

Adhesion Molecule Polymorphisms and Pulse Wave Velocity in American Youth

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Background: Our research group recently reported that aorto-radial (radial) and aorto-dorsalis-pedis (foot) pulse wave velocity (PWV) as proxies of arterial stiffness are substantially heritable in healthy youth. This article aimed at uncovering the genetic contributions of adhesion molecules, key members in the inflammatory process, to PWV in these young individuals. **Methods:** Radial and foot PWV were non-invasively measured with applanation tonometry in 702 black and white subjects (42% blacks, mean age 17.7 ± 3.3 years) from the Georgia Cardiovascular Twin Study. Eight functional polymorphisms from genes for E-selectin (SELE), P-selectin (SELP), intercellular adhesion molecules-1 (ICAM1), and vascular cell adhesion molecules-1 (VCAM1) were genotyped. **Results:** Youth with Ser290Asn or Asn290Asn genotype (SELP) compared to those with Ser290Ser had an increase in both radial and foot PWV (6.61 ± 0.07 vs. 6.41 ± 0.05 m/s, $p = .026$; 7.22 ± 0.05 vs. 7.04 ± 0.04 m/s, $p = .007$). TT homozygotes of rs2244529 (SELP) had higher foot PWV (7.28 ± 0.07 vs. 7.06 ± 0.03 m/s, $p = .002$) than CT heterozygotes and CC homozygotes. There appeared to be a decrease in foot PWV in youth with the 241Arg allele (ICAM1) as compared to those without (6.96 ± 0.08 vs. 7.14 ± 0.03 m/s, $p = .005$). For the Asp693Asp (C to T) polymorphism (VCAM1), CC genotype had higher foot PWV than CT and TT genotypes (7.18 ± 0.04 vs. 6.95 ± 0.06 m/s, $p < .0001$). There was an epistatic interaction between Ser290Asn, Gly241Arg, and Asp693Asp on foot PWV ($p = .017$), explaining 3.6% variance of the foot PWV. **Conclusion:** Genetic variation of adhesion molecules may be implicated in the development of arterial stiffness. Screening for adhesion molecule polymorphisms may help identify high-risk youth.

Keywords: adhesion molecules, polymorphisms, PWV, arterial stiffness, youth

arteries (Nichols & O'Rourke, 1998). As a non-invasive measure of arterial stiffness, PWV has been demonstrated to provide prognostic information above and beyond traditional risk factors such as age, gender, elevated blood pressure (BP), high cholesterol, and smoking (Bhuiyan et al., 2006; Li et al., 2004; Mattace-Raso et al., 2006; Oren et al., 2003; Willum-Hansen et al., 2006).

Genome-wide linkage and whole-genome scan studies have shown several suggestive genetic loci or chromosomal regions linked to arterial stiffness in adult populations (Mitchell et al., 2005; Sherva et al., 2007). In fact, at least three categories of candidate genes for PWV have been investigated in adults: genes involved in BP regulation such as the sympathetic and renin-angiotensin-aldosterone systems; genes related to the structure of the arterial wall and extracellular matrix including elastin, collagens, and matrix metalloproteinases; and genes mediating cell signaling (Ge et al., 2007a; Laurent et al., 2005). However, although it is well recognized that arterial damage tracks from late childhood into adulthood with a relatively consistent progression (Berenson et al., 1992; Newman et al., 1986) little is known about the genetic determinants of arterial stiffness at a preclinical stage in healthy youth. Recently, our research group reported that individual differences in PWV are substantially heritable in American youth, i.e., 43% to 53% for aorto-radial (radial) and aorto-dorsalis-pedis (foot) PWV, respectively (Ge et al., 2007a). This finding warrants the search for candidate genes underlying arterial stiffness and PWV in these youth.

Arterial stiffness has been considered to be a low-grade inflammatory condition (Blankenberg et al., 2003; Laurent et al., 2005). Adhesion molecules are

When the heart contracts it generates a pulse or energy wave that travels through the circulation. The speed of travel of this pulse wave, referred to as pulse wave velocity (PWV), is related to the stiffness of

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transmembrane receptors typically located on the cell surface; once expressed, these molecules attract leukocytes to the endothelium. Leukocyte rolling and platelet-leukocyte interaction is mediated by selectins including E-selectin (SELE) and P-selectin (SELP), whereas firm attachment to, and subsequent migration through, the endothelium requires the expression of intracellular adhesion molecule-1 (ICAM1) and vascular cell adhesion molecule-1 (VCAM1; Blankenberg et al., 2003). Gene deficiency in knockout mice reveals that these adhesion molecules are implicated in the formation and progression of atherosclerotic lesions (Blankenberg et al., 2003). In the literature, common single nucleotide polymorphisms (SNPs) of the adhesion molecules were previously studied in relation to hypertension, coronary artery calcification, coronary artery disease, angiographically proven atherosclerosis, and myocardial infarction (Blankenberg et al., 2003). However, to the best of our knowledge, associations between adhesion molecule SNPs and arterial stiffness or PWV have not been investigated, especially in healthy youth. Therefore, this study aimed at uncovering the roles of SNPs of adhesion molecule genes (SELE, SELP, ICAM1 and VCAM1) in arterial stiffness measured by radial and foot PWV in our young twin subjects. Twins can be efficiently used to study specific candidate genes underlying complex traits such as PWV. Means and ranges of quantitative phenotypes in twins, including cardiovascular traits, have shown to be similar to age-matched individuals from the general population (Andrew et al., 2001; Kupper et al., 2005). Therefore, our large twin cohort of apparently healthy youth provided an opportunity to explore the role of genetic determinations for pre-clinical cardiovascular disease such as arterial stiffness.

Methods

Study Population

PWV data for this study was available from 702 twins (41% blacks) in the Georgia Cardiovascular Twin Study, including monozygotic (MZ) and dizygotic (DZ) pairs of same- as well as opposite-gender (Table 1). Plasma buffy coat or buccal swabs were collected for genomic DNA extraction. Zygosity determination, the criteria to classify subjects as black or white Americans, and recruitment into the Georgia Cardiovascular Twin Study have been described previously (Snieder & Treiber, 2002). All participants were apparently healthy based upon parental report of the child's medical history. Informed consent was provided by all participants and by parents if participants were < 18 years. The Institutional Review Board at the Medical College of Georgia had given approval for the study.

The smoking status was determined by self-report using a questionnaire based survey (<http://www.cdc.gov/mmwr/>). Smokers were defined as those who reported smoking any cigarettes in the past 30 days; and nonsmokers were defined as those who reported smoking 0 cigarettes/day.

Measures

Aorto–radial (radial) PWV and aorto–dorsalis–pedis (foot) PWV were measured noninvasively with applanation tonometry (Millar Instruments)(Nichols & O'Rourke, 1998) and analysis software (SphygmoCor, AtCor Medical, Sydney, Australia). Pressure waves were recorded at the common carotid and radial arteries for the radial PWV and at the common carotid and dorsalis–pedis arteries for the foot PWV. Applanation tonometry records fluctuations in the pressure wave by a high-fidelity transducer incorporated into the tip of a pencil-shaped probe. PWV was then automatically calculated from measurements of pulse transit time and the distance traveled by the pulse between the two recording sites: $PWV = \text{Distance (meters)}/\text{Transit Time (seconds)}$. Systolic BP (SBP) and diastolic BP (DBP) measurements (Dinamap 1846 SX; Critikon Incorporated, Tampa, FL) were taken at 11, 13 and 15 minutes, during a 15-minute supine relaxation period. The average was used to represent SBP and DBP.

SNP Selection and Genotyping

DNA was extracted by using the QiaAmp DNA Blood Mini Kit (Qiagen, Valencia, CA) or QuickExtract DNA Extraction Kit (Epicentre, Madison, WI). A total of eight SNPs from genes of SELE, SELP, ICAM1 and VCAM1 were selected based on prior evidence of potential functionality, validated allele frequency, and sequence proven allelic variation (Table 2). The polymorphisms were genotyped by allelic discrimination Taqman assays and genotypes were analyzed using Sequence Detection Software 1.3 (Applied Biosystems, Foster City, CA). Genotyping quality control procedures included genotyping 10% duplicates for accuracy checking; inclusion of both positive and non-template controls in each 96-well plate.

Analytical Approach

Hardy-Weinberg equilibrium and ethnic differences in allele and genotype frequencies were tested by χ^2 tests in subjects including only one of each twin pair chosen randomly to prevent inflated significance. Association analyses were performed using Generalized Estimating Equations (GEE). For related individuals, conventional statistical analyses lead to inflated significance. Dependency of the observations within pairs was accounted for by use of the GEE procedure (Tregouet et al., 1997) in which both monozygous (MZ) and dizygous (DZ) twins can be used in tests of association. The approach accounts for dependency of the observations within pairs and yields unbiased standard errors and *P* values. The effects of age, gender, and ethnicity on radial and foot PWV were first modeled. After arriving at the most parsimonious full 'environmental' model including only significant terms, SNPs or haplotypes were then added to test for main effects and interactions. Simultaneously modeling gender and ethnicity as factors in the model is more powerful than subgroup analyses, and it also allows for statistically testing gender and ethnic spe-

Table 1

General Characteristics of Study Subjects

Characteristics	Whites		Blacks		P value	Gender
	Males	Females	Males	Females		
N, subjects ^a	214	199	129	160	—	—
Age, years	18.2 ± 3.5	17.9 ± 3.2	17.5 ± 3.1	17.0 ± 3.4	< .05	.969
Height (m)	1.74 ± 0.09	1.63 ± 0.07	1.73 ± 0.10	1.62 ± 0.07	.260	< .001
BMI, kg/m ²	23.8 ± 5.6	22.6 ± 5.0	23.8 ± 5.4	25.9 ± 6.7	.001 ^b	.220
SBP, mm Hg	114 ± 10	108 ± 9	117 ± 11	111 ± 10	< .001	< .001
DBP, mm Hg	57 ± 6	59 ± 6	59 ± 7	61 ± 7	< .001	< .001
PP, mm Hg	57 ± 10	48 ± 8	58 ± 10	49 ± 8	.236	< .001
Radial PWV, m/s	6.4 ± 1.1	6.3 ± 1.0	6.7 ± 1.0	6.7 ± 1.0	< .001	0.755
Foot PWV, m/s	7.1 ± 0.9	7.0 ± 0.8	7.1 ± 0.8	7.2 ± 0.8	< .05	.716
Smokers, %	20.1	21.5	14.5	6.7	< .001	.169

Note: Values are mean ± SD. ^a Maximum number of subjects is shown for each ethnicity-by-gender group, but total number were slightly lower for radial PWV (*n* = 699) and foot PWV (*n* = 677). ^b Females only.

cific effects of SNPs and haplotypes. To reduce the numbers of tests, a 2-df overall test (co-dominant model) of genotypic association was first performed. Only in the presence of a significant association, 1-df models including additive, dominant and recessive effects were further tested to find the best mode of inheritance. The haplotype trend regression (HTR) approach was used to test for associations of statistically inferred haplotypes with PWV (Dong et al., 2004; Zaykin et al., 2002; Zhu et al., 2005). Single locus association analyses and HTR were performed with STATA 8 (StataCorp, College Station, Texas).

As previously demonstrated (Ge et al., 2007b), Multivariate Adaptive Regression Splines (MARS) was performed for modeling epistatic interaction between SNPs. One of the advantages of this approach is that MARS can search through all possible models composed of main effects and/or interactions, and

automatically arrives at a potentially optimal solution. Because MARS allows the interaction terms in the absence of the main effects (Salford-Systems, 2001) for the final step we verified the models selected by MARS and kept only significant interactions in the model after adjusting for all the main effects involved. GEE was then used, and a final predictive model for each dependent variable was eventually constructed. A naïve *P* value of .05 was called statistically significant in all the analyses.

Results

General Characteristics

Table 1 shows mean values of general characteristics and PWV for all four ethnicity-by-gender groups. The mean age of the sample was 17.7 ± 3.3 years, with black youth slightly younger than white youth. Height, and pulse pressure were similar for black and

Table 2

Allele Frequencies of the Eight SNPs in Adhesion Molecule Genes in Black and White Youth

Gene	Chromosome	SNPs	Nucleotide substitution	Whites	Blacks
				MAF(95%CI)	MAF(95%CI)
SELP	1q22-q25	Ser290Asn (rs6131)	G/A	0.22(0.02)	0.26(0.02)
		rs2244529	C/T	0.41(0.02)	0.46(0.02)
SELE	1q22-q25	Ser128Arg (rs5361)	A/C	0.05(0.01)	0.07(0.01)
		Tyr468His (rs5368)	T/C	0.10(0.01)	0.08(0.01)
		Gly241Arg (rs1799969)	G/A	0.08(0.01)	0.12(0.01)
ICAM1	19 p13.3-p13.2	Lys29Met	A/T	0.10(0.01)	0.12(0.01)
		Lys469Glu (rs5498)	A/G	0.32(0.02)	0.31(0.12)
VCAM1	1p32-p31	Asp693Asp (rs3176878)	C/T	0.19(0.02)	0.16(0.02)

Note: Based on tests in one twin of each pair, there was no statistically difference between black and white youth in the minor allele frequency (MAF) of any of the eight SNPs. SELP: P-selectin gene; SELE: E-selectin gene; ICAM1: intercellular adhesion molecule-1 gene; and VCAM1: vascular cell adhesion molecule-1 gene.

Table 3

PWV and Adhesion Molecule SNPs

Phenotype	Gene	SNP	Mode	Number of Genotype	Mean(SE)	VE (%)	P value	
Foot PWV, m/s	SELP	Ser290Asn	GG/(GA+AA)	377/232	7.04(0.04)	7.22(0.05)	0.98	.007
		rs2244529	(CC+CT)/TT	479/127	7.06(0.03)	7.28(0.07)	1.33	.002
	ICAM1	Gly241Arg	GG/(GA+AA)	510/97	7.14(0.03)	6.96 (0.08)	0.77	.005
	VCAM1	Asp693Asp	CC/(CT+TT)	425/181	7.18(0.04)	6.95(0.06)	1.57	<.0001
Radial PWV, m/s	SELP	Ser290Asn	GG/(GA+AA)	384/242	6.41(0.05)	6.61(0.07)	0.62	.026

Note: VE: explained variance; SELP: P-selectin gene; SELE: E-selectin gene; ICAM1: intercellular adhesion molecule-1 gene; VCAM1, vascular cell adhesion molecule-1 gene. Age, gender, and ethnicity were adjusted for all analyses.

white youth, while SBP, DBP and both radial and foot PWV levels were significantly higher in black than in white youth. Body mass index (BMI) was higher in black compared to white females. Males were taller and heavier, and had higher SBP and PP but lower DBP levels than females. There were more smokers in whites than blacks.

Single-Locus Analyses

All the minor allele frequencies of the eight adhesion molecule SNPs were greater than 5%, and similar between black and white youth (Table 2). Table 3 presents the single locus tests of the SNPs for radial and foot PWV, and the statistically significant findings. After adjustments of age, gender, and ethnicity, four out of the eight SNPs genotyped were significantly associated with radial and/or foot PWV. Youth with Ser290Asn or Asn290Ser genotype (SELP) compared to those with Ser290Ser had increased radial and foot PWV (6.61 ± 0.07 vs. 6.41 ± 0.05 m/s, $p = .026$; 7.22 ± 0.05 vs. 7.04 ± 0.04 m/s, $p = .007$). For rs2244529 (SELP) polymorphism, TT homozygotes compared to CT heterozygotes and CC homozygotes had higher foot PWV (7.28 ± 0.07 vs. $7.06 \pm .03$ m/s, $p = .002$). There appeared to be a decrease in foot PWV in youth with the 241Arg allele (ICAM1) as compared to those without (6.96 ± 0.08 vs. $7.14 \pm .03$ m/s, $p = .005$). For the Asp693Asp (C to T) polymorphism (VCAM1), CC genotype showed higher foot PWV than CT and TT genotypes (7.18 ± 0.04 vs. 6.95 ± 0.06 m/s, $p < .0001$). No gene-ethnicity or gene-gender interactions were detected. After adjustment of mean arterial pressure (MAP) and/or heart rate, the associations between rs2244529 (SELP) and foot PWV ($p = .032$), as well as Asp693Asp (VCAM1) and foot PWV remained significant ($p = .002$). Findings of haplotype analyses for the genes with more than one SNP were not more informative, and therefore, were not presented.

Multilocus Analyses

The MARS program was used to evaluate both main-effect and two-way interaction models. For each independent variable, one optimal model was selected by MARS. GEE was used to verify whether the interaction terms uncovered by MARS were solely

attributable to the main effects (2001) that MARS did not include in the predictive models. Under a dominant mode, there was an epistatic interaction between Ser290Asn (SELP), Gly241Arg (ICAM1) and Asp693Asp (VCAM1) on foot PWV ($p = .017$). Collectively, these three SNPs explained 3.6% of the variance of foot PWV. There were no statistically significant interactions between the SNPs and ethnicity or gender.

Discussion

Inflammation is known to stiffen arteries through various mechanisms including endothelial dysfunction by decreasing endothelial-derived nitric oxide, structural changes in the arterial wall by altering the balance between elastin breakdown and synthesis, medial calcification, and cellular infiltration around the vasa vasorum leading to vessel ischemia (Blankenberg et al., 2003; Laurent et al., 2005). The correlations between circulating levels of inflammatory factors and arterial stiffness have been reported in the literature (Blankenberg et al., 2003; Laurent et al., 2005). We hypothesized that genetic variability of adhesion molecules, a group of key components in the inflammatory process, could explain variation in arterial stiffness early in life.

This study measured radial PWV reflecting distal muscular arteries, and foot PWV representing a mixture of proximal elastic arteries, mainly the aorta, and distal muscular arteries such as femoral, sural, and dorsalis-pedis arteries. Genetic components for radial and foot PWV may overlap, or substantially differ. We found that individuals with the 290Asn allele (SELP) had an increase in both radial and foot PWV. The Ser290Asn polymorphism is located within the consensus repeat domain of the SELP protein, which seemed to be of particular importance for the binding of SELP to its ligand on leukocytes. The presence of an asparagine at position 290 was speculated to result in a protein that is more efficient to recruit leukocytes to the endothelium (Blankenberg et al., 2003; Tregouet et al., 2002). Indeed, the Ser290Asn polymorphism was previously associated with an increased risk of myocardial infarction in adults (Tregouet et al., 2002). In the Atherosclerosis Risk In Communities (ARIC) Study,

Volcik et al. recently showed that the Ser290Asn polymorphism was associated with increased risk of incident coronary heart disease (Volcik et al., 2007). The present study also identified another polymorphism of the SELP gene, rs2244529, which could confer a risk for arterial stiffness. SELP stored in α -granules of platelets and the Weibel-Palade bodies of vascular endothelial cells mediates the interaction of activated endothelial cells or platelets with leukocytes. SELP expression was significantly increased in endothelium overlying atherosclerotic plaques, and it was focally expressed in the aorta of hypercholesterolemic rabbits (Collins et al., 2000). Clearly, the SELP gene is a plausible candidate for arterial stiffness. Of interest, there appeared to be a reduction of foot PWV in youth with 241Arg (ICAM1) or 693T (VCAM1) allele, indicating the possibility that these alleles might be protective for arterial stiffness in youth. ICAM1 and VCAM1 both are cell surface glycoproteins belonging to the immunoglobulin superfamily (Blankenberg et al., 2003). ICAM1 is involved in the firm attachment of leukocytes to endothelium, and VCAM1 coordinates the inflammatory response by recruiting leukocytes and in turn, activating lymphocytes. While ICAM1 was abundantly expressed at lesion-prone areas in mice with normal cholesterol levels, VCAM1 was specifically up-regulated in arterial endothelial cells at lesion-prone areas in hypercholesterolemic mice and rabbits (Bourdillon et al., 2000; Dansky et al., 2001). Variants in genes for ICAM1 and VCAM1 were previously associated with coronary heart disease, peripheral arterial occlusive disease, and symptomatic stroke in sickle cell disease (Blankenberg et al., 2003).

Several studies demonstrated that variation of the SELE gene, mainly the Ser128Arg polymorphism, were previously associated with early severe coronary heart disease, coronary calcification, and restenosis after angioplasty (Blankenberg et al., 2003). The 128Arg allele displayed decreased binding specificity and increased affinity for additional ligands, and the range of lymphocytes recruited by SELE was extended. (Wenzel et al., 1999) These effects might provide a mechanistic link between this polymorphism and arterial stiffness. However, in this study, Ser128Arg was not associated with either radial or foot PWV, which could be partly due to the relatively low frequency of the 128Arg allele in this cohort (5% in white and 7% in black youth). Further studies are needed to explore the genetic role of SELE on early arterial stiffness measured by PWV.

The additional information in this study was that although haplotypes for each individual gene was not more informative, the multilocus models by MARS disclosed that the synergetic effects of Ser290Asn (SELP), Gly241Arg (ICAM1) and Asp693Asp (VCAM1) could explain a greater portion (3.6%) of the foot PWV than any single SNPs. This suggests that complex genetic interactions among adhesion

molecules might be more critical for the observed PWV phenotypes than the independent effects of single gene or SNP. Our data also support the notion that PWV is a polygenic phenotype.

The potential weakness of this study is that the candidate SNPs were selected based on potential functionality and documented evidence of association, and information from other variants that were not included could have been missed. We call on additional studies that will potentially replicate these findings, either through association analyses, longitudinal study design, or functional examinations, based on the demonstrated or hypothesized role of adhesion molecule pathway as a whole. Second, the use of the dorsalis-pedis (foot) site as opposed to the femoral site is more readily accepted in youth, although it has issued a concern. Foot PWV represents a mixture of both proximal elastic arteries and distal muscular arteries. Some differences from other reports that typically measured carotid-femoral PWV are thus expected. However, there was a strong correlation between foot PWV and age, and a moderate correlation between foot and radial PWV (Ge et al., 2007a). In an independent study including 102 unrelated youth, we found that foot PWV was better correlated with femoral PWV ($r = .56, p = .0002$) than radial PWV ($r = .38, p = .02$) (unpublished data). Thus, foot PWV could be considered at least another reasonable surrogate of arterial stiffness, if not interchangeable with femoral PWV. Last, we did not measure plasma levels of the adhesion molecules and other inflammatory markers, which would provide more insight of the roles of these adhesion molecules on the development of arterial stiffness.

In conclusion, the current study suggests that adhesion molecule gene variance could be informative modifiers of early arterial stiffness in youth. Screening for adhesion molecule SNPs may help identify a subset of youth at risk of cardiovascular disease.

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