Adiponectin and renal risk in type 2 diabetes


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**Association of ADIPOQ genetic variants and plasma adiponectin isoforms with the risk of incident renal events in type 2 diabetes**

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Abstract

**Background.** Adiponectin levels are high in cases of diabetic nephropathy, but it remains unclear whether these high levels are a cause or a consequence of the disease. We investigated the possible association of polymorphisms in the adiponectin gene and baseline adiponectin levels with the incidence of renal events in subjects with type 2 diabetes.

**Methods.** We studied three adiponectin polymorphisms (−11391G > A, +45T > G and +276G > T) in 3086 subjects with type 2 diabetes and high levels of albumin excretion from the diabetes, hypertension, microalbuminuria or proteinuria, cardiovascular events and ramipril (DIABHYCAR) trial. Baseline concentrations of total adiponectin and of adiponectin isoforms were determined in cases with incident renal events and in controls matched for sex, age, body mass index (BMI) and adiponectin genotype. We used another cohort of type 2 diabetes patients—the survice, diabete de type 2 et génétique(SURDIAGENE) study (n = 1004)—for the replication of genetic data.

**Results.** In DIABHYCAR, the −11391A and +45G alleles were associated with a higher incidence of renal events [hazard ratio (HR) = 1.73; 95% confidence interval (CI), 1.10–2.71; and HR = 1.68; 95% CI, 1.14–2.47, respectively]. The haplotype containing susceptibility alleles, −11391A/+45G/+276G, was more frequent in cases with renal events (5.1% vs. 1.9% in those without, P = 0.005). In SURDIAGENE, the −11391A/+45G/+276G haplotype was also associated with renal events (5.6% vs. 1.9% in those without, P = 0.03). In DIABHYCAR, all isoforms were more abundant in subjects carrying the −11391A or +45G alleles. Medium- (MMW) and low-molecular weight (LMW) isoforms were more abundant in cases with renal events.

**Conclusions.** In subjects with type 2 diabetes and early renal dysfunction, adiponectin gene variants are determinants of the renal risk. The −11391A and +45G alleles may affect renal risk by leading to high circulating adiponectin concentrations, at least those of MMW and LMW isoforms.

**Keywords:** adiponectin; genetic polymorphisms; nephropathy; prospective study; type 2 diabetes

Introduction

Subjects with type 2 diabetes, particularly those with high urinary albumin concentrations, are at high risk of renal disease [1]. Adiponectin is an anti-inflammatory adipokine with a low circulating concentration in cases of insulin resistance, type 2 diabetes and coronary heart disease [2,3]. Polymorphisms in the adiponectin gene have been associated with these conditions [2,3]. By contrast, adiponectin levels have been found to be high in subjects with kidney disease [4,5], diabetic nephropathy in particular [6–9], but have generally been interpreted as a consequence of renal failure. However, we found that high total adiponectin levels were predictive of microalbuminuria development in cases of type 1 diabetes [10], suggesting a causal association. Adiponectin is present in the bloodstream as trimers [low-molecular weight (LMW)], hexamers [medium-molecular weight (MMW)] and 12-mers to 18-mers [high-molecular weight (HMW)] [11]. Interest in the role of these complexes in metabolism and disease is growing, but the biological activities of these isoforms remain a matter of debate.

We therefore wanted to clarify the consequences of variants of the adiponectin gene and their plasma concentrations on the risk of renal failure in patients with type 2 diabetes. We investigated the association of three adiponectin gene (ADIPQ) polymorphisms [−11391G > A, +45 T > G and +276G > T single-nucleotide polymorphisms (SNPs)] and the various isoforms of circulating adiponectin with the risk of new renal events in 3086 French subjects participating in the genetic substudy of DIABHYCAR [12], a trial conducted on patients with type 2 diabetes and high urinary albumin concentrations. We also analysed an independent cohort of French type 2 diabetes patients, SURDIAGENE—a single-centre follow-up study—to replicate our findings [13].

Materials and methods

**Primary cohort**

The results of the DIABHYCAR trial have been reported elsewhere [10]. The participants had type 2 diabetes, high urinary albumin levels (76% micro- and 24% macroalbuminuric patients), were at least 50 years old and had serum creatinine concentrations ≤150 μmol/l (1.7 mg/dl). The drug tested against placebo was low-dose ramipril (1.25 mg/day), which decreases microalbuminuria in diabetic subjects. The trial was a 4-year, double-blind, parallel study. The French participants (3137 of the 4912 subjects) gave written informed consent for enrolment in the genetic sub-study. The study protocol was approved by the Angers University Hospital Ethics Committee. Renal events were defined as a doubling of serum creatinine concentration or end-stage renal disease. An independent Event Committee blinded to treatment group and genotyping data was responsible for adjudication concerning all the recorded events. This trial showed no effect of the drug on the incidence of renal events [12].

**Replication cohort**

SURDIAGENE is a prospective, single-centre follow-up study of patients with type 2 diabetes regularly followed up at the Diabetes Department of Poitiers University Hospital between 2001 and 2007. This study was designed to identify the genetic determinants of microvascular and macrovascular complications of type 2 diabetes (n = 1017, mean follow-up = 3 years) [13]. Renal events were defined exactly as for DIABHYCAR. The main exclusion criteria were residence outside the Poitiers area and/or evidence of non-diabetic kidney disease. The SURDIAGENE study was approved by the Poitiers University Ethics Committee. All participants gave written informed consent.

**Genotyping**

SNPs studied were chosen because they belong to the two linkage disequilibrium blocks of the adiponectin gene, and because their relationship to adiponectin levels or diabetes-related phenotypes has been extensively studied [3]. The −11391 G > A (rs17300539) and +276 G > T (rs1501299) SNPs were genotyped with the Assay On Demand (AOD) kit (Applied Biosystems, Foster City, CA). The conditions for the TaqMan reaction were: 50°C for 2 minutes, 95°C for 10 minutes, and 40 cycles of 95°C for 15 s and 60°C for 1 minute. The +45 T > G SNP (rs2241766) was genotyped with a PCR-molecular beacon technique [2]. The three SNPs were genotyped in 98% of the subjects in DIABHYCAR (n = 3086) and 99% of those in SURDIAGENE (n = 1004).

**Biochemical measurements**

Samples collected from fasted subjects were analysed centrally. An HPLC method was used to determine glycated haemoglobin (HbA1c) levels with a Biorad DIAMAT analyser (Bio-Rad, Richmond, CA, range of normal va-
Adiponectin and renal risk in type 2 diabetes

The baseline characteristics of subjects with \( n = 40, 22 \) doublings of serum creatinine concentration, and 18 cases of end-stage renal failure) and without renal events \( (n = 964) \) in SURDIAGENE are provided in Table A1, online appendix. The genotypes of the three SNPs were in Hardy–Weinberg equilibrium. In the whole SURDIAGENE cohort, no significant association was found between renal events and any of the SNPs (Table A2, online appendix). Nevertheless, the \(-11391A/+45G/+276G\) haplotype was significantly associated with a higher risk of renal events, as in DIABHYCAR \([OR = 2.99 (1.09–8.18)](\text{Table 3})\). The risk associated with the haplotype \(-11391G/+45T/+276G\) was similar to that observed in DIABHYCAR, although not significant \([OR = 0.70 (0.45–1.11)]\).

When analysing the pooled DIABHYCAR and SURDIAGENE data, no heterogeneity in allelic effects was found across the populations. The association of the \(-11391G > A\) and \(+45T > G\) SNPs with the incidence of renal events was significant in both the crude and adjusted models (Table A3, online appendix). Adjustment for renal parameters at entry increased the statistical significance further (Table A3, online appendix). The odds ratios associated with the various haplotypes remained unchanged when data for the two cohorts were combined, but the statistical significance of the associations was greater than for DIABHYCAR or SURDIAGENE alone \([ORs = 0.71 (0.55–0.93)\) and 2.80 (1.53–5.13), respectively, for G/T/G and A/G/G haplotypes, respectively\] (Table 3).

Data from the SURDIAGENE cohort

The baseline characteristics of subjects with \( n = 40, 22 \) doublings of serum creatinine concentration, and 18 cases of end-stage renal failure) and without renal events \( (n = 964) \) in SURDIAGENE are provided in Table A1, online appendix. The genotypes of the three SNPs were in Hardy–Weinberg equilibrium. In the whole SURDIAGENE cohort, no significant association was found between renal events and any of the SNPs (Table A2, online appendix). Nevertheless, the \(-11391A/+45G/+276G\) haplotype was significantly associated with a higher risk of renal events, as in DIABHYCAR \([OR = 2.99 (1.09–8.18)](\text{Table 3})\). The risk associated with the haplotype \(-11391G/+45T/+276G\) was similar to that observed in DIABHYCAR, although not significant \([OR = 0.70 (0.45–1.11)]\).

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Statistical analysis

The differences in baseline characteristics (other than adiponectin levels) as a function of the presence or absence of renal event were evaluated with a two-tailed \( t \)-test for continuous variables, and a \( \chi^2 \) test for categorical variables. Variables with a skewed distribution (serum creatinine concentration, urinary albumin excretion, total triglyceride concentration, C-reactive protein concentration and the concentrations of total adiponectin and adiponectin isoforms) were log-transformed before analysis. A \( \chi^2 \) test was used to determine whether genotype distributions were in Hardy–Weinberg equilibrium. We compared the baseline adiponectin levels of cases with a new renal event in DIABHYCAR with those of their matched controls, by paired \( t \)-tests. The associations between genotypes and baseline adiponectin levels were tested by analysis of covariance adjusted for age, sex, BMI and disease status. The relationship between \( ADIPOQ\) SNPs and the incidence of renal events was first assessed by \( \chi^2 \) tests. We assessed the effects of \( ADIPOQ\) SNPs on the incidence of renal events, by carrying out survival analyses, using Cox proportional hazard ratio models to adjust for covariates. The homogeneity of allelic effects across the DIABHYCAR and SURDIAGENE populations was checked by calculating the Mantel–Haenszel statistic, making it possible to evaluate genetic effects in a population consisting of DIABHYCAR and SURDIAGENE patients. All calculations, other than those for haplotype data, were performed with SYSTAT 11 for Windows (Systat Software Inc, San Jose, CA, USA).

Adiponectin levels in DIABHYCAR

At baseline, the MMW and LMW isoforms were significantly more abundant in subjects who suffered renal events than in the corresponding controls \(+25.5%\) and \(+12.2\%, respectively\) (Table 4). Two of the three SNPs were significantly associated with adiponectin levels (Table 4). Total, HMW and MMW adipo-
Table 1. Baseline characteristics of the genotyped French participants in the DIABHYCAR genetic substudy, as a function of the incidence of renal events

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All (n = 3086)</th>
<th>Without (n = 75)</th>
<th>Incident renal event</th>
</tr>
</thead>
<tbody>
<tr>
<td>With (n = 3011)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Women</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>26.9</td>
<td>24.0</td>
<td>27.0</td>
</tr>
<tr>
<td>65.7 ± 8.3</td>
<td>67.1 ± 7.5</td>
<td>65.6 ± 8.3</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.4 ± 4.6</td>
<td>28.6 ± 5.3</td>
<td>29.4 ± 4.6</td>
</tr>
<tr>
<td>Current smoking</td>
<td>14.4</td>
<td>20.0</td>
<td>14.2</td>
</tr>
<tr>
<td>Prior myocardial infarction</td>
<td>5.4</td>
<td>12.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>10.3 ± 7.7</td>
<td>12.4 ± 8.7</td>
<td>10.2 ± 7.7</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>7.87 ± 1.77</td>
<td>8.44 ± 2.15</td>
<td>7.85 ± 1.75</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.80 ± 1.07</td>
<td>5.91 ± 1.15</td>
<td>5.79 ± 1.07</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>3.53 ± 0.88</td>
<td>3.49 ± 1.06</td>
<td>3.53 ± 0.88</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.32 ± 0.36</td>
<td>1.21 ± 0.35</td>
<td>1.32 ± 0.36</td>
</tr>
<tr>
<td>Triglyceride (mmol/l)</td>
<td>1.83 (1.30–2.67)</td>
<td>2.56 (1.67–3.28)</td>
<td>1.81 (1.30–2.65)</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>144.3 ± 13.2</td>
<td>146.5 ± 11.8</td>
<td>144.2 ± 13.2</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>81.6 ± 8.1</td>
<td>82.5 ± 7.5</td>
<td>81.6 ± 8.1</td>
</tr>
<tr>
<td>Albuminuria (mg/l)</td>
<td>75.5 (40.0–184.0)</td>
<td>512.0 (128.4–1122.8)</td>
<td>74.5 (39.5–174.9)</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l)</td>
<td>88.4 (75.1–100.8)</td>
<td>97.2 (87.0–115.6)</td>
<td>88.4 (75.1–99.9)</td>
</tr>
<tr>
<td>CRP (mg/l)</td>
<td>3.10 (1.50–6.80)</td>
<td>4.00 (2.10–8.90)</td>
<td>3.10 (1.45–6.70)</td>
</tr>
</tbody>
</table>

**a**Data are means ± SD, or %, or medians (interquartile range).

**b**P-values for the differences in each characteristic between the groups with and without renal events. P-values were obtained in two-tailed t-tests or χ² tests, as appropriate.

**c**Skewed data were log-transformed before analysis.

**SBP** systolic blood pressure; **DBP** diastolic blood pressure; **CRP** C-reactive protein.

Table 2. DIABHYCAR: analysis of associations of ADIPOQ SNPs with incident renal events

<table>
<thead>
<tr>
<th>SNP</th>
<th>Genotype</th>
<th>With (n) (%)</th>
<th>Without (n) (%)</th>
<th>P allelic</th>
<th>Hazard ratio (95% CI) a</th>
<th>Hazard ratio (95% CI) b</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>−11391G &gt; A</td>
<td>GG</td>
<td>53 (70.7)</td>
<td>2488 (82.6)</td>
<td>0.01</td>
<td>1.73 (1.10–2.71)</td>
<td>1.54 (0.98–2.43)</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>GA</td>
<td>21 (28.0)</td>
<td>493 (16.4)</td>
<td></td>
<td>1.89 (1.14–3.13)</td>
<td>1.70 (1.02–2.83)</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>AA</td>
<td>1 (1.3)</td>
<td>30 (1.0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+45T &gt; G</td>
<td>TT</td>
<td>46 (61.3)</td>
<td>2223 (73.8)</td>
<td>0.006</td>
<td>1.68 (1.14–2.47)</td>
<td>2.06 (1.38–3.07)</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>TG</td>
<td>25 (33.3)</td>
<td>728 (24.2)</td>
<td></td>
<td>1.76 (1.10–2.83)</td>
<td>2.13 (1.32–3.44)</td>
<td>0.002</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>4 (5.3)</td>
<td>60 (2.00)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+276G &gt; T</td>
<td>TT</td>
<td>40 (53.3)</td>
<td>1570 (52.1)</td>
<td>0.85</td>
<td>0.95 (0.67–1.35)</td>
<td>0.78 (0.48–1.38)</td>
<td>0.85</td>
</tr>
<tr>
<td></td>
<td>GG</td>
<td>27 (36.0)</td>
<td>1197 (39.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>GT</td>
<td>8 (10.7)</td>
<td>244 (8.1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**a**Hazard ratios are adjusted for sex, diabetes duration, history of myocardial infarction, baseline values of age, BMI, HbA1c, total cholesterol, HDL cholesterol, systolic blood pressure, Log (TG), Log (CRP) and for the use of drugs at baseline: lipid-lowering treatments, treatments for hypertension, various antidiabetic treatments.

**b**As for **a** + adjustment for Log (albuminuria) and Log (creatinine).

Discussion

In the DIABHYCAR study of patients with micro- or macroalbuminuria, we found that the −11391A allele and +45G allele of the adiponectin gene were associated with a higher risk of renal events during a 4-year follow-up period. The haplotype containing these two alleles was also associated with a higher renal risk, whereas the frequent haplotype containing the other two alleles was associated with a lower risk. In SURDIAGENE, another cohort of type 2 diabetes patients without selection for urinary albumin levels, the haplotype containing the two high-risk alleles was also associated with renal events. A pooled analysis of the data for both studies gave a high statistical significance for associations between the ADIPOQ SNPs and the incidence of renal events. High MMW and LMW adiponectin concentrations were also predictive of renal events. The −11391A and +45G alleles were associated with high adiponectin concentrations. We therefore suggest that the high adiponectin levels associated with these two gene variants may be the cause, rather than a consequence of renal failure in these subjects.

In a large European case-control and family study, Vionnet et al. [15] reported an association of the
Table 3. Haplotype analysis of risk for incident renal events in DIABHYCAR, SURDIAGENE and combination of the two study populations

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>DIABHYCAR With</th>
<th>Without</th>
<th>P</th>
<th>SURDIAGENE With</th>
<th>Without</th>
<th>P</th>
<th>Both combined With</th>
<th>Without</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>G/T/G</td>
<td>0.493</td>
<td>0.576</td>
<td>0.04</td>
<td>0.513</td>
<td>0.595</td>
<td>0.12</td>
<td>0.500</td>
<td>0.581</td>
<td>0.01</td>
</tr>
<tr>
<td>G/T/T</td>
<td>0.185</td>
<td>0.210</td>
<td>0.43</td>
<td>0.281</td>
<td>0.203</td>
<td>0.10</td>
<td>0.217</td>
<td>0.209</td>
<td>0.80</td>
</tr>
<tr>
<td>G/G/G</td>
<td>0.168</td>
<td>0.121</td>
<td>0.09</td>
<td>0.082</td>
<td>0.107</td>
<td>0.45</td>
<td>0.140</td>
<td>0.118</td>
<td>0.33</td>
</tr>
<tr>
<td>A/T/T</td>
<td>0.102</td>
<td>0.068</td>
<td>0.12</td>
<td>0.069</td>
<td>0.068</td>
<td>0.99</td>
<td>0.092</td>
<td>0.068</td>
<td>0.18</td>
</tr>
<tr>
<td>A/G/G</td>
<td>0.051</td>
<td>0.019</td>
<td>0.005</td>
<td>0.056</td>
<td>0.019</td>
<td>0.03</td>
<td>0.052</td>
<td>0.019</td>
<td>0.0005</td>
</tr>
</tbody>
</table>

*Obtained with the SHESIS programme (haplotypes with a frequency <0.03 in both controls and cases were not taken into account).

Table 4. Baseline adiponectin levels as a function of the incidence of renal events and as a function of genotype for the three ADIPOQ SNPs in DIABHYCAR

<table>
<thead>
<tr>
<th>Renal event</th>
<th>Total (ELISA)</th>
<th>HMW</th>
<th>MMW</th>
<th>LMW</th>
<th>Total (RIA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cases (n = 74)</td>
<td>6.15 (5.00–10.07)</td>
<td>2.80 (1.70–4.46)</td>
<td>1.66 (1.25–2.45)</td>
<td>2.20 (1.62–2.81)</td>
<td>11.12 (7.91–16.89)</td>
</tr>
<tr>
<td>Controls (n = 74)</td>
<td>6.06 (4.49–7.64)</td>
<td>2.58 (1.57–3.80)</td>
<td>1.37 (1.04–1.76)</td>
<td>1.94 (1.49–2.37)</td>
<td>10.71 (7.89–15.93)</td>
</tr>
<tr>
<td>G/T/A</td>
<td>0.13</td>
<td>0.63</td>
<td>0.02</td>
<td>0.05</td>
<td>0.71</td>
</tr>
<tr>
<td>G/G/A (n = 250)</td>
<td>5.72 (4.12–7.79)</td>
<td>2.33 (1.47–3.58)</td>
<td>1.36 (0.97–1.87)</td>
<td>1.93 (1.48–2.38)</td>
<td>9.54 (6.86–14.46)</td>
</tr>
<tr>
<td>G/G/A (n = 72)</td>
<td>7.42 (5.37–9.97)</td>
<td>3.55 (2.15–5.18)</td>
<td>1.69 (1.23–2.13)</td>
<td>2.16 (1.68–2.65)</td>
<td>15.17 (9.90–19.58)</td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>&lt;0.001</td>
<td>0.03</td>
<td>0.06</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>+45T &gt; G</td>
<td>TT (n = 206)</td>
<td>5.73 (4.23–8.00)</td>
<td>2.40 (1.41–3.77)</td>
<td>1.36 (0.97–1.91)</td>
<td>1.95 (1.49–2.46)</td>
</tr>
<tr>
<td>+45T &gt; G</td>
<td>TG (n = 100)</td>
<td>6.12 (4.47–8.46)</td>
<td>2.63 (1.69–4.07)</td>
<td>1.47 (1.07–2.03)</td>
<td>1.93 (1.55–2.49)</td>
</tr>
<tr>
<td>+45T &gt; G</td>
<td>GG (n = 16)</td>
<td>8.89 (6.11–9.92)</td>
<td>3.10 (2.71–4.99)</td>
<td>2.05 (1.22–2.61)</td>
<td>2.52 (1.79–3.01)</td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.05</td>
<td>0.60</td>
</tr>
<tr>
<td>+11389A</td>
<td>TT (n = 192)</td>
<td>6.05 (4.23–8.53)</td>
<td>2.58 (1.45–3.81)</td>
<td>1.45 (0.99–2.15)</td>
<td>1.95 (1.47–2.52)</td>
</tr>
<tr>
<td>+11389A</td>
<td>GT (n = 104)</td>
<td>6.06 (4.49–8.08)</td>
<td>2.55 (1.63–4.02)</td>
<td>1.34 (1.07–1.94)</td>
<td>1.97 (1.62–2.52)</td>
</tr>
<tr>
<td>+11389A</td>
<td>TT (n = 26)</td>
<td>5.73 (4.14–8.18)</td>
<td>2.23 (1.55–4.14)</td>
<td>1.39 (0.98–1.67)</td>
<td>1.99 (1.68–2.49)</td>
</tr>
<tr>
<td>P&lt;0.001</td>
<td>0.58</td>
<td>0.51</td>
<td>0.71</td>
<td>0.85</td>
<td>0.16</td>
</tr>
</tbody>
</table>

aData are presented as medians (interquartile range).

bHMW, high molecular weight; MMW, medium molecular weight; LMW, low molecular weight.

cPaired t-test on log-transformed values for the comparison of cases (presenting a renal event during follow-up) and controls (who did not present such an event) matched for sex, age, BMI and SNP genotypes.

dAnalysis of covariance on log-transformed values, adjusted for sex, age, body mass index and disease status, global model.

Adiponectin and renal risk in type 2 diabetes

−11391A allele with diabetic nephropathy in type 1 diabetes. Two other case-control studies replicated this result in type 1 diabetes [16,17], consistent with our findings for type 2 diabetes. However, Jorsal et al. [16] failed to confirm this result in their prospective data. The +276G > T SNP was not associated with renal events in our type 2 diabetes patients. This was also the case in the previous study on type 1 diabetes [15].

Nephropathy has been associated with high adiponectin levels in type 1 and type 2 diabetes [6–10], chronic kidney disease and renal failure [5]. Renal failure per se may lead to the stimulation of adiponectin production as a physiological counter-regulatory response to restrict endothelial damage [7]. It may also decrease adiponectin clearance [6], and the kidney may develop secondary resistance to adiponectin [5]. However, we have previously shown that high adiponectin levels precede nephropathy in type 1 diabetes [10], and our results for type 2 diabetes are consistent with this finding, at least for MMW and LMW isoforms. A causal relationship is thus plausible. It could be argued that all the patients included in DIABHYCAR already presented some stage of diabetic nephropathy on inclusion. This was not the case in SURDIAGENE. However, if patients from SURDIAGENE were selected on the basis of DIABHYCAR criteria for albuminuria and creatininaemia (n = 443, 15 incident renal events), the −11391A allele was significantly associated with renal events (P = 0.02) (data not shown). Nevertheless, the −11391A allele has repeatedly been reported to be associated with high adiponectin levels in the healthy general population [2,3], and a transcriptional effect of this allele has been shown in vitro [18]. We therefore suggest that the functional −11391A allele variant resulting in high adiponectin levels favours renal failure, at least in patients with type 2 diabetes. This may also be the case for the +45G allele, which was found to be independently and additively associated with high adiponectin levels and with the risk of renal events in the DIABHYCAR study population. The +45G allele had also been reported to be associated with high adipo-
nnectin levels in healthy Caucasians [19]. This is at contrast with a study showing a beneficial effect of adipectin on albuminuria and podocyte function, low adiponectin levels being associated with early albuminuria [20]. Taking this study into account, our hypothesis is that the ADIPOQ SNPs associated with high levels of adiponectin may affect the severity of nephropathy rather than being involved in its onset, as their effects were observed principally in patients with albuminuria.

High adiponectin levels may favour glomerulosclerosis by inducing endothelial nitric oxide (eNOS) production [21] because NO dilates preglomerular vessels and potentiates postglomerular constriction due to angiotensin II [22]. The activation of nuclear factor-kappaB and enhancement of angiotensin II action by globular adiponectin are therefore important [23]. Adiponectin is generally considered to be anti-inflammatory, due to its inhibition of the nuclear factor-kappaB signalling pathway [24], but in vitro experiments with various types of cells have suggested that this molecule may increase nuclear factor-kappaB (NFkB) production, thereby playing a proinflammatory role. The type of cellular effect observed may depend on the isoform [23,25,26]. Our findings suggest that the LMW and MMW adiponectin isoforms may activate inflammation, contributing to sclerosis within the kidneys. The HMW isoform inhibited cytokine-induced NFkB activation, whereas globular adiponectin strongly activated NFkB and had a proinflammatory effect on cytokines in the same study [27]. Thus, cleaved forms may favour the inflammatory process whereas HMW may protect against inflammatory stimuli. The HMW isoform has generally been associated with higher insulin sensitivity which also could protect against renal risk. Unfortunately, insulin levels were not measured in our study. Nevertheless, we observed that only HMW isoform levels were significantly negatively associated with triglyceride levels (Table A4, online appendix), which could indicate a lower insulin resistance associated with HMW. Nevertheless, the mechanism underlying the association observed here between ADIPOQ SNPs and renal events is somewhat unclear since these SNPs are associated with all isoforms of adiponectin. Moreover, a positive association has recently been shown between adiponectin levels and insulin levels, insulin resistance [28].

The key strength of the DIABHYCAR study is its prospective follow-up design, with assessment of the studied events as part of a clinical trial [12]. However, this study is subject to several limitations. Its statistical power for the detection of significant associations with genetic variation was limited by the small number of incident cases. The adiponectin assay for isoforms (ELISA) yields lower values for total adiponectin than the other method using RIA. These two assays are highly correlated but it is likely that they do not measure exactly the same epitopes, which could explain the lack of association between the +45T > G SNP and the adiponectin levels measured by RIA. Nevertheless, this association has already been found in other studies including a large one (n = 1727) [19]. The participants in the DIABHYCAR study had type 2 diabetes with albuminuria. The findings of this study may therefore not be generally applicable to non-albuminuric diabetic patients or to non-diabetic subjects. The haplotype effect was replicated in the SURDIAGENE cohort of patients with type 2 diabetes but no selection for urinary albumin levels, but the allelic effect of the −11391A allele was observed only in a selection of albuminuric patients in this cohort. The first difference between the cohorts concerns the criteria of albuminuria and creatininæmia. Other differences in the selection of patients can also explain the lack of complete replication between the cohorts, mainly concerning treatments. In DIABHYCAR, patients on insulin and/or angiotensin-converting enzyme (ACE) inhibitor treatments were excluded, whereas in SURDIAGENE, 64.5% of patients were on insulin and 36.6% on ACE inhibitor.

In conclusion, the −11391A and +45G alleles of the adiponectin gene are associated with the risk of new renal events in type 2 diabetic patients with albuminuria. This might be due to an effect on circulating adiponectin concentrations, at least on the LMW and MMW isoforms.

### Supplementary data

Supplementary data are available online at http://ndt.oxfordjournals.org

### References


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Initiation of anaemia management in patients with chronic kidney disease not on dialysis in the Veterans Health Administration

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Abstract

Background. Erythropoiesis-stimulating agents (ESAs) are frequently used to treat anaemia of chronic kidney disease (CKD) in the dialysis setting; however, few data are available regarding factors influencing initiation of ESAs and other therapies in non-dialysis patients.

Methods. A retrospective cohort study of Veterans Health Administration data from 2003 to 2005 for 89 585 patients...