Effect of a COL1A1 Sp1 Binding Site Polymorphism on Arterial Pulse Wave Velocity
An Index of Compliance

David J. Brull, Liam J. Murray, Colin A. Boreham, Stuart H. Ralston, Hugh E. Montgomery, Alison M. Gallagher, Fiona E.A. McGuigan, George Davey Smith, Maurice Savage, Steve E. Humphries, Ian S. Young

Abstract—Reduced arterial compliance precedes changes in blood pressure, which may be mediated through alterations in vessel wall matrix composition. We investigated the effect of the collagen type I-α1 gene (COL1A1) +2046G>T polymorphism on arterial compliance in healthy individuals. We recruited 489 subjects (251 men and 238 women; mean age, 22.6±1.6 years). COL1A1 genotypes were determined using polymerase chain reaction and digestion by restriction enzyme Bal1. Arterial pulse wave velocities were measured in 3 segments, aortoiliac (PWVA), aorta-radial (PWVB), and aorto-dorsalis-pedis (PWVF), as an index of compliance using a noninvasive optical method. Data were available for 455 subjects. The sample was in Hardy-Weinberg equilibrium with genotype distributions and allele frequencies that were not significantly different from those reported previously. The T allele frequency was 0.22 (95% confidence interval, 0.19 to 0.24). Two hundred eighty-three (62.2%) subjects were genotype GG, 148 (35.5%) subjects were genotype GT, and 24 (5.3%) subjects were genotype TT. A comparison of GG homozygotes with GT and TT individuals demonstrated a statistically significant association with arterial compliance: PWVF 4.92±0.03 versus 5.06±0.05 m/s (ANOVA, P=0.009), PWVB 4.20±0.03 versus 4.32±0.04 m/s (ANOVA, P=0.036), and PWVA 3.07±0.03 versus 3.15±0.03 m/s (ANOVA, P=0.045). The effects of genotype were independent of age, gender, smoking, mean arterial pressure, body mass index, family history of hypertension, and activity scores. We report an association between the COL1A1 gene polymorphism and arterial compliance. Alterations in arterial collagen type 1A deposition may play a role in the regulation of arterial compliance. (Hypertension. 2001;38:444-448.)

Key Words: compliance ■ genetics ■ polymorphism ■ hypertension

It has long been noted that even modest elevations in human blood pressure are associated with substantial rises in cardiovascular risk.1,2 For this reason, there has been increasing interest in the mechanisms that might modulate rises in blood pressure within the normotensive range, and not just in the development of an extreme hypertensive phenotype. Numerous such factors, including those that influence salt and water balance3 and small vessel vascular resistance,4 may be involved. In addition, it now seems likely that the mechanical compliance of large arteries may significantly influence blood pressure through alterations in both elastic absorbance of stroke volume and pressure-wave conductance.5,6 There appears to be a bidirectional relationship between pressor burden and aortic compliance. Raised intra-aortic pressure stimulates alterations in vessel wall structure that reduce compliance. Meanwhile, reduced compliance leads to elevations in intra-aortic pressure.

Reduced arterial compliance has been observed in many individuals at high risk for the development of cardiovascular disease, including patients with familial hypercholesterolemia,7 those with borderline hypertension,8 smokers,9 and those with type 2 diabetes.10 Reduced compliance has also been shown with advancing age11,12 and in obesity.13 It is now clear that small reductions in compliance may precede changes in measured blood pressure.14 As such, reduced arterial compliance may represent an important intermediate phenotype in the development of cardiovascular disease. Studies are thus warranted of the mechanisms that regulate alterations in large vessel compliance.

Received February 8, 2001; first decision March 7, 2001; revision accepted March 13, 2001.

From the Division of Cardiovascular Genetics, Department of Medicine, Royal Free and UCL Medical School (D.J.B., H.E.M., S.E.H.), London, United Kingdom; Departments of Epidemiology and Public Health (L.J.M.), Child Health (M.S.), and Clinical Chemistry (I.S.Y.), The Queen’s University of Belfast, Belfast, United Kingdom; Department of Sport and Exercise (C.A.B.) and School of Biomedical Sciences (A.M.G.), University of Ulster, Ulster, United Kingdom; Departments of Medicine and Therapeutics, University of Aberdeen Medical School (S.H.R., F.E.A.M.), Aberdeen, United Kingdom; and Department of Social Medicine, University of Bristol (G.D.S.), Bristol, United Kingdom.

Correspondence to Dr David Brull, Division of Cardiovascular Genetics, Department of Medicine, Royal Free and UCL Medical School, The Rayne Institute, London, UK WC1E 6JU. E-mail D.Brull@ucl.ac.uk

© 2001 American Heart Association, Inc.

Hypertension is available at http://www.hypertensionaha.org
One such mechanism might be mediated through alterations in the matrix composition of the vessel wall. Type I collagen is an important constituent of extracellular matrix and is abundant in bone, connective tissues, and arterial vessel walls. Several previous studies have looked at the effect of genetic polymorphisms on arterial compliance,\textsuperscript{10,15-19} but none have examined the effect of the collagen gene. Recent work has identified a G>T (guanine for thymidine) substitution in the collagen type I-a1 gene (COL1A1) at the first base of a consensus site for the transcription factor Sp1. This has been associated with reduced bone density and osteoporotic fracture.\	extsuperscript{20} In this study, we sought to determine whether the COL1A1 Sp1 polymorphism was associated with differences in arterial compliance in a group of young healthy subjects.

**Methods**

This study was part of The Young Hearts Project, which initially examined the prevalence of coronary risk factors in a sample of adolescents in Northern Ireland. The study design and response rates of the first 2 screening phases (YH1 and YH2) are described elsewhere.\textsuperscript{21,22} Ethical approval was obtained from the Medical Ethical Committee, Queen’s University of Belfast, and written informed consent was given by all subjects.

Standing height, weight, and a mean of 2 recordings of resting blood pressure were obtained for all participants. Detailed socioeconomic and dietary information\textsuperscript{23} was obtained by questionnaire. Details regarding usual physical activity were obtained with a modified Baecke questionnaire.\textsuperscript{24} A 5-point Likert scale was used to calculate a total physical activity score.

**Fitness Testing**

All subjects underwent fitness testing, with the physical work capacity (PWCT:\textsubscript{1ml}) cycle ergometer test,\textsuperscript{26} calculated as the workload that corresponds to a heart rate of 170 beats per minute.\textsuperscript{26} Maximum oxygen consumption (V\textsubscript{O}\textsubscript{2max}) was calculated from the extrapolation of V\textsubscript{O}\textsubscript{2} at predicted maximum heart rate against PWCT\textsubscript{1ml}.

**Arterial Compliance**

Arterial pulse wave velocity (PWV) characteristics were used to determine arterial compliance with a noninvasive optical method.\textsuperscript{27} The technique was a modification of that developed by Greenwald et al\textsuperscript{28} to determine the transit time of the wave of dilatation propagating from the pressure wave generated by left ventricular contraction. PWV is calculated as the time taken to travel a known distance, timed from the ECG R wave, to the arrival of the pressure wave at a distal site, using a photoplethysmographic probe. PWV is inversely related to the square root of vessel wall compliance, so a high PWV indicates a stiffer arterial wall. This method yields results\textsuperscript{29,30} similar to those of Doppler ultrasonography,\textsuperscript{26} which provides reproducible estimates of arterial compliance.\textsuperscript{31}

PWVs were measured in 3 arterial segments: aortoiliac (PWV\textsubscript{A}), from the proximal common carotid into the femoral artery at the inguinal ligament; aorto-adial (PWV\textsubscript{B}), from the carotid into the radial artery; and aorto–dorsalis-pedis segment (PWVF), from the carotid into the posterior tibial artery posterior to the dorsalis-pedis artery.

All subjects were assessed by 1 skilled observer who was blind to genotype. Estimations of PWV based on <10 cycles or those in which the coefficient of variance of arterial transit times was >20% were rejected.

**COL1A1 Genotyping**

DNA was extracted from whole blood.\textsuperscript{32} COL1A1 2046G>T genotypes were determined using polymerase chain reaction in which a mismatched primer introduces a restriction site for the enzyme BulI in the T allele.\textsuperscript{30} Genotypes were resolved by agarose gel electrophoresis.

<table>
<thead>
<tr>
<th>Study Variable</th>
<th>Men (n=238)</th>
<th>Women (n=217)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>22.4 (0.10)</td>
<td>22.7 (0.12)</td>
<td>NS</td>
</tr>
<tr>
<td>Height, m</td>
<td>1.78 (0.004)</td>
<td>1.64 (0.004)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>75.3 (0.7)</td>
<td>64.6 (0.8)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Body mass index, kg/m\textsuperscript{2}</td>
<td>23.6 (0.20)</td>
<td>23.4 (0.26)</td>
<td>NS</td>
</tr>
<tr>
<td>Total physical activity score</td>
<td>7.96 (0.09)</td>
<td>7.41 (0.08)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>125.3 (1.0)</td>
<td>107.0 (0.8)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>74.3 (0.8)</td>
<td>70.7 (0.4)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>PWVA, m/s</td>
<td>3.26 (0.03)</td>
<td>2.92 (0.02)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>PWVB, m/s</td>
<td>4.33 (0.04)</td>
<td>4.15 (0.04)</td>
<td>0.0006</td>
</tr>
<tr>
<td>PWVF, m/s</td>
<td>5.16 (0.04)</td>
<td>4.77 (0.04)</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>V\textsubscript{O}\textsubscript{2max}</td>
<td>29.2 (0.6)</td>
<td>26.4 (0.4)</td>
<td>&lt;0.0005</td>
</tr>
</tbody>
</table>

Values are given as mean (SEM).

**Statistical Analysis**

Variation in arterial compliance between genotypes was assessed by ANOVA and by Student’s t tests for unpaired data. One-way ANCOVA was performed with age, gender, body mass index (BMI), smoking, mean arterial pressure (MAP), fitness and activity scores, V\textsubscript{O}\textsubscript{2max}, and family history of hypertension as covariates. Allele frequencies were estimated by gene counting. A \(\chi^2\) test was used to test for the presence of Hardy-Weinberg equilibrium.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

**Results**

Two hundred fifty-one men (48.7% of the male members of the cohort) and 238 women (51.3% of the original female members of the cohort) participated in the present study. Response rates were higher in non–manual-work social classes than in manual-work social classes. Of the subjects who attended the final screening (YH3), 52.7% (n=228) were from non–manual-work social classes defined at the initial screening visit (YH1) and 47.3% (n=205) were from manual work social classes (\(\chi^2=18.6, 1 df, P<0.01\)). Nonattending men, but not women, were heavier and fatter and had a higher saturated fat intake at YH1 than did attending men.

Of 489 subjects recruited into the Young Hearts Study, data for compliance and genotype were available for 455. The sample was in Hardy-Weinberg equilibrium with genotype distributions and allele frequencies not significantly different from those reported previously in whites.\textsuperscript{33,34} The \(T\) allele frequency was 0.22 (95% confidence interval, 0.19 to 0.24): 283 (62.2%) subjects were genotype GG, 148 (35.5%) subjects were genotype GT, and 24 (5.3%) subjects were genotype TT.

The physical characteristics of the study population and summary data on the PWVs are presented in Table 1. Satisfactory PWV recordings were obtained in 425 (86.9%) subjects for PWVA, 431 (88.1%) subjects for PWVB, and 428 (87.5%) subjects for PWVF. Measurements of arterial compliance were higher in women than in men in all 3 arterial segments. There were no significant differences in blood pressure among genotypes (Table 2). Genotype distributions for volunteers included within the analysis matched those for whom data were excluded.
Table 3 shows a comparison of the unadjusted and adjusted PWVs by genotype for all 3 arterial segments. Individuals homozygous for the G allele had the greatest arterial compliance (lowest PWVs), whereas TT homozygotes were the least compliant. The Figure shows the mean±SEM PWVs for all 3 arterial segments according to the presence of the T allele (GG versus GT+TT). A comparison of the compliance measures for individuals with ≥1 T allele with the wild-type GG homozygotes demonstrated a statistically significant association with genotype (Figure): PWVF 4.92±0.03 versus 5.06±0.05 m/s (ANOVA, P=0.009), PWVB 4.2±0.03 versus 4.32±0.04 m/s (ANOVA, P=0.036), and PWVA 3.07±0.03 versus 3.15±0.03 m/s (ANOVA, P=0.045). There was no loss of statistical significance after ANCOVA (P=0.001, P<0.05, and P=0.05, respectively). Mean PWVs were higher in all 3 arterial segments in the presence of ≥1 T allele (2.6% for PWVA, 2.9% for PWVB, and 2.9% for PWVF). The effects of COL1A1 genotype remained when the data were analyzed separately for men and women, with the genotype differences of the same order of magnitude in both genders.

Several of the covariates used in the ANCOVA had a statistically significant effect on arterial compliance, but only MAP and BMI significantly influenced all 3 measures of PWV. The important covariates for PWVA were gender (P<0.0005), BMI (P=0.003), MAP (P=0.06), VO2max (P<0.01), and smoking (P=0.06). For PWVB, the important covariates were MAP (P=0.0005), BMI (P=0.05), and family history of hypertension (P<0.05), and for PWVF, the important covariates were MAP (P<0.0005), gender (P<0.00005), VO2max (P<0.005), BMI (P=0.066), and Baecke work index (P<0.04).

Discussion

We report for the first time an association between the COL1A1 gene polymorphism and genotype-related changes in arterial compliance. Although there have been earlier reports that describe the effect of other common genetic polymorphisms on arterial compliance, ours is the first study to demonstrate an effect in a healthy study group. Previous work has shown the effect of polymorphisms in the angiotensin II type 1 receptor, ACE insertion/deletion, and aldosterone synthetase genes in hypertensive persons and the ACE gene in diabetic persons, who are at a much higher risk for the development of cardiovascular disease. The study by Hanon et al demonstrated an effect of the apolipoprotein E polymorphism on carotid intima-medial hypertrophy but not on arterial compliance.

In the present study, individuals with ≥1 rare T allele of the COL1A1 gene have a higher aortic PWV (and therefore lower arterial compliance) than do subjects of genotype GG. The effects of genotype are independent of age, gender, MAP, smoking, BMI, fitness and activity scores, and family history of hypertension. As such, it seems likely that alterations in arterial collagen type 1A deposition play a role in the regulation of such compliance.

The identification of mechanisms that influence arterial compliance may have clinical implications far beyond the more modest scale of the genotype effect. Reduced arterial compliance precedes the development of hypertension, with a fall in arterial elasticity of 1 SD being associated with a 15% greater risk of hypertension independent of established risk factors for hypertension. Indeed, this is reflected in our finding of no apparent effect of COL1A1 genotype on blood pressure, in contrast to the more pronounced effect on arterial compliance. Understanding the factors that affect compliance is vital, because hypertension and left ventricular hypertrophy are important complications of long-standing elevations of arterial compliance. These in turn are associated with the development and progression of atherosclerosis and increased cardiovascular risk.

There is evidence that reductions in systemic arterial compliance are detectable in patients with coronary artery disease. This observation might suggest the presence of a systemic process that affects arterial structure and function throughout the body rather than just influencing changes in peripheral or systemic compliance, as measured in studies such as the present one.

One possible mechanism could be mediated through changes in collagen deposition. The explanation for this is unclear, because extracellular matrix plays a more important role in the determination of elastic properties of central than of peripheral arteries, where vascular smooth muscle tone is a major determinant.

There is a wealth of experimental work regarding the evolution of hypertension. It has been demonstrated that functional changes in coronary artery wall elasticity in spontaneously hypertensive rats are associated with an increase in the proportion of collagen in the arterial wall. Furthermore, decreased...

### Table 2. Blood Pressure According to Genotype

<table>
<thead>
<tr>
<th>COL1A1 Genotype</th>
<th>Systolic Blood Pressure, mm Hg</th>
<th>Diastolic Blood Pressure, mm Hg</th>
<th>Mean Arterial Pressure, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>117±16</td>
<td>72±9</td>
<td>87±10</td>
</tr>
<tr>
<td>GT</td>
<td>117±16</td>
<td>73±9</td>
<td>88±10</td>
</tr>
<tr>
<td>TT</td>
<td>114±16</td>
<td>72±8</td>
<td>87±10</td>
</tr>
</tbody>
</table>

### Table 3. Adjusted and Unadjusted Pulse Wave Velocities by Genotype

<table>
<thead>
<tr>
<th>COL1A1 Genotype</th>
<th>PWVA</th>
<th>PWVB</th>
<th>PWVF</th>
<th>Adjusted PWVA</th>
<th>Adjusted PWVB</th>
<th>Adjusted PWVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>GG</td>
<td>3.07 (0.03)</td>
<td>3.03 (0.02)</td>
<td>4.20 (0.03)</td>
<td>4.21 (0.03)</td>
<td>4.92 (0.03)</td>
<td>4.91 (0.03)</td>
</tr>
<tr>
<td>GT</td>
<td>3.16 (0.03)</td>
<td>3.11 (0.03)</td>
<td>4.30 (0.05)</td>
<td>4.30 (0.04)</td>
<td>5.60 (0.05)</td>
<td>5.05 (0.04)</td>
</tr>
<tr>
<td>TT</td>
<td>3.10 (0.08)</td>
<td>3.08 (0.09)</td>
<td>4.43 (0.14)</td>
<td>4.50 (0.11)</td>
<td>5.11 (0.16)</td>
<td>5.15 (0.11)</td>
</tr>
<tr>
<td>P</td>
<td>0.11</td>
<td>0.12</td>
<td>0.06</td>
<td>0.03</td>
<td>0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>PT</td>
<td>&lt;0.05</td>
<td>0.05</td>
<td>&lt;0.04</td>
<td>&lt;0.05</td>
<td>0.009</td>
<td>0.001</td>
</tr>
</tbody>
</table>

*ANOVA by COL1A1.
†ANOVA by GG vs GT+TT.
arterial distensibility and compliance appear to precede the development of hypertension. In addition, aging is associated with a reduction in the elastin content of arterial walls, which results in aortic wall stiffening. Collagen accumulation may contribute to the increases in aortic wall stiffness, which would in turn accelerate the aging process.

Our study data support an important role for type 1A collagen in influencing large vessel compliance, although the exact mechanism of action is not known. The COL1A1 gene polymorphism occurs in a recognition site for the transcription factor Sp1. Preliminary data suggest that the polymorphism alters Sp1 binding and allele-specific transcription in GT heterozygotes. The consequences of these observations on collagen synthesis, however, have yet to be clarified in vivo. To date, there have been no other studies on the effects of the COL1A1 polymorphism on arterial compliance, although there is a body of work regarding its effect on bone metabolism. Individuals with genotype TT are at an increased risk of osteoporotic bone fracture, an effect that is in part independent of bone mineral density. There also are data to suggest a role of the T allele in increased bone turnover and in the regulation of bone mass. If the COL1A1 polymorphism is indeed associated with changes in collagen metabolism, altered collagen deposition might be the mechanism behind the effects seen on arterial compliance.

The present study does have its limitations. The response rate in YH3 was low, but agreement to participate is very unlikely to be associated with COL1A1 genotype and therefore will not have compromised the internal validity of this study. Examination of a young healthy population-based sample is a major advantage of this study; however, in common with all associative mechanistic studies of cardiovascular genetics, it might prove difficult to detect physiological influences of a gene in older and more heterogeneous subject cohorts. Such groups have already been subjected to potentially genotype-dependent mortality skews. Furthermore, multiple complex and interrelated gene/environment interactions may mask the recognition of the effect of a single gene, especially if the genetic effect is slight. Nevertheless, further studies are evidently required to confirm the association in other groups.

The apparent low PWV values obtained mainly reflect methodological differences from other studies but may also be related to population characteristics: our subjects were young and healthy. The values we report are consistent, however, with other studies that have used the same methodology, such as the Barry Caerphilly study. This was a population-based study of young adults of very similar age as our subjects that showed almost identical values for PWV measured at the groin and foot. In addition, a recent study of middle-aged Indian men and women, also using the ECG approach, again found “low” mean values (between 3.3 and 5.7 m/s).

In the method we used, data were sampled every millisecond, whereas Doppler ultrasound devices sample the data every 4 milliseconds. Therefore, it was possible to detect differences in transit times of 1 or 2 milliseconds. This is different from studies that calculate the time for the pulse wave to travel between 2 probes, with the proximal probe usually being placed at either the sternal notch or the carotid artery. The latter method excludes the time (t) from left ventricular contraction to arrival of the pressure wave at the proximal. It will always be greater than the estimate of PWV obtained from the method we used because the transit time will be shorter (−t) and the distance traveled is the same as that using the ECG approach.

In conclusion, we describe for the first time the association of the T allele of the COL1A1 gene and reduced arterial compliance in healthy young volunteers. This finding awaits confirmation in other populations, but if repeated, this finding may provide a means of further stratifying cardiovascular risk in healthy individuals.
Acknowledgments

The research is supported by British Heart Foundation grants FS 99025, RG 95007, and SP98003 and by the Wellcome Trust. We thank members of the Young Hearts Cohorts for agreeing to participate in the screening procedures and Dr Chris Martyn, University of Southampton, for providing the arterial compliance equipment, training in its use, and technical support.

References

42. Grant SFA. Studies on the Genetic Susceptibility to Osteoporosis: Analysis of cis-Acting Sequences in the Collagen Type 1A1 Gene. Adelaide, Australia: Aberdeen; 1996.
Effect of a COL1A1 Sp1 Binding Site Polymorphism on Arterial Pulse Wave Velocity: An Index of Compliance

David J. Brull, Liam J. Murray, Colin A. Boreham, Stuart H. Ralston, Hugh E. Montgomery, Alison M. Gallagher, Fiona E.A. McGuigan, George Davey Smith, Maurice Savage, Steve E. Humphries and Ian S. Young

Hypertension. 2001;38:444-448
doi: 10.1161/01.HYP.38.3.444

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2001 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/38/3/444

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2001/09/16/38.3.444.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/