Carotid and Femoral Artery Stiffness in Relation to Three Candidate Genes in a White Population

Elisabeth J. Balkestein, Jan A. Staessen, Ji-Guang Wang, Janneke J. van der Heijden-Spek, Luc M. Van Bortel, Cristina Barlassina, Giuseppe Bianchi, Eva Brand, Stefan-Martin Herrmann, Harry A. Struijker-Boudier

Abstract—Different genetic polymorphisms influence cardiovascular disease. We recently discovered a relationship between the intima-media thickness of the muscular femoral artery, but not the elastic common carotid artery, and the combined ACE (ACE, I/D), α-adducin (Gly460Trp), and aldosterone synthase (AS, C−344T) gene polymorphisms. To investigate the relationship between these polymorphisms and functional properties of the carotid artery and femoral artery, a sample of 756 subjects enrolled in a population study were genotyped for the presence of the ACE D, α-adducin 460Trp, and aldosterone synthase −344T alleles. Vessel wall properties were assessed using a vessel wall movement detector system in combination with applanation tonometry. Statistical analysis allowed for confounders and interaction among genes. Cross-sectional compliance of the common carotid artery was negatively associated with the ACE D allele. ACE II versus ACE DD homozygotes differed, expressed as a percentage of the population mean (7.0%; 95% confidence interval [CI], 1.6% to 12.4%; P=0.02). In multigene analysis, ACE DD subjects also deviated significantly from the population mean for the distensibility coefficient of the common carotid artery when carrying the AS/T allele (−5.5%; 95% CI, −9.3% to −1.7%; P<0.01), without a change in cross-sectional compliance. ACE DD subjects, when homozygote for α-adducin Gly460, had a lower femoral cross-sectional compliance (−10.4%; 95% CI, −1.9% to −18.9%; P<0.03) and a lower distensibility (−9.7%; 95% CI, −2.1% to −17.3%; P<0.02) compared with the population mean. These data show that functional large artery properties are influenced by the ACE I/D polymorphism. Cross-sectional compliance and distensibility coefficients are influenced by the ACE I/D genotype, but this influence depends on the vascular territory and genetic background. (Hypertension. 2001;38:1190-1197.)

Key Words: angiotensin-converting enzyme • genes • polymorphism • race • arteries • population

Numerous studies have examined the effects of different genetic polymorphisms on cardiovascular disease, with emphasis on the genes coding for components of the renin-angiotensin-aldosterone system (RAAS). We recently observed that the ACE insertion/deletion (I/D) polymorphism (ACE I/D) was related to increased intima-media thickness of the large muscular femoral artery and that this relationship was strongest when the α-adducin 460Trp allele or the aldosterone synthase gene polymorphisms. In hypertensive patients, a positive association with PWV was found for the angiotensin II type 1 receptor (A1166C), in both patients with and without treatment, and for the aldosterone synthase gene polymorphisms. In hypertensive patients, a positive association with PWV was found for the angiotensin II type 1 receptor (A1166C), in both patients with and without treatment, and for the aldosterone synthase gene polymorphisms. In hypertensive patients, a positive association with PWV was found for the angiotensin II type 1 receptor (A1166C), in both patients with and without treatment, and for the aldosterone synthase gene polymorphisms. In hypertensive patients, a positive association with PWV was found for the angiotensin II type 1 receptor (A1166C), in both patients with and without treatment, and for the aldosterone synthase gene polymorphisms.

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stiffness in patients with type 2 diabetes and age-matched control subjects. In type 2 diabetics, both PWV and carotid stiffness were negatively associated with the I allele of the ACE I/D polymorphism. However, like Benetos et al., 13 no association was found in healthy control subjects. In the present study, we investigated the single-gene effects and the interactions of the ACE I/D, a-adducin, and aldosterone synthase gene polymorphisms on arterial function of the elastic common carotid artery and the muscular femoral artery in a general population.

Methods

Study Population

The Flemish Study on Environment, Genes and Health Outcomes (FLEMENGHO) was started in 1985. The Ethics Committee of the University of Leuven approved its protocol. From August 1985 until November 1990, a random sample of the households living in a geographically defined area of northern Belgium was recruited. 18 To further investigate the role of genetic factors, from June 1996 until January 1999, the study population was enlarged with nuclear families including children who were at least 10 years old, using the former participants as index persons. 18 The participants or their parents gave informed consent. The participation rate among all subjects contacted was 64.3%. The study population for whom all significant covariables and outcome variables were present consisted of 756 persons.

Measurements

Before the participants were examined at our field center, they refrained from smoking, heavy exercise, and drinking alcohol or caffeine-containing beverages for ≥3 hours. Their blood pressure was measured 5 times consecutively after they had rested for 5 minutes and were sitting. Hypertension was diagnosed if the average of the 5 blood pressure readings was ≥140 mm Hg systolic or ≥90 mm Hg diastolic or when the subjects were on antihypertensive medication. A venous blood sample was obtained for measurement of serum lipids and determination of genotypes.

Vascular Measurements

All subjects were examined in a quiet room after 15 minutes of supine rest. Two observers (J.J. van der H.-S. and E.J.B.) performed measurements at the common carotid artery 2 cm proximal from the bulb and measurements at the femoral site 1 cm proximal to the bifurcation into the profound and superficial branches. To examine vascular wall properties of the arteries, a pulsed ultrasound echotripping system was used, which is based on the radiofrequency signal and was described and validated earlier by Hoeks et al. 19 Diameter and diameter changes throughout the cardiac cycles were measured on average for 15 heartbeats, and the mean of these measurements was taken as the subject’s reading. Local pulse pressure was calculated after assessment of the tonometric signal at the artery of interest with a pencil-shaped probe (Millar Instruments) 20 by calibration of the pulse wave contour with diastolic and mean arterial pressure, which is assumed to be equal over the arterial tree. 31 The mean of an average of 15 heartbeats was taken as the subject’s reading. If tonometry was not possible due to obesity or the presence of arterial plaque, the vessel wall movement contour was described and validated earlier by Hoeks et al. 19

Results

Characteristics of the Participants

The 756 participants included 379 men (50.1%) and 377 (49.1%) women (Table 1). The sample consisted of 272 subjects belonging to households or nuclear families with ≥1 related individual (82 families), 33 spouse-spouse pairs (33 families), and 418 single individuals. Age ranged from 12 to 79 years. One hundred fifty-four subjects (20.4%) were hypertensive, and of those, 49 were receiving antihypertensive treatment (31.8%). One hundred twenty male participants (31.7%) reported current smoking, and 192 (50.7%) reported intake of alcohol. For female participants, 107 (28.4%) reported current smoking, and 91 (24.1%) reported intake of alcohol. Ninety-two female subjects (24.4%) were using oral contraceptives. The characteristics of the carotid and femoral arteries by gender and age are presented in Figure 1.
**TABLE 1. Characteristics of Study Participants**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Male (n=379)</th>
<th>Female (n=377)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>44.5±15.1</td>
<td>44.6±14.8</td>
<td>0.91</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.3±3.5</td>
<td>24.6±4.1</td>
<td>0.02</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>129±14</td>
<td>125±16</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>82±11</td>
<td>79±11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean arterial pressure,* mm Hg</td>
<td>90±9</td>
<td>84±10</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Heart rate,* bpm</td>
<td>60±9</td>
<td>64±9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>80 (21.1)</td>
<td>74 (19.6)</td>
<td>0.61</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>120 (31.7)</td>
<td>107 (28.4)</td>
<td>0.33</td>
</tr>
<tr>
<td>Alcohol, n (%)</td>
<td>192 (50.7)</td>
<td>91 (24.1)</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Common carotid artery**

- Diameter, mm: Male 7.6±0.8, Female 6.9±0.8, P <0.001
- Distensibility coefficient, 10⁻³/kPa: Male 24.7±11.2, Female 26.2±13.6, P = 0.11
- Cross-sectional compliance, mm²/kPa: Male 1.07±0.40, Female 0.93±0.43, P <0.001
- Local pulse pressure, mm Hg: Male 48±12, Female 47±11, P = 0.04

**Femoral artery**

- Diameter, mm: Male 10.2±1.2, Female 8.7±1.1, P <0.001
- Distensibility coefficient, 10⁻³/kPa: Male 9.7±6.3, Female 11.1±7.0, P <0.01
- Cross-sectional compliance, mm²/kPa: Male 0.78±0.51, Female 0.64±0.40, P <0.001
- Local pulse pressure, mm Hg: Male 54±12, Female 52±13, P <0.01

| Total cholesterol, mmol/L     | Male 5.32±1.07 | Female 5.36±1.03 | 0.62 |
| HDL cholesterol, mmol/L       | Male 1.23±0.30  | Female 1.48±0.36  | <0.001|
| LDL cholesterol, mmol/L       | Male 3.08±1.01  | Female 3.11±0.95  | 0.60 |
| Triglycerides, mmol/L         | Male 2.19±1.40  | Female 1.65±0.96  | <0.001|

*Measured during vascular measurements with Dinamap. Data are mean±SD or n (%).

### Single-Gene Effects

Genotype frequencies of the **ACE I/D (P=0.06)**, **α-adducin (Gly460Trp, P=0.48)**, and aldosterone synthase (**C−344T (P=0.17)** polymorphisms did not significantly deviate from Hardy-Weinberg equilibrium.

In stepwise regression analysis, cross-sectional compliance, distensibility coefficient, and diameter of the carotid and femoral arteries were significantly correlated with age, with the exception of femoral artery compliance (Table 2). Other covariables that entered the models on ≥1 occasions were gender, body mass index, mean arterial pressure, current treatment for hypertension, current smoking, and observer. With cumulative adjustment for all these covariables, we found a significant association between the cross-sectional compliance of the common carotid artery and the **ACE I/D genotype** (Figure 2). Cross-sectional compliance decreased with the number of **ACE D alleles**. Indeed, compared with the population mean, **ACE II subjects** had a cross-sectional compliance of, expressed as a percentage of the population mean, 3.9% (95% CI, 0.1% to 7.7%; P=0.04) higher. In contrast, in single-gene analyses, the cross-sectional compliance and distensibility coefficients of both the common carotid and femoral arteries were not significantly associated with the **α-adducin** or aldosterone synthase genotypes.

### Multigene Effects

In multigene analyses, with adjustments for the same covariables as applied in the single-gene approach, we found significant differences in the arterial wall properties associated with the **ACE DD genotype**. The analysis showed that the influence of the **ACE DD genotype** depended on the vascular territory and individual genetic background. Indeed, compared with the population mean (expressed as percentage of the population mean), the diameter of the common carotid artery was significantly larger (1.8%; 95% CI, 0.1% to 3.5%, P=0.03) in the presence of the aldosterone synthase **−344T allele** (Figure 3). This increase in diameter was associated with a decreased distensibility of the common carotid artery in **ACE DD homozygotes**, who also carried the aldosterone synthase **−344T allele (−5.5%; 95% CI, −1.7% to 9.3%, P=0.005 versus the population mean)**. However, in the latter subgroup, no change was observed in the cross-sectional compliance of the common carotid artery (Figure 3). Furthermore, compared with the population mean (Figure 4), the cross-sectional compliance of the femoral artery was significantly smaller in **ACE DD subjects** not carrying the **α-adducin 460Trp allele (−10.4%; 95% CI, −1.9% to −18.9%, P=0.02)**. This was accompanied by a decrease in the distensibility coefficient of the femoral artery (−7%; 95% CI, −2.1% to −17.3%, P=0.01 versus the population mean) but not with a change in diameter (Figure 4).
Discussion

The results of the present study show that combinations of genetic polymorphisms of the ACE I/D, α-adducin, and aldosterone synthase gene influence local arterial function.

A single-gene effect was observed for the ACE I/D polymorphism on the compliance of the elastic common carotid artery. In multigene analysis, a significant interaction between ACE I/D and α-adducin Gly460Trp was detected at the femoral artery. ACE DD subjects homozygote for α-adducin (GlyGly) deviated significantly from the population mean for both distensibility coefficient and cross-sectional compliance. With respect to the carotid artery,
interaction between the ACE I/D and the aldosterone synthase C—344T polymorphism was significant. ACE DD subjects carrying the aldosterone synthase −344T allele had a lower distensibility coefficient, with a larger diameter and no change in cross-sectional compliance.

Our finding of a single-gene effect on carotid artery stiffness in the general population is in contrast with previous observations on the influence of the ACE I/D polymorphism in healthy subjects.13,17 The variance of our data with those of observations on the influence of the ACE I/D polymorphism was significant. In 13,17 The variance of our data with those of observations on the influence of the ACE DD allele in 216 hypertensive subjects, whereas in the present study, local stiffness was measured. PWV may reflect other aspects of vascular function than those we have measured.

In previous studies, we35 and others36 showed that the interaction of various genes might provide a stronger basis for the genetic determination of cardiovascular diseases than single-gene effects. In our previous work, we showed the particular significance of such interactions between components of the RAAS and the α-adducin Gly460Trp polymorphism.33 There are various potential physiological interactions between these systems. On the one hand, at the level of the kidney, both the RAAS and α-adducin contribute to sodium handling and volume homeostasis.35 At the vascular level, interactions are more complex and may depend on the vessel wall component that is involved. Vessel wall elasticity depends on vascular smooth muscle cell mass and tone as well as on the composition of extracellular matrix material. There are marked differences between various types of arteries with respect to the contribution of each of these components. Thus, gene–gene interactions may have a different outcome according to the type of artery studied.

In the present study, we noted an interaction between aldosterone synthase gene and ACE I/D polymorphisms at the level of the carotid artery. The occurrence of the aldosterone synthase −344T allele seems necessary to bring about the negative association with the ACE D allele on distensibility of the common carotid artery. Our data are compatible with a decrease in distensibility due to an increase in diameter.

At the level of the femoral artery, we noted a significant interaction between the α-adducin 460Trp allele and the ACE

### Table 2: Determinants of Characteristics of Common Carotid and Femoral Arteries in Multiple Regression Analysis

<table>
<thead>
<tr>
<th>Determinant</th>
<th>Common Carotid Artery</th>
<th>Femoral Artery</th>
<th>Common Carotid Artery</th>
<th>Femoral Artery</th>
<th>Common Carotid Artery</th>
<th>Femoral Artery</th>
</tr>
</thead>
<tbody>
<tr>
<td>R²</td>
<td>0.54</td>
<td>0.27</td>
<td>0.74</td>
<td>0.30</td>
<td>0.45</td>
<td>0.42</td>
</tr>
<tr>
<td>Intercept</td>
<td>2.95</td>
<td>1.51</td>
<td>95.16</td>
<td>26.02</td>
<td>5.01</td>
<td>8.15</td>
</tr>
<tr>
<td>Regression coefficients (SE)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>−0.032±0.004 §</td>
<td>0.004±0.005 $</td>
<td>−1.315±0.084 §</td>
<td>0.029±0.074 $</td>
<td>0.014±0.008 §</td>
<td>0.004±0.014 §</td>
</tr>
<tr>
<td>Age²/10³</td>
<td>0.185±0.042 §</td>
<td>−0.054±0.057 §</td>
<td>8.410±0.920 §</td>
<td>−0.405±0.806 §</td>
<td>0.116±0.092 §</td>
<td>0.186±0.151 §</td>
</tr>
<tr>
<td>Gender (1, male; 2, female)</td>
<td>−0.18±0.02§ $</td>
<td>−0.19±0.03§ $</td>
<td>−0.13±0.49 §</td>
<td>0.44±0.43</td>
<td>−0.62±0.05§</td>
<td>−1.41±0.08§</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>−0.006±0.003*</td>
<td>−0.004±0.004</td>
<td>−0.140±0.070†</td>
<td>−0.194±0.061†</td>
<td>0.040±0.007§</td>
<td>0.08±0.01§</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>−0.011±0.001§</td>
<td>−0.012±0.002§ $</td>
<td>0.310±0.028§</td>
<td>−0.172±0.025§</td>
<td>0.009±0.003§</td>
<td>−0.0006±0.005</td>
</tr>
<tr>
<td>Current smoking (0, 1)</td>
<td>0.06±0.02†</td>
<td>0.07±0.03†</td>
<td>1.12±0.52†</td>
<td>1.29±0.46‡</td>
<td>0.09±0.05*</td>
<td>−0.15±0.09*</td>
</tr>
<tr>
<td>On antihypertensive drugs (0, 1)</td>
<td>−0.02±0.03</td>
<td>−0.07±0.05</td>
<td>0.04±0.74</td>
<td>−0.39±0.65</td>
<td>0.06±0.07</td>
<td>−0.30±0.12†</td>
</tr>
<tr>
<td>Observer (1, J.J. van der H.-S.; 2, E.J.B.)</td>
<td>0.08±0.02§ $</td>
<td>0.37±0.03§ $</td>
<td>0.43±0.53</td>
<td>3.76±0.46§</td>
<td>0.30±0.05§</td>
<td>0.71±0.09§</td>
</tr>
</tbody>
</table>

*0.1<P<0.05; †P<0.05; ‡P<0.01; §P<0.001.
I/D polymorphism. In a previous study, we found that the presence of the 460Trp allele in combination with the ACE D allele causes enhanced intima-media thickening of the muscular femoral artery (Balkestein EJ, Wang JG, Staessen JA, Barlassina C, Bianchi G, Birkenhager WH, Brand E, Den Hond E, Fagard R, Herrmann S-M, Van Bortel LM, Struijker Boudier HAJ, unpublished data, 2001). If this hypertrophy were primarily due to an increased vascular smooth muscle cell mass, it would lead to an increased distensibility of the vessel wall. On the other hand, an ACE D allele may have a more pronounced effect on extracellular matrix synthesis due to higher local levels of angiotensin II.36,37 This would result in a decreased distensibility of the femoral artery in ACE DD persons who lack the α-adducin 460Trp allele. The present data suggest that α-adducin can override the increased femoral stiffness in ACE DD subjects by increasing smooth muscle cell mass. Before such conclusions can be drawn more firmly, additional research should be performed on the mechanisms whereby ACE and α-adducin influence the structure and function of various components of the vessel wall.

In summary, gene–gene interactions between components of the RAAS and α-adducin seem to play a role in large artery function and structure. Similarly, they contribute to the development of hypertension.39 The unraveling of the cellular mechanisms underlying these interactions poses a challenge for future hypertension research.

Acknowledgments

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Figure 2. Cross-sectional compliance of the common carotid artery by ACE genotype. Data are presented as means adjusted for age, age², gender, body mass index, mean arterial pressure, current treatment for hypertension, smoking, and observer, with 95% CI adjusted for multiple comparisons. For each genotype, the number of subjects is given. *P<0.03 vs population mean.

Figure 3. Characteristics of the common carotid artery of ACE DD subjects by aldosterone synthase genotype. Data are presented as mean deviations from the population mean with 95% CI, adjusted for age, age², gender, body mass index, mean arterial pressure, current treatment for hypertension, smoking, and observer. For each genotype, the number of subjects is given. ○, ACE DD subjects with aldosterone synthase CC genotype; ●, ACE DD subjects carrying the aldosterone synthase T allele. *P<0.05, **P<0.01.
Figure 4. Characteristics of the femoral artery of ACE DD subjects by a-adducin genotype. Data are presented as mean deviation from the population mean with 95% CI, adjusted for age, gender, body mass index, mean arterial pressure, current treatment for hypertension, smoking, and observer. For each genotype, the number of subjects is given. ○, ACE DD subjects with a-adducin GlyGly genotype; ●, ACE DD subjects carrying the a-adducin Trp allele. * P<0.05, ** P<0.01.

References


Carotid and Femoral Artery Stiffness in Relation to Three Candidate Genes in a White Population

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