Influence of fibrillin-1 genotype on the aortic stiffness in men

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Influence of fibrillin-1 genotype on the aortic stiffness in men. J Appl Physiol 99: 1036–1040, 2005; doi:10.1152/japplphysiol.00554.2004.—Aortic stiffness is a predictor of cardiovascular mortality. The mechanical properties of the arterial wall depend on the connective tissue framework, with variation in fibrillin-1 and collagen I genes being associated with aortic stiffness and/or pulse pressure elevation. The aim of this study was to investigate whether variation in fibrillin-1 genotype was associated with aortic stiffness in men. The mechanical properties of the abdominal aorta of 79 healthy men (range 28–81 yr) were investigated by ultrasonographic phase-locked echo tracking. Fibrillin-1 genotype, characterized by the variable tandem repeat in intron 28, and collagen type I alpha 1 gene characterized by the 2,064 G>T polymorphism, were determined by using DNA from peripheral blood cells. Three common fibrillin-1 genotypes, 2-2, 2-3, and 2-4, were observed in 50 (64%), 10 (13%), and 11 (14%) of the men, respectively. Those of 2-3 genotype had higher pressure strain elastic modulus and aortic stiffness compared with men of 2-2 or 2-4 genotype (P = 0.005). Pulse pressure also was increased in the 2-3 genotype (P = 0.04). There was no significant association between type 1 collagen genotype and aortic stiffness in this cohort. In conclusion, the fibrillin-1 2-3 genotype in men was associated with increased aortic stiffness and pulse pressure, indicative of an increased risk for cardiovascular disease.

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was included to enable simultaneous monitoring of the electrocardiogram, arterial blood pressure, and vessel diameter. Briefly, two electronic markers automatically locked to the luminal interface of the echoes from the anterior and posterior vessel wall and followed the pulsatile movements of the vessel wall. The repetition frequency of the echo-tracking loops was 870 Hz, with a time resolution of ~1.2 ms.

All measurements were performed with the subject in the supine position after at least 15 min of rest. Differences in blood pressure between left and right arm were excluded before the investigation. From the real-time picture, the abdominal aorta distal to the renal arteries was insonated in longitudinal section, and the echoes from the aortic wall were optimized before pulsatile diameter changes were recorded, ~3.5 cm proximal to the iliac bifurcation. A sequence of at least five representative consecutive-diameter cycles was chosen manually, and mean vessel diameters and diameter changes were calculated. Indirect blood pressure measurements were performed immediately after measurements of the pulsatile aortic diameter with a sphygmomanometer and a standard cuff on the left arm. Mean arterial blood pressure (MAP) was taken as the brachial diastolic pressure obtained by the auscultatory method. Mean values for each subject were calculated.

Pressure strain elastic modulus and stiffness.

Pressure strain elastic modulus (Ep) was defined according to Peterson et al. (26) as:

\[ Ep = K \times \frac{P_{\text{syst}} - P_{\text{diast}}}{D_{\text{syst}} - D_{\text{diast}}/D_{\text{diast}}} \]  

(1)

Stiffness (\(\beta\)) was defined according to Kawasaki et al. (14) as

\[ \beta = \ln \left( \frac{P_{\text{syst}}/P_{\text{diast}}}{D_{\text{syst}} - D_{\text{diast}}/D_{\text{diast}}} \right) \]  

(2)

In these equations, \(P_{\text{syst}}\) and \(P_{\text{diast}}\) are the maximal systolic and end-diastolic blood pressure levels (mmHg), \(D_{\text{syst}}\) and \(D_{\text{diast}}\) are the corresponding vessel diameters (mm). Ep is measured in newtons per square meter. The factor (K) for converting millimeters of mercury to newtons per square meter is 133.3.

Each subject was examined three times with calculation of pressure strain elastic modulus and stiffness from the corresponding diameter, pulsatile diameter change, and blood pressures obtained by the auscultatory method. Mean values for each subject were calculated. By this technique, the variability for repeated measurements of the aortic diameter and pulsatile diameter changes were 5 and 15%, respectively (10).

Preparation and analysis of genomic DNA. Isolation of genomic DNA was performed according to routine protocols, and DNA samples were typed for the (TAAA)<sub><sup>n</sup></sub> repeat in intron 28 of the fibrillin-1 gene on chromosome 15, using the primers CCT GGC TAC CAT TCA ACT CCC and GAG TAC ATA GAG TGT TTT AGGG as described previously (27). Polymorphism in the Sp1 binding site (in the first intron, 2064 G>T) of the COL1A1 gene was determined as described previously, using the polymerase chain reaction with a mismatched primer that introduces a diallelic restriction site for BglI (9).

Statistics. Data are presented as means ± SD for all data, because these approximated to a normal distribution. Comparisons were performed by \(\chi^2\) tests for categorical variables. Analysis of variance was used for the three-group analysis of continuous variables. Associations among genotype, aortic diameter, and aortic stiffness were adjusted for age, body mass index, mean blood pressure, and heart rate in a regression model.

RESULTS

The 79 men (mean age 55.3 yr, range 28–81) had mean aortic diameter 1.84 ± 0.16 cm and mean systolic pressure 138 ± 13 mmHg; 17 (22%) were current smokers, but no subject had diabetes.

The distribution of the fibrillin-1 genotypes for the (TA-AAA)<sub><sup>n</sup></sub> repeat was 1-2 (4), 1-3 (1), 1-4 (1), 2-2 (50), 2-3 (10), 2-4 (11), 3-3 (1), and 3-4 (1). Associations between genotype and aortic stiffness were investigated by using only the three common genotypes 2-2, 2-3, and 2-4 (total of 71 men, of whom 47 were middle-aged, 40–59 yr). The mean aortic stiffness was 13.30 ± 7.62. The demographic and measured data, according to fibrillin-1 genotype, are shown in Table 1. Seven subjects were being treated for hypertension (four 2-2, two 2-3, one 2-4). Subjects of 2-3 genotype had the highest pulse pressure, 61 ± 9, vs. 52 ± 9 and 51 ± 11 mmHg for those of 2-2 and 2-4 genotype, respectively (\(P = 0.04\)). Subjects of 2-3 genotype also had the highest aortic stiffness 18.66 ± 7.10, vs. 12.85 ± 5.09, and 11.49 ± 6.88 for those of 2-2 and 2-4 genotype, respectively (\(P = 0.001\)). Interestingly, the subject of 3-3 genotype had the highest aortic stiffness (\(\beta = 38.9\)). The subjects of 2-3 genotype also had the highest systolic blood pressure, pulse pressure, and heart rate (Table 1). The association between aortic stiffness and 2-3 genotype remained highly significant after adjustment for

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Table 1. Characteristics according to fibrillin-1 genotype

<table>
<thead>
<tr>
<th>Fibrillin-1 Genotype</th>
<th>2-2 (n = 50)</th>
<th>2-3 (n = 10)</th>
<th>2-4 (n = 11)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>54.7±3.2</td>
<td>56.0±1.7</td>
<td>55.4±2.4</td>
<td>0.91</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25.3±2.1</td>
<td>26.9±2.2</td>
<td>25.3±1.8</td>
<td>0.25</td>
</tr>
<tr>
<td>Current smokers, %</td>
<td>10 (20)</td>
<td>2 (20)</td>
<td>4 (36)</td>
<td>0.42</td>
</tr>
<tr>
<td>Systolic pressure, mmHg</td>
<td>140±15</td>
<td>158±9</td>
<td>139±16</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>88±10</td>
<td>95±5</td>
<td>87±7</td>
<td>0.08</td>
</tr>
<tr>
<td>Mean pressure, mmHg</td>
<td>105±11</td>
<td>117±8</td>
<td>105±11</td>
<td>0.03</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>52±9</td>
<td>61±9</td>
<td>51±11</td>
<td>0.04</td>
</tr>
<tr>
<td>Aortic diameter, cm</td>
<td>1.8±0.2</td>
<td>1.9±0.2</td>
<td>1.9±0.2</td>
<td>0.81</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>72±10</td>
<td>80±11</td>
<td>64±12</td>
<td>0.01</td>
</tr>
<tr>
<td>Pressure strain elastic modulus (\times 10^{-5}), N/m²</td>
<td>1.8±0.7</td>
<td>3.0±0.7</td>
<td>1.7±0.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Aortic stiffness ((\beta))</td>
<td>12.9±7.1</td>
<td>18.7±5.1</td>
<td>11.5±6.9</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means ± SD; \(n\), number of subjects.
age, body mass index, mean blood pressure, and heart rate ($P = 0.005$).

Because aortic stiffness increases markedly with age, we analyzed separately the results for the 47 middle-aged (40–59 yr) men of 2-2 ($n = 33$), 2-3 ($n = 7$), and 2-4 ($n = 7$) genotypes. There was no difference between the groups in mean age, smoking habit, body mass index, and aortic diameter. Subjects of 2-3 genotype had the highest MAP, pressure strain elastic modulus, and aortic stiffness, but pulse pressure was not significantly higher. The MAPs were $103 \pm 1$ (2-2), $116 \pm 9$ (2-3), and $102 \pm 10$ mmHg (2-4) ($P = 0.02$). The mean pulse pressures were $53 \pm 13$ (2-2), $60 \pm 9$ (2-3), and $52 \pm 12$ mmHg (2-4) ($P = 0.38$). The mean aortic stiffnesses were $10.63 \pm 4.34$ (2-2), $17.99 \pm 4.05$ (2-3), and $10.69 \pm 4.67$ (2-4) ($P = 0.001$), and these findings were unchanged after adjustment for age, body mass index, MAP, and heart rate ($P = 0.008$).

For the COL1A1 genotype, 58 men (74%) were homozygous for the G allele, 20 (25%) men were GT heterozygotes, and one man (1%) was homozygous for the T allele. Mean aortic stiffness for men of GG genotype was $12.67 \pm 6.89$ compared with $13.54 \pm 7.20$ for men of GT genotype ($P = 0.35$). Adjustment for age, body mass index, and heart rate had little effect ($P = 0.32$).

**DISCUSSION**

This study has extended current information about the genetic contribution to aortic stiffness. Using phase-locked echo tracking to noninvasively measure aortic stiffness, we have shown that in men (predominantly middle-aged) those of fibrillin-1 2-3 genotype had stiffer abdominal aortas than men of the two other common genotypes (2-2 and 2-4). The strength of the association in a small population indicates the importance of this effect. In contrast, the polymorphism in the COL1A1 promoter had a nonsignificant effect on the stiffness of the abdominal aorta.

Expression and turnover of the elastic connective tissue matrix in the arterial wall may influence the elastic properties. As a constituent of extensible microfibrils, fibrillin-1 might be of importance. These microfibrils are associated with the elastin fibers and arranged in concentric elastic lamellae. By directing the orientation of the elastin fibers, fibrillin-1 microfibrils may play a vital role in load bearing (28). There are several indications that fibrillin-1 is an important factor in the regulation of mechanical properties in central arteries. Mutations in the fibrillin-1 gene have been associated with increased aortic stiffness as well as increase in central pulse pressure in Marfan syndrome (12, 13, 31). Furthermore, an association has been established between the fibrillin-1 polymorphism used in this study, with pulse pressure and aortic impedance, even though this polymorphism has not been reported to be functional (21, 27).

Because the pressure-diameter relationship of the arterial wall is nonlinear, pressure strain elastic modulus is pressure dependent (see Eq. 1). This might explain why the pressure strain elastic modulus value was ~80% higher in the subjects of fibrillin-1 2-3 genotype than in those of fibrillin-1 2-2 and 2-4 genotypes, because mean blood pressure was higher in the 2-3 genotype (Table 1). However, stiffness index ($\beta$) is based on the observed linear relation between the logarithm of relative pressure and distention ratio (Eq. 2) and has been shown to be less pressure dependent (15). Nevertheless, stiffness was ~60% higher in subjects of fibrillin-1 2-3 genotype than in those of fibrillin-1 2-2 and 2-4 genotypes. Furthermore, the association between aortic stiffness and 2-3 genotype remained highly significant also after adjustment for mean pressure, indicating the possibility of a structural difference in the aortic wall between the groups (Table 1).

Calculation of impedance provides an estimate of average stiffness distal to the point of measurement. In contrast, phase-locked echo tracking measures stiffness at a single point in the artery, the abdominal aorta in the present investigations. This differentiates our study from other recent studies (5, 20, 21). The cohorts also had very different characteristics: older patients with coronary artery disease and healthy young adults (5, 21). We confined our study to men because of the gender difference in aortic stiffness (29). Furthermore, because aortic stiffness also is dependent on age, we separately evaluated the middle-aged men (40–59 yr). Although the associations between aortic stiffness and fibrillin-1 genotype were demonstrated in both the complete cohort as well as the more restricted group, the association between pulse pressure and aortic stiffness only was significant in the complete cohort, probably because of sample size limitations.

All the men were selected by being first-degree relatives of patients with abdominal aortic aneurysm. It might be argued that an underlying genetic predisposition to aneurysmal disease might confound our results, because abdominal aortic aneurysms have a higher prevalence in near relatives of patients with abdominal aortic aneurysms than in the general population (34). Also, in patients with abdominal aortic aneurysm, those of fibrillin-2 2-3 genotype have highest systolic and pulse pressures and these patients have stiff aortic walls (16, 23). However, the frequency of the fibrillin-1 genotypes in our study was similar to those earlier described, both in healthy middle-aged men and in patients with coronary artery disease (21, 27). Furthermore, none of the subjects in our study had a dilated aorta, and aortic stiffness, diameter, blood pressure, and heart rate were within the ranges reported earlier (Table 1) (32). Therefore, any underlying genetic or cardiovascular disease bias to our study was likely to be minimal.

We observed a surprising, novel association between increased heart rate and the fibrillin-1 2-3 genotype in our cohort, the significance of which is uncertain (Table 1). An increased heart rate might alter cardiovascular regulation in several ways. It may lead to decreased pulse pressure due to a combination of an increase in diastolic decay and reduced diastolic time. An increase in sympathetic tone often accompanies an increase in heart rate, which leads to increased peripheral resistance (24). This, in turn, may result in changed pulse pressure amplification between central and peripheral arteries owing to a modification in the location of reflection sites, with an overestimation of central aortic pressure as consequence when only the peripheral blood pressure is measured (as in this study) (1, 35). Several recent papers have addressed the issue whether increased heart rate affects arterial stiffness and peripheral blood pressure. The pulse wave velocity, as well as the peripheral pulse pressure, were unaffected or lower in the studies by Albaladejo et al.
REFERENCES