Angiotensinogen gene M235T polymorphism and reduction in wall thickness in response to antihypertensive treatment

Erwan BOZEC*, Céline FASSOT*, Anne-Isabelle TROPEANO*, Pierre BOUTOUYRIE*, Xavier JEUNEMAITRE†, Patrick LACOLLEY*, Hubert DABIRE* and Stéphane LAURENT*

*Department of Pharmacology, INSERM EMI 0107, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France, and †Department of Molecular Biology, INSERM U36, Hôpital Européen Georges Pompidou, 20 rue Leblanc, 75015 Paris, France

Abstract

The angiotensinogen M235T polymorphism has been linked to hypertension and cardiovascular disease. Carotid intima-media thickness (IMT) is an early marker of atherosclerosis. The objectives of the present study were to determine in previously untreated essential hypertensive patients whether carotid IMT was associated with the M235T polymorphism, and to determine whether the M235T polymorphism could influence the reduction of carotid IMT by antihypertensive treatment. Common carotid artery IMT was determined with a high-definition echotracking system in 98 previously untreated hypertensive patients in a cross-sectional study. A subgroup of 56 patients was included in a randomized double-blind parallel group study comparing the effect of the angiotensin-converting-enzyme-inhibitor enalapril with that of the β-blocker celiprolol during a 5 month period. In the cross-sectional study, a multivariate analysis showed that the M235T genotype was a significant independent determinant of carotid IMT, explaining 7% of the variance. Carotid IMT was higher in patients homozygous for the T allele than in MM patients. In the longitudinal study, the reduction in carotid IMT after antihypertensive treatment was significantly (P < 0.01) higher in patients carrying the TT genotype than in patients carrying the MM genotype, despite similar reductions in blood pressure and independently of drug type. In conclusion, these data suggest that the angiotensinogen TT genotype at position 235 is a genetic marker for early carotid atherosclerosis in a hypertensive population and its regression under antihypertensive treatment.

Introduction

In hypertensive patients, increased large artery wall thickness contributes to the normalization of circumferential wall stress [1,2], parallels the development of left ventricular hypertrophy [3] and potentiates the development of atherosclerosis [4,5]. An increase in carotid intima-media thickness (IMT) is generally considered as a marker of early atherosclerosis and a cardiovascular risk factor in older adults [6]. Several clinical trials have shown that a slower progression or even a reduction in carotid IMT could be obtained in response to long-term antihypertensive treatment [5,7–10]. However, the mean amplitude of the regression was limited and inter-individual variability was high among the study populations, suggesting an individual susceptibility that may account for the heterogeneity of drug response [5,7–10]. To our knowledge, whether the

Key words: angiotensinogen, essential hypertension, genetic polymorphism, intima-media thickness, pharmacogenetics.

Abbreviations: ACE, angiotensin-converting enzyme; AGT, angiotensinogen; BP, blood pressure; DBP, diastolic BP; IMT, intima-media thickness; MBP, mean BP; PR, pulse pressure; SBP, systolic BP.

Correspondence: Professor Stéphane Laurent (e-mail stephane.laurent@egp.ap-hop-paris.fr).
effectiveness of antihypertensive treatment on carotid IMT can be predicted by gene polymorphism has never been investigated.

An increasing body of evidence suggests that the M235T polymorphism of the angiotensinogen (AGT) gene could influence baseline arterial wall thickness and drug-induced changes. The molecular variant (M235T) of the AGT gene, consisting of the thymine → cytosine substitution at nucleotide 704 and encoding the presence of a threonine instead of a methionine at residue 235 of mature AGT (T235 compared with M235), has been correlated with plasma AGT concentrations, with circulating levels 11–39% higher in patients homozygous for the T allele [11–13] and associated with hypertension and cardiovascular diseases [11,14–16]. The concentration of AGT is a rate-limiting factor in the generation of angiotensin II [17]. Local renin–angiotensin systems within the arterial wall may regulate smooth muscle cell growth through the local generation of angiotensin II [18].

In hypertensive patients, pulsatile mechanical load is a major determinant of baseline carotid artery wall thickness [19] and its reduction under antihypertensive treatment [8]. In cultured vascular smooth muscle cells, mechanical stretch increases protein kinase C activity [20], potentiates the mitogenic activity of angiotensin [21] and involves angiotensin II type 1 receptors in collagen synthesis [22]. No influence of M235T polymorphism on carotid IMT was reported in candidate gene studies [23–25], except through an interaction with blood pressure (BP) [26].

The objectives of the present study were (i) to determine, in previously untreated essential hypertensive patients, whether carotid IMT was associated with the M235T polymorphism of the AGT gene, and (ii) to determine whether the M235T polymorphism could influence the reduction of carotid IMT by antihypertensive treatment.

**METHODS**

**Patients**

Ninety-eight previously untreated hypertensive patients, all Caucasians, from 24–80 years of age, were included in the study. This hypertensive population consisted of ambulatory patients referred to the Hypertension Unit of Broussais Hospital. The diagnosis of essential hypertension was established by the presence of a sustained increase in BP, i.e. as casual sitting systolic BP (SBP) ≥ 140 mmHg and diastolic (DBP) ≥ 90 mmHg, measured by sphygmomanometer and auscultatory methods (Phase I and V of Korotkoff sounds), and the absence of clinical or laboratory evidence suggestive of secondary forms of hypertension. All subjects were free of clinical evidence of coronary artery or cerebrovascular disease. No patients with valvular heart disease, arrhythmia or renal disease were included. None of the patients had atherosclerotic plaques on the common carotid artery and only two patients had a plaque on the carotid bifurcation or the internal carotid. The study was approved by the Institutional Review Committee of Broussais Hospital and the subjects gave informed consent.

**Non-invasive arterial measurements**

The non-invasive investigation was performed in a controlled environment at 22 ± 1 °C after subjects had reclined at rest for 15 min. Senior physician (P.B.) and technician (B.L.), trained and certified in vascular echocardiography, performed BP and arterial measurements. Brachial BP was measured with a mercury sphygmomanometer.

Carotid internal diameter and wall thickness were measured on the right common carotid artery and 2 cm beneath the carotid bifurcation with a 7.5 MHz pulsed ultrasound echotracking system [27] (Wall Track System; Neurodata, Maastricht, The Netherlands) analysing the radiofrequency signal originating from an M line perpendicular to the longitudinal and transversal axes of the artery, selected on the two-dimensional B-mode image (Sigma 44 KONTRON). This system has been validated and described in detail and used for various clinical studies [8,19]. Measurements of carotid IMT were performed only on the far wall, and the value retained for calculation was the mean of 3–4 measurements, as previously described [8,19].

Mean circumferential wall stress (σθ in kPa) was calculated according to Lame’s equation as $\sigma_\theta = MBP \times Di / 2h$, where MBP is mean BP, Di is mean internal diameter, and h is wall thickness [1].

Common carotid artery pressure waveforms were determined non-invasively with aplanation tonometry, using a pencil-type probe incorporating a high-fidelity strain-gauge transducer (SPT-301; Millar Instruments, Houston, TX, U.S.A.), as described previously and validated in vitro and in vivo in humans [8,19].

**Genotyping**

After DNA purification from peripheral blood with the use of a standard protocol, 80 ng of genomic DNA was subjected to the genotyping process as described previously [11]. Determination of M235T allele status was successfully performed in all 98 subjects.

**Pharmacological study**

A subgroup of 56 patients was included in a randomized double-blind parallel group study [8] comparing the effect of an angiotensin-converting-enzyme (ACE)-inhibitor (enalapril) with that of a β-adrenoceptor antagonist with β-2 agonist properties (celiprolol) on carotid IMT. Firstly, patients entered a single-blind placebo
washed-out period which lasted 4 weeks. After the placebo wash-out period, patients with a DBP $\geq 90$ mmHg and $\leq 120$ mmHg were included in the study and randomized to receive treatment for 5 months with either enalapril (10 mg; $n = 30$) or celiprolol (200 mg; $n = 26$) each morning in a double-blind fashion. After 1 month, the dose was doubled if DBP remained $\geq 90$ mmHg. If DBP remained $\geq 90$ mmHg at the third monthly visit, celiprolol dosage was increased from 200 mg twice a day to 200 mg three times a day and enalapril dosage from 20 mg once a day to 20 mg twice a day. Arterial parameters were determined at baseline and after 5 months [8]. The protocol was approved by the ethical committee of Broussais Hospital. Written informed consent to participate in the study was obtained from each subject.

Statistical analysis
Data are expressed as means $\pm$ S.D. Quantitative variables were compared by means of an unpaired Student’s $t$ test and categorical variables by means of a $\chi^2$ test. Alleles and genotype frequencies were determined at baseline and after 5 months [8]. ANOVA was used to study the relationships between genotypes and phenotypes. The genotype effect was assumed to be additive (MM = 1, MT = 2 and TT = 3). Multivariate regression models [28] were constructed in the whole population and systematically included MBP. The other variables included in the model were chosen with a multivariate variable selection procedure among the following: age, gender, MBP, carotid pulse pressure (PP), M235T genotype, total cholesterol, smoking and plasma glucose. The algorithm allows the selection of the set of variables among all possible pertinent ones on the basis of the maximization of $R^2$. Up to five variables were kept until $R^2$ reached a plateau. Once the set of variables was determined for the parameter of interest, a robust multiple stepwise regression analysis was performed. This procedure has been shown to be more robust to marginal violation of normality assumption and to the presence of outliers. A value of $P < 0.05$ was considered significant. The statistical analysis was performed by means of a NCSS 6.0 package software (Hintze JL, Kaysville, UT, U.S.A.).

RESULTS
Cross-sectional study
The frequency of the M235T genotype distribution in the hypertensive population was found to conform the Hardy–Weinberg equilibrium, with a $T$ allele frequency of 0.34. No significant differences between the three genotypes were observed concerning baseline values of BP, heart rate, total cholesterol, plasma glucose, smoking and carotid artery parameters (Table 1).

In the multivariate analysis (Table 2), the M235T genotype was a significant independent determinant of carotid IMT, explaining 7% of the variance, with patients homozygous for the 235T genotype having a higher carotid IMT than patients homozygous for the M genotype. Besides M235T, some atherosclerosis risk factors were found to independently and significantly affect carotid IMT (gender, carotid PP and age), whereas others (MBP, total cholesterol, plasma glucose and smoking) did not. Carotid internal diameter was not influenced by the M235T genotype.

Pharmacological study
The 30 and 26 patients randomized to enalapril and celiprolol respectively, were similar in relation to age, body mass index, BP and heart rate before treatment and blood biochemistry (results not shown). Antihypertensive treatment for 5 months produced a significant reduction in SBP, DBP, MBP and the following carotid artery parameters: internal diameter, IMT, wall cross-sectional area, local PP and circumferential wall stress (Table 3). No significant difference between enalapril and celiprolol was observed concerning the changes in BP, heart rate and carotid parameters during the 5 month treatment period (results not shown).

Table 1 Clinical characteristics and carotid artery parameters in 98 previously untreated essential hypertensive patients according to the M235T polymorphism of the AGT gene

<table>
<thead>
<tr>
<th>Parameters</th>
<th>MM (n = 42)</th>
<th>MT (n = 35)</th>
<th>TT (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>51 $\pm$ 9</td>
<td>51 $\pm$ 11</td>
<td>49 $\pm$ 12</td>
</tr>
<tr>
<td>Sex ratio (male/female)</td>
<td>26/16</td>
<td>25/10</td>
<td>10/11</td>
</tr>
<tr>
<td>Body mass index (kg $\cdot$ m$^{-2}$)</td>
<td>26 $\pm$ 3</td>
<td>25 $\pm$ 3</td>
<td>25 $\pm$ 3</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>158 $\pm$ 12</td>
<td>155 $\pm$ 10</td>
<td>162 $\pm$ 15</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>97 $\pm$ 11</td>
<td>99 $\pm$ 10</td>
<td>102 $\pm$ 9</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>118 $\pm$ 13</td>
<td>118 $\pm$ 13</td>
<td>122 $\pm$ 9</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>73 $\pm$ 11</td>
<td>77 $\pm$ 10</td>
<td>75 $\pm$ 11</td>
</tr>
<tr>
<td>Total cholesterol (mg/dl)</td>
<td>245 $\pm$ 40</td>
<td>233 $\pm$ 33</td>
<td>225 $\pm$ 35</td>
</tr>
<tr>
<td>Plasma glucose (g/l)</td>
<td>0.98 $\pm$ 0.11</td>
<td>1.0 $\pm$ 0.12</td>
<td>0.97 $\pm$ 0.05</td>
</tr>
<tr>
<td>Smoking (yes/no)</td>
<td>9/33</td>
<td>7/28</td>
<td>7/14</td>
</tr>
</tbody>
</table>

BP, heart rate, total cholesterol, plasma glucose, smoking and carotid artery parameters (Table 1).
Table 2 Multiple stepwise robust regression analysis of the determinants of carotid artery parameters in 98 hypertensive patients according to the M235T polymorphism of the AGT gene

The influence of gender was determined as M = 1 and F = 2. The influence of M235T polymorphism was determined as MM = 1, MT = 2 and TT = 3. In, In the model.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>In</th>
<th>Variable</th>
<th>$r$</th>
<th>$R^2$ increment</th>
<th>$β$ coefficient</th>
<th>T</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT</td>
<td>Yes</td>
<td>Gender</td>
<td>−0.27</td>
<td>0.07</td>
<td>−62 ± 16</td>
<td>−3.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Carotid PP</td>
<td>0.43</td>
<td>0.17</td>
<td>1.9 ± 0.3</td>
<td>5.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Age</td>
<td>0.44</td>
<td>0.17</td>
<td>5.0 ± 0.8</td>
<td>6.1</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>M235T</td>
<td>0.27</td>
<td>0.07</td>
<td>30 ± 10</td>
<td>3.8</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>MBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adjusted global $R^2 = 0.52$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal diameter</td>
<td>Yes</td>
<td>Gender</td>
<td>−0.45</td>
<td>0.21</td>
<td>−0.580 ± 0.080</td>
<td>−7.0</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>Carotid PP</td>
<td>0.63</td>
<td>0.39</td>
<td>0.014 ± 0.001</td>
<td>9.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>Age</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>M235T</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>MBP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Adjusted global $R^2 = 0.60$</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3 BP and carotid artery parameters at baseline and after antihypertensive treatment for 5 months in 56 hypertensive patients

Values are means ± S.D. NS, not significant.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline</th>
<th>After 5 months</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP (mmHg)</td>
<td>161 ± 18</td>
<td>146 ± 24</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>100 ± 9</td>
<td>90 ± 11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>MBP (mmHg)</td>
<td>120 ± 11</td>
<td>109 ± 14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>74 ± 11</td>
<td>70 ± 9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Carotid parameters</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal diastolic diameter (mm)</td>
<td>5.63 ± 0.61</td>
<td>5.35 ± 0.68</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>IMT (µcm)</td>
<td>575 ± 126</td>
<td>540 ± 120</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Wall cross-sectional area (mm²)</td>
<td>11.4 ± 3.3</td>
<td>10.2 ± 3.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Wall/lumen ratio (h/R)</td>
<td>0.20 ± 0.04</td>
<td>0.20 ± 0.04</td>
<td>NS</td>
</tr>
<tr>
<td>Carotid PP (mmHg)</td>
<td>72 ± 24</td>
<td>61 ± 20</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Circumferential wall stress (kPa)</td>
<td>81 ± 18</td>
<td>74 ± 17</td>
<td>&lt; 0.05</td>
</tr>
</tbody>
</table>

After antihypertensive treatment, patients carrying the TT genotype had a higher percentage reduction in carotid IMT and wall cross-sectional area than patients carrying the MM genotype ($P < 0.01$), despite similar reductions in BP (Table 4). MT subjects had intermediate reductions in carotid IMT. This was confirmed in each group of treatment (Table 5). After treatment with either enalapril or celiprolol, carotid IMT was reduced to a larger extent in TT patients than in MM patients, MT subjects being intermediate, without significant difference between the enalapril and celiprolol groups (no 'drug' effect). The contribution of the M235T genotype to the reduction in carotid IMT was tested in a multivariate analysis (Table 6): the M235T genotype explained 7% of the variance of carotid IMT reduction, independently of baseline IMT, and carotid PP and MBP changes in a model explained 38% of the variance (Table 6). Similar results were observed when carotid IMT at the fifth month was introduced into the model instead of IMT changes. The M235T polymorphism only marginally influenced ($P = 0.053$) the changes in wall cross-sectional area under treatment and did not influence the changes in internal diameter.

We also took into account the influence of carotid PP reduction on carotid IMT reduction in the analysis of genotype–carotid IMT interactions. Although the
percentage reduction in carotid PP was not significantly different among the three genotypes after treatment for 5 months (Table 4), a significant (P < 0.01) genotype–drug interaction was observed (Table 5). In TT patients, carotid PP decreased after enalapril, but not after celiprolol, whereas, in patients carrying the M allele, carotid PP decreased to a larger extent after celiprolol than after enalapril treatment. These differential changes did not affect the significant influence of M235T genotype on carotid IMT reduction after treatment, which was demonstrated in the multivariate model described above (Table 6) after inclusion of PP change as an independent covariate.

No significant genotype–treatment interaction was observed for the decrease in MBP (Table 5).

**DISCUSSION**

The present study is the first control-blinded study showing an influence of a genetic polymorphism of the renin–angiotensin system on drug-induced changes in arterial wall thickness. A significant association between the M235T polymorphism of the AGT gene and carotid IMT was observed not only in a cross-sectional study, but also during a longitudinal trial. In a multivariate analysis, carotid IMT was higher in previously untreated hypertensive patients homozygous for the T allele than in MM patients, and was reduced to a larger extent after treatment in TT than in MM patients.

Because various factors are known to simultaneously affect carotid IMT, it is not surprising that a significant difference occurred only when the effect of the AGT polymorphism on carotid IMT was tested in a multivariate analysis. Using a similar methodology, we have shown previously [19] that age and local carotid PP were independent determinants of carotid IMT. In the present studies, we show that, in addition to these factors, the M235T AGT polymorphism was a significant independent determinant of carotid IMT, explaining 7% of the variance, with patients homozygous for the 235T genotype having a higher carotid IMT than patients homozygous for the M genotype. Interestingly, other classical atherosclerosis risk factors (total cholesterol, plasma glucose and smoking) were not independently and significantly associated with carotid IMT.

An increased carotid IMT has been linked previously to genetic factors such as ACE gene polymorphisms [29,30]. However, to our knowledge, the influence of the AGT gene M235T polymorphism on carotid IMT...
has not yet been reported in candidate gene studies [23–25, 31]. The finding of positive results in the present study may have been favoured by a careful selection of hypertensive patients that had not been treated previously and devoid of cardiovascular complications. Previously, Jeng [24] included more than two-thirds of treated hypertensive patients, and Schimdt et al. [23] and Barley et al. [25] included patients who had previously suffered strokes. This may have increased the number of confounding variables. Tabara et al. [26] reported a significant influence of M235T polymorphism on carotid IMT but occurred only through an interaction between SBP and TT genotype in healthy subjects: the influence of SBP on carotid IMT was higher in TT subjects than in MT and MM patients [26]. Unmasking an influence of M235T polymorphism on carotid IMT in the present study may also have been possible through the use of a high-definition echotracking system [27] able to measure carotid IMT more accurately [32] than the classical bi-dimensional ecosystems used in most association studies [23–25, 31].

Our cross-sectional findings on AGT polymorphism and carotid IMT in previously untreated hypertensive patients were strengthened by the results of the pharmacological study. We analysed the 56 patients from the previously reported CELIMENE study [8] possessing the M235T polymorphism and limited the analysis to the first 5 months to retain only the influence of the monotherapy on carotid IMT. Although treatment for 5 months is a short time for observing significant changes in carotid IMT, and the present study included only a limited number of patients, our results were significant and consistent with those of a larger cohort reported previously [8]. Patients homozygous for the T allele had a larger reduction in carotid IMT than MM patients in response to a 5 month BP-lowering treatment, with intermediate values in MT patients. Because the changes in carotid IMT were opposite between TT and MM patients (+11 μm in MM patients compared with −89 μm in TT patients; Table 4) and because the high-resolution echotracking system allowed the measurement of carotid IMT to be performed with high accuracy and small S.D. changes, the present study was satisfactorily powered (86 %) to detect a significant association between the genetic polymorphism and the change in carotid IMT.

In a multivariate analysis (Table 6), the influence of the M235T polymorphism on carotid IMT reduction was independent of baseline value and reduction in MBPs or PPs. In addition, we did not observe a significant interaction between the pharmacological class (either the ACE inhibitor enalapril or the β-blocker celioprolo) and M235T polymorphism on carotid IMT reduction (Table 5). However, the study was not powered for this objective and a much larger number of patients should have been included in each genotype group (MM, MT and TT) to demonstrate a significant drug–genotype interaction.

To our knowledge, this is the first control-blinded study showing an influence of a genetic polymorphism of the renin–angiotensin system on drug-induced changes in arterial wall thickness. Two limitations should be discussed. Firstly, these results have been observed in a limited number of patients and should, therefore, be confirmed in larger trials. However, the present study used a homogenous group of patients carefully selected as Caucasian hypertensives who had never been treated previously and who underwent a follow-up long enough to unmask significant changes in arterial remodelling. Second, because TT patients had a larger carotid IMT at baseline than MM patients, a ‘regression to the mean’ phenomenon [28] may be evoked. This was not the case, since adjustment of baseline IMT did not change the results.

We observed an interaction between drugs and M235T polymorphism concerning the reduction in PP during treatment (Table 5). Indeed, the largest reduction under celioprolo treatment was observed in patients carrying the M allele, whereas the largest reduction under enalapril treatment was observed in patients homozygous for the T allele (Table 5). No significant interaction between genotype and SBP, MBP or DBP was observed. To our knowledge, no significant influence of the M235T AGT gene polymorphism on BP reduction under treatment has been reported in previously published pharmacogenetic studies using blockers of the renin–angiotensin system, including the ACE inhibitors captopril [33] and lisinopril [34] and the angiotensin II antagonist irbesartan [35]. The discrepancies between previous studies [31–35] and ours may be explained by the direct measurement of PP at the site of the carotid artery in the present study, which is more representative of the pulsatile load exerted on the carotid wall [8, 19]. However, these differential changes in carotid PP did not affect the significant influence of M235T genotype on carotid IMT reduction after treatment, since the latter was shown in a multivariate model including PP change as an independent covariate.

The mechanism responsible for the effect of the M235T AGT gene polymorphism on carotid IMT in untreated patients and its regression under antihypertensive treatment remains speculative. Higher AGT plasma and tissue levels were found in patients carrying the T allele of the M235T polymorphism [11–13]. Although no influence of the M235T polymorphism has been shown on plasma angiotensin II levels [13], we cannot exclude that higher AGT plasma and tissue levels may lead to increased angiotensin II formation within the arterial wall, activating smooth muscle cell growth and production of extracellular matrix [18]. In cultured vascular smooth muscle cells, mechanical stretch increases protein kinase C activity [20] and potentiates the mitogenic activity [21] and collagen production of
angiotensin II [22]. Numerous in vitro studies show that cyclic strain exerts a greater influence than static load on phenotype and growth of vascular smooth muscle cells (for instance synthesis of DNA, smooth muscle myosin and collagen) [36,37]. In humans, angiotensin II increases arterial stiffness and, thus, central PP [38], which is a major determinant of carotid IMT in untreated hypertensive patients [19]. In humans and animals, ACE inhibitors reduce BP, arterial stiffness and aortic PP [39,40]. Thus arterial wall thickening may be influenced by a genetically determined overactivity of circulating or autocrine/paracrine renin–angiotensin system, including interactions between the growth-promoting effect of angiotensin II and pressure load, either steady or pulsatile. Unfortunately, in the present study, we did not determine the AGT plasma levels.

IMT of the carotid arteries is a reliable marker for an early phase of the generalized atherosclerotic process and is strongly correlated with the presence of coronary artery disease [41]. The predictive value of carotid IMT on cardiovascular events remains significant after adjustment to classical cardiovascular risk factors, such as age, cigarette smoking, BP and cholesterol level [6]. Interestingly, in the present study, carotid IMT remained significantly associated with the M235T polymorphism after adjustment of these risk factors. Although the degree of statistical significance is quite high ($P < 0.001$) in the multivariate analysis (Table 2), it is still difficult to judge the putative clinical relevance of a 8.4 % ($+46 \mu m$) increase in carotid IMT in TT patients compared with MM patients. A comparative estimation with age can be done: 46 $\mu m$ is equivalent to 9 years of age-induced wall thickening.

Interestingly, Kurland et al. [42] showed that patients carrying the T allele of the M235T polymorphism responded with a greatest reduction in left ventricular mass index when treated with the angiotensin II receptor antagonist irbesartan. Thus the M235T polymorphism may influence the effect of antihypertensive treatment on the heart and the large artery, which are both target organs for hypertension and are damaged in a correlated manner.

In conclusion, we observed a significantly higher common carotid IMT in previously untreated hypertensive patients homozygous for the T allele than in MM patients. Carotid IMT was reduced to a larger extent after treatment in TT than in MM patients. The pathophysiological relevance of such findings still needs to be assessed by larger and prospective-association studies or linkage-based family studies.

REFERENCES


© 2003 The Biochemical Society
42 Kurland, L., Melhus, H., Karlsson, J. et al. (2002) Polymorphisms in the angiotensinogen and angiotensin II type 1 receptor gene are related to change in left ventricular mass during antihypertensive treatment: results from the Swedish Irbesartan Left Ventricular Hypertrophy Investigation versus Atenolol (SILVHIA) trial. J. Hypertens. 20, 657–663

Published as Immediate Publication 12 August 2003, DOI 10.1042/CS20030156