Association of Soluble Cell Adhesion Molecules with Ankle-Brachial Index in a Biethnic Cohort of Predominantly Hypertensive Individuals

Mahyar Khaleghi,1 Zeenat Ali,1 Thomas H. Mosley Jr.,2 Stephen T. Turner,3 and Iftikhar J. Kullo1*

BACKGROUND: Higher plasma concentrations of soluble adhesion molecules have been shown to be associated with increased risk of cardiovascular events. We investigated the association of soluble intercellular adhesion molecule-1 (sICAM-1) and soluble vascular cell adhesion molecule-1 (sVCAM-1) with ankle-brachial index (ABI), a measure of peripheral arterial disease (PAD), in a biethnic cohort of adults without known coronary heart disease or stroke.

METHODS: Participants included 1102 blacks (mean 63 years old, 74% women) and 1013 non-Hispanic whites (mean 58 years old, 59% women) belonging to hypertensive sibships. We measured plasma concentrations of sICAM-1 and sVCAM-1 using high-sensitivity immunoassays and ABI using a standard protocol; PAD was defined as ABI <0.9. We used generalized estimating equations to assess whether sICAM-1 and sVCAM-1 were associated with ABI and PAD, independently of conventional risk factors.

RESULTS: After adjustment for conventional risk factors, blacks with sICAM-1 and sVCAM-1 concentrations in the highest quartiles had lower ABIs than those in the lowest quartiles (mean ABI 1.02 vs 0.98, P = 0.007, vs 1.02 vs 0.99, P = 0.003, respectively). In multivariable logistic regression analysis, sICAM-1 and sVCAM-1 concentrations in the highest quartiles were each associated with a higher odds ratio of having PAD, compared with the lowest quartiles: odds ratio (95% CI): 5.2 (1.8–15.2) and 2.2 (1.0–4.8), respectively. In contrast, in non-Hispanic whites, sICAM-1 and sVCAM-1 concentrations were not associated with ABI or PAD.

CONCLUSIONS: Higher sICAM-1 and sVCAM-1 concentrations were independently associated with lower ABI and PAD in blacks, but not in non-Hispanic whites. © 2008 American Association for Clinical Chemistry

Hypertension is a risk factor for endothelial dysfunction and progression of atherosclerosis. Endothelial dysfunction results in pathological activation of endothelial cells to express adhesion molecules (1) that facilitate the recruitment of circulating leukocytes to sites of inflammation (2), a crucial step in the initiation and progression of atherosclerotic lesions (3, 4). Previous studies have shown increased expression of cellular adhesion molecules in several components of atherosclerotic plaque (5, 6). Circulating concentrations of soluble adhesion molecules such as soluble intercellular adhesion molecule 1 (sICAM-1)4 and soluble vascular cell adhesion molecule 1 (sVCAM-1)4 have been associated with carotid atherosclerosis (7, 8), as well as with cardiovascular events (9, 10). Whether these adhesion molecules are related to lower-extremity atherosclerosis in hypertensive participants, especially in black adults with hypertension, is not known.

A noninvasive measure of lower-extremity atherosclerosis is the ankle-brachial index (ABI), the ratio of systolic blood pressure (SBP) in the posterior tibial artery at the ankle to the SBP in the brachial artery at the elbow. The ABI is normally >1.0, but in the setting of atherosclerotic peripheral arterial disease (PAD), ankle blood pressure is reduced and ABI falls to <1.0. An ABI of <0.9 is used to make the diagnosis of PAD in the clinical setting (11, 12). Prospective studies have shown that ABI <0.9 predicts fatal and nonfatal cardiovascular events and all-cause mortality (13–15).

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4 Nonstandard abbreviations: sICAM-1, soluble intercellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; ABI, ankle-brachial index; SBP, systolic blood pressure; PAD, peripheral arterial disease; CRP, C-reactive protein; BP, blood pressure; BMI, body mass index; SNP, single nucleotide polymorphism.
We investigated whether plasma concentrations of soluble cell adhesion molecules were associated with ABI and PAD (ABI < 0.9) in blacks and non-Hispanic whites belonging to sibships ascertained on the basis of hypertension. We sought to determine whether any detected association was independent of conventional risk factors and circulating levels of established markers of inflammation—namely, C-reactive protein (CRP) and fibrinogen.

Materials and Methods

Study participants were enrolled in the Genetic Epidemiology Network of Arteriopathy (GENOA) study, a multicenter community-based study of hypertensive sibships that aims to identify genes influencing blood pressure (BP) levels and the development of target-organ damage due to hypertension. Recruitment and subject characteristics in the initial phase of this study have been described (16). ABI and soluble adhesion molecules were measured in 2406 participants. We excluded 265 participants with history of myocardial infarction, stroke, or coronary artery bypass surgery, 10 participants with ABI > 1.5 (as they may have noncompressible arteries due to medial arterial calcification), and 16 participants with missing values for CRP or fibrinogen. The final study sample therefore included 1102 blacks and 1013 non-Hispanic whites. The study was approved by the Institutional Review Boards of the University of Mississippi Medical Center, Jackson, MS, and Mayo Clinic, Rochester, MN. Written informed consent was obtained from each participant.

Height was measured by stadiometer and weight by electronic balance, and body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Resting SBP and diastolic BP (DBP) were measured in the supine position by random zero sphygmomanometer. The diagnosis of hypertension was established based on BP levels measured at the study visit (≥140/90 mm Hg) or a prior diagnosis of hypertension and current treatment with antihypertensive medications. Diabetes was considered to be present if the subject was being treated with insulin or oral agents or had a fasting glucose concentration ≥ 7 mmol/L. “Ever smoking” was defined as having smoked more than 100 cigarettes. Information about the use of BP medications and statins was obtained from the participants at the time of the study visit.

Blood was drawn by venipuncture after an overnight fast, serum total and HDL cholesterol were measured using standard enzymatic methods (17), and the total/HDL cholesterol ratio was calculated. We measured plasma concentrations of sICAM-1 and sVCAM-1 using quantitative 2-site enzyme immunoassay (R&D Systems). Interassay CVs were as follows: sICAM-1, 14.1% and 11.4% at 284 and 645 μg/L, respectively; sVCAM-1, 11.0%, 5.3%, 9.8%, and 8.5% at 25.2, 62.6, 115.2, and 868 μg/L. We measured plasma CRP using a highly sensitive immunoturbidimetric assay. Interassay CVs were 14%, 3.2%, 3.4%, and 3.6% at 0.33, 1.05, 9.07, and 23.8 mg/L. We measured plasma concentrations of fibrinogen using the Clauss (clotting time-based) method. Interassay CVs were 3.5% and 2.5% at 1.1 and 2.9 g/L.

ANKLE-BRACHIAL INDEX

At each center, ABI was measured by examiners who had undergone training in Mayo Clinic’s noninvasive vascular laboratory in Rochester, MN. An identical, standardized protocol was used at both centers. Following a 5-min rest, participants were evaluated in the supine position. Appropriately-sized BP cuffs were placed on each arm and ankle, and a Doppler ultrasonic instrument (Medisonics) was used to detect arterial signals. The cuff was inflated to 10 mmHg above SBP and deflated at 2 mmHg/s. The first reappearance of the arterial signal was taken as the SBP. To calculate the ABI, the SBP at each ankle site (posterior tibial and dorsalis pedis arteries) was divided by the higher of the 2 brachial pressures. The average of the ABIs at each ankle was calculated, and the lower of the average ABIs from the 2 legs was used in analyses. An ABI < 0.9 was used to indicate the presence of PAD.

SINGLE NUCLEOTIDE POLYMORPHISM GENOTYPING

After we had completed the assays for sICAM-1 and sVCAM-1, Register et al. (18) reported that a nonsynonymous single nucleotide polymorphism (SNP) (rs5491 A/T) in ICAM1 may result in epitope change and poor recognition by BBE1B antibody used in the commonly used immunoassay for sICAM-1 (R&D Systems). We therefore genotyped this SNP in the study participants to evaluate its association with plasma concentration of sICAM-1. We isolated DNA using the PureGene DNA Isolation Kit from Gentra Systems. Genotyping, based on PCR amplification techniques, was conducted at the University of Texas Health Sciences Center at Houston using the TaqMan assay and ABI Prism Sequence Detection System (Applied Biosystems). Primers and probes are available from the authors on request. Quality control measures for genotyping assays included robotic liquid handling; separate pre- and post-PCR areas; and standard protocols and quality control analyses including 5% dupli-

Human gene: ICAM1, intercellular adhesion molecule 1.
cates, positive and negative controls, computerized sample tracking, and data validity checks.

STATISTICAL METHODS

Continuous data are summarized as either mean (SD) or median (interquartile range). Between-group differences were assessed by an unpaired 2-tailed Student $t$ test or Wilcoxon rank sum test. Categorical data were expressed as percentages, and between-group differences were assessed by $\chi^2$ test. We estimated allele frequencies at SNP rs5491 in ICAM1 by gene counting. In each ethnic group, to evaluate the association of sICAM-1 and sVCAM-1 with ABI and PAD (ABI $\leq 0.9$), we divided the participants according to quartiles of sICAM-1 and sVCAM-1, with participants in the lowest quartiles of ICAM-1 and VCAM-1 considered the referent group. We constructed multivariable linear and logistic regression models that adjusted for conventional risk factors and other potential confounding variables to assess whether sICAM-1 or sVCAM-1 was independently associated with ABI. Adjustments were performed for (a) age and sex; (b) a plus BMI, BMI$^2$, statin use, smoking history, diabetes, hypertension, and total/HDL cholesterol ratio; and (c) b plus plasma CRP and fibrinogen concentrations. In each model, the mean ABI for each quartile of both adhesion molecules was estimated using least squares means. We also checked for interactions between conventional risk factors and adhesion molecules in the prediction of ABI. Because the study participants belonged to sibships, regression analyses were performed using generalized estimating equations (GEEs). A 2-sided $P$-value of $<0.05$ was deemed statistically significant. Statistical analyses were carried out using SAS v. 8.2 (SAS Institute) software package.

Results

There was a greater proportion of women in both cohorts (Table 1), and blacks were older than non-Hispanic white participants. Blacks also had a higher prevalence of diabetes than their non-Hispanic white counterparts; use of statins was less frequent in black men and women than their non-Hispanic white counterparts, and 78% of black and 70% of non-Hispanic white participants were hypertensive.

<table>
<thead>
<tr>
<th></th>
<th>Blacks</th>
<th>Non-Hispanic whites</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>1102</td>
<td>1013</td>
<td></td>
</tr>
<tr>
<td>Mean age, years (SD)</td>
<td>63.3 (9.2)</td>
<td>57.6 (10.0)</td>
<td>$&lt;0.001$</td>
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<tr>
<td>Men, n (%)</td>
<td>302 (27.4)</td>
<td>412 (40.7)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Mean BMI, kg/m$^2$ (SD)</td>
<td>31.5 (6.5)</td>
<td>30.9 (6.4)</td>
<td>0.033</td>
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<tr>
<td>Mean total cholesterol, mmol/L (SD)</td>
<td>5.2 (1.0)</td>
<td>5.1 (0.9)</td>
<td>0.065</td>
</tr>
<tr>
<td>Mean HDL cholesterol, mmol/L (SD)</td>
<td>1.5 (0.5)</td>
<td>1.3 (0.4)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Smoking, n (%)</td>
<td>421 (38.2)</td>
<td>474 (46.8)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Diabetes, n (%)</td>
<td>305 (27.7)</td>
<td>134 (13.2)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>858 (77.9)</td>
<td>705 (69.6)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Statin use, n (%)</td>
<td>184 (16.7)</td>
<td>237 (23.4)</td>
<td>$&lt;0.001$</td>
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<td>Median C-reactive protein, mg/L (interquartile range)</td>
<td>3.4 (1.6–7.2)</td>
<td>2.5 (1.2–5.0)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Mean fibrinogen, g/L (SD)</td>
<td>3.67 (0.84)</td>
<td>3.16 (0.76)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Median sICAM-1, $\mu$g/L (interquartile range)$^a$</td>
<td>279 (235–320)</td>
<td>281 (238–336)</td>
<td>0.21</td>
</tr>
<tr>
<td>Median sVCAM-1, $\mu$g/L (interquartile range)</td>
<td>566 (465–699)</td>
<td>671 (548–800)</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>Mean ankle-brachial index (SD)</td>
<td>1.00 (0.12)</td>
<td>1.15 (0.13)</td>
<td>$&lt;0.001$</td>
</tr>
</tbody>
</table>

$^a$n = 565 in blacks; n = 1003 in non-Hispanic whites [due to exclusion of participants with A/T and T/T genotypes at SNP rs5491 (A/T) in ICAM1].
The prevalence of PAD was 10.0% and 3.4% in blacks and non-Hispanic whites, respectively. Black participants with PAD had higher median concentrations of sICAM-1 (309 vs 275 μg/L, P < 0.001) and sVCAM-1 (631 vs 559 μg/L, P < 0.001) than those without PAD. Blacks in the highest quartiles for plasma concentrations of sICAM-1 and sVCAM-1 had a significantly higher prevalence of PAD than those in the lowest quartiles (15.8% vs 3.5%, P < 0.001, and 13.9% vs 3.6%, P < 0.001) (Fig. 2A).

In separate age- and sex-adjusted linear regression models, blacks in the highest quartiles for sICAM-1 and sVCAM-1 concentrations had significantly lower ABIs compared to those in the lowest quartiles (mean ABI, 1.023 vs 0.978, P = 0.001, and 1.020 vs 0.986, P = 0.001, respectively). After additional adjustment for conventional risk factors, being in the highest quartile of sICAM-1 or sVCAM-1 was significantly associated with lower ABI, compared to being in the lowest quartile of each marker (mean ABI, 1.019 vs 0.982, P = 0.007, and 1.018 vs 0.989, P = 0.003). These associations remained significant after additional adjustment for plasma concentrations of CRP or fibrinogen (Table 2). As shown in Fig. 2B, the association between plasma concentrations of sVCAM-1 and ABI was not linear throughout the range of sVCAM-1 concentrations. Instead, there appeared to be a threshold effect, and blacks with plasma concentrations of sVCAM-1 above the 25th percentile had significantly lower ABIs than those in the first quartile. We noted a significant interaction (P < 0.001) between sICAM-1 and sex in the prediction of ABI, indicating that the association was present in men only (analysis not shown). Sex-stratified analyses confirmed this interaction and showed that higher plasma concentrations of sICAM-1 were significantly associated with lower ABI in men but not in women.
In multivariable logistic regression models, sICAM-1 and sVCAM-1 concentrations were also associated with the presence of PAD in blacks (Table 3). In age- and sex-adjusted models, the odds ratios (95% CIs) for PAD for being in the highest quartiles of sICAM-1 or sVCAM-1 compared to those in the lowest quartiles were 5.6 (2.0–16.1) and 2.5 (1.2–5.1), respectively. These associations remained significant even after further adjustment for BMI, BMI², statin use, history of smoking, diabetes, hypertension, and total/HDL cholesterol ratio: 5.2 (1.8–15.2) and 2.2 (1.0–4.8) for sICAM-1 and sVCAM-1. After additional adjustment for plasma concentrations of CRP or fibrinogen, the association between sICAM-1 and the presence of PAD remained significant [odds ratio (95% CI) 4.9 (1.6–15.3)], whereas sVCAM-1 was marginally associated with the presence of PAD [2.0 (0.9–4.4)].

In contrast, in non-Hispanic whites, concentrations of sICAM-1 and sVCAM-1 were not significantly different between participants with PAD and participants with ABI >0.90 (sICAM-1, 285 vs 281 g/L, P = 0.52; sVCAM-1, 721 vs 669 g/L, P = 0.52). Furthermore, multivariable linear and logistic regression analysis showed...
yses showed no significant association of sICAM-1 or sVCAM-1 with ABI or presence of PAD (analyses not shown).

Discussion

In a biethnic, predominantly hypertensive cohort, we found that higher plasma concentrations of sICAM-1 and sVCAM-1 were associated with lower ABI and presence of PAD among blacks without known coronary heart disease or stroke. This association was independent of conventional risk factors as well as 2 established markers of inflammation: CRP and fibrinogen. In contrast, plasma concentrations of sICAM-1 and sVCAM-1 were not related to ABI or PAD in non-Hispanic whites. These findings indicate a cross-sectional association between soluble cell adhesion molecules and systemic atherosclerotic burden in blacks.

The association of higher sICAM-1 and sVCAM-1 concentrations with lower ABI and the presence of PAD in blacks was independent of conventional risk factors as well as 2 established markers of inflammation: CRP and fibrinogen. In contrast, plasma concentrations of sICAM-1 and sVCAM-1 were not related to ABI or PAD in non-Hispanic whites. These findings indicate a cross-sectional association between soluble cell adhesion molecules and systemic atherosclerotic burden in blacks.

The association of higher sICAM-1 and sVCAM-1 concentrations with lower ABI and the presence of PAD among blacks was independent of the inflammatory markers CRP and fibrinogen, suggesting a key role of adhesion molecules in the arterial immune response. Atherosclerosis is a chronic inflammatory process (4), and migration of leukocytes across vascular endothelium is mediated by interaction of cell adhesion molecules on endothelial cells with integrin receptors on leukocytes (19). Several inflammatory cytokines induce cell adhesion molecules on endothelial cells (20). In addition, conventional risk factors that impair endothelial function, such as hypertension and diabetes, can increase the expression of adhesion molecules on the luminal surface of endothelial cells. In contrast to VCAM-1, which is expressed exclusively in the vessel wall, ICAM-1 is expressed additionally by fibroblasts and hematopoietic cells (21), and soluble forms of cell-adhesion molecules are detectable in plasma owing to shedding from the endothelial cells (22). It has been shown that sICAM-1 concentrations are correlated with the surface expression of ICAM-1 in endothelial cells in culture (23), and a correlation has been reported between plasma concentrations of sVCAM-1 and VCAM-1 mRNA in human atherosclerotic aorta (24).

Several, but not all, studies have found soluble adhesion molecules to be markers of cardiovascular risk (25). Higher plasma concentrations of sICAM-1 have been associated with an increased risk of myocardial infarction (9) and coronary heart disease (26), extent of carotid atherosclerosis (8), and PAD (27). Higher plasma concentrations of sVCAM-1 have also been associated with greater risk of cardiovascular events (28), increased carotid intima-media thickness (8), and the extent of angiographic disease in patients with known PAD (29). Our results add to previous reports, as black participants with higher sVCAM-1 concentrations had significantly lower ABI and higher prevalence of PAD. In the Edinburgh Artery Study sample (n = 1592, almost entirely white participants), there was no association between sICAM-1, sVCAM-1, and baseline ABI after adjustment for cardiovascular risk factors (30). However, sICAM-1 concentrations (not sVCAM-1 concentrations) were significantly associated with change in ABI over a 12-year follow-up. In a prospective, nested case-control study conducted among 14,916 middle-aged men, median concentrations of sICAM-1, but not sVCAM-1, were significantly higher at baseline among men (n = 140) who developed PAD than among those who did not (140 control participants selected at random from the remaining study participants and matched with cases on the basis of age, smoking status, and year of follow-up) (sICAM-1, 285 vs 268 µg/L, P = 0.005; sVCAM-1, 701 vs 709 µg/L, P = 0.80) (27). This association remained significant after additional adjustment for lipid and nonlipid risk factors, including CRP.

We observed a significant interaction between sICAM-1 and sex in the prediction of ABI in blacks, indicating the association was significant in men only. Although there are no previous data regarding the effect of sex on the association between soluble cell adhesion molecules and atherosclerotic burden, the results from 2 large cohorts are suggestive of a sex-specific effect. In the Women’s Health Study (n = 23984), Albert et al. (31) found no association between baseline plasma concentrations of sICAM-1 and myocardial infarction, stroke, or cardiovascular death over a 10-year follow-up. In contrast, in the Physician Health Study, of initially healthy male physicians, sICAM-1 was associated with risk of myocardial infarction, particularly among those physicians with sICAM-1 concentrations in the highest quartile.16

Register et al. (18) noted that a nonsynonymous SNP in ICAM1 (rs5491 A/T) results in epitope change and poor recognition by BBE1B antibody employed in a commonly used ELISA for sICAM-1 (R&D Systems), and this could be corrected using an alternative commercial human sICAM-1 antibody (BMS201INST; Bender MedSystems). There are 2 alleles of rs5491 (A/T): the codon AAG encodes lysine (K), and ATG encodes methionine (M). This amino acid occupies residue 56 in the ICAM1 reference protein sequence (NP_000192) in the precursor peptide, which contains a 27–amino acid leader sequence. Our results confirm this finding in a large cohort of blacks. The frequency of T allele was 23.9% in blacks and 0.1% in non-Hispanic whites, similar to the prevalence noted by Register et al. (18) and in the Women’s Health Study. Therefore, at least in blacks, an immunoassay using the BBE1B antibody may not be reliable, and an alternative antibody
should be used for measurement of sICAM-1 concentrations.

Previous studies have also shown that blacks have lower concentrations of sICAM-1 than whites (7, 32, 33). We believe that this may be largely due to higher prevalence of the above polymorphism in blacks, leading to lower detectability of sICAM-1 concentration in immunoassays using the BBE1B antibody. In our analyses, before excluding black participants with A/T (n = 448) and T/T (n = 57) genotypes in blacks, the median plasma concentrations of sICAM-1 were significantly lower in blacks than in non-Hispanic whites (231 vs 289 μg/L, P < 0.001), but after exclusion of these participants, the groups had similar plasma concentrations of sICAM-1 (Table 1).

Our results are consistent with previous studies in which plasma concentrations of sVCAM-1 were significantly higher in whites than in blacks (7, 33). Overall, however, there was no association between soluble cell adhesion molecules and ABI in non-Hispanic whites. It is not clear what accounts for ethnicity-specific associations found between soluble adhesion molecules and ABI. These 2 ethnic groups may vary substantially in the extent of prevalent atherosclerotic vascular disease. Ethnic differences in ABI and PAD have been previously described by us and others (34). The mean ABI was >1 SD higher in non-Hispanic whites compared to blacks, and the prevalence of PAD was nearly a third of that in blacks. Therefore, our null findings for sICAM-1 and sVCAM-1 in non-Hispanic whites do not rule out a pathophysiological role of soluble adhesion molecules in this ethnic group. Further research at the molecular and the population level is essential to clarify the role of cell adhesion molecules as markers of atherosclerotic vascular disease.

A strength of the present study is the inclusion of a large biethnic cohort of individuals—most of them hypertensive—from the general population. In addition, plasma concentrations of sICAM-1 and sVCAM-1 were measured using the same assay in 1 central laboratory. Although the study was population-based, the majority of the study participants were hypertensive, and therefore the findings may not be generalizable to the entire hypertensive blacks (n = 858), the results were similar (analyses not shown). The study is cross-sectional, hence causality is difficult to infer. Confounding by unknown potentially causal factors cannot be ruled out. Finally, nearly a fifth of the study participants were on statins, and these medications could differentially affect levels of inflammatory markers (35). However, the correlation between systemic markers of inflammation (CRP and fibrinogen) and cell adhesion molecules did not differ in strata defined by use or nonuse of statins (analyses not shown).

**Author Contributions:** Each author confirmed he or she has contributed to the intellectual content of this paper and has met the following three requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data, (b) drafting or revising the article for intellectual content, and (c) final approval of the published article.

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**Expert Testimony:** Not Applicable

**Role of Sponsor:** The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, preparation or approval of manuscript.

**References**

7. Huang SJ, Ballantyne CM, Sharrett AR, Smith LC, Davis CE, Gotto AM Jr, Boerwinkle E. Circulating adhesion molecules VCAM-1, ICAM-1, and E-