Lack of Association Between Renin-Angiotensin System,
Gene Polymorphisms, and Wall Thickness of the Radial
and Carotid Arteries

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Abstract—To investigate the relationship between polymorphisms of the angiotensin-converting enzyme (ACE) and the angiotensin II type 1 receptor (AT1R) genes and structural phenotypes of arteries, we studied a cohort of 340 subjects (aged 49±12 years) without evidence of cardiovascular disease and who had never been treated previously with any cardiovascular treatments. Structural phenotypes (wall thickness and internal diameter) were evaluated for the common carotid and the radial arteries using high-resolution echo-tracking devices (NIUS-02 and Wall Track System). The influence of ACE insertion/deletion (I/D) and AT1R A/C1166 polymorphism genotypes on structural parameters was tested by ANOVA and logistic regression analysis. For the radial artery, mean wall thickness among subjects according to the ACE I/D or AT1R A/C1166 genotypes was not different. This lack of association persisted in a logistic regression analysis or when the comparison was restricted to a subgroup of subjects potentially at high genetic risk (DD and CC or AC) compared with subjects at low genetic risk (AA and II or ID). Also, no association was observed between the carotid artery intima-media thickness and the 2 polymorphisms. In conclusion, the ACE I/D and the AT1R A/C1166 gene polymorphisms are not markers of vascular hypertrophy in subjects with no evidence of cardiovascular disease. These results suggest that these gene polymorphisms have an undetectable role in the geometry of the radial and carotid arteries compared with usual determinants such as blood pressure and age. (Hypertension. 1998;32:579-583.)

Key Words: genes • angiotensin • arteries • wall thickness

Since the detection of an insertion/deletion (I/D) polymorphism in intron 16 of the gene encoding angiotensin-converting enzyme (ACE),1 and the original report by Cambien et al2 describing an association between the DD genotype and myocardial infarction in the Etude Cas-Temoins sur l’Infarctus du Myocardie (ECTIM) study, several studies have attempted to demonstrate relationships between the ACE I/D polymorphism and various cardiovascular phenotypes. Associations with myocardial infarction,3 ischemic heart disease,4,5 coronary atherosclerosis,6 coronary artery restenosis,7 cardiac hypertrophy,8-10 and carotid wall thickness11-13 have been reported. However, these studies yielded conflicting results: some found positive associations with ACE genotype and others did not.

Other polymorphisms of genes coding for components of the renin-angiotensin system have been detected. The angiotensin II type 1 receptor (AT1R) appears to be the primary receptor that mediates the vasoconstrictor and growth-promoting effects of angiotensin II (Ang II) in humans. The A/C1166 transversion of the AT1R gene has been detected in the 3’ untranslated part of the gene, and a significant increase of the C allele frequency was observed by Bonnardeaux et al14 in hypertensive subjects with a positive family history of hypertension compared with normotensive control subjects. Recently, Benetos et al15 reported a positive association with aortic pulse wave velocity, an index of aortic stiffness. Conversely, in a study including subjects selected from a general population, Castellano et al16 reported no association with other cardiovascular phenotypes such as left ventricular mass and carotid artery wall thickness. However, Tiret et al17 found a significant interaction between the ACE I/D and the AT1R A/C1166 polymorphisms. The odds ratio for myocardial infarction associated with the DD genotype was 4-fold higher in AT1R CC homozygotes than in AA homozygotes. No data have been reported thus far on the possible influence of AT1R A/C1166 gene polymorphism combined with ACE I/D polymorphism on vascular structural alterations.

In the present study, vascular properties of the carotid and radial arteries were studied. These 2 sites do not provide the same information: the carotid artery acts as a proximal elastic large artery, whereas the radial artery acts as a muscular medium-sized artery. Morphological studies have shown that for the carotid artery, wall hypertrophy is a marker of atherosclerotic lesions and reflects intima thickening.18 For
the radial artery, wall hypertrophy is mainly dependent on structural changes of the media.\textsuperscript{19} We hypothesized that the associations between ACE I/D or AT\textsubscript{1}R A/C\textsuperscript{1166} gene polymorphisms and vascular hypertrophy might be different according to arterial site. To investigate these associations, we studied a cohort of 340 random subjects without history of any cardiovascular disease and pharmacological treatment that could potentially interfere with the vascular phenotypes under investigation.

Methods

Study Population

The study group was a part of a cohort of ambulatory subjects referred to our atherosclerosis prevention clinic because of risk factors or symptomatic vascular disease. Subjects underwent ultrasound examination of the carotid arteries and were classified into 3 strata on the basis of ultrasound morphology: stratum 1, subjects with a stenosis that reduced the diameter of the carotid artery by 50\%; stratum 2, subjects with at least 1 plaque in the right or left common carotid artery (defined as a discernible focal thickening of the arterial wall with an intima-media thickness (IMT) >1.3 mm at this level); and stratum 3, subjects with a normal carotid wall (defined as an absence of stenosis or plaque). A complete medical history and physical examination were undertaken for all patients. Between November 1995 and December 1996, we selected 340 subjects from the cohort of patients referred to our prevention clinic. Patients were eligible for the study if they (1) were in stratum 3 of our ultrasound classification; (2) had no history or clinical evidence of cardiovascular disease; (3) had never been treated with antihypertensive drugs; and (4) had technically satisfactory carotid ultrasound studies. Blood samples were obtained for analysis of serum glucose and lipid profile with standard methods, as well as DNA. Systolic and diastolic blood pressures were determined automatically every 3 minutes on the left arm with a Dynamap oscillometric blood pressure recorder. The average of the 5 following measurements determined the blood pressure level. Of the subjects examined, 18\% had essential hypertension, 18\% had a total cholesterol level >6.4 mmol/L, and 22\% were current or past smokers. All the subjects gave written informed consent to participate in the study, which was approved by the local ethics committee.

Radial Artery Parameters

The ultrasound system used in the present study has been previously described and validated for the measurements of radial artery IMT and internal diameter and its systolic-diastolic variation in humans.\textsuperscript{20} A high-resolution echo-tracking device (NIUS-02) with a 10-MHz probe was used to acquire backscattered radiofrequency (RF) data from the radial artery at the wrist. The internal diameter was computed from the anterior and posterior RF echo signal, and the arterial wall thickness was calculated from the posterior RF echo signal. The examination was carried out with a standardized protocol that included measurement of the mean IMT and the diastolic internal diameter.

Carotid Artery Parameters

We used a high-resolution echo-tracking system (Wall Track System) coupled with a conventional 2-dimensional vascular echograph (sigma 44 Kontrom) equipped with a 7.5-MHz probe. The details of this method, based on the RF signal analysis, have been described and validated elsewhere.\textsuperscript{21} Measurements were performed in the right and left common carotid arteries, 1 cm below the bifurcation at the site of the distal wall. From the RF signal, it is possible to determine the signals corresponding to the proximal and the distal walls and therefore to measure the posterior wall thickness. For statistical analysis, only the value obtained for the right common carotid artery was taken.

Determination of Genotypes

ACE I/D Polymorphism

Genomic DNA was extracted from peripheral blood samples according to standard protocol.\textsuperscript{22} The I/D polymorphism of the ACE gene was identified with polymerase chain reaction (PCR) using a set of oligonucleotide primers flanking the polymorphic site according to the method described by Rigat et al.\textsuperscript{1} In brief, a set of primers was designed to encompass the polymorphic region in intron 16 of the ACE gene (sense primer 5'-CTGGAGACCTGCTCCATCCTTTCT-3' and antisense primer 5'-GATGTGGCCATCACCATGTCGAT-3'). The PCR reaction contained 100 ng of DNA template, 5 pmol/\mu L of each primer, 1 \mu L of 2 mmol/L DNTP, 1 \mu L of 5\% DMSO, 0.04 \mu L of ampli Taq DNA polymerase ATGC, and 2 \mu L of PCR Buffer Ampligene (500 mmol/L KC1, 100 mmol/L Tris-HCl, pH 9, 15 mmol/L MgCl\textsubscript{2}). DNA was amplified for 30 cycles; each cycle was composed of denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 1 minute, with a final extension time of 7 minutes. The PCR products were separated by electrophoresis on 2\% agarose gel.

AT\textsubscript{1}R A/C\textsuperscript{1166} Polymorphism

The detection of the AT\textsubscript{1}R A/C\textsuperscript{1166} polymorphism was accomplished by allele-specific oligonucleotide hybridization as previously reported.\textsuperscript{23} After enzymatic amplification of genomic DNA, PCR products were denatured in 0.4 mol/L NaOH and 25 mmol/L EDTA, blotted in duplicate on nylon membranes, neutralized (3 mmol/L Na acetate), and cross-linked with UV light. Each membrane was then hybridized for 12 hours in 7\% PEG and 10\% SDS with \gamma\textsuperscript{32}P\textsuperscript{32}ATP end-labeled 15-mer oligonucleotide probes. The probes were 3'-AATGAGCATTAGCTA-5' for the A\textsuperscript{1166} allele, and 3'-AATGAGCTTAGCTA-5' for the C\textsuperscript{1166} allele. The membranes were washed twice at room temperature in 2\times SSC and 0.1\% SDS, and for 10 minutes in 1\times SSC at 42°C (A\textsuperscript{1166} or 46°C (C\textsuperscript{1166}), respectively. The PCR results were scored by 2 independent investigators. No intraobserver variability was found on repeated readings of the same gel, and the interobserver variability was <1%.

Statistical Analysis

Quantitative results are expressed as mean±SD. Repeatability of the measurement of the artery diameter and IMT was investigated in 20 different subjects through a calculation of the repeatability coefficient as defined by the British Standards Institution.\textsuperscript{21} Allele and genotype frequencies were analyzed by the gene counting method, and the Hardy-Weinberg equilibrium was checked by a \chi\textsuperscript{2} test. ANOVA was used to study the relations between genotypes and phenotypes. All the inheritance models, recessive (DD versus ID+II), dominant (DD+ID versus II), and additive (DD versus ID versus II), were considered. Relations between arterial wall hypertrophy (carotid or radial) and independent variables (genotypes, age, body mass index, gender, systolic blood pressure, total cholesterol, smoking habits) were evaluated by logistic regression analysis. Presence of radial hypertrophy was considered if the wall thickness was >260 \mu m (the value of the 95th percentile of a random population studied in our laboratory). Carotid hypertrophy was defined as a wall thickness >660 \mu m (the value of the 95th percentile of a random population studied in our laboratory). For the regression model, several analyses were performed with the genotype effect assumed to be additive (II=1, ID=2, DD=3), dominant (II=0, ID and DD=1), or recessive (ID and II=0, DD=1). Odds ratios were calculated as the measure of the association of the ACE genotype or the AT\textsubscript{1}R genotype with the phenotype of vascular hypertrophy. The II genotype was taken as the reference group for ACE I/D analysis, and AA genotype was taken as the reference group for AT\textsubscript{1}R A/C\textsuperscript{1166} analysis. A value of P<0.05 was considered statistically significant.

Results

Study Population

In the overall sample, the mean age of the subjects was 49±12 years; 181 were men (53\%) and 95\% were white. The...
demographic characteristics according to the ACE I/D and AT,R A/C1166 genotypes are summarized in Table 1.

The frequencies of the D and I alleles in the overall sample were 0.59 and 0.41, respectively. Genotypes frequencies (II=16.76%, ID=48.53%, DD=34.71%) were in agreement with the Hardy-Weinberg equilibrium ($\chi^2=0.001$, $P=0.99$). The frequencies of the A1166 and C1166 alleles in the overall sample were 0.71 and 0.29, respectively, and genotype frequencies (AA=50.7%, AC=39.6%, CC=9.6%) were in agreement with the Hardy-Weinberg equilibrium ($\chi^2=0.21$, $P=0.91$).

The clinical and biological characteristics were similar across genotypes, except for a higher weight in DD subjects ($P=0.04$).

Associations Between Genotype and Phenotype
No association was observed between the ACE I/D polymorphism and wall thickness, internal diameter, or thickness/ radius and either radial artery or carotid artery (Table 2). Further analyses were carried out separately for men and women or when genotype effect was assumed to be inherited as additive (CC versus AA versus AC), dominant (AA versus AC+CC), or recessive (CC versus AC+AA).

Because a positive interaction between these 2 polymorphisms had been observed previously in myocardial infarction,15 we compared a subgroup of subjects at "high genetic risk" (subjects with DD and CC or AC genotypes) with a subgroup of subjects at "low genetic risk" (subjects with AA and II or ID genotypes). No difference in the vascular phenotypes could be observed (Table 4).

Finally, we performed an analysis in which radial artery hypertrophy or carotid hypertrophy was included as a dichotomous variable (presence or absence of hypertrophy). Table 5 shows the odds ratio calculated as the measure of the association of the ACE genotype or the AT,R genotype with the phenotype of vascular hypertrophy. This analysis confirmed the absence of association between these polymorphisms and vascular hypertrophy of either the radial or the carotid artery. Logistic regression analysis indicates that age, systolic blood pressure, gender, and smoking are independent determinants for radial artery hypertrophy, but ACE I/D or AT,R A/C1166 are not (Table 6). For the carotid artery, age, systolic blood pressure, gender, smoking, and total cholesterol were found to be independent determinants, although ACE I/D and AT,R A/C1166 were not statistically associated with carotid hypertrophy (Table 6). Thus, these results suggest that these gene polymorphisms have an undetectable role in the

**TABLE 1. Demographic Data According to ACE I/D and AT,R A/C1166 Genotypes**

<table>
<thead>
<tr>
<th></th>
<th>ACE I/D</th>
<th>AT,R A/C1166</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>II (n=57)</td>
<td>ID (n=165)</td>
</tr>
<tr>
<td>Male/female</td>
<td>24/33</td>
<td>93/72</td>
</tr>
<tr>
<td>Age, y</td>
<td>48±12</td>
<td>49±13</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>68±12</td>
<td>72±16</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>25±4</td>
<td>26±4</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>145±18</td>
<td>145±19</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>85±12</td>
<td>85±11</td>
</tr>
<tr>
<td>Cholesterol, mmol/L</td>
<td>5.6±1.0</td>
<td>5.6±1.0</td>
</tr>
</tbody>
</table>

*P<0.05, II vs DD.

**TABLE 2. Structural Phenotypes for Radial or Carotid Artery According to ACE I/D Genotype**

<table>
<thead>
<tr>
<th></th>
<th>ACE I/D Genotype</th>
<th>AT,R A/C1166 Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artery</td>
<td>II (n=57)</td>
<td>ID (n=165)</td>
</tr>
<tr>
<td>Radial</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wall thickness, μm</td>
<td>227±46</td>
<td>241±56</td>
</tr>
<tr>
<td>Diameter, μm</td>
<td>2225±441</td>
<td>2329±445</td>
</tr>
<tr>
<td>Thickness/radius</td>
<td>0.21±0.06</td>
<td>0.21±0.06</td>
</tr>
<tr>
<td>Carotid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wall thickness, μm</td>
<td>536±103</td>
<td>538±129</td>
</tr>
<tr>
<td>Diameter, μm</td>
<td>6123±986</td>
<td>6326±917</td>
</tr>
<tr>
<td>Thickness/radius</td>
<td>0.17±0.04</td>
<td>0.17±0.04</td>
</tr>
</tbody>
</table>
structure of the radial and the carotid artery compared with usual determinants such as blood pressure, age, and gender.

Discussion

The main finding of our study is the absence of association between vascular phenotypes (internal diameter, wall thickness) and the ACE I/D polymorphism or the AT1R A/C1166 gene polymorphism. Association studies are exposed to the risk of bias, but the design of the present study should have avoided several common sources of error. In this respect, its strength includes the selection of a large sample of a general population rather than a case-control design, the careful inclusion of subjects without any evidence of cardiovascular disease or pharmacological treatment potentially interfering with the phenotypes under investigation, and the adoption of quantitative criteria for structural parameters.

Because Ang II is a very potent vasoconstrictor and promotes vascular hypertrophy and hyperplasia, it has been hypothesized that polymorphisms of the renin-angiotensin system could play a role in the development of arterial wall thickening. In a group of 189 subjects, Castellano et al found an association between the ACE I/D polymorphism and common carotid IMT. However, this association was limited to subjects who were not receiving chronic drug therapy and was only statistically significant for the common carotid segment. Our study, which included 340 subjects, was unable to duplicate this finding and is in agreement with other reports that failed to demonstrate any association between carotid wall thickness and ACE I/D polymorphism.

In our study, we limited the analysis to those subjects with less evidence of vascular damage, and this point differs from other studies reporting an association between carotid wall thickness and ACE I/D genotype. We justified this restriction in subjects with less evidence of vascular damage because Kauma et al had demonstrated that a positive relationship between I/D polymorphism and carotid wall thickness was observed only in subjects without carotid plaques. These authors suggested that the lack of association between atherosclerotic plaques and ACE genotypes may reflect different mechanisms for plaque development and early arterial wall thickening. Our study, which included 340 subjects, was unable to duplicate this finding.

Association between AT1R A/C1166 gene polymorphism and vascular structure has been less frequently tested. Castellano et al in a sample of 212 subjects selected from a general population, investigated the association of AT1R A/C1166 gene polymorphism with carotid wall thickness. No statistically significant difference among AT1R A/C1166 genotypes was observed for the carotid artery. Our study confirms this previous report in a larger group of subjects. Because Benetos et al reported that patients with a CC genotype had increased aortic stiffness, and because we did not observe any association with structural phenotypes of 2 different arteries, it appears that genotype-phenotype studies with arteries should include parameters describing the structure as well as the distensibility.

One of the most obvious explanations for the negativity of genotype-phenotype studies is the low informativeness car-
ried by the study of only 1 genetic marker in a multigenic disease. One way to avoid this limitation is to study subgroups of subjects selected according to a combination of polymorphisms. This strategy was used by Tiret et al., who demonstrated that the association between the ACE DD genotype and myocardial infarction was restricted to a subset of individuals, also carriers of the AT1R A/C1166 allele. However, a large number of subjects in the initial population is necessary to avoid a too small sample size for each subgroup.

The design of our study allowed us to select 2 subgroups of subjects: 1 defined as high genetic risk that included subjects with DD and CC or AC genotypes, and 1 defined as low genetic risk for subjects with AA and II or ID genotypes. The comparison between the 2 groups did not show any difference in the structure of the radial or carotid artery.

In conclusion, in the present study of 340 subjects, we were unable to identify the ACE I/D or the AT1R A/C1166 gene polymorphism as a marker for vascular hypertrophy in subjects with no evidence of cardiovascular disease. Furthermore, the subjects defined as having a high genetic risk did not show any difference in the structure of the radial or carotid artery compared with subjects defined as a low genetic risk, suggesting that these gene polymorphisms have an undetectable role in the structure of the radial and carotid arteries compared with usual determinants such as blood pressure and age.

Acknowledgments

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