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 A1166C 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-medial thickness of the large muscular femoral artery and that this relationship was strongest when the α-adducin 460Trp allele or the aldosterone synthase −344T allele was present as well. The ACE I/D polymorphism has previously been studied in relation to other manifestations of cardiovascular disease, such as myocardial infarction,1 atherosclerosis,2–5 left ventricular hypertrophy,6–8 and hypertension.9–11 Only few studies have investigated the effect of candidate genes on more specific cardiovascular phenotypes, such as large artery function. Benetos and colleagues12–14 studied pulse wave velocity (PWV), a marker of aortic stiffness,15 in relation to ACE I/D, angiotensin II type 1 receptor, and 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,12,13,16 and for ACE I/D13 and aldosterone synthase C→344T in treated hypertensive subjects.14 In a recent study, Benetos and colleagues16 did not confirm the relationship between the aldosterone synthase gene and PWV in untreated hypertensives, nor did they find a relationship with the ACE I/D or 2 angiotensinogen gene (AGT) polymorphisms (T174M, M235T). Two variants of the angiotensin II type 1 receptor gene, A→153G and A1166C, were, however, reported to be related to PWV, whereas in normotensive control subjects, no association for the A1166C variant has been observed.13 Taniwaki et al17 studied PWV and local carotid

Received April 28, 2001; first decision June 18, 2001; revision accepted July 2, 2001.
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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., no association was found in healthy control subjects. In the present study, we investigated the single-loci effects and the interactions of the ACE I/D, α-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. 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. The participants or their parents gave informed consent. The participation rate among all families contacted was 64.3%. The study population for whom all significant covariates 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 echotrack- ing system was used, which is based on the radiofrequency signal and was described and validated earlier by Hoeks et al. 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) by calibration of the pulse wave contour with diastolic and mean arterial pressure, which is assumed to be equal over the arterial tree. 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 used as a surrogate for the tonometrically derived pulse pressure contour with calibration as described above. Recently, this method was described in detail.

Blood pressure and heart rate were measured every 3 minutes with a semiautomated device (Dinamap model 845; Critikon), and the within-subject mean of the vascular measurements was used for descriptive analysis and calibration of local pulse pressure. Densitability coefficient (DC) was calculated from the diastolic cross-sectional area (A) and change in cross-sectional area (ΔA) and local pulse pressure (ΔP) with the formula DC=(ΔA/A)/ΔP. A and ΔA were calculated as A=π·(D/2)^2 and ΔA=π·[((D+ΔD)/2)^2−(D/2)^2]. Cross-sectional compliance (CC) was calculated as CC=ΔA/ΔP.

Determination of Genotypes

Genotyping of ACE I/D Polymorphism

The ACE I/D polymorphism was detected as previously described by Lindpaintner et al. All samples genotyped as DD underwent a second polymerase chain reaction (PCR) using insertion-specific primers. PCR conditions were as before except for an annealing temperature of 67°C. The sense primer was 5’-TGCGACACACGGCGCCACTAC-3’ and the antisense primer was 5’-TCGCCAGCCCCATGCCCATAA-3’.

Genotyping of Gly460Trp α-Adducin Polymorphism

Allelic discrimination of the Gly460Trp α-adducin polymorphism was carried out using a 5’ nuclelease assay on an ABI Prism 7700 apparatus (Perkin-Elmer) as described by Cusi et al. The forward and reverse primers and the 460Gly and 460Trp probes used in the TaqMan assay were 5’-CGTCCACACTTGCTTGCCACT-3’, 5’-GGAGAAGACAGATGCTGGAACCT-3’, 5’-FAM-TTCCA-TTCTGCTTCTCCGGA-TAMRA-3’, and 5’-TET-TTCCA-TTCTGCAATTCTGCGAAT-TAMRA-3’, respectively.

Genotyping of CYP11B2 Polymorphism

For determination of the C–344T aldosterone synthase gene variants, PCR and subsequent genotyping were performed as described by Brand et al.

Statistical Analysis

For statistical analysis, we used SAS version 8.1 (SAS Institute). Significant covariates for cross-sectional compliance and distensibility coefficient for the common carotid and femoral arteries were traced by stepwise linear regression. In addition, potenti- ally important covariates were forced into the models regardless of statistical significance. In multiple linear regression, the genotypes were first represented by dummy variables using the deviation from mean coding approach, which does not imply any genetic hypothesis. In single-gene analyses in which we tested independent hypotheses, we adjusted α levels and confidence intervals (CIs) for multiple testing using Bonferroni’s method. Because family members are more likely to share identical alleles than are randomly selected subjects, we performed the analysis using generalized estimating equations to account for the possible nonindependence of vessel wall properties within families. P<0.05 were considered significant for the analysis of interaction among genes.

Results

Characteristics of the Participants

The 756 participants included 379 men (50.1%) and 377 women (49.1%) (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 (24.1%) used 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>
<tr>
<td>Common carotid artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>7.6±0.8</td>
<td>6.9±0.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distensibility coefficient, 10⁻³/kPa</td>
<td>24.7±11.2</td>
<td>26.2±12.6</td>
<td>0.11</td>
</tr>
<tr>
<td>Cross-sectional compliance, mm²/kPa</td>
<td>1.07±0.40</td>
<td>0.93±0.43</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Local pulse pressure, mm Hg</td>
<td>48±12</td>
<td>47±11</td>
<td>0.04</td>
</tr>
<tr>
<td>Femoral artery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diameter, mm</td>
<td>10.2±1.2</td>
<td>8.7±1.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Distensibility coefficient, 10⁻³/kPa</td>
<td>9.7±6.3</td>
<td>11.1±7.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Cross-sectional compliance, mm²/kPa</td>
<td>0.78±0.51</td>
<td>0.64±0.40</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Local pulse pressure, mm Hg</td>
<td>54±12</td>
<td>52±13</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.32±1.07</td>
<td>5.36±1.03</td>
<td>0.62</td>
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<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.23±0.30</td>
<td>1.48±0.36</td>
<td>&lt;0.001</td>
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<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.08±1.01</td>
<td>3.11±0.95</td>
<td>0.60</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>2.19±1.40</td>
<td>1.65±0.96</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*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 Taniwaki et al17 may be due to the exclusion in the latter study of hypertensives (systolic blood pressure > 160 mm Hg); patients with a history of peripheral vascular disease, myocardial infarction, or cerebrovascular disease; and patients who used any medication. The same holds for the study of Benetos et al,13 who excluded subjects on the basis of a history or presence of lower limb arterial disease, stroke, or heart failure or a blood pressure of 145 mm Hg systolic and > 90 mm Hg diastolic. Furthermore, the number (n=260 and 128) of subjects was much smaller in their study than our study. Our data suggest that in a general population, the single-gene effect on carotid artery distensibility observed in the more muscular femoral artery. Previous research has shown that the different structural bases of elastic and muscular arteries may have important implications for their adaptive behavior.32

In our study population, no single-gene effect was found for the aldosterone synthase gene polymorphism. Benetos et al14 reported an increased PWV for the C−344 allele in 216 hypertensive patients, although this was not confirmed in their most recent study in 441 untreated hypertensives.16 There were clear differences in the experimental approaches and populations studied by us and by Benetos et al.14,16 PWV is a general marker of arterial stiffness in essential hypertension, 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, we33 and others9,34 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
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, Struijk-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 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.38 The unraveling of the cellular mechanisms underlying these interactions poses a challenge for future hypertension research.

Acknowledgments
The FLEMENGHO Study was supported by research grants G.0174.97 and G.0291.98 from the Fonds voor Wetenschappelijk Onderzoek Vlaanderen (Brussels, Belgium) and by a special research grant from the Katholieke Universiteit Leuven (Onderzoekstoelage OT99/28). Nuclear families were recruited in the framework of the European Project on Genes in Hypertension, which is supported by the European Union (contracts IC15-CT98-0329-EPOLIG and QLG1-CT-2000-01137-EURNETGEN). The Flemish population study would not have been possible without the collaboration of the family physicians of the participants. The municipality Hechtel-Eksel (Belgium) provided logistic support. The authors acknowledge the expert assistance of Leszek Bieniaszewski, MD; Rina Bollen; Lut

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. o, ACE DD subjects with aldosterone synthase CC genotype; ●, ACE DD subjects carrying the aldosterone synthase T allele. *P<0.05, **P<0.01.
De Pauw, RN; Hilde Celis, MD; Primož Dolenc, MD; Paul Drent, RN; Dmitri Emelianov, MD; Jerzy Gasowski, MD; Heng Fan; Lieve Gijsbers; Alda Hermans; Tatjana Kuznetsova, MD; Tim Nawrot, BSc; Lutgarde Thijs, BSc; Yvette Toremans; Sylvia Van Hulle, RN; and Renilde Wolfs (Study Coordinating Center, Leuven, Belgium). Genetic studies in Berlin, Germany, were supported by grants from the Deutsche Forschungsgemeinschaft to Eva Brand (BR 1589/1–1) and Stefan-Martin Herrmann (HE 2852/1–1) and by a grant from the Société Française d’Hypertension Arterielle to Dr Brand. Brigitte Eghers (Berlin) determined the aldosterone synthase genotypes.

References


Figure 4. Characteristics of the femoral artery of ACE DD subjects by α-adducin genotype. Data are presented as mean deviation 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 α-adducin GlyGly genotype; ●, ACE DD subjects carrying the α-adducin Trp allele. *P<0.05, **P<0.01.


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*Hypertension*. 2001;38:1190-1197
doi: 10.1161/hy1101.095992

*Hypertension* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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