sBP (mm Hg)	145 ± 22	134 ± 19	<0.001
dBP (mm Hg)	83 ± 12	76 ± 10	<0.001
UAER (mg per 24 h)	593 (3–14,545)[a]	7 (1–30)	—
S-creatinine (mmol/l)	103 (52–706)	79 (53–134)	<0.001
GFR (crEDTA, ml/min per 1.73 m^2)	74 ± 34	—	—
eGFR (ml/min per 1.73 m^2)	66 ± 28	87 ± 16	<0.001
S-cholesterol (mmol/l)	5.6 ± 1.2	4.9 ± 1.0	<0.001
S-HDL cholesterol (mmol/l)	1.5 ± 0.6	1.6 ± 0.5	0.002
S-triglycerides (mmol/l)	1.3 (0.3–9.9)	0.8 (0.3–5.4)	<0.001
Smoking (%)	45.8	39.5	0.08
Retinopathy (0/SR/PR)	7/133/298	159/163/118	<0.001
Myocardial infarction (%)	4.5	1.8	0.03
Stroke (%)	7.0	1.6	<0.001
S-adiponectin (mg/l)[b]	24.2 (7.6–117.5)	17.3 (6.1–48.6)	<0.001

BMI, body mass index; dBP, diastolic blood pressure; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; PR, proliferative retinopathy; sBP, systolic blood pressure; SR, simplex retinopathy; UAER, urinary albumin excretion rate.
[a]Some patients with previously persistent macroalbuminuria receiving antihypertensive treatment had values <300 mg per 24 h at the time of investigation.
[b]*N*=198 patients with diabetic nephropathy and *N*=175 patients with normoalbuminuria. Data are expressed as *N*, means ± s.d., medians (range).

Table 2 | Allele frequencies (%) of the 878 patients divided according to nephropathy status

Polymorphism	Allele	Nephropathy (*N*=438)	Normoalbuminuria (*N*=440)	*P*-value
−11422	G	9.5	9.0	0.81
(rs16861194)	A	90.5	91.0	
−11387	G	90.6	93.4	0.014
(rs17300539)	A	9.4	6.6	
−11373	C	76.8	73.8	0.33
(rs266729)	G	23.2	26.2	
−10065	A	32.9	35.0	0.51
(rs182052)	G	67.1	65.0	
+45 (rs2241766)	G	10.5	10.0	0.81
	T	89.5	90.0	
+276	G	68.6	72.5	0.22
(rs1501299)	T	31.4	27.5	
+1242	C	2.9	3.0	1.00
(rs17366743)	T	97.1	97.0	
+2033	A−	37.0	41.3	0.050
	AA	63.0	58.7	

(fatal and non-fatal CVD events) ($N=107$) in a univariate proportional hazards model (HR 1.50 (1.26–1.78, $P<0.001$). However, this association disappeared when adjusting for confounding factors ($P=0.59$). No further analyses were performed regarding this end point.

Progression of renal disease. During follow-up, only one patient with normoalbuminuria developed macroalbuminuria and none progressed to ESRD. Nevertheless, 40 (23.2%) patients with diabetic nephropathy reached ESRD (Table 3). Progressors received more antihypertensive treatment, had higher urinary albumin excretion rate, and serum adiponectin concentrations, but lower GFR (Table 4). Cox regression analysis revealed an unadjusted HR for ESRD of 2.09 (1.59–2.75, $P<0.001$) for a change of one s.d. in $\log_{10}$ of serum adiponectin; covariate-adjusted (sex, age, ± nephropathy, systolic blood pressure, HbA_{1c}, serum creatinine, serum cholesterol, and antihypertensive treatment) HR of 1.45 (1.01–2.09, $P=0.047$). This association was linear and the interaction term for case–control group and serum adiponectin was significant ($P=0.003$). We then examined the risk estimates in the group of patients with diabetic nephropathy separately ($N=198$). These patients were divided into two groups according to the median level of serum adiponectin (24.2 (7.6–117.5) mg/l). During follow-up, 12 patients (12%) with adiponectin levels below the median and 28 patients (30%) with levels above the median reached ESRD ($P=0.001$; Figure 1). Cox regression analysis revealed an unadjusted HR for ESRD in patients with adiponectin levels above the median of 3.44 (1.75–6.78, $P<0.001$); covariate-adjusted (sex, age, systolic blood pressure, HbA_{1c}, GFR, serum cholesterol, and antihypertensive treatment) HR of 2.72 (1.27–5.84, $P=0.010$). Similar results were found when analyzing adiponectin as a continuous variable. In addition, the result did not change when adjusting for high-density lipoprotein cholesterol instead of total cholesterol. Furthermore, rate of decline in kidney function was explored in

Table 3 | Clinical characteristics of 373 patients with adiponectin measurements classified according to guidelines of American clinical practice (K/DOQI)

eGFR stage	Number of patients (%)	UAER (mg per 24 h)	Progression to ESRD (*N* (%))	S-adiponectin (mg/l)[a]
Nephropathy (N=*198*)				
I	22	532 (16–5145)	2 (5)	21.6 (7.7–54.6)
II	37	736 (35–8824)	8 (11)	20.4 (7.6–64.0)
III	30	1444 (46–14,545)	18 (31)	26.9 (8.9–111)
IV	9	1904 (43–6735)	11 (65)	33.9 (15.6–117.5)
V	2	1208 (452–4099)	1 (20)	56.4 (32.6–109.2)
Normoalbuminuria (N=*176*)				
I	59	7 (1–30)	0	17.0 (8.8–37.8)
II	39	8 (1–30)	0	17.9 (6.1–45.2)
III	2	15 (7–21)	0	35.6 (12.1–48.6)
IV	0	—	—	—
V	0	—	—	—

ANOVA, analysis of variance; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; UAER, urinary albumin excretion rate.
The different stages of renal insufficiency are based on eGFR calculated by the modification of diet in renal disease method.
[a]A statistically significant difference in adiponectin concentration was seen in patients with nephropathy (*P*=0.034 (ANOVA)). No association was seen in patients with normoalbuminuria (*P*=0.67). Data are expressed as *N*, medians (range). The five stages based on eGFR are as follows: I ⩾ 90, II=60–89, III=30–59, IV=15–29, and V < 15.

Table 4 | Clinical characteristics of the198 patients with adiponectin measurements and diabetic nephropathy divided according to ESRD status

	Progressor (*N*=40)	Non-progressor (*N*=158)	*P*-value
Sex (men/women)	27/13	92/66	0.09
Age (years)	41.2 ± 8.6	40.8 ± 9.8	0.81
BMI (kg/m^2)	24.4 ± 4.5	23.9 ± 2.8	0.39
HbA_{1c} (%)	10.0 ± 1.5	9.4 ± 1.5	0.019
Antihypertensive treatment (%)	91.3	66.4	0.001
sBP (mm Hg)	162 ± 23	148 ± 21	<0.001
dBP (mm Hg)	93 ± 12	84 ± 12	<0.001
UAER (mg per 24 h)	2191 (35–14545)	644 (16–8824)	<0.001
GFR (ml/min per 1.73 m^2)	44 ± 26	83 ± 30	<0.001
S-adiponectin (mg/l)	29.0 (7.7–117.5)	22.5 (7.6–109.2)	0.012

BMI, body mass index; dBP, diastolic blood pressure; ESRD, end-stage renal disease; GFR, glomerular filtration rate; sBP, systolic blood pressure; UAER, urinary albumin excretion rate.
Data are expressed as *N*, means ± s.d., medians (range).

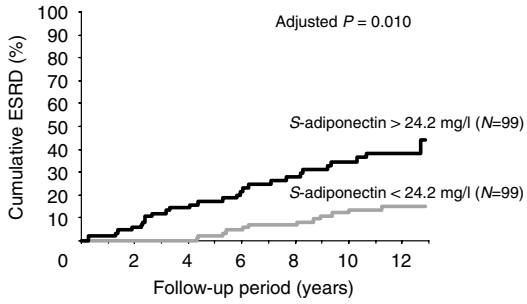

Figure 1 | Kaplan–Meier curves of end-stage renal disease (ESRD) in 198 type I diabetic patients with diabetic nephropathy ($P=0.010$). Grey line, serum adiponectin levels below the median; black line, serum adiponectin levels above the median.

patients with diabetic nephropathy using annually measured GFR ($N = 186$ patients). Mean rate of decline in GFR differed significantly according to adiponectin levels: low levels 3.6 (3.8) ml/min per 1.73 m^2 per year and high levels 5.0 ml/min per 1.73 m^2 per year, $P = 0.045$. This effect persisted after correction for sex and age; however, this association disappeared when adjusting for well-known progression promoters ($P = 0.36$).

A prospective follow-up analysis was performed to investigate the common polymorphisms in the *ADIPOQ* gene in relation to development of ESRD, CVD events, and mortality in type I diabetic patients with and without diabetic nephropathy ($N = 878$). A total of 90 (10.3%) patients progressed to ESRD, 211 (24.0%) patients had a fatal or non-fatal CVD event and 176 (20.0%) patients died during follow-up. However, none of the polymorphisms predicted renal/cardiovascular outcome.

DISCUSSION

The current case–control study with 8.1 years of follow-up demonstrated that high levels of serum adiponectin predict all-cause mortality and progression to ESRD in patients with type I diabetes. This effect persisted after adjustment for sex, age, established progression promoters, and antihypertensive treatment. Furthermore, two polymorphisms in the *ADIPOQ* gene were associated with serum adiponectin levels, but no associations were seen when adjusting for multiple testing. None of the eight polymorphisms investigated were associated with renal/cardiovascular outcome.

Adiponectin appears to play a major role in fatty acid and glucose metabolism through a change in insulin sensitivity by stimulating peroxisome proliferator-activated receptor-γ and the AMP-activated protein kinase.[2] In previous studies, insulin resistance has been linked to microalbuminuria in type I diabetes,[19] but as insulin resistance was not evaluated in the current population of type I diabetic patients we cannot evaluate the relationship among the levels of adiponectin, insulin sensitivity, and diabetic nephropathy. Nevertheless, it is possible that the elevation of adiponectin is unrelated to the insulin sensitivity. Apart from being an endogenous insulin sensitizer, adiponectin exerts a plethora of anti-inflammatory, antiatherogenic, and cardioprotective properties.[2] Adiponectin modulates endothelial function by inhibition of the deleterious effects of tumor necrosis factor-α[10,20,21] and increases the activity of endothelial nitric oxide synthase.[22] Endothelial nitric oxide synthase is an enzyme responsible for NO synthesis leading to increased NO levels. Furthermore, adiponectin reduces expression of the vascular cell adhesion molecule-1, E-selectin, and intracellular adhesion molecule-1.[10]

In previous studies, low concentrations of adiponectin have been associated with obesity,[7,8] type II diabetes,[7,9] and coronary artery disease.[9–12] In addition, with respect to kidney disease in type I diabetes, the present study demonstrated that levels of adiponectin are increased in type I diabetic patients with diabetic nephropathy and predict development of ESRD. This is in accordance with previous studies[11,13–15] in which high concentrations of adiponectin were demonstrated in type I diabetic patients with diabetic nephropathy. Furthermore, a recent study identified elevated levels of adiponectin as a novel predictor for chronic kidney disease progression in male non-diabetic patients.[23]

An essential question to be answered is why coexisting type I diabetes and diabetic kidney disease is associated with increased concentrations of adiponectin. Clearance of adiponectin from the circulation is mainly renal dependent and deteriorated kidney function increases levels of adiponectin in both type I and II diabetes.[13,14,24] Consequently, differences in kidney function need to be taken into consideration when comparing different study groups. In addition, ACE inhibitors have been shown to increase serum adiponectin in non-diabetic patients with essential hypertension.[25] In the current study, treatment with ACE inhibitors[13] were not associated with serum adiponectin and all analyses were adjusted for antihypertensive treatments and serum creatinine or actual GFR measured with a clearance technique. Thus, mechanisms other than kidney function must be responsible for the elevated levels of serum adiponectin and progression to ESRD in type I diabetes.

The adiponectin protein has three distinct domains corresponding to a signal sequence with a variable region, a collagenous triple helix, and a globular head domain.[2] Functional studies have shown that four lysine residues in the collagenous domain of adiponectin can be both hydroxylated and glycosylated.[26] These post-translational modifications lead to a changed three-dimensional structure and might be a consequence of hyperglycemia, oxidative stress, and chronic low-grade inflammation that are a part of type I diabetes and diabetic nephropathy. This hypothesis is supported by recent data. Adiponectin multimer composition is affected by physiological factors, and most notably, even a relatively short period of high plasma glucose leads to an increase in the production of high-molecular-weight polymers of adiponectin.[27] Kollerits *et al.*[23] suggest that the post-translational modifications affect receptor affinity and result in adiponectin resistance. For this reason, it is conceivable that the increased levels of adiponectin is a counter-regulatory response to metabolic derangements in type I diabetes and renal failure.[28] Whether this phenomenon is present in chronic hyperglycemia in type I diabetic patients with diabetic nephropathy is unknown.

In the present study, we have determined total adiponectin levels without differentiating among the various isoforms. However, recent data indicate that it is the plasma fraction of high-molecular-weight polymers rather than the total concentration of adiponectin that is associated with insulin sensitivity in *db/db* mice as well as in type II diabetic patients treated with thiazolidinedione.[29] On the other hand, in patients with polycystic ovary syndrome treated with thiazolidinedione, total adiponectin levels yielded stronger correlations with changes in insulin sensitivity than the high-molecular adiponectin isoform as determined with fast

protein liquid chromatography.[30] Future studies estimating different isoforms of adiponectin are needed to elucidate the paradoxically elevated adiponectin levels in type I diabetes as well as their exact role in relation to metabolism and development of late diabetic complications.

It has been suggested in several studies that genetic factors may influence the risk of developing micro- and macrovascular complications in type I diabetes.[31,32] We have previously shown that genetic variations in the *ADIPOQ* gene are associated with the risk of diabetic nephropathy.[17] The present study demonstrated that patients carrying the minor allele in −11387 and the non-A-allele in +2033 had significant elevated serum adiponectin levels. This finding is in accordance with a large study in non-diabetic individuals ($N = 1727$).[16] Thus, data confirm that the association with diabetic nephropathy is likely to be due to the effect of the polymorphisms on adiponectin levels. However, our study did not show any relationship between genetic variants in the *ADIPOQ* gene and the development of ESRD, CVD events, or mortality. This might be a matter of lack of statistical power to detect an impact of the relatively infrequent genetic variants.

In conclusion, in this well-characterized population of type I diabetic patients we demonstrated that adiponectin predicts all-cause mortality and ESRD. Future studies are needed to elucidate the exact roles of different isoforms of adiponectin and insulin resistance in relation to metabolism, development, and progression of late-diabetic complications.

MATERIALS AND METHODS

Patients

Since 1993, all adult Danish Caucasian patients with type I diabetes and diabetic nephropathy attending the outpatient clinic at Steno Diabetes Center have been invited to participate in a study of genetic risk factors for the development of diabetic micro- and macrovascular complications. Of these, 73% accepted. Type I diabetes was considered present if the age at onset of diabetes was ⩽35 years and time to definite insulin therapy ⩽1 year. Established diabetic nephropathy (cases) was defined by persistent albuminuria (>300 mg per 24 h) in two out of three consecutive measurements in sterile urines, presence of retinopathy, and absence of other kidney or urinary tract disease.[33] In four cases without retinopathy, the diagnosis of diabetic glomerulopathy was verified by a kidney biopsy. Absence of diabetic nephropathy (controls) was defined as persistent normoalbuminuria (<30 mg per 24 h) after at least 15 years of type I diabetes in patients not treated with ACE inhibitors or angiotensin II receptor blockers. In total, 76% of patients approached as controls. No patients were treated with thiazolidinedione.

Baseline clinical and laboratory investigations

All patients had blood samples and phenotypic characteristics collected as part of the EURAGEDIC project.[18] Blood pressure was measured twice in the resting state. From venous samples, plasma lipid levels were determined by standard methods. HbA_{1c} was determined by standard high-performance liquid chromatography techniques with normal values in the range from 4.1 to 6.4%. Urinary albumin excretion rate was measured in 24-h urine collections by an enzyme immunoassay. Serum creatinine concentration was determined by a modified Jaffe's method. GFR was measured annually in patients with diabetic nephropathy after a single injection of 3.7 MBq ^{51}Cr-EDTA by determination of radioactivity in venous blood samples taken 180, 200, 220, and 240 min after injection.[34] Linear regression analysis of the GFR determinations in each individual was used to estimate the rate of decline in kidney function. In patients with normoalbuminuria, the GFR was estimated by the modification of diet in renal disease equation.[35] ESRD was defined as kidney transplantation or dialysis. Diabetic retinopathy was assessed by fundus photography after pupillary dilatation and graded nil, simplex, and proliferative retinopathy. On the basis of standardized questionnaires, current smokers of one or more cigarettes/cigars/pipes per day were classified as smokers and all others as non-smokers. Major CVD events were diagnosed as stroke, myocardial infarction, coronary artery bypass graft, and/or percutanous coronary intervention.

The genotyping was performed as part of the EURAGEDIC study using standard methods as recently described.[17] The following eight polymorphisms in the *ADIPOQ* gene—listed according to the first position of the translation starting point ATG—were analyzed: −11422 (rs16861194), −11387 (rs17300539), −11373 (rs266729), −10065 (rs182052), +45 (rs2241766), +276 (IVS2G62T, rs1501299), +1242 (Y111H, rs17366743), and +2033.

Serum adiponectin was measured in a subgroup of 373 patients (175 patients with normoalbuminuria and 198 patients with macroalbuminuria). These patients did not differ from the whole study population with respect to sex, age, and duration of diabetes. Serum adiponectin was determined by an in-house time-resolved immunofluorometric assay based on commercial reagents (from R&D Systems, Abingdon, UK) as recently described.[13]

Follow-up

In a prospective observational study design, the patients were followed until an end point was reached, to the last visit at Steno Diabetes Center or until the 1st of September 2006. The end points were ESRD, major cardiovascular events, cardiovascular mortality, and all-cause mortality.

All patients were traced through the National Register during autumn 2006. If a patient died before the 1st of September 2006, the date of death was recorded and information on cause of death was obtained from the death certificate. Two observers reviewed all death certificates independently and the primary cause of death was recorded. Additional available information from necropsy reports was included. All deaths were classified as cardiovascular deaths unless an unequivocal non-cardiovascular cause was established.[36] Information about date of ESRD and non-fatal CVD events was obtained from patient records or discharge letters from other hospitals.

The study was performed in accordance with the Helsinki Declaration, the local ethics committee approved the study and all patients gave their informed consent.

Statistical analysis

Normally distributed variables are given as means ± s.d., whereas non-normally distributed variables were $\log_{10}$ transformed before analysis and are given as medians (range). Comparisons between groups were performed by an unpaired Student's *t*-test, analysis of variance, or linear regression as appropriate. The χ^2 test was used to compare non-continuous variables. A two-tailed *P*-value of 0.05 or less was considered statistically significant.

All time-to-end point variables were analyzed using a log-rank test and displayed in Kaplan–Meier plots. Cox regression models were used to estimate the unadjusted and adjusted HRs with 95% confidence interval. The models were tested for a nonlinear relationship with serum adiponectin by evaluation of the significance of adding a quadratic term. All patients were analyzed together, but as the study was initially a case–control study we added an interaction term for case–control group and adiponectin level, if significant we evaluated cases and controls separately.

All calculations were performed using a commercially available program (SPSS for Windows, version 14.0, Chicago, IL, USA).

DISCLOSURE

All the authors declared no competing interests.

ACKNOWLEDGMENTS

The EURAGEDIC study was supported by the European Commission (contract QLG2-CT-2001-01669), the Danish Medical Research Council, and the Eva and Henry Fraenkels Memorial Foundation. We acknowledge the assistance of Birgitte V Hansen, Anne-Kathrine Holstein, Berit R Jensen, Tina R Juhl, Karen Mathiassen, Hanne Petersen, Lotte Pietraszek, and Ulla M Smidt.

REFERENCES

1. Borch-Johnsen K, Andersen PK, Deckert T. The effect of proteinuria on relative mortality in type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1985; **28**: 590–596.
2. Gable DR, Hurel SJ, Humphries SE. Adiponectin and its gene variants as risk factors for insulin resistance, the metabolic syndrome and cardiovascular disease. *Atherosclerosis* 2006; **188**: 231–244.
3. Yamauchi T, Kamon J, Waki H *et al.* The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med* 2001; **7**: 941–946.
4. Berg AH, Combs TP, Du X *et al.* The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001; **7**: 947–953.
5. Yamauchi T, Kamon J, Ito Y *et al.* Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature* 2003; **423**: 762–769.
6. Yamauchi T, Kamon J, Minokoshi Y *et al.* Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8**: 1288–1295.
7. Weyer C, Funahashi T, Tanaka S *et al.* Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab* 2001; **86**: 1930–1935.
8. Arita Y, Kihara S, Ouchi N *et al.* Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun* 1999; **257**: 79–83.
9. Hotta K, Funahashi T, Arita Y *et al.* Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol* 2000; **20**: 1595–1599.
10. Ouchi N, Kihara S, Arita Y *et al.* Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. *Circulation* 1999; **100**: 2473–2476.
11. Costacou T, Zgibor JC, Evans RW *et al.* The prospective association between adiponectin and coronary artery disease among individuals with type 1 diabetes. The Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetologia* 2005; **48**: 41–48.
12. Maahs DM, Ogden LG, Kinney GL *et al.* Low plasma adiponectin levels predict progression of coronary artery calcification. *Circulation* 2005; **111**: 747–753.
13. Frystyk J, Tarnow L, Hansen TK *et al.* Increased serum adiponectin levels in type 1 diabetic patients with microvascular complications. *Diabetologia* 2005; **48**: 1911–1918.
14. Saraheimo M, Forsblom C, Fagerudd J *et al.* Serum adiponectin is increased in type 1 diabetic patients with nephropathy. *Diabetes Care* 2005; **28**: 1410–1414.
15. Hadjadj S, Aubert R, Fumeron F *et al.* Increased plasma adiponectin concentrations are associated with microangiopathy in type 1 diabetic subjects. *Diabetologia* 2005; **48**: 1088–1092.
16. Heid IM, Wagner SA, Gohlke H *et al.* Genetic architecture of the APM1 gene and its influence on adiponectin plasma levels and parameters of the metabolic syndrome in 1,727 healthy Caucasians. *Diab* 2006; **55**: 375–384.
17. Vionnet N, Tregouet D, Kazeem G *et al.* Analysis of 14 candidate genes for diabetic nephropathy on chromosome 3q in European populations: strongest evidence for association with a variant in the promoter region of the adiponectin gene. *Diab* 2006; **55**: 3166–3174.
18. Tarnow L, Groop PH, Hadjadj S *et al.* European rational approach for the genetics of diabetic complications EURAGEDIC: patient populations and strategy. *Nephrol Dial Transplant* 2008; **23**: 161–168.
19. Groop PH, Forsblom C, Thomas MC. Mechanisms of disease: pathway-selective insulin resistance and microvascular complications of diabetes. *Nat Clin Pract Endocrinol Metab* 2005; **1**: 100–110.
20. Kawanami D, Maemura K, Takeda N *et al.* Direct reciprocal effects of resistin and adiponectin on vascular endothelial cells: a new insight into adipocytokine–endothelial cell interactions. *Biochem Biophys Res Commun* 2004; **314**: 415–419.
21. Malyszko J, Malyszko JS, Brzosko S *et al.* Adiponectin is related to CD146, a novel marker of endothelial cell activation/injury in chronic renal failure and peritoneally dialyzed patients. *J Clin Endocrinol Metab* 2004; **89**: 4620–4627.
22. Chen H, Montagnani M, Funahashi T *et al.* Adiponectin stimulates production of nitric oxide in vascular endothelial cells. *J Biol Chem* 2003; **278**: 45021–45026.
23. Kollerits B, Fliser D, Heid IM *et al.* Gender-specific association of adiponectin as a predictor of progression of chronic kidney disease: the Mild to Moderate Kidney Disease Study. *Kidney Int* 2007; **71**: 1279–1286.
24. Looker HC, Krakoff J, Funahashi T *et al.* Adiponectin concentrations are influenced by renal function and diabetes duration in Pima Indians with type 2 diabetes. *J Clin Endocrinol Metab* 2004; **89**: 4010–4017.
25. Furuhashi M, Ura N, Higashiura K *et al.* Blockade of the renin–angiotensin system increases adiponectin concentrations in patients with essential hypertension. *Hypertension* 2003; **42**: 76–81.
26. Wang Y, Xu A, Knight C *et al.* Hydroxylation and glycosylation of the four conserved lysine residues in the collagenous domain of adiponectin. Potential role in the modulation of its insulin-sensitizing activity. *J Biol Chem* 2002; **277**: 19521–19529.
27. Richards AA, Stephens T, Charlton HK *et al.* Adiponectin multimerization is dependent on conserved lysines in the collagenous domain: evidence for regulation of multimerization by alterations in posttranslational modifications. *Mol Endocrinol* 2006; **20**: 1673–1687.
28. Zoccali C, Mallamaci F, Panuccio V *et al.* Adiponectin is markedly increased in patients with nephrotic syndrome and is related to metabolic risk factors. *Kidney Int Suppl* 2003; **84**: S98–S102.
29. Pajvani UB, Hawkins M, Combs TP *et al.* Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem* 2004; **279**: 12152–12162.
30. Glintborg D, Frystyk J, Hojlund K *et al.* Total and high molecular weight (HMW) adiponectin levels and measures of glucose and lipid metabolism following pioglitazone treatment in a randomized placebo-controlled study in polycystic ovary syndrome. *Clin Endocrinol (Oxf)* 2007; **68**: 165–174.
31. Seaquist ER, Goetz FC, Rich S *et al.* Familial clustering of diabetic kidney disease: evidence of genetic susceptibility to diabetic nephropathy. *N Engl J Med* 1989; **320**: 1161–1165.
32. Clustering of long-term complications in families with diabetes in the diabetes control and complications trial. The Diabetes Control and Complications Trial Research Group. *Diab* 1997; **46**: 1829–1839.
33. Parving H-H, Mauer M, Ritz E. Diabetic nephropathy, Chapter 39. In: Brenner BM (ed). *Brenner and Rector's the Kidney*, 7th edn. WB Saunders (publ.): Boston, USA, 2004, pp 1777–1818.
34. Bröchner-Mortensen J, Rödbro P. Selection of routine method for determination of glomerular filtration rate in adult patients. *Scand J Clin Lab Invest* 1976; **36**: 35–45.
35. Levey AS, Bosch JP, Lewis JB *et al.* A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999; **130**: 461–470.
36. Pfeffer MA, Swedberg K, Granger CB *et al.* Effects of candesartan on mortality and morbidity in patients with chronic heart failure: the CHARM-Overall programme. *Lancet* 2003; **362**: 759–766.