Heritability and a Genome-Wide Linkage Scan for Arterial Stiffness, Wave Reflection, and Mean Arterial Pressure

The Framingham Heart Study

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Background—Arterial stiffness and mean arterial pressure variably contribute to systolic hypertension and increased cardiovascular risk. However, few prior community-based studies have evaluated the genetics of arterial stiffness and separate mean and pulsatile components of blood pressure.

Methods and Results—Using arterial tonometry, we evaluated heritability and linkage of forward and reflected wave amplitude, mean arterial pressure, and carotid-femoral pulse wave velocity (CFPWV) in 1480 participants representing 817 pedigrees in the Framingham Study offspring cohort. In 204 families with tonometry data, a genome-wide scan was performed with microsatellite markers that covered the genome at 10-cM intervals. Heritability estimates were moderate for reflected wave amplitude (h^2=0.48), forward wave amplitude (h^2=0.21), CFPWV (h^2=0.40), and mean arterial pressure (h^2=0.33). Variance components linkage analysis identified 2 regions of linkage for reflected wave amplitude: chromosome 4 at 181 cM (logarithm of odds [LOD]=4.93, permuted P=0.002) and chromosome 8 at 33 cM (LOD=3.27, permuted P=0.058). There was 1 region of linkage for forward wave amplitude on chromosome 7 at 174 cM (LOD=2.88, permuted P=0.017). There were several regions of suggestive linkage for CFPWV: chromosome 2 at 94 cM (LOD=2.46), chromosome 7 at 29 cM (LOD=2.50), chromosome 13 at 108 cm (LOD=2.10), and chromosome 15 at 108 cM (LOD=2.48). There was 1 region of suggestive linkage for mean arterial pressure on chromosome 1 at 192 cM (LOD=2.18).

Conclusions—Arterial stiffness measures and mean and pulsatile components of blood pressure are heritable and appear to have genetic determinants that may be linked to separate genetic loci in humans. **(*Circulation*. 2005;112:194-199.)**

Key Words: genetics ■ blood pressure ■ arteries ■ arteriosclerosis ■ elasticity

Pulse pressure, an indicator of arterial stiffness, has emerged as a cardiovascular disease risk factor.[1,2] However pulse pressure provides only an approximation of arterial stiffness, because it may be influenced by other factors such as heart rate, stroke volume, and myocardial contractility. Recently, carotid-femoral pulse wave velocity (CFPWV), a more direct measure of aortic stiffness, has been shown to predict adverse clinical events in hypertensive[3] and elderly[4] cohorts. These adverse prognostic implications have stimulated interest in defining potential genetic and environmental determinants of arterial stiffness. Three recent reports evaluated heritability and linkage for pulse pressure and demonstrated modest heritability and regions of suggestive linkage.[5–7] Two additional studies found moderate heritability for augmentation index, which is a measure of reflected wave amplitude that depends, in part, on arterial stiffness.[8,9] Several studies have evaluated polymorphisms in potential candidate genes for vascular phenotypes in various cohorts, with some showing evidence of association between specific genes and vascular properties,[10–13] whereas others have not.[14] To the best of our knowledge, no prior study has evaluated genetic determinants of direct measures of arterial stiffness, such as CFPWV, using an unbiased, genome-wide approach.

Several prior studies evaluated potential genetic determinants of brachial blood pressure.[15–22] However, the prior studies evaluated blood pressure in traditional (systolic and diastolic) terms and did not separately consider true mean arterial pressure and the components (forward and reflected

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wave) of pulse pressure. Mean and pulsatile components of blood pressure have separate physical determinants, so it is reasonable to suspect that different genes may influence them. Indeed, studies in an animal model of genetic hypertension have demonstrated that diastolic and pulse pressure map to distinct loci.[23] To the best of our knowledge, however, no prior study has separately evaluated heritability and linkage for the individual components of mean and pulsatile load in humans. Therefore, we assessed heritability and genome-wide linkage for arterial stiffness measures and mean arterial pressure in a community-based sample.

Methods

Study Participants

The design of the Framingham Offspring Study has been described.[24] Arterial tonometry was performed routinely in participants undergoing their seventh examination cycle (1998–2001). The Boston Medical Center Institutional Review Board approved the protocol, and each participant gave written informed consent. Of the 3539 participants who were seen during this examination cycle, 2636 had tonometry data.

Noninvasive Hemodynamic Data Acquisition

Participants were studied in the supine position after several minutes of rest as previously described in detail.[25] Arterial tonometry, with simultaneous ECG, was obtained from the brachial, radial, femoral, and carotid arteries. Transit distances were assessed by body-surface measurements from the suprasternal notch to each pulse recording site.

Tonometry Data Analysis

Tonometry waveforms were analyzed as described previously.[25] Carotid-brachial pulse wave velocity and CFPWV were calculated from tonometry waveforms and body-surface measurements.[26] Reflected wave transit time was assessed from the carotid waveform.[25] The central forward wave amplitude was defined as the difference between pressure at the waveform foot and pressure at the first systolic inflection point or peak of the carotid pressure waveform. Reflected wave pressure was defined as the difference between central systolic pressure and pressure at the forward wave peak. As reported recently, analysis reproducibility for these measures is high; correlation coefficients for key variables were as follows: CFPWV, $r=0.972$; reflected wave transit time, $r=0.968$; augmentation index, $r=0.997$; and SEP, $r=0.999$.[25]

Genotyping

DNA was extracted from whole blood or buffy coat specimens by a standard protocol. A 10-cM density genome scan was performed by the National Heart, Lung, and Blood Institute's Mammalian Genotyping Service laboratory at the Marshfield Clinic (Marshfield, Wis; marker set 8A, average heterozygosity 0.77) on individuals with DNA available from the 330 largest Framingham Heart Study families. Participants were not selected for any trait values. Details about markers and primers are available from Research Genetics (http://www.marshmed.org/genetics/default.htm). Genotype data cleaning consisted of 2 steps. Family relationships were verified on the basis of all available markers with the sib_kin program of the ASPEX (ftp://lahmed.stanford.edu/pub/aspex/ index.html) package. Mendelian inconsistencies were detected and eliminated with the GENTEST program (http://www.sfbr.org/sfbr/public/software/software.html).

Statistical Analyses

Regression analysis was used to create standardized residuals for tonometry variables, adjusted for age, age squared, height, and weight with SAS version 8.1. Residuals were computed separately for men and women. Standardized residuals with values >3.5 or less than −3.5 were set equal to 3.5 or −3.5, respectively. Heritability was estimated by fitting a polygenic model that included a term for additive genetic variance as well as environmental variance. Variance component estimates were obtained with the program SOLAR.[27] Multipoint linkage analysis was conducted with the variance component approach as implemented in the program GENEHUNTER,[28,29] which has been shown to provide more exact multipoint identity by descent estimates than the SOLAR algorithm.[30] For heritability estimation, which does not require genotype information, tonometry data were available for 1480 individuals in 817 pedigrees. For linkage analysis, tonometry data were available for 590 of 1138 individuals in 204 families with genome scan data.

A constrained permutation test, which maintains the additive genetic variance of the phenotype,[31] was used to obtain a genome-wide empirical probability value for the 3 highest logarithm of odds (LOD) scores observed in the present study. The test was implemented by simulating a normally distributed trait with the same heritability as the observed trait (either forward or reflected wave amplitude) with the SIMQTL command in SOLAR. Observations were deleted to maintain the missing phenotype pattern observed in the true sample. The simulated phenotypes were paired with the observed phenotypes on the basis of rank, and the observed phenotypes were permuted on the basis of this pairing. A genome-wide linkage analysis was performed with GENEHUNTER using the original observed genotype data and the permuted observed phenotypes. The simulation, permutation, and linkage analysis was repeated 1000 times for each trait. The genome-wide probability value for a specific LOD score was computed as the proportion of permutation-based maximum LOD scores that exceeded the observed LOD score.

TABLE 1. Characteristics of Participants

Variable	Heritability Sample (n=1480)	Linkage Sample (n=590)
Age, y	60±10	58±10
Height, cm	168±10	168±10
Weight, kg	77±16	77±16
Body mass index, kg/m^2	27.4±4.6	27.4±4.7
Brachial systolic pressure, mm Hg	124±18	122±17
Brachial diastolic pressure, mm Hg	71±11	71±11
Brachial pulse pressure, mm Hg	53±15	51±14
Heart rate, bpm	64±11	64±11
Total/HDL cholesterol, ratio	4.0±1.4	4.1±1.4
Triglycerides, mm/L	1.5±1.0	1.5±1.0
Fasting glucose, mm/L	5.7±1.4	5.6±1.3
Cardiovascular disease, %	12	10
Antihypertensive medication, %	30	26

Values are mean±SD.

Results

Clinical characteristics of the study sample are presented in Table 1, and tonometry values are presented in Table 2. Heritability estimates were moderate for CFPWV ($h^2=0.40\pm0.09$), central pulse pressure ($h^2=0.35\pm0.08$), forward wave amplitude ($h^2=0.21\pm0.09$), reflected wave transit time ($h^2=0.28\pm0.09$), reflected wave amplitude ($h^2=0.48\pm0.09$), and mean arterial pressure ($h^2=0.33\pm0.08$) but were low for carotid-brachial pulse wave velocity ($h^2=0.09\pm0.09$).

A summary of all multipoint LOD scores ≥1.5 is presented in Table 3. Full multipoint linkage results for selected chromosomes are presented in the figures. There was 1 region of linkage for forward wave amplitude on chromosome 7 at 174 cM (LOD=2.88, permuted $P=0.017$; Figure 1A). There

TABLE 2. Tonometry Variables

Variable	Heritability Sample (n=1480)	Linkage Sample (n=590)
Central systolic pressure, mm Hg	121±20	120±19
Central pulse pressure, mm Hg	51±17	49±16
Mean arterial pressure, mm Hg	92±12	92±12
Forward wave amplitude,* mm Hg	41±13	40±12
Reflected wave amplitude, mm Hg	8±8	8±7
Reflected wave transit time, ms	126±30	127±29
CFPWV, m/s	9.9±3.4	9.4±3.0
Carotid-brachial pulse wave velocity, m/s	9.0±1.8	8.9±1.8

Values are mean±SD.
*First pressure peak or inflection point minus foot pressure.

were 2 additional regions of possible linkage on chromosome 3 at 79 cM and chromosome 15 at 122 cM (Table 3). There were 2 regions of linkage for reflected wave amplitude on chromosome 4 at 181 cM (LOD=4.93, permuted $P=0.002$) and chromosome 8 at 33 cM (LOD=3.27, permuted $P=0.058$), although the chromosome 8 peak failed to achieve genome-wide significance (Figure 1B). There were additional regions of suggestive linkage on chromosome 1 at 27 cM and chromosome 9 at 22 and 164 cM (Figure 1B; Table 3). There were 4 regions of suggestive linkage for CFPWV on chromosome 2 at 94 cM, chromosome 7 at 29 cM, chromosome 13 at 108 cM, and chromosome 15 at 108 cM (Figure 2A; Table 3). There was 1 region of suggestive linkage for mean arterial pressure on chromosome 1 at 192 cM (Figure 2B; Table 3). There were no regions of suggestive or significant linkage for reflected wave transit time or carotid-brachial pulse wave velocity.

TABLE 3. Multipoint LOD Scores >1.5 for Tonometry Variables

Phenotype	Location, cM	LOD Score	Permuted *P*
Forward wave amplitude			
Chromosome 3	79	1.87	...
Chromosome 7	174	2.88	0.017
Chromosome 15	122	1.80	...
Reflected wave amplitude			
Chromosome 1	27	2.34	...
Chromosome 4	181	4.93	0.002
Chromosome 5	175	1.70	...
Chromosome 8	33	3.27	0.058
Chromosome 9	22	2.09	...
Chromosome 9	164	2.04	...
Chromosome 15	119	1.51	...
Chromosome 18	22	1.78	...
CFPWV			
Chromosome 2	94	2.46	...
Chromosome 4	29	1.55	...
Chromosome 7	29	2.50	...
Chromosome 11	19	1.62	...
Chromosome 13	108	2.10	...
Chromosome 15	108	2.48	...
Chromosome 17	88	1.82	...
Mean arterial pressure: chromosome 1	192	2.18	...

Ellipses (...) indicate that a permuted *P* was not evaluated.

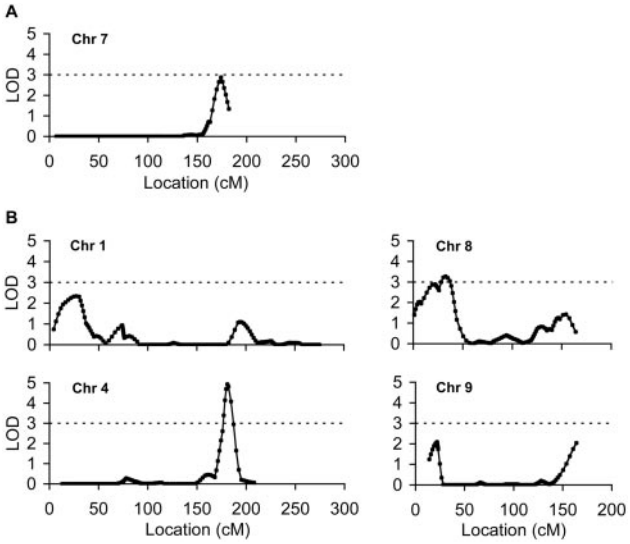

Figure 1. Multipoint linkage results for (A) forward wave amplitude and (B) reflected wave amplitude. Multipoint LOD score is on vertical axis and centimorgan distance from p-terminus of chromosome is on horizontal axis. Chr indicates chromosome.

As a secondary analysis, we evaluated linkage for central pulse pressure, which represents the aggregate effects of amplitude and timing of forward and reflected waves. We found 3 regions of suggestive linkage (multipoint LODs): on chromosome 7 at 172 cM (LOD=2.85), in the region of a forward pressure wave peak; chromosome 9 at 160 cM (LOD=2.72), near a reflected pressure wave peak; and chromosome 15 at 122 cM (LOD=2.92), adjacent to forward wave, reflected wave, and CFPWV peaks (Table 3).

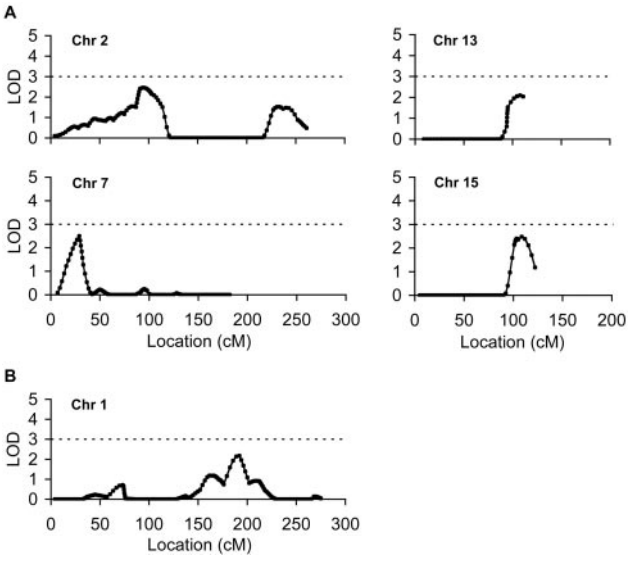

Figure 2. Multipoint linkage results for (A) CFPWV and (B) mean arterial pressure. Formatting is as per Figure 1. Chr indicates chromosome.

Discussion

We evaluated heritability and linkage for key blood pressure components and arterial stiffness measures and found that individual elements of hemodynamic load were moderately heritable and had distinct regions of significant or suggestive genetic linkage. Thus, specific components of blood pressure and arterial load may have separate genetic determinants. The finding of suggestive evidence of linkage for mean arterial pressure in a region not related to pulsatile measures suggests that the steady component of load, which depends on cardiac output and peripheral resistance, may be modulated by a different set of genes from those that are related to large-artery stiffness. Because systolic and diastolic blood pressure represent variable contributions from forward and reflected pressure wave amplitude and mean arterial pressure, evaluation of distinct components of hemodynamic load may lead to novel insights into the genetic determinants of hypertension and various other diseases related to blood pressure and vascular function, such as atherosclerosis, heart failure, and stroke.

Few prior studies have evaluated heritability and linkage for arterial stiffness and the pulsatile component of hemodynamic load. Prior analyses of brachial pulse pressure found modest heritability (h^2=0.13 to 0.26),[5,6,21] with higher estimates in 1 twin study (h^2=0.54).[22] A recent report from Framingham evaluated long-term pulse pressure and found relatively high heritability (h^2=0.52).[7] Three of the foregoing studies included genome scans.[5–7] The prior study from Framingham identified several regions of suggestive linkage for long-term pulse pressure, including 2 regions of potential overlap with the present findings. The largest linkage peak from the prior study (LOD=2.94) was on the distal long arm of chromosome 15, where the present study has concordant suggestive evidence of linkage for forward and reflected wave amplitude, CFPWV, and central pulse pressure, as detailed below. The other area of possible overlap was on chromosome 7 at 152 cM (LOD=1.55), which is just upstream of the forward wave linkage peak at 174 cM (LOD=2.88) in the present study. Genes for endothelial nitric oxide synthase (*NOS3*) and the γ-2 subunit of the AMP-activated protein kinase (*PRKAG2*) are located directly beneath this forward wave linkage peak.

We did not find any regions of overlap between our findings and the pulse-pressure linkage analyses in Mexican Americans reported by Atwood et al.[5] There were 2 regions of potential overlap between our results and the pulse-pressure linkage results of Camp et al[6] They reported an LOD of 2.89 at 54 cM on chromosome 8, which is 21 cM downstream from the reflected wave peak in the present study (LOD=3.27). There was also a prior report of suggestive linkage (LOD=2.72) for systolic blood pressure at 43 cM.[32] Thus, there are 3 studies with suggestions of linkage for various blood pressure components in this 21-cM region of chromosome 8 spanning from 33 to 54 cM. The present data suggest that this region may contain genes that modulate reflected wave properties. Positional candidates within this region include genes for lipoprotein lipase (*LPL*) and the α-1A adrenergic receptor (*ADRA1A*). Camp et al[6] also reported possible linkage on chromosome 9 for pulse pressure (LOD=1.81 at 14 cM) and systolic pressure (LOD=1.70 at 22 cM), which is adjacent to the reflected wave peak (LOD=2.09 at 22 cM) in the present study, which again suggests that genes within this region may modulate wave reflection.

We found additional regions of linkage for reflected wave amplitude on chromosome 4 at 181 cM (LOD=4.93) and suggestive linkage on chromosomes 1 and 9. The gene for vascular endothelial growth factor-C (*VEGFC*) lies directly beneath the relatively narrow linkage peak on chromosome 4. The region of suggestive linkage on chromosome 1 also contains a number of potentially important candidate genes, including those for urotensin-2 (*UTS2*), methylene tetrahydrofolate reductase (*MTHFR*), and atrial (*NPPA*) and brain (*NPPB*) natriuretic peptides. The gene for endothelin converting enzyme (*ECE1*) is located just downstream from the main peak. At the opposite end of chromosome 1, we found a region of suggestive linkage for mean arterial pressure. The selectin gene cluster and the gene for fas ligand (*TNFSF6*) are in the vicinity of this linkage peak.

We found 4 regions of suggestive linkage for CFPWV. The region on distal chromosome 15 was particularly interesting because of suggestive linkage for central pulse pressure (LOD=2.92), possible linkage for forward and reflected wave amplitude (Table 3), and prior evidence of linkage for long-term pulse pressure[7] in this region. This concordance of findings suggests that genes in this region may have an effect on the stiffness of the wall of the aorta, because characteristic impedance, which largely determines forward pressure wave amplitude,[33] and pulse wave velocity both appear to be affected. The insulin-like growth factor-1 receptor gene (*IGF1R*) is a potentially important candidate gene in this region. Additional candidates include genes for the myocyte-specific enhancer factor 2A (*MEF2A*),[34,35] chondroitin synthase (*CHSY1*),[36,37] and genes for the related proprotein convertases, *PACE4* and *FURIN*.[38–41] Potential candidates on chromosome 2 include β-adducin (*ADD2*), the neurokinin-1 receptor (*TACR1*), and the α-2B adrenergic receptor (*ADRA2B*). The linkage peak on chromosome 7 at 29 cM includes interleukin-6 (*IL6*), a proinflammatory cytokine with plasma levels that are related to pulse pressure.[42] The genes under linkage peaks for blood pressure phenotypes are the focus of additional ongoing and planned genotyping studies in the Framingham Heart Study.

Prior studies have found relations between measures of arterial stiffness and polymorphisms in various candidate genes, including genes for the angiotensin II type 1 receptor,[10] fibrillin-1,[11] angiotensin converting enzyme, α-adducin, aldosterone synthase,[13,43] endothelin A and B receptors,[12] and matrix metalloproteinases 3 and 9.[44,45] The present unbiased, genome-wide scan in an unselected, community-based sample found no evidence of linkage in the vicinity of these various candidate genes, which suggests that positive associations in prior studies may be specific to the disease populations that were studied. Alternatively, these loci may represent false-positive associations in the prior studies or false-negative associations in the present study. Additionally, it is important to note that 2 of the prior studies evaluated central aortic pressure and flow,[44,45] which provides an assessment of proximal aortic properties, whereas CFPWV provides an estimate of stiffness averaged over the full length of the aorta. These technical differences may have

contributed to the differences in findings between these studies and the present study.

We found supportive evidence for linkage in the vicinity of the lipoprotein lipase gene, which has been related to systolic and pulse pressure.[32,46] We also found regions of potential overlap with a prior study that related CFPWV to gene expression profiles using aortic samples taken at the time of cardiac surgery.[47] Genes that were overexpressed or underexpressed in that study that lie in the vicinity of CFPWV peaks identified in the present study include serum amyloid A1 (*SAA1*) on chromosome 11, aggrecan 1 (*AGC1*) on chromosome 15, and CDC42 effector protein 4 (*CDC42EP4*) on chromosome 17. Genes falling under the forward wave peak on chromosome 3 (Table 3) include the monocyte chemotactic protein-1 receptor (*CCR2*) and chondroitin sulfate proteoglycan 5 (*CSPG5*). Genes related to reflected wave peaks include microfibrillar-associated protein 2 (*MFAP2*), phospholipase A2, group IIA (*PLA2G2A*), and heat shock protein 70B′ (*HSPA6*) on chromosome 1, calcineurin A γ-subunit (*PPP3CC*) on chromosome 8, and protein tyrosine phosphatase, nonreceptor type 2 (*PTPN2*) on chromosome 18.

Several studies that evaluated linkage for standard brachial systolic and diastolic blood pressure identified regions that overlap with our findings. For example, 2 studies found linkage peaks for either systolic blood pressure[16] or diastolic blood pressure[25] near our CFPWV peak on chromosome 2 at 94 cM. Another study found suggestive evidence for linkage near the p-terminus of chromosome 1, where we have a broad linkage peak for reflected pressure wave.[18] Finally, a qualitative trait study of hypertension found an LOD of 2.8 at 192 cM on chromosome 1, which is the location of our mean pressure peak (LOD=2.18), and at 103 cM on chromosome 15 (LOD=2.4), which is the location of our concordant peaks for forward wave amplitude (LOD=1.80), CFPWV (LOD=2.48), and central pulse pressure (LOD=2.92).[19] On the basis of the results of our analysis of individual components of hemodynamic load (forward wave, reflected wave, mean arterial pressure, and CFPWV), it seems reasonable to speculate that the foregoing studies may have detected varying effects of differing components of blood pressure. These individual blood pressure components, which are affected by separate genetic loci, may combine in variable proportions within individuals or cohorts to produce the observed brachial systolic and diastolic blood pressure. Because the relative proportions of systolic and diastolic pressure that are attributable to differences in forward or reflected wave or mean arterial pressure may vary depending on the cohort studied, linkage results may also vary. An approach that evaluates the individual hemodynamic components of blood pressure circumvents this potentially unrecognized interaction between loci and may therefore increase the consistency of observations among studies.

The limitations and strengths of the present study must be acknowledged. Because the present study cohort is largely white and middle-aged to elderly, our findings may not be generalizable to other ethnicities or younger individuals. Our measures of arterial stiffness were noninvasive; estimates of forward wave amplitude were derived from the carotid pressure waveform alone owing to the unfeasibility of measuring aortic flow directly during this examination cycle. The strengths of the present study include the single-site, community-based cohort, with routinely ascertained tonometry with adherence to a rigorous quality control protocol, which thereby minimized variability in the phenotype measurement.

In summary, we have performed an analysis of heritability and a genome-wide scan for separate components of blood pressure and arterial stiffness. Measures of central arterial stiffness (forward pressure wave amplitude and CFPWV), reflected wave amplitude, and mean arterial pressure were heritable and mapped to separate locations in the genome in the vicinity of a number of credible candidate genes, which suggests that distinct genes may modulate these various components of hemodynamic load. The approach that we have used may prove useful in future studies aimed at defining the genetics of complex polygenic traits related to arterial function, such as hypertension, atherosclerosis, and heart failure.

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Disclosure

Dr Mitchell is owner of Cardiovascular Engineering, Inc, a company that designs and manufactures devices that measure vascular stiffness. The company uses these devices in clinical trials that evaluate the effects of diseases and interventions on vascular stiffness. No other author has any ownership rights or other financial relationship with Cardiovascular Engineering, Inc.

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