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

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Powell, J. T., R. J. Turner, M. Sian, R. Debasso, and T. Länne. 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 genotype 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 I 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.

blood pressure; collagen; elastin; mechanics

The mechanical properties of the aorta and large distribution arteries are important determinants of pulse pressure. Important to the mechanisms underlying this association are factors controlling the stiffness of the aorta and other large arteries. The stiffness of the aorta increases with age and is higher in men than women (29). The stiffness of the aorta also depends on the distribution of biological components, particularly the extracellular matrix, with the proteins elastin, collagen, and fibrillin having an important role. Fibrillin-1 is the Marfan gene, and in the Marfan syndrome both arterial stiffness and elevated pulse pressure are important factors leading to aortic dilatation (12, 13).

In a previous study, our laboratory demonstrated an association between arterial pulse pressure and variation in the fibrillin-1 gene in healthy middle-aged men (27). This study used the variable tandem nucleotide repeat in intron 28 of the fibrillin-1 gene to describe three common genotypes, 2-2 (present in over half the population), 2-3, and 2-4. Men of 2-3 genotype had the highest pulse pressure. Pulse pressure is an independent predictor of cardiovascular outcomes (2). Arterial stiffness is an independent determinant for pulse pressure, and recently evidence has begun to emerge that aortic stiffness itself is an independent predictor of cardiovascular mortality in hypertensive patients (3, 4, 18, 20). These findings have awakened interest in genetic determinants of aortic stiffness. Recently, Medley and coworkers showed that fibrillin-1 genotype was associated with increased aortic stiffness in patients with coronary artery disease (21). In particular, using the same variable tandem nucleotide repeat (27), they showed, as a measure of aortic stiffness, that patients of the 2-3 fibrillin genotype had significantly higher input impedance and characteristic impedance than patients of 2-2 or 2-4 genotype (21). Similarly, pulse-wave velocity measurements have been used to demonstrate an association between a polymorphism in a Sp1 binding site of the first intron of the COL1A1 (type 1 collagen) gene and aortic stiffness in healthy young adults (5).

We have used a different, noninvasive technique, phase-locked echo tracking, to measure the stiffness of the abdominal aorta in healthy men. The aim of this study was to determine whether variation in the fibrillin-1 or type 1 collagen genes was associated with aortic stiffness in this cohort.

MATERIALS AND METHODS

The majority of subjects studied (n = 50) were healthy male volunteers, aged 40–59 yr, who were first-degree relatives of patients with abdominal aortic aneurysm. In addition, 4 persons <40 yr and 25 persons >59 yr were investigated. Subjects with peripheral arterial disease (signified by an ankle brachial systolic pressure index <0.9) and maximum abdominal aortic diameter more than 2.5 cm were excluded. A questionnaire was administered to determine the history of previous myocardial infarction, angina, hypertension, diabetes, and smoking, and the use of any regular medications was noted. A blood sample was taken for the measurement of cholesterol and to provide peripheral blood cells for the preparation of genomic DNA. The staff performing ultrasonographic investigations were blinded to the clinical data and to the genotypes, which were evaluated later. Staff undertaking the genotyping were blinded to the physiological measurements. Each subject gave informed consent to the experiments, which were approved by the Ethics Committee at Lund University, Lund, Sweden.

Ultrasonic measurements of aortic distensibility. The diameter and pulsatile diameter changes of the abdominal aorta were measured by means of echo-tracking ultrasonography (29). We used an electronic echo-tracking instrument (Diamove, Teltec AB, Lund, Sweden) capable of detecting vessel wall movements of <10 μm (19). With this equipment, pulsatile vessel diameter changes can be measured and, in combination with blood pressure measurements, form the basis for calculation of vessel wall stiffness (inversely related to vascular distensibility). The instrument was interfaced with a B-mode real-time ultrasound scanner (EUB-240, Hitachi, Tokyo, Japan) and fitted with a 3.5-MHz linear array transducer. A data acquisition system containing a personal computer type 386 (Express, Tokyo, Japan) and a 12-bit analog-to-digital converter (Analog Devices, Norwood, MA)

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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 \( \sim 1.2 \text{ ms} \).

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, \( \sim 3.5 \text{ cm} \) 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 plus one-third of the pulse pressure and assumed to be equal to the intra-arterial pressure in the abdominal aorta. Comparison between intra-arterial pressure in abdominal aorta and the brachial blood pressure obtained by the auscultatory method has shown a slight systematic underestimation of pulse pressure, which does not affect comparative studies of aortic stiffness between different groups of subjects (30).

Distensibility was expressed as the inverse relation of pressure strain elastic modulus and stiffness.

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

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

(1)

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

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

(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). \( E_p \) 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 (TAAAA)\(_n\) 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 TTG AGGG as described previously (27). Polymorphism in the Sp1 binding site (in the first intron, 2064 G\( \rightarrow \)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 Ball1 (9).

Statistics. Data are presented as means \( \pm SD \) 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 \( \pm 0.16 \) cm and mean systolic pressure 138 \( \pm 13 \) mmHg; 17 (22\%) were current smokers, but no subject had diabetes.

The distribution of the fibrillin-1 genotypes for the (TAAAA)\(_n\) 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 \( \pm 7.62 \). 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 \( \pm 9 \), vs. 52 \( \pm 9 \) and 51 \( \pm 11 \) 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 vs. 12.85 \( \pm 7.10 \) and 11.49 \( \pm 6.88 \) 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, pressure strain elastic modulus, and heart rate (Table 1). The association between aortic stiffness and 2-3 genotype remained highly significant after adjustment for

### 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 ( \pm ) 3.2</td>
<td>56.0 ( \pm ) 1.7</td>
<td>55.4 ( \pm ) 2.4</td>
<td>0.91</td>
</tr>
<tr>
<td>Body mass index, kg/m(^2)</td>
<td>25.3 ( \pm ) 2.1</td>
<td>26.9 ( \pm ) 2.2</td>
<td>25.3 ( \pm ) 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 ( \pm ) 15</td>
<td>158 ( \pm ) 9</td>
<td>139 ( \pm ) 16</td>
<td>0.04</td>
</tr>
<tr>
<td>Diastolic pressure, mmHg</td>
<td>88 ( \pm ) 10</td>
<td>95 ( \pm ) 5</td>
<td>87 ( \pm ) 7</td>
<td>0.08</td>
</tr>
<tr>
<td>Mean pressure, mmHg</td>
<td>105 ( \pm ) 11</td>
<td>117 ( \pm ) 8</td>
<td>105 ( \pm ) 11</td>
<td>0.03</td>
</tr>
<tr>
<td>Pulse pressure, mmHg</td>
<td>52 ( \pm ) 9</td>
<td>61 ( \pm ) 9</td>
<td>51 ( \pm ) 11</td>
<td>0.04</td>
</tr>
<tr>
<td>Aortic diameter, cm</td>
<td>1.8 ( \pm ) 0.2</td>
<td>1.9 ( \pm ) 0.2</td>
<td>1.9 ( \pm ) 0.2</td>
<td>0.81</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>72 ( \pm ) 10</td>
<td>80 ( \pm ) 11</td>
<td>64 ( \pm ) 12</td>
<td>0.01</td>
</tr>
<tr>
<td>Pressure strain elastic modulus ( \times 10^{-5} ), N/m(^2)</td>
<td>1.8 ( \pm ) 0.7</td>
<td>3.0 ( \pm ) 0.7</td>
<td>1.7 ( \pm ) 0.6</td>
<td>0.001</td>
</tr>
<tr>
<td>Aortic stiffness (( \beta ))</td>
<td>12.9 ( \pm ) 7.1</td>
<td>18.7 ( \pm ) 5.1</td>
<td>11.5 ( \pm ) 6.9</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Values are means \( \pm SD; n, \) 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 ± 11 (2-2), 116 ± 9 (2-3), and 102 ± 10 mmHg (2-4) \( (P = 0.02) \). The mean pulse pressures were 53 ± 13 (2-2), 60 ± 9 (2-3), and 52 ± 12 mmHg (2-4) \( (P = 0.38) \). The mean aortic stiffnesses were 10.63 ± 4.34 (2-2), 17.99 ± 4.05 (2-3), and 10.69 ± 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 ± 6.89 compared with 13.54 ± 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 \( (\text{see Eq. 1}) \). 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 \( (\text{Table 1}) \). However, stiffness index \( (\beta) \) is based on the observed linear relation between the logarithm of relative pressure and distension ratio \( (\text{Eq. 2}) \) 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 \( (\text{Table 1}) \).

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 \( (40–59 \text{ yr}) \). 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 2-3 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 \( (\text{Table 1}) \) \( (32) \). 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 unclear \( (\text{Table 1}) \). 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 \( \text{(as in this study)} \) \( (1, 35) \). 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 Albala...
of synergistic mechanisms increasing their risk of cardiovascular events. In contrast, Lantelme et al. (17) found a significant increase in pulse-wave velocity but without any change in pressure. Thus the effect on arterial stiffness appears unresolved, and, when we adjusted for heart rate, the significance of the association between fibrillin-1 genotype and aortic stiffness remained. A high resting heart rate has been related to the development of coronary atherosclerosis and of cardiovascular events and death in several studies (8, 25, 33). In fact, the findings of both increased aortic stiffness with concomitant increase in pulse pressure and a significant increase in heart rate might indicate that individuals with the fibrillin-1 2-3 genotype are at additional high risk for cardiovascular events.

The mechanical properties of the wall of the aorta are determined mainly by elastin and collagens. The distensible elastin is load bearing at low pressures and the much stiffer collagen at high pressures, giving rise to a nonlinear pressure diameter curve, and it is clear that the collagen-to-elastin ratio is the principal determinant of wall mechanics (6). Because only a minor part of the collagen is load bearing in the physiological pressure range, however, the amount and function of elastin will probably have the largest impact on the outcome of the mechanics of the aortic wall (7, 11). Thus it is not surprising that, with a normal distribution of genotypes, no significant difference in aortic stiffness was found between COL1A1–2064 GT heterozygotes and GG homozygotes, although the trend was in the same direction as reported previously (5). This collagen polymorphism may be functional, though the trend was in the same direction as reported previously (5). This collagen polymorphism may be functional, though the trend was in the same direction as reported previously (5).

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