Genetic polymorphisms of the renin–angiotensin system and complications of insulin-dependent diabetes mellitus

Frans J. van Ittersum1, Angelique M. E. de Man1, Sandra Thijssen2, Peter de Knijff2, Eline Slagboom2, Yvo Smulders1, Lise Tarnow3, Ab J. M. Donker1, Henk J. G. Bilo4 and Coen D. A. Stehouwer1

1Department of Medicine, Institute for Cardiovascular Research, Vrije Universiteit Amsterdam, The Netherlands, 2Gaubius Laboratory TNO-PG, Leiden, The Netherlands, 3Steno Diabetes Center, Gentofte, Denmark and 4Department of Internal Medicine, Isala Clinics, Weezenlanden Location, Zwolle, The Netherlands

Abstract

Objective. Patients with insulin-dependent diabetes mellitus (IDDM) have a high risk of developing diabetic nephropathy, retinopathy and cardiovascular diseases. The contribution of gene polymorphisms of the renin angiotensin system to these complications is controversial and may differ among populations.

Methods. In 257 Dutch IDDM patients (188 with urinary albumin excretion (UAE) <30 mg/24 h), logistic regression analysis was used to study the relationships among, on the one hand, the insertion/deletion gene polymorphism of the angiotensin-converting enzyme gene (ACE-ID), the M235T gene polymorphism of the angiotensinogen gene (AGT-M235T), and the A1166C gene polymorphism of the angiotensin type 1 receptor gene (AT1-A1166C), and, on the other hand, UAE, retinopathy, hypertension, and coronary heart disease.

Results. The T-allele of the AGT-M235T polymorphism was associated with an increased risk of an elevated UAE (odds ratio (OR) 3.03; 95% confidence interval (CI) 1.06–8.61), but only when interaction with the D-allele of the ACE-ID polymorphism was considered. A previously described positive interaction between the T-allele of the AGT-M235T polymorphism and the D-allele of the ACE-ID polymorphism could not be confirmed. The T-allele was also associated with an increased risk of retinopathy (OR 3.89, 95% CI 1.79–8.47). The CC-genotype of the AT1-A1166C polymorphism was associated with hypertension (OR 3.58; 95% CI 1.23–10.37).

Conclusions. In a Dutch IDDM population, including 69 patients with (incipient) diabetic nephropathy, the T-allele of the AGT-M235T polymorphism is associated with an elevated UAE and diabetic retinopathy and the CC-genotype of the AT1-A1166C polymorphism is associated with hypertension. A previously described interaction between the AGT-M235T and the ACE-ID polymorphisms could not be confirmed. Since the number of nephropathic patients in this study is small, these conclusions must be interpreted with caution.

Keywords: insulin-dependent diabetes mellitus; complications; angiotensin-converting enzyme gene polymorphism; angiotensinogen gene polymorphism; angiotensin II type 1 receptor gene polymorphism

Introduction

Patients with insulin-dependent diabetes mellitus (IDDM) have a high risk of developing severe complications, such as diabetic nephropathy, retinopathy and cardiovascular disease. Studies demonstrating familial clustering of diabetic nephropathy, cardiovascular disease and hypertension [1–3] suggest that, in addition to poor glycaemic control, genetic factors may affect susceptibility to the development of diabetic micro- and macroangiopathy. In this context, genetic polymorphisms of the renin–angiotensin system (RAS) are attractive candidates to be studied, since inhibition of the activity of this system has shown to retard the development of diabetic complications, such as nephropathy and retinopathy [4,5].

Three genetic polymorphisms of the RAS have been studied. One meta-analysis of the insertion/deletion gene polymorphism of the angiotensin-converting enzyme gene (ACE-ID) has shown that, in IDDM patients, the D-allele is associated with diabetic nephropathy in a dominant model [6], although two other meta-analyses showed only a tendency towards this relationship [7] or severe heterogeneity impairing pooling of the data available [8]. The DD-genotype has been shown to be associated with an increased risk of coronary heart disease in IDDM patients, and an
increased risk of myocardial infarction in NIDDM patients [9], whereas the excess risk for myocardial infarction due to this genotype in a large general population was at most 10% [10]. Another gene polymorphism of the RAS, the angiotensinogen gene M235T polymorphism (AGT-M235T), has not been associated with an increased risk of diabetic nephropathy in a recent meta-analysis [11]. However, for this meta-analysis, the results of just six studies in IDDM patients and six studies in NIDDM patients were available and the association between nephropathy and the AGT-M235T polymorphism was not analysed in IDDM patients separately. The available studies of this polymorphism in IDDM patients showed an increased risk of diabetic nephropathy in some studies [12,13], but not in all [14–16]. In addition, a third gene polymorphism, the A1166C polymorphism of the angiotensin-II-type 1 receptor gene (AT1-A1166C), was not associated with diabetic nephropathy or retinopathy in Caucasian IDDM patients [17]. However, this genotype was associated with coronary artery stenosis in another study of diabetic patients (probably NIDDM) [18]. Interactions between the D-allele of the ACE-ID polymorphism and the T-allele of the AGT-M235T polymorphism have been found to be associated with nephropathy in IDDM patients with proliferative retinopathy [12], but have not been studied extensively.

Thus, with regard to the major complications of IDDM, the contribution of and the interactions among several gene polymorphisms of the RAS have not been fully elucidated, and may in fact differ among populations. Therefore, we analysed the associations of three gene polymorphisms and diabetic complications in a group of Caucasian IDDM patients in The Netherlands.

Methods

All Caucasian IDDM patients older than 17 years who visited the outpatient clinic of the Department of Medicine of the Isala Clinics, Weezenlanden Location in Zwolle between September 1993 and March 1994 were asked to participate in the study. A total of 268 patients gave informed consent. Clinical characteristics were gathered from the records and from interviewing the patients. IDDM was defined as onset of diabetes before the age of 30 years and necessity of insulin therapy within 6 months after the onset of the disease. Patients were classified according to the amount of albuminuria using the median result of three 24-h urine collections. Urinary albumin excretion (UAE) was determined with a nephelometric technique (Beckmann Instrument Inc., Brea, CA, USA). Normoalbuminuria was defined as UAE < 30 mg/24 h; microalbuminuria as UAE 30–300 mg/24 h, and macroalbuminuria as UAE ≥ 300 mg/24 h. Smoking was defined as more than one cigarette, cigar or pipe per day. Retinopathy was assessed by an experienced ophthalmologist and graded as: none, background, non-proliferative or proliferative. Coronary heart disease was defined as history of treatment or admission for myocardial infarction, unstable angina pectoris (diagnosis made by an experienced cardiologist), percutaneous transluminal coronary angioplasty or coronary artery bypass graft, or stable angina pectoris with angiographic evidence of significant (>50%) coronary artery stenosis or positive exercise ECG-test result. Data on stroke and transient ischaemic attacks were not collected. The study was approved by the medical ethics committee of the Weezenlanden Ziekenhuis and all patients gave informed consent.

On the day of the study visit, patients took their regular diet and medication. In the hospital height and weight were determined to calculate the body mass index (BMI). Blood pressure was taken at the right arm in the sitting position after 5 min of rest using a sphygmomanometer with appropriate cuff size. The first Korotkoff sound was taken as the systolic and the fifth Korotkoff sound as the diastolic blood pressure. Patients were considered hypertensive if blood pressure exceeded the limit of 140/90 mmHg at two or more occasions and/or if they used antihypertensive medication. Blood samples were drawn for measurement of serum creatinine, serum cholesterol, HbA1c and plasma ACE level, and for the determination of the genotypes.

Serum creatinine was determined with the Jaffé reaction with a Hitachi 1717 automatic analyser; HbA1c with high-pressure liquid chromatography (normal range 3.4–6.5%). Serum ACE levels were measured spectrophotometrically based on the decrease of extension at a wavelength of 340 nm [19].

Determination of genotypes

Genomic DNA was obtained from the white cell pellets using an isoamylalcohol–chloroform extraction. To determine the ACE-ID genotype, DNA (100 ng) was amplified using the polymerase chain reaction (PCR). Thirty-two cycles of amplification were performed with a High Bau DNA Thermal Cycler with denaturation for 1 min at 94°C, annealing for 1.5 min at 58°C, and extension for 2 min at 72°C. Each 50 μl reaction mixture contained 1.0 μl 50 mM magnesium chloride, 5.0 μl 1% W-1, 2.5 μl DMSO, 5.0 μl 2 mM dNTP polymerization mix, 0.2 μl 10 μl Taq DNA polymerase and 100 ng unlabelled primers. The PCR primers with the sequences reported by Tiret [20] were used: ACE-1 24-mer Isogen Bioscience (5'-CTGGAGAGCCTCACCACCCCTTCTT-3') and ACE-2 25-mer Isogen Bioscience (5'-GATGTG-GCCATCAGATACGGTATC-3'). The reaction products were electrophorized on 2% agarose gels and stained with ethidium bromide. Under ultraviolet light two bands, insertion (I; 490 bp) and deletion (D; 190 bp) were visible. Preferential amplification of the IDD homozygotes has led to their mistyping as DD homozygotes [21]. To exclude this possibility, all DD homozygotes were retyped using an I-specific primer (5'-TTTGGAGACGGAGTCTCGCT-3') with modification in reaction conditions such that denaturation took place at 93°C for 1 min, annealing at 68°C for 1.5 min, and extension at 72°C for 2 min. When a ‘DD’ sample amplified using the I-specific primer, it was recorded ‘ID’. Determination of the AGT-M235T genotype was performed by enzymatic amplification of DNA with the PCR described above using the unlabelled primers ANGOL 1C Isogen Bioscience (5'-GCTGTCACAAGTGACGCCACCC-3') and ANGOL 2D Isogen Bioscience (5'-GTGCAG-GGCCTGCTCTCTTCT-3'). The PCR products were digested with the restriction enzyme Aspl at 37°C for 2 h. DNA fragments were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide and were visualized using visualizing.
ultraviolet light. The PCR-amplified region was 165 bp long and contained the Apo restriction site. Digestion with Apo yielded 141 and 24 bp products according to the presence of the T→C mutation at nucleotide 704 [22]. Genotypes were coded as MM, MT or TT.

The determination of the AT1-A1166C genotype started with the synthesis of two primers as described by Bonnardeaux [23]: U6 (5'-AGAAGCCTGCACCATTGTTTGGAG-3') and Lx (5'-CAACAAGACAAAGACAAAGCC-3'). PCR was performed with an initial denaturation at 93°C for 3 min, followed by 32 cycles (93°C for 45 s, 58°C for 45 s, 72°C for 45 s). The PCR products were digested with 1.5 U Dde I overnight at 37°C and the digestion products were separated on 3% agarose gels and visualised with ethidium bromide. The genotype C1166 was identified if digestion of the PCR-products was observed. The genotypes were coded as AA, AC, and CC.

**Statistical analysis**

Data are given as mean (SD) unless otherwise indicated. Logistic regression was performed with elevated UAE (>30 mg/24 h), retinopathy, cardiovascular disease and hypertension as dependent variables. Independent variables were selected for inclusion in the model because of their pathophysiological relevance. Therefore, all selected variables were kept in the regression model. In case of elevated UAE, analyses were performed twice: first, with elevated UAE defined as UAE≥30 mg/24 h and second, defined as UAE≥30 mg/24 h plus any retinopathy. The latter analysis was added to study a combination of diabetic complications, which might reflect a phenotype which is highly vulnerable to diabetic nephropathy, these analyses were repeated with UAE≥30 mg/24 h replaced by UAE≥30 mg/24 h or the use of an ACE-inhibitor. All analyses were adjusted for age, sex, diabetes duration, HbA1c, and smoking (yes or no). In the analyses of elevated UAE, additional independent variables were the presence of hypertension (yes or no) and total serum cholesterol (mmol/l). In the analysis of retinopathy, additional independent variables were hypertension, total serum cholesterol and UAE≥30 mg/24 h (yes or no). In case of coronary heart disease, additional independent variables were hypertension (yes or no), total serum cholesterol, diabetic retinopathy and/or UAE≥30 mg/24 h. In the analyses with hypertension as dependent variable, additional analyses were performed with diabetic retinopathy and/or UAE≥30 mg/24 h as independent variables. In all analyses, the genotypes and the alleles of the three genetic polymorphisms were studied both in a dominant and in a non-dominant model (dominant: DD and ID vs II for the ACE-ID polymorphism; TT and MT vs MM for the AGT-M235T polymorphism; and CC and AC vs AA for the AT1-A1166C polymorphism; non-dominant: ID vs II and DD vs II for the ACE-ID polymorphism; MT vs MM and TT vs MM for the AGT-M235T polymorphism; and AC vs AA and CC vs AA for the AT1-A1166C polymorphism).

Interactions were studied in a dominant model using interaction variables D*T, D*C and T*C, where D is ‘1’ in case of DD or ID and ‘0’ in case of II; T is ‘1’ in case of MT or TT and ‘0’ in case of MM and C is ‘1’ in case of AC or CC and ‘0’ in case of AA. Interactions in a non-dominant model were studied using all 27 genotypes and taking the HIMMA genotype as a reference for the other 26 genotypes. Odds ratios (ORs) are provided with their 95% confidence intervals (95% CIs) in parentheses.

Expected and observed frequencies of genotypes were compared with χ2-analysis. All analyses were performed with the SPSS 7.5 for Windows 95 and SPSS 9.01 for Windows 95, 98 & NT software packages (SPSS Inc., Chicago, IL, USA).

**Results**

All 268 invited patients were included in the study. Patient characteristics are given in Table 1. UAE could not be determined in 11 patients (three men), because 24-h urine collections were not handed in or were lost. Data on retinopathy were not available in 16 patients. The distribution of the ACE and the angiotensinogen genotypes were similar in subgroups with a normal or increased UAE, but the AA genotype of the AT1-A1166C polymorphism tended to be more frequent among patients with macroalbuminuria (AA 60%, AC 37%, CC 3%) when compared to normalalbuminuria (AA 37%, AC 53%, CC 10%, P=0.06). Allele and genotype frequencies were in Hardy-Weinberg equilibrium in all subgroups, except for the M235T genotypes in the subgroup with retinopathy (Table 2).

**ACE levels and ACE-ID genotypes**

ACE levels, obtained in 248 patients, differed significantly between the groups (II-genotype, [n=77], 36.6 (17.7) U/l; ID-genotype, [n=116], 48.0 (21.8) U/l; DD-genotype, [n=55], 51.0 (20.5) U/l; P (ANOVA)<0.0001).

**Elevated UAE**

When elevated UAE defined as UAE≥30 mg/24 h was taken as dependent variable, significant associations were found with diabetes duration (OR 1.22 [1.03–1.45] per 5 years) and hypertension (OR 5.92 [2.66–13.17]). Univariate analyses of the alleles and elevated UAE did not reveal statistically significant associations (data not shown).

When the interaction variable between the D-allele (ACE-ID) and the T-allele (AGT-M235T) was introduced into the logistic regression model, the T-allele showed a significant association with an elevated UAE (OR 3.03 [1.06–8.61]), whereas the interaction between the alleles itself was inversely associated with an elevated UAE (OR 0.14 [0.02–1.13]). After addition of retinopathy to this model (which itself had an OR 2.69 [1.15–6.28]), the statistically significant association of the T-allele (AGT-M235T) with elevated UAE was similar (OR 2.95 vs 3.03); analysis of the interaction of the D-allele (ACE-ID) and the genotypes of the AGT-M235T polymorphism again showed an inverse association of the interaction between the D-allele and the TT-genotype with an elevated UAE (OR 0.05 [0.003–0.79]). Interactions with the AT1-A1166C polymorphism were not observed (P values for I/D and AT1-A1166C polymorphism interaction 0.2 and for
### Table 1. Patient characteristics in 268 IDDM patients

<table>
<thead>
<tr>
<th></th>
<th>All (m/f)</th>
<th>Normo-albuminuria</th>
<th>Micro-albuminuria</th>
<th>Macro-albuminuria</th>
<th>ANOVA/Chi-square</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (m/f)</td>
<td>268 (153/115)</td>
<td>188 (109/79)</td>
<td>39 (21/18)</td>
<td>30 (20/10)</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>45.9 (15.2)</td>
<td>42.2 (13.7)</td>
<td>50.6 (15.3)</td>
<td>57.7 (11.8)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>17.1 (11.4)</td>
<td>15.5 (11.3)</td>
<td>20.6 (9.8)</td>
<td>20.8 (11.2)</td>
<td>0.006</td>
</tr>
<tr>
<td>Retinopathy (none, background, preproliferative, proliferative) (%)</td>
<td>151/35/22/44</td>
<td>130/20/11/20</td>
<td>15/10/3/7</td>
<td>3/3/6/15</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>UAE (median, range)</td>
<td>11 (2 to 2300)</td>
<td>8 (2 to 29)</td>
<td>64 (30 to 271)</td>
<td>689 (337 to 2300)</td>
<td></td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>44.0</td>
<td>30.9</td>
<td>59.0</td>
<td>86.7</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Use of antihypertensive drugs (%)</td>
<td>18.3</td>
<td>6.9</td>
<td>30.8</td>
<td>60.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Use of ACE-inhibitors (%)</td>
<td>14.9</td>
<td>4.8</td>
<td>30.8</td>
<td>50.0</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Creatinine (µmol/l) median (range)</td>
<td>88 (58–1470)</td>
<td>86 (58–120)</td>
<td>90 (67–138)</td>
<td>592 (68–1470)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/l)</td>
<td>5.5 (1.1)</td>
<td>5.3 (1.1)</td>
<td>5.9 (1.1)</td>
<td>5.7 (1.1)</td>
<td>0.009</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.3 (2.0)</td>
<td>8.2 (2.0)</td>
<td>8.5 (1.8)</td>
<td>8.9 (2.1)</td>
<td>0.3</td>
</tr>
<tr>
<td>Serum ACE (U/l)</td>
<td>45.2 (21.1)</td>
<td>46.9 (21.2)</td>
<td>42.1 (17.6)</td>
<td>38.3 (23.9)</td>
<td>0.09</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>25.8</td>
<td>24.3</td>
<td>33.8</td>
<td>34.6</td>
<td>0.3</td>
</tr>
<tr>
<td>Cardiovascular events (n (%))</td>
<td>38 (14.2)</td>
<td>17 (9.3)</td>
<td>5 (13.5)</td>
<td>12 (41.4)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>ACE-ID-genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I-allele/D-allele (%)</td>
<td>53/47</td>
<td>52/48</td>
<td>59/41</td>
<td>55/45</td>
<td></td>
</tr>
<tr>
<td>II/ID/DD (%)</td>
<td>30/47/23</td>
<td>28/46/26</td>
<td>33/51/16</td>
<td>33/43/24</td>
<td>0.7</td>
</tr>
<tr>
<td>AT1-A1166C-genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-allele/C-allele (%)</td>
<td>65/35</td>
<td>64/36</td>
<td>56/44</td>
<td>78/22</td>
<td></td>
</tr>
<tr>
<td>AA/AC/CC (%)</td>
<td>40/50/10</td>
<td>37/53/10</td>
<td>31/51/18</td>
<td>60/37/3</td>
<td>0.06</td>
</tr>
</tbody>
</table>

#Some data were missing, so that numbers do not add up in all cases (see Methods). #Data are number, mean (SD) or frequency, unless otherwise indicated. Five patients had blood pressures <140/<90 mmHg and used an ACE-inhibitor as the only antihypertensive drug. These patients were classified as having hypertension. Exclusion of these patients from the analysis did not materially affect the results.
AGT-M235T and AT1-A1166C interaction 0.4). Interactions among the genotypes in a non-dominant model were not observed (P values > 0.8).

When elevated UAE was defined as UAE ≥ 30 mg/24 h or use of an ACE-inhibitor, the univariate analyses did not show statistically significant associations (Table 3). The results of the multivariate analyses were even more pronounced than with the previous definition (Table 3). In addition to diabetes duration and hypertension, total serum cholesterol was found to be statistically significantly associated with an elevated UAE (OR 1.57 [1.08–2.29]). When the interaction variable between the D-allele (ACE-ID) and the T-allele (AGT-M235T) was introduced into the logistic regression model, both the T-allele (AGT-M235T) and the interaction variable were statistically significantly associated with an elevated UAE (ORs 4.45 and 0.07; Table 3). With respect to the genotypes, the MT-genotype (AGT-M235T) and the interaction between the I/D (ACE-ID) and the MT (AGT-M235T) genotypes were statistically significantly related to an elevated UAE (ORs 3.00 [1.15–7.87] and 0.02 [0.0007–0.54]). Other interactions were not observed in this analysis.

When elevated UAE was defined as UAE ≥ 30 mg/24 h plus retinopathy, the associations with diabetes duration and hypertension remained. In the model without any interaction term, the presence of the T-allele of the AGT-M235T polymorphism was significantly associated with elevated UAE (OR 2.91 [1.04–8.19]). In the analysis of the genotypes, AGT-M235T heterozygosity was associated with elevated UAE (OR, 2.70; P=0.07). With this definition of elevated UAE, interactions among the genes and genotypes were not found (P values > 0.3).

Defining an elevated UAE as UAE ≥ 30 mg/24 h plus retinopathy or use of an ACE-inhibitor plus retinopathy revealed similar results as using the previous definition (data not shown). The relationship with the T-allele (AGT-M235T) was even stronger (OR 4.04 [1.41–11.6]).

### Retinopathy (Table 3)

In the univariate analysis, the T-allele was associated with retinopathy (OR 2.17). In the multivariate analyses, the presence of retinopathy was associated with diabetes duration (OR 1.76 [1.44–2.16] per 5 years), hypertension (OR 2.91 [1.38–6.12]) and the presence...
of the T-allele of AGT-M235T (OR, 3.89; Table 3). Retinopathy was also associated with the MT genotype of AGT-M235T (OR, 4.53). When added to the model, elevated UAE (UAE ≥ 30 mg/24 h) was significantly and independently from the T-allele of AGT-M235T associated with retinopathy (OR 2.86 [1.23–6.66]). Interactions among the alleles and genotypes were not observed (P-values > 0.5).

**Coronary heart disease (Table 4)**

In the univariate analyses, the alleles and genotypes of the gene polymorphisms studied were not associated with coronary heart disease. In the multivariate analyses, coronary heart disease was associated with age (OR 1.84 [1.41–2.41] per 5 years), gender (OR for females 0.34 [0.12–0.95]) and cholesterol (OR 1.61 [1.02 to 2.56] per 1 mmol/l). Addition of retinopathy (defined as any retinopathy) or UAE ≥ 30 mg/24 h (yes or no) as independent variable did not change these results. Interactions among the alleles and genotypes were not observed (P-values > 0.4).

**Hypertension (Table 4)**

In the univariate analyses, the alleles and the genotypes were not associated with hypertension. In the multivariate analyses, hypertension was associated with age (OR 1.24 [1.09–1.41] per 5 years) and, inversely, with smoking (OR 0.39 [0.19–0.79]). In a dominant model, associations of the alleles with hypertension were not found, whereas in a non-dominant model the CC genotype of AT1-A1166C polymorphism was significantly associated with hypertension (OR 3.58 [1.23–10.37]). UAE ≥ 30 mg/24 h (yes or no) was significantly associated with hypertension (OR 3.01 [2.35–10.71]) in both the dominant and the non-dominant model, as was retinopathy (OR 2.91), but the addition of these variables did not affect the association between hypertension and the CC-genotype of the AT1-A1166C polymorphism (OR, 2.98). Interactions among the alleles and the genotypes were not found (P-values > 0.4).

In all analyses, the results were similar when men and women were analysed separately.

**Discussion**

Recent meta-analyses have shown controversial results with regard to the relationship between the D-allele of the ACE-ID gene polymorphism and the risk of diabetic nephropathy in IDDM patients [6,8,10]. The present cross-sectional study in 268 IDDM patients does not find a statistically significant association among the D-allele or DD genotype (ACE-ID) and diabetic nephropathy in the dominant, non-dominant or interaction models. This might be due to a lack of statistical power. The main findings of this study are the associations of the AGT-M235T polymorphism with both elevated UAE and retinopathy in Dutch IDDM patients, and the absence of a positive interaction between the ACE-ID and the AGT-M235T polymorphisms, thus not confirming a previous study [12]. Our results were robust in that the AGT-M235T polymorphism was consistently associated with risk of retinopathy and elevated UAE in all analyses, although these associations were not always statistically significant (Table 3).

Previous studies in IDDM patients showed an association between diabetic nephropathy and the T-allele of the AGT-M235T polymorphism [13], and, among patients with proliferative retinopathy, an association with an interaction between the D-allele (ACE-ID) and the T-allele (AGT-M235T) [12]. In the present study, the T-allele (AGT-M235T) was also associated with an elevated UAE (OR 3.03 [1.06–8.61]). However, the interaction between the D-allele (ACE-ID) and the T-allele (AGT-M235T) differed from previous findings: in the initial observation the interaction between both alleles increased the risk for diabetic nephropathy [12], whereas in the present study, if anything, it tended towards protection. The reported interaction between these two RAS gene polymorphisms amplifying the risk for diabetic nephropathy, therefore, could not be confirmed in the population of the present study. The 95% CI suggests that a type II statistical error is unlikely; therefore, we suggest that the presence of this interaction may differ among populations.

Since it has been reported that the combination of retinopathy and microalbuminuria reflects a state in which patients are highly vulnerable to diabetic complications [24], we performed an additional analysis.
with elevated UAE defined as UAE ≥ 30 mg/24 h plus retinopathy. With this definition, the association between nephropathy and the T-allele (AGT-M235T) was present as well. However, the associations were of similar strength, suggesting that our definition does not define a complication-prone phenotype that is a consequence of one of the gene polymorphisms studied.

In the present study the T-allele of the AGT-M235T polymorphism was associated with retinopathy. In patients with retinopathy, the Hardy-Weinberg equilibrium for the M235T gene polymorphism was disturbed. Retinopathy itself is not a fatal complication of IDDM, but may reflect a complicated state in which patients have an elevated risk of lethal events. However, as we tested genotype distributions with regard to a number of clinical variables, we cannot exclude a chance finding. A recent meta-analysis, including two studies in IDDM patients [13,25], did not find an association between the AGT-M235T polymorphism and diabetic retinopathy [11]. Nevertheless, associations between RAS activity and retinopathy have been reported previously: an elevation of the circulating and intraocular levels of prorenin, possibly due to an increased intraocular production, has been associated with retinopathy [26,27], and blockade of the RAS with an ACE-inhibitor has shown beneficial effects on the progression of retinopathy in IDDM patients [5].

Recently, the presence of the I-allele of the ACE-ID polymorphism has been associated with protection against cardiovascular diseases in IDDM patients with nephropathy [28]. In the present study, we did not find any such association. However, our study probably lacked sufficient statistical power, since only 69 patients with elevated UAE were included, whereas the initial observation by Tarnow et al. was done in 198 IDDM patients with nephropathy [28].

In the present study, hypertension was associated with the CC genotype of the AT1-A1166C polymorphism, independent of the presence of an elevated UAE. Previous studies revealed associations of the C-allele (AT1-A1166C) with the severity of coronary artery stenosis in a population with coronary artery disease [18], increased artery stiffness in never-treated hypertensive patients, and a reduction of pulse wave velocity after treatment with an ACE-inhibitor in patients with hypertension [29]. In another study, lower office blood pressures were found in the CC group when compared with the AC and AA groups, and differences in left ventricular mass or carotid arterial compliance were not found [30]. In a study on NIDDM patients, an association between hypertension and the AT1166C gene polymorphisms was not found [31]. In IDDM patients, associations of this polymorphism with hypertension have not been studied; an association with diabetic nephropathy was not found [17]. Although these data suggest that the CC genotype (AT1-A1166C) is related to cardiovascular abnormalities, definite conclusions cannot be derived from these data.

A limitation of the present study is the low number of patients with micro- or macroalbuminuria (69 plus 9 normoalbuminuric patients using an ACE-inhibitor).

However, in our opinion it is important to present all available results on studies of genetic polymorphisms to avoid publication bias in future meta-analyses.

In conclusion, in the present study on three gene polymorphisms of the RAS, the AGT-M235T polymorphism was associated with an elevated UAE and retinopathy in a Dutch Caucasian IDDM population. In the same group, the CC genotype of the AT1-A1166C polymorphism was associated with hypertension. A previously described interaction [12] between the D-allele of the ACE-ID polymorphism and the T-allele of the AGT-M235T polymorphism that increased the risk for an elevated UAE could not be confirmed and thus apparently is not a consistent phenomenon.

References
Genetic polymorphisms of the renin–angiotensin system


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