Angiotensin-II type 1 receptor gene polymorphism and diabetic microangiopathy

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Abstract

Background. Genotypic abnormalities of the renin-angiotensin system have been suggested as risk factors for the development of hypertension, diabetic nephropathy and proliferative retinopathy. Most of the known actions of angiotensin-II are exerted through the angiotensin-II type 1 receptor, which is present particularly in vascular smooth muscle cells, myocardium and the kidney. A transversion of adenine to cytosine at nucleotide position 1166 in the gene coding for the angiotensin-II type 1 receptor has been associated with hypertension in the non-diabetic population.

Methods. We studied the relationship between the A1166→C polymorphism in the angiotensin-II type 1 receptor gene in insulin dependent diabetes mellitus (IDDM) and diabetic nephropathy (121 men, 77 women, age 41±10 years, diabetes duration 27±8 years) and in IDDM patients with normoalbuminuria (116 men, 74 women, age 43±10 years, diabetes duration 27±8 years). 156 patients (40%) had proliferative retinopathy, 67 patients (17%) had no diabetic retinopathy.

Results. There was no difference in genotype distribution between IDDM patients with diabetic nephropathy and normoalbuminuria: 103 (52%) / 81 (41%) / 14 (7%) vs. 97 (51%) / 80 (42%) / 13 (7%) had AA/AC/CC genotypes, respectively. The allele frequencies (A/C) in patients with nephropathy (0.73/0.27) and patients with normoalbuminuria (0.72/0.28) were also similar. No difference in genotype distribution between IDDM patients with proliferative retinopathy and without diabetic retinopathy was found either: 77 (50%) / 66 (42%) / 13 (8%) vs. 42 (63%) / 22 (33%) / 3 (4%) had AA/AC/CC genotypes, respectively.

Conclusions. The A1166→C polymorphism in the angiotensin-II type 1 receptor gene does not contribute to the genetic susceptibility to diabetic nephropathy or proliferative retinopathy in Caucasian IDDM patients.

Key words: angiotensin-II type 1 receptor gene polymorphism; diabetic nephropathy; IDDM; proliferative retinopathy

Introduction

A number of factors has been considered to contribute to the initiation and progression of diabetic nephropathy, including genetic and racial predispositions, glycaemic and other metabolic abnormalities, alterations in systemic and renal hemodynamics and various cytokines and growth factors, as reviewed by Parving et al. [1]. Abnormalities in the renin-angiotensin system have been suggested to play a role in the development of diabetic nephropathy and retinopathy [2-5]. Nearly all known actions of angiotensin-II are mediated through the angiotensin-II type 1 (AT1) receptor, a G-protein coupled receptor, which is particularly present in vascular smooth muscle, myocardium, adrenal cortex and the kidney [6-8]. Thus the gene coding for the AT1 receptor (AGT1R gene) could be a candidate gene for diabetic nephropathy. Recently the cDNA encoding the human AT1 receptor has been cloned [9,10]. A polymorphism in the AGT1R gene, corresponding to a transversion of adenine to cytosine (A→C) at nucleotide position 1166 of the mRNA sequence has been identified and associated with essential hypertension in a non-diabetic population [11]. Furthermore, genetic predisposition to hypertension has been suggested as a risk marker for diabetic nephropathy in patients with insulin dependent diabetes mellitus (IDDM) [12,13].

We have studied the relationship between the A1166→C polymorphism in the AGT1R gene in two large groups of IDDM patients with and without diabetic nephropathy, respectively. Since previous studies have suggested a potential role for the renin-angiotensin system in the development of proliferative retinopathy [14], we furthermore assessed the possible link between the AGT1R gene polymorphism and proliferative diabetic retinopathy.
Subjects and methods

The patients participating in this study have been described in details previously [15,16]. All albuminuric IDDM patients attending the outpatient clinic at Steno Diabetes Center in 1993, who were more than 18 years of age and had their glomerular filtration rate measured during the same year were invited to participate in the study. Two hundred patients (83%) accepted the invitation and were enrolled. The AGT\textsubscript{R} gene polymorphism was determined in 198 nephropathic patients and in 190 IDDM patients with persisting normoalbuminuria recruited from our outpatient clinic and matched for sex, age and duration of diabetes (Table 1).

Lymphocytes were isolated from peripheral blood and DNA prepared by standard techniques [17]. The A\textsuperscript{1166->C} polymorphism was assayed by allele-specific oligonucleotide hybridisations of PCR products [11]. The primers used to amplify the AGT\textsubscript{R} region encompassing the A\textsuperscript{1166->C} polymorphism were 5'-AATGCTTGTAGCCAAAGTCACCT-3' and 5'-GGCTTTGCTTTGTCTT-GTTG-3'. The hybridization procedure [11] was slightly modified: Each allele was detected by preincubating the amplification products for 2 h with 50 pmoles of unlabelled oligonucleotide probe specific for the other allele and then incubating for 4 h with 10 pmoles of labelled probe specific for the allele (sequences published previously [11]); the membranes were then washed and autoradiographed. Patients were classified as AA/AC/CC according to the absence/presence of the A\textsuperscript{1166->C} mutation.

Urinary albumin concentration was determined by enzyme immunoassay [18] from 24 h urine collections. Arterial blood pressure was measured twice, on the right arm, after at least 10 min rest in the supine position and averaged. The measurements were performed with a Hawksley random zero sphygmomanometer (Hawksley & Sons Ltd, Lancing, Sussex, UK) and appropriate cuff size. Diastolic blood pressure was recorded at the disappearance of Korotkoff sounds (phase V). Hypertension was diagnosed according to the WHO criteria: systolic blood pressure $\geq$ 160 mm Hg and/or diastolic blood pressure $\geq$ 95 mm Hg and/or if antihypertensive medication was being prescribed. Retinopathy was assessed by fundus photography after pupillary dilatation and graded: nihil, simplex or proliferative diabetic retinopathy.

Table 1. Clinical characteristics of 198 IDDM patients with diabetic nephropathy and 190 IDDM patients with normoalbuminuria

<table>
<thead>
<tr>
<th></th>
<th>Nephropathy (n = 198)</th>
<th>Normoalbuminuria (n = 190)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (men/women)</td>
<td>121/77</td>
<td>116/74</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>40.9 ± 9.6</td>
<td>42.5 ± 10.0</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>26.7 ± 7.9</td>
<td>26.8 ± 8.5</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.0 ± 3.3</td>
<td>23.6 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>HbA\textsubscript{lc} (%)</td>
<td>9.6 ± 1.5</td>
<td>8.5 ± 1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>50%</td>
<td>42%</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary albumin excretion rate* (mg/24 h)*</td>
<td>796 (16–14545)</td>
<td>8 (1–30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine* (umol/l)</td>
<td>103 (54–684)</td>
<td>76 (40–116)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>151 ± 23</td>
<td>132 ± 18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>86 ± 13</td>
<td>76 ± 10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prevalence of antihypertensive treatment (%)</td>
<td>76%</td>
<td>11%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Retinopathy, (numbers [%])</td>
<td>nihil 0</td>
<td>67 (35%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>simplex 61 (31%)</td>
<td>104 (55%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>proliferative 137 (69%)</td>
<td>19 (10%)</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± SD, *Median (range), or n(%). Some patients with previously persistent albuminuria receiving antihypertensive medication have UAER < 300 mg/24 h.
AGT1-R-gene polymorphism and diabetic microangiopathy

Table 2. Distribution of AGT1-R genotypes in 198 IDDM patients with diabetic nephropathy and 190 IDDM patients with normoalbuminuria

<table>
<thead>
<tr>
<th>Genotype</th>
<th>IDDM patients with nephropathy</th>
<th>IDDM patients with normoalbuminuria</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>103 (52%)</td>
<td>97 (51%)</td>
</tr>
<tr>
<td>AC</td>
<td>81 (41%)</td>
<td>80 (42%)</td>
</tr>
<tr>
<td>CC</td>
<td>14 (7%)</td>
<td>13 (7%)</td>
</tr>
<tr>
<td>Allele frequency</td>
<td>0.73/0.27</td>
<td>0.72/0.28</td>
</tr>
</tbody>
</table>

Diabetic nephropathy (Table 2). The distribution of AGT1-R genotypes in cases and controls were in Hardy-Weinberg equilibrium (NS). The prevalence of hypertension was 81% in patients with nephropathy. No difference in genotype distribution was observed between nephropathic patients with and without hypertension: 83 (52%) / 65 (40%) / 13 (8%) vs. 20 (54%) / 16 (43%) / 1 (3%) had AA/AC/CC genotypes, respectively (NS). Furthermore, no difference in genotype distribution was observed, when the study population was dichotomized according to sex and above or below the median value either with respect to age, duration of diabetes or HbA1c (NS).

Patients with nephropathy had a much higher prevalence of proliferative retinopathy (137 [69%]) compared to patients with normoalbuminuria (19 [10%], P<0.001). Thus, when patients were stratified according to retinopathy status diabetic nephropathy was present in 137 (88%) of patients with proliferative retinopathy. Clinical data for these patients are shown in Table 3. There was no difference in AGT1-R genotype distribution and no difference in allele frequency between patients with proliferative retinopathy and without diabetic retinopathy (Table 4).

Discussion

In our cross-sectional case control study of Caucasian IDDM patients the genotype and allele frequencies of the AGT1-R gene polymorphism did not differ between patients with and without diabetic nephropathy. In addition, no difference in AGT1-R genotype distribution was observed between nephropathic patients with and without hypertension. The distribution of the AGT1-R genotypes in cases and controls were in Hardy-Weinberg equilibrium, and furthermore in accordance with the allele frequencies (A/C: 0.71/0.29) found in the control group of Caucasian subjects (n = 723) in the Etude Cas-Témoin de l'Infarctus du Myocarde study (ECTIM) [19].

Our cohorts were sufficiently large to yield 80% power to detect a 10% deviation of CC genotype frequency with P<0.05 and had 99.5% power to detect a 20% deviation. The negative finding is thus not explained by insufficient statistical power.

Systemic and glomerular hypertension play a role in the initiation and progression of experimental and human diabetic glomerulopathy [20-22]. Predisposition to systemic hypertension has been reported increased in some [12,13] but not all studies [23] dealing with IDDM patients suffering from diabetic nephropathy. In contrast, all studies reporting on presence or absence of familial clustering in diabetic nephropathy have found it to be present [24,25]. Some of the above mentioned abnormalities may be explained by alterations in the renin-angiotensin system. In addition, angiotensin-II acts as a growth

Table 3. Clinical characteristics of 156 IDDM patients with proliferative retinopathy and 67 patients with no signs of diabetic retinopathy

<table>
<thead>
<tr>
<th></th>
<th>Proliferative retinopathy (n=156)</th>
<th>No signs of diabetic retinopathy (n=67)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>89/67</td>
<td>41/26</td>
<td>NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>41.0±8.6</td>
<td>42.0±9.3</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>29.1±7.9</td>
<td>25.2±9.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.9±3.3</td>
<td>23.4±2.4</td>
<td>NS</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.6±1.6</td>
<td>8.4±0.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>46%</td>
<td>50%</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary albumin excretion rate* (mg/24 h)</td>
<td>733 (4-14545)</td>
<td>9 (2-30)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum creatinine* (μmol/l)</td>
<td>99 (59-684)</td>
<td>76 (40-103)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>151±22</td>
<td>132±18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>86±13</td>
<td>76±11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Prevalence of antihypertensive treatment (%)</td>
<td>72%</td>
<td>7%</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Mean ± SD, *Median (range), or n(%).
promoter by enhancing mesangial cell proliferation and formation of mesangial extracellular matrix [26,27], abnormalities characteristically found in diabetic glomerulopathy [28,29].

The cellular effects of angiotensin-II are mediated by two structurally distinct receptor subtypes, AT₁ and AT₂ [6,30]. In adult humans, most of the known actions of angiotensin-II are exerted through the AT₁ receptor, which is present in vascular smooth muscle, myocardium and in the kidney (6–8). Therefore, the gene coding for AGT,R could be a candidate gene for diabetic nephropathy.

Originally, Bonnardeaux et al. [11] identified five polymorphisms in the gene coding for the AT₁ receptor, though no mutation that alters the encoded amino acid sequence was detected. A case control study, performed on white hypertensive and normotensive subjects, revealed a significant increase in frequency of the C¹¹⁶⁶ allele in hypertensive non-diabetic subjects.

Recently, the same group has demonstrated that the association between the DD-genotype of the ACE/ID polymorphism in the ACE gene and myocardial infarction is restricted to a subset of individuals, also carriers of AGT,R C¹¹⁶⁶ allele [19]. Though we have studied the ACE/ID polymorphism in the present patient population previously [15,16], no valid analysis of interaction between the ACE/ID and the AGT,R polymorphisms in relation to coronary heart disease in IDDM patients can be performed presently due to the small number of affected patients (n=53/388). No interaction between the two above mentioned gene polymorphisms was found, comparing patients with and without diabetic nephropathy (Table 5).

Other genotypic abnormalities of the renin-angiotensin system have been suggested as risk factors for the development of diabetic nephropathy [31,32]: originally, Marre et al. [31] reported that the II genotype of the ACE/ID polymorphism is a marker for reduced risk for diabetic nephropathy in IDDM patients. Patients with the II genotype having the lowest level of plasma ACE [31]. Conversely, we found that raised plasma ACE concentration may play a role in the initiation and progression of diabetic nephropathy in Caucasian IDDM patients [15]. This suggestion is in accordance with the beneficial effects of ACE inhibition on the development and progression of experimental and human diabetic glomerulopathy [33–38]. Subsequent studies have not been able to confirm the initial finding of an association between the ACE/ID polymorphism and diabetic nephropathy [15,39].

Also the gene coding for angiotensinogen could be implicated in the genetic basis of diabetic microangiopathy. Recent studies imply a strong linkage to essential hypertension of regions within or close to the angiotensinogen gene [40]. None of the investigated gene polymorphisms contributes to the genetic susceptibility to diabetic nephropathy in IDDM patients [41].

Studies suggest, that prorenin serve as a risk-factor/marker for diabetic microvascular disease [2]. Elevated plasma prorenin is associated with increased risk of development and presence of diabetic retinopathy [2,3]. Furthermore, raised prorenin levels have been demonstrated in eyes with proliferative retinopathy, thus indicating an activation of the intraocular renin-angiotensin system [14]. Angiotensin-II is known to stimulate new vessel formation in the eye [42] and finally, IDDM patients with diabetic retinopathy have enhanced pressor response to intravenously infused angiotensin-II [43], suggesting an enhanced AT₁ receptor mediated vascular responsiveness. Genetic abnormalities in the AGT,R gene could thus be responsible for a possible constitutive activation of the AT₁ receptor in patients with proliferative retinopathy in IDDM.

In the present study no difference in AGT,R genotype distribution was observed between IDDM patients with proliferative and patients without retinopathy. Due to our method of patient selection there was a preponderance of patients with diabetic nephropathy among patients with proliferative retinopathy. In the normoalbuminuric group the AGT,R genotype distribution did not differ significantly between 19 patients with proliferative retinopathy as compared to patients without retinopathy, but numbers were small. Thus, the A¹¹⁶⁶→C polymorphism does not seem to be involved in the pathogenesis of proliferative retinopathy in IDDM.

We conclude that the A¹¹⁶⁶→C polymorphism in the AGT,R gene does not contribute to the genetic susceptibility to diabetic nephropathy or proliferative retinopathy in Caucasian IDDM patients.

Acknowledgements. We acknowledge the assistance of Ms B. R. Jensen and Ms U. M. Smidt in conducting this study.

References


Received for publication: 27.12.95
Accepted in revised form: 15.2.96