Aging, Carotid Artery Distensibility, and the Ser422Gly Elastin Gene Polymorphism in Humans

Olivier Hanon, Vu Luong, Jean Jacques Mourad, Luiz A. Bortolotto, Xavier Jeunemaitre, Xavier Girerd

Abstract—Elastin is a protein of the extracellular matrix that forms the major component of elastic fibers from the arterial wall thickness and plays an important role in elastic properties of large blood vessels. To study the relationships between the Ser422Gly polymorphism in exon 16 of the gene-encoding elastin and the distensibility of 2 different arteries, the radial artery (a muscular artery) and the common carotid artery (an elastic artery), we studied a cohort of 320 subjects (49±12 years of age) without evidence of cardiovascular disease and who had never been treated with cardiovascular drugs. Distensibility and elastic modulus were evaluated for the common carotid and the radial arteries with high-resolution echo-tracking devices (NIUS-02 and Wall Track System). The A-to-G nucleotide change corresponding to the Ser422Gly amino acid change was studied by digestion of polymerase chain reaction products with BstNI.

Results indicate that genotype frequencies (AA=10%, AG=51%, GG=39%) were in agreement with the Hardy-Weinberg equilibrium. For the carotid artery, a significant decrease in distensibility was observed in subjects carrying the A allele (with AA+AG genotypes) compared with subjects with the GG genotype (13.8±6.4 kPa⁻¹ · 10⁻³ versus 15.9±6.2 kPa⁻¹ · 10⁻³, P<0.01), assuming a dominant effect of the A allele. Moreover, the presence of the A allele was associated with a significant increase in elastic modulus (0.98±0.40 kPa · 10³ in subjects with AA+AG genotypes versus 0.83±0.41 kPa · 10³ in subjects with GG genotypes, P<0.01). Multivariate analysis indicated that these results were observed after adjustment for age, gender, and mean arterial blood pressure (P<0.01). In contrast, no association was found between arterial parameters and genotypes for the radial artery. The 2-way analysis of covariance adjusted for mean arterial blood pressure indicated that the association between the A allele and distensibility of the carotid artery was observed only in subjects >50 years of age, assuming for carotid distensibility a significant age effect (P<0.01), genotype effect (P=0.01), and age-genotype interaction (P=0.04). The present results indicate a relationship between the Ser422Gly polymorphism and the distensibility of elastic arteries but not of muscular arteries and suggest that there is an age-genotype interaction for carotid artery distensibility. (Hypertension. 2001;38:1185-1189.)

Key Words: carotid artery ■ distensibility ■ aging ■ elastin gene polymorphism

Morphological studies have indicated that arterial distensibility depends on different factors such as blood pressure and age.¹ Aging is the main variable responsible for functional changes in the arterial wall leading to an increase in arterial stiffness.² Aging changes in the human arterial wall are especially apparent in the load-bearing media in which there is progressive disorganization, with loss of the orderly arrangement of elastin fibers. Furthermore, morphological studies have indicated that the effects of aging are different on proximal elastic arteries than on distal muscular arteries.³-⁴

Some other clinical⁵ and experimental⁶ studies have also suggested that genetic factors may be implicated in the mechanism of increased pulse pressure, which is a marker of arterial stiffness. Elastin is one of the major determinants of arterial distensibility of large blood vessels⁷ that forms the principal component of elastic fibers from the media of arteries.⁸ An elastin gene has been located on chromosome 7 in humans.⁹ Several polymorphisms of the elastin gene have been described,¹⁰,¹¹ but no intermediate phenotype has been demonstrated for these genetic variants.¹² However, a deletion involving 7q11.23 and resulting in hemizygosity of the elastin gene has been identified as the mechanism responsible for the Williams and Buren syndrome,¹³ which is characterized by supravalvular aortic stenosis, hypertension, or peripheral stenoses. Furthermore, Tromp et al¹⁴ identified an A-to-G polymorphism of the elastin gene in exon 16 resulting from a variant that converted the codon -AGT-(serine) at amino acid position 422 to -GGT-(glycine). Until now, no quantified phenotype has been associated with this polymorphism of the elastin gene.

Recently, it has been suggested that genetic factors may influence the effects of age on arterial stiffness. This relationship between angiotensin II type 1 receptor genotypes and aortic stiffness was observed only in subjects >50 years of age, suggesting an interaction between aging and genetic variants.¹⁵

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The main objective of this study was to evaluate the relationships between the Ser422Gly polymorphism of the gene-encoding elastin and arterial distensibility at the level of 2 different vascular sites: the common carotid artery, an elastic large artery, and the radial artery, which is a muscular, medium-sized artery. Moreover, we examined the role of this polymorphism in the relationship between age and arterial distensibility. We studied a cohort of 320 subjects with no history of cardiovascular disease and no pharmacological treatment that would potentially interfere with the vascular phenotypes under investigation.

**Methods**

**Study Population**

We selected 320 subjects (180 men, 140 women) from a cohort of patients referred to the Atherosclerosis Prevention Clinic because of cardiovascular risk factors or symptomatic vascular disease. Patients were eligible for the study if they (1) had a normal carotid wall (defined as the absence of stenosis or plaque) after ultrasound examination, (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 pressure was measured automatically every 3 minutes on the left arm with a Dinamap oscillometric blood pressure recorder (model 845, Critikon). Blood samples were obtained to analyze serum glucose, lipid profile, and DNA with standard methods. All the subjects were white; 58% had essential hypertension (BP<90 mm Hg diastolic pressure and the absence of clinical or laboratory evidence suggesting secondary forms of hypertension); 22% had a body mass index (BMI) and/or were smokers; and 22% had a body mass index (BMI) >30 kg/m². Patients with diabetes mellitus were excluded. All subjects gave written, informed consent to participate to the study, which was approved by the local ethics committee.

**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, which is based on radiofrequency signal analysis, have been described and validated elsewhere. Measurements were performed on the right and left common carotid arteries 1 cm below the bifurcation at the site of the distal wall. For statistical analysis, only the value obtained for the right common carotid artery was used.

**Radial Artery Parameters**

The ultrasound system used in the present study (NIUS 02) has been described and validated for measurement of radial artery intima-media thickness and internal diameter and its systolic diastolic variations in humans. Determination of Genotypes

Genomic DNA was extracted from peripheral blood samples according to standard protocol. The A-to-G gene polymorphism was studied by use of a polymerase chain reaction–radiofrequency technique as previously described by Tromp et al.

**Statistical Analysis**

All results given in the tables and text are expressed as mean±SD. Allele and genotype frequencies were analyzed by the gene counting method, and the Hardy-Weinberg equilibrium was checked by a χ² test. ANOVA was used to study the relations between genotypes and arterial phenotypes. The relation between arterial distensibility or elastic modulus (carotid or radial) and independent variables (genotypes, age, BMI, gender [male=0, female=1], mean arterial blood pressure, total cholesterol) was evaluated by a multivariate regression analysis.

Two-way ANCOVA with mean arterial blood pressure as the covariate was used to evaluate whether the associations between arterial distensibility and genotypes were modified by age (>50 years) independently of blood pressure level.

Statistical analysis was performed with the General Linear Models package from NCSS 6.0 software (Statistical Solutions Ltd). A value of P<0.05 was considered statistically significant.

**Results**

**Study Population**

The demographic characteristics according to genotypes are summarized in Table 1. Allele frequencies were 36% for the A allele and 64% for the G allele. Genotypes frequencies (AA=10%, AG=51%, GG=39%) were in agreement with the Hardy-Weinberg equilibrium (χ²=0.02, P=0.99). No significant differences between genotypes were found in terms of gender distribution, age, BMI, systolic blood pressure, mean arterial blood pressure, pulse pressure, serum glucose, and total cholesterol.

**Arterial Parameters According to Genotypes**

Arterial parameters of structure (wall thickness, internal diameter) and function (distensibility, elastic modulus) for the carotid and radial arteries according to genotype are summarized in Table 2. No association was found between arterial parameters of structure and the polymorphism. In contrast, for the carotid artery, a significant decrease in distensibility was observed in subjects carrying the A allele (with AA+AG genotypes) compared with GG subjects, assuming a dominant effect of the A allele. Moreover, the presence of the A allele was associated with a significant increase in elastic modulus.

A multivariate regression analysis was conducted to assess the independent effects of usual factors and the polymorphism on distensibility and elastic modulus of the carotid artery. This analysis indicated that carotid distensibility was significantly lower (P<0.01) and elastic modulus was significantly higher (P<0.01) in

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**TABLE 1. Demographic and Biological Characteristics of Patients According to Genotype**

<table>
<thead>
<tr>
<th></th>
<th>AA (n=134)</th>
<th>AG (n=163)</th>
<th>GG (n=123)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female, n</td>
<td>17/17</td>
<td>98/65</td>
<td>65/58</td>
<td>0.50</td>
</tr>
<tr>
<td>Age, y</td>
<td>48±11</td>
<td>49±13</td>
<td>49±13</td>
<td>0.82</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26±3.7</td>
<td>25.8±3.9</td>
<td>25.9±4.5</td>
<td>0.79</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>147±18</td>
<td>146±20</td>
<td>144±18</td>
<td>0.44</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>88±11</td>
<td>86±13</td>
<td>87±13</td>
<td>0.43</td>
</tr>
<tr>
<td>MAP, mm Hg</td>
<td>108±13</td>
<td>106±14</td>
<td>106±14</td>
<td>0.57</td>
</tr>
<tr>
<td>PP, mm Hg</td>
<td>59±18</td>
<td>60±15</td>
<td>57±12</td>
<td>0.30</td>
</tr>
<tr>
<td>HR, bpm</td>
<td>70±11</td>
<td>71±11</td>
<td>71±11</td>
<td>0.82</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>5.2±0.9</td>
<td>5.5±0.9</td>
<td>6.0±0.9</td>
<td>0.66</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.9±1.0</td>
<td>5.8±0.8</td>
<td>5.8±0.7</td>
<td>0.80</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial blood pressure; PP, pulse pressure; and HR, heart rate.
subjects carrying the A allele after adjustment for age and mean arterial blood pressure.

In contrast, no association was found between functional parameters (distensibility and elastic modulus) and genotype at the level of the radial artery.

**Influence of Age on the Relationship Between Genotypes and Carotid Artery Elasticity**

The 2-way analysis of covariance adjusted for mean arterial blood pressure indicated that the association between the A allele and distensibility of the carotid artery was observed only in older subjects (>50 years of age). Moreover, the elastic modulus of the carotid artery was significantly higher in subjects carrying the A allele in subjects who were >50 years of age. In contrast, no relationship between genotype and carotid artery distensibility or elastic modulus in subjects <50 years of age was observed (Table 4). Furthermore, the 2-way analysis of covariance adjusted for mean arterial blood pressure showed a significant age-gene interaction ($P = 0.04$) for the distensibility and elastic modulus of the carotid artery.

**Discussion**

The main finding of our study is the presence of an association between the A allele and distensibility of the carotid artery after adjustment for age and blood pressure. Moreover, this association is apparent only in patients >50 years of age, suggesting an age-genotype interaction for carotid artery elasticity. Association studies are exposed to the risk of bias, but we designed the present study to avoid several common sources of error. In this respect, some of its strengths include the selection of a large sample of a general population rather than a case-control design, the careful inclusion of subjects with less evidence of vascular damage without any evidence of symptomatic cardiovascular disease or pharmacological treatment that could potentially interfere with the phenotypes under investigation, and the adoption of quantitative criteria to evaluate arterial functional parameters.

The present results indicated that mutations affecting the overall architecture of the elastic network could be involved in the vascular changes in the arterial wall. Vascular abnormalities resulting from mutations of other structural components of the extracellular matrix have previously been described. Several studies have reported a relationship between the Williams and Buren syndrome and the deletion involving 7q11.23, resulting in hemizygosity of the elastin gene. Mutations in the fibrillin-1 gene located on human chromosome 15 cause Marfan syndrome, and a morphological study has shown an increase in large artery stiffness in these patients, suggesting the role of the deficit of fibrillin in arterial wall properties. In our study, we evaluated the relationships between the Ser422Gly elastin gene polymorphism described by Tromp et al and the elastic properties of arteries. To the best of our knowledge, no study has ever described an association between this polymorphism and quantified vascular phenotypes.

We observed a significant association between the Ser422 allele and elasticity of the carotid artery after adjustment for age and mean arterial blood pressure, suggesting that the relationship was independent of these usual determinants.

The investigation was designed to evaluate the elastic response of arteries via distensibility and the elastic properties of the arterial wall via elastic modulus. In contrast to distensibility, which provides information about elasticity of the artery as a hollow structure, the incremental modulus of elasticity provides direct information on the elastic properties of the wall material independent of the vessel geometry.

The present study indicates that the Ser422 allele was associated with a decrease in distensibility and an increased elastic modulus at the site of an elastic artery. In contrast, no relationship was found between the arterial parameters of elasticity and genotypes at the level of the radial artery. These 2 arterial sites do not provide the same information. The carotid artery is an elastic artery with high amounts of elastin and collagen fibers, whereas the radial artery is a muscular artery composed principally of arterial smooth muscle. Morphological studies indicated that during hypertension elastic properties of the carotid and radial arteries are not the same. In hypertensive subjects, distensibility of the carotid artery decreased, whereas for the radial artery, the distensibility was not significantly different from that of normotensive control subjects when the 2 groups were compared at their respective mean arterial pressures.
The aging process is the main determinant responsible for arterial wall changes leading to arterial stiffening. With increasing age, arteries progressively stiffen and dilate. These changes are due to a degeneration of elastin fibers associated with an increase in collagenous material. Furthermore, it has been observed during aging that elastic fibers undergo thinning, splitting, fraying, and fragmentation. Moreover, an increase in stiffness with age is observed for the carotid artery, whereas there is little or no consistent change in distensibility of the radial artery with age.

A recent study suggested that genetic factors may influence the magnitude of the effects of age on large artery stiffness. The authors reported that AT1 gene polymorphisms (153 A/G and 1166 A/C) could influence arterial aging in hypertensive subjects. Moreover, the effect of the AT1 153 A/G polymorphism on aortic stiffness became apparent only in patients >55 years of age, and subjects with 153 G and 1166 C haplotypes had both the highest and the steepest increase in arterial stiffness after 50 years of age. Similarly, our results indicate that the associations between the Ser422 allele and carotid distensibility and modulus elastic are apparent only in patients >50 years of age. These findings illustrate that arterial aging might be influenced by genetic factors, suggesting an acceleration of the vascular aging effects in Ser422 allele carriers, and/or that the phenotypic modifications bound to this genotype express themselves later.

Experimental and morphological studies have suggested that genetic factors could play a role in the distensibility of the large arteries. It has been reported that the stiffness of the carotid artery in genetic Dahl rats was increased independently of the height of arterial pressure and the level of sodium intake but could be related to the genetic trait. Additionally, it has been observed during aging that elastic fibers undergo thinning, splitting, fraying, and fragmentation. Moreover, an increase in stiffness with age is observed for the carotid artery, whereas there is little or no consistent change in distensibility of the radial artery with age.

In conclusion, the present results indicate a relationship between the Ser422Gly polymorphism and the distensibility of elastic arteries but not of muscular arteries and suggest that there is an age-genotype interaction for carotid artery distensibility.

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