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ORIGINAL ARTICLE

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Gender- and age-dependent relationships between the E-selectin S128R polymorphism and coronary artery calcification

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Abstract Development and progression of atherosclerosis involves recruitment and binding of circulating leukocytes to areas of inflammation within the vascular endothelium mediated by a diverse array of cellular adhesion molecules. A polymorphism in the endothelial-leukocyte adhesion molecule 1 (E-selectin) gene has been implicated in early-onset, angiographically defined, severe atherosclerotic disease because it profoundly affects ligand recognition and binding specificity, resulting in a significant increase in cellular adhesion. Relationships between the E-selectin S128R polymorphism and coronary artery calcification (CAC), a marker of atherosclerosis detected with noninvasive electron beam computed tomography, were examined in 294 asymptomatic women aged 40–88 years and 314 asymptomatic men aged 30–80 years from the Epidemiology of Coronary Artery Calcification Study. The E-selectin polymorphism was not associated with presence of CAC in men of any age or in women over age 50. In women 50 years of age or younger the E-selectin polymorphism was significantly associated with presence of CAC after adjustment for age, body mass index, systolic blood pressure, ratio of total cholesterol to high-density lipoprotein cholesterol, and smoking. The significant association between E-selectin and CAC in women 50 years of age or younger may suggest that the 128R allele is a risk factor for coronary atherosclerosis in younger asymptomatic women, who typically have lower levels of traditional risk factors and reduced adhesion molecule expression due to the presence of higher levels of endogenous hormones.

Keywords Atherosclerosis (coronary) · Calcium · Cellular adhesion molecules · Computed tomography · Genetics · Heart disease

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Abbreviations *BMI:* Body mass index · *CAC:* Coronary artery calcification · *CAD:* Coronary artery disease · *EBCT:* Electron beam computed tomography · *E-selectin:* Endothelial-leukocyte adhesion molecule 1 · *HDL:* High-density lipoprotein

Introduction

Coronary artery disease (CAD) attributable to atherosclerosis is a leading cause of death for both men and women in the United States and other Westernized countries [1, 2]. Biochemical and cellular interactions that define the atherosclerotic process are believed to reflect bodily responses to injury or assault on the vascular endothelium [3]. A key component in the development and progression of atherosclerosis involves recruitment and binding of circulating leukocytes (primarily monocytes) to the vascular endothelium mediated by a diverse array of cellular adhesion molecules [4, 5].

The selectins comprise a family of carbohydrate-binding proteins (lectins) that participate in the accumulation of leukocytes at areas of arteriole damage or inflammation. Selectin molecules facilitate adhesive interactions between leukocytes and endothelial cells that cause circulating leukocytes to slow and roll along the endothelium. Leukocyte rolling is believed to be the initial phase in a cascade of events that leads to firm adhesion to the endothelium followed by migration into the subendothelial space. Monocytes may then differentiate into macrophages that ingest oxidized lipids and become visible as foam cells comprising the fatty streaks characteristic of early atherosclerotic lesions [6, 7].

Selectin glycoproteins contain an amino-terminal lectin-like domain and an epidermal growth factor-like domain that enable the molecules to bind specific carbohydrate ligands [8] and are important in mediating cellular adhesion [9]. Endothelial-leukocyte adhesion molecule 1 (E-selectin) is expressed on endothelial cells at sites of inflammation and plays a crucial role in the process of monocyte trafficking [10, 11].

A polymorphism within the epidermal growth factor-like domain of the human E-selectin gene results in the substitution of arginine (R) for serine (S) at position 128 (S128R) of the mature protein [12]. The S128R polymorphism has a profound effect on ligand recognition and binding. The 128R allele, which exhibits decreased binding specificity and increased affinity for additional ligands [13], has been associated with early-onset, angiographically defined, severe atherosclerotic disease in persons younger than 50 years of age [14, 15, 16, 17].

In the present study we examined relationships between the E-selectin S128R polymorphism and coronary artery calcification (CAC), a reliable marker of atherosclerosis, in 294 asymptomatic women aged 40–88 years and 314 asymptomatic men aged 30–80 years from the Epidemiology of Coronary Artery Calcification Study. The study participants were not referred nor considered at high risk for CAD. Asymptomatic coronary atherosclerosis was detected with noninvasive electron beam computed tomography (EBCT) that assessed presence of CAC. CAC is hypothesized to be an organized and regulated attempt to strengthen atherosclerotic plaques that are prone to rupture [18]. EBCT is an accurate method for detecting CAC, which is a predictor of future clinical events [19]. After adjustment for verification bias, the sensitivity and specificity of any CAC (detected by EBCT) to detect at least 50% angiographic stenosis is 97% and 72%, respectively [20].

CAD risk factor profiles and the frequency of coronary events differ by gender [21]. In addition, estrogens have been shown to modulate the expression of cellular adhesion molecules (including E-selectin) from the endothelium [22], which may lead to differences in phenotypic effects of the S128R polymorphism between men and women. Since the association between the S128R polymorphism and angiographically defined, severe atherosclerosis has been reported only in persons 50 years of age or younger [15, 16], we investigated associations separately in men and women and stratified participants within each gender into two groups, those over the age of 50 years and those 50 years of age or younger. Our objectives were to: (a) investigate gender- and age-specific associations between the E-selectin S128R polymorphism and presence of CAC as a marker of asymptomatic atherosclerosis in a community-based study, and (b) evaluate the ability of the S128R polymorphism to predict presence of CAC beyond that afforded by established risk factors for CAD.

Material and methods

Sample

Participants in the Epidemiology of Coronary Artery Calcification Study were recruited from the Rochester Family Heart Study, a community-based cross-sectional investigation of the genetic epidemiology of essential hypertension and coronary disease in Rochester, Minn., USA [23, 24]. Individuals at least 20 years of age (n=1173) were recruited independently of disease or risk factor status, but some were ineligible to participate (n=44) due to pregnancy, lactation, or history of heart surgery [25]. The participation rate among those eligible was 91% (1024/1129). To assess relationships between E-selectin and CAC, 96 men under the age of 30 years and 155 women under the age of 40 years were excluded due to the low prevalence of CAC in these age groups [26]. Additional exclusions resulting from the unavailability of DNA or missing genotype data for the E-selectin polymorphism (n=160), invalid measures of CAC (n=3), or missing risk factor information (n=2) produced a final study group of 608 participants (294 white women aged 40–88 years and 314 white men aged 30–80 years).

Coronary artery disease risk factors

Information on selected risk factors for CAD including age, gender, measures of body size, arterial blood pressure, lipid metabolism, and cigarette smoking [25] was obtained during a clinical examination with appropriate informed consent following protocols approved by the Mayo Clinic Institutional Review Board.

Body mass index (BMI; kg/m^2) was calculated from height and weight measurements on participants wearing light clothing. Systolic blood pressure values were measured in triplicate at least 2 min apart from the right arm of seated subjects with a random-zero sphygmomanometer and then averaged. History of diabetes mellitus, myocardial infarction, stroke, and hypertension was extracted from medical records. Plasma total cholesterol was measured by standard enzymatic methods [27], and high-density lipoprotein (HDL) cholesterol was determined after precipitation of lipoproteins containing apolipoprotein B [28]. The ratio of total cholesterol/HDL cholesterol was calculated as a summary measure of lipid metabolism. Cigarette consumption was quantified from participant reports as the average number of cigarette packs smoked per day multiplied by the number of years of smoking. The resulting variable (cigarette pack years+1) was log-transformed to reduce skewness. Menopausal status in women was based on whether a woman had a menstrual period within the past 12 months not including periods brought on by use of hormones after menopause.

Electron beam computed tomography

Presence of CAC was detected with an Imatron C-100 or C-150 EBCT scanner (Imatron, South San Francisco, Calif., USA). A scan run consisted of 40 contiguous 3-mm-thick tomograms commencing at the root of the aorta and proceeding caudally through the entire coronary arterial tree (12 cm). All images were acquired in late diastole using electrocardiographic gating. Exposure time was 100 ms per tomogram. Radiation exposure was 10 mGy (1 rad) per scan.

Tomograms were first scored by a radiological technologist using an automated scoring system [29]. Calcification was defined as a hyperattenuating focus four or more adjacent pixels in size (1.04 mm^2 under a field of view of 26 cm; 1.38 mm^2 under a field of view of 30 cm) with a computed tomography number above 130 HU within 5 mm of the arterial midline. We chose to use the four-pixel definition of calcification because hyperattenuating foci of this size are more repeatable and less likely to be noise than smaller foci [30]. An experienced radiologist then interpreted the findings of each examination after inspecting the technical quality and scoring accuracy of each tomogram.

The E-selectin S128R polymorphism

The E-selectin S128R polymorphism was genotyped by subjecting 20-ng aliquots of genomic DNA to PCR amplification using the oligonucleotide primers 5′-AGTAATAGTCCTCCTCATCATG-3′ and 5′-ACCATCTCAAGTGAAGAAAGAG-3′ [12]. A *Pst*I restriction fragment length polymorphism within the 186-bp amplification product was identified from published sequence [14]. Fragments resulting from restriction enzyme digestion were subsequently resolved on ethidium bromide stained 10% vertical acrylamide gels. Samples initially scored as heterozygotes (SR genotypes) were subjected to successive enzyme digestions and re-scored to minimize the possibility of mistypings due to incomplete digestions. Genotypes were scored independently by trained laboratory personnel and any discrepant typings were adjudicated by a third party. All laboratory personnel were blind to the CAC and risk factor status of individual samples.

Statistical analysis

The distribution of E-selectin genotypes in a subsample of 500 unrelated participants was tested for conformance to Hardy-Weinberg equilibrium by a χ^2 goodness-of-fit test. Contingency χ^2 tables and Fisher's exact test were used to compare allele and genotype frequencies between all of the men and all of the women in this study and to compare frequencies between present study participants aged 50 years or younger and those observed in a study group aged similarly examined by Wenzel et al. [16]. Other statistical analyses were performed separately for men and women. Since a previous study found an association between the E-selectin polymorphism and severe angiographic CAD among individuals 50 years of age or younger, participants within each gender were stratified by age into two groups, those over the age of 50 years and those 50 years of age or younger. Risk factor variables were compared between individuals with the E-selectin SS genotype and those carrying at least one copy of the 128R allele (SR or RR genotypes) using a χ^2 statistic (categorical variables) or *t* test (continuous variables).

Within the four gender and age strata, logistic regression models were used to relate the selected CAD risk factors and the E-selectin polymorphism to presence of CAC. Maximum likelihood estimates were generated from models containing information on age, systolic blood pressure, total cholesterol/HDL cholesterol, BMI, ln(pack years+1), and the E-selectin polymorphism (full model) as well as models containing all covariates except one (reduced model). The maximum likelihood estimates for the full and the six reduced models were used in likelihood ratio tests to assess whether information on each risk factor and the E-selectin polymorphism improved the ability to predict presence of CAC when contributions of the other predictors were considered. The fit of the final models was assessed with the Hosmer-Lemeshow goodness-of-fit statistic, and the area under the receiver operator curves was calculated.

Parameter estimates from the full models were used to calculate the probability (95% confidence interval) of having detectable CAC based on the full logistic regression model for individuals with mean age, BMI, systolic blood pressure, total cholesterol/HDL cholesterol and ln(pack years+1) and the SS versus the SR or RR E-selectin genotypes in each gender and age group.

The likelihood ratio test was also used to assess the significance of interaction terms between the E-selectin polymorphism and each of the risk factors in each group by comparing a full logistic regression model with all of the risk factors, polymorphism, and the interaction term to a reduced model with only the risk factors and polymorphism. All statistical analyses considered a *P* value less than 0.05 statistically significant.

Results

E-selectin allele and genotype frequencies

The observed allele and genotype frequency distributions in Epidemiology of Coronary Artery Calcification participants are presented in Table 1. Neither allele nor genotype proportions differed significantly between the genders (χ^2=1.09, *P*=0.296 for allele proportions; *P*=0.479, Fisher's exact test, for genotype proportions). The frequencies for men and women under the age of 50 years (*n*=286) combined were not significantly different from frequencies reported for a study group of 216 white Germans examined by Wenzel et al. [16] (*P*=0.250, Fisher's exact test). Genotype frequencies in the subsample of 500 unrelated men and women with 340 SS, 156 SR, and 4 RR genotypes did not conform to Hardy-Weinberg expectations (χ^2=9.48, *P*=0.002), primarily due to observing fewer RR homozygotes than expected (observed=4; expected=13).

Coronary artery disease risk factors

There were no significant differences in risk factor variables for CAD or measures of CAC between those par-

Table 1 E-selectin genotype and allele frequencies in younger (≤50 years) and older (>50 years) men and women from the Epidemiology of Coronary Artery Calcification Study

			Men				Women			
	Total (*n*=608)		Older (*n*=151)		Younger (*n*=163)		Older (*n*=171)		Younger (*n*=123)	
	Count	%	Count	%	Count	%	Count	%	Count	%
Genotype										
SS	427	70.2	112	74.2	115	70.6	110	64.3	90	73.2
SR	175	28.8	38	25.2	46	28.2	58	33.9	33	26.8
RR	6	1.0	1	0.6	2	1.2	3	1.8	0	0.0
Allele										
S	1029	84.6	262	86.8	276	84.7	278	81.3	213	86.6
R	187	15.4	40	13.2	50	15.3	64	18.7	33	13.4

Table 2 Risk factor variables in younger (≤50 years) and older (>50 years) men and women from the Epidemiology of Coronary Artery Calcification Study by E-selectin genotype

Variable	Older			Younger		
	Genotype		*P*[a]	Genotype		*P*[a]
	SS	SR or RR		SS	SR or RR	
Men	(*n*=112)	(*n*=39)		(*n*=115)	(*n*=48)	
Age	59.4±7.1	59.5±8.2	0.958	41.7±5.8	43.2±5.8	0.126
Systolic blood pressure	125.6±16.6	125.0±19.0	0.864	115.7±11.0	119.0±13.3	0.140
Total:HDL cholesterol	5.3±1.7	5.2±1.3	0.630	5.2±1.6	5.6±1.6	0.152
Body mass index	27.6±3.5	27.5±4.3	0.858	27.6±3.8	28.0±4.9	0.611
Pack years	22.7±32.0	14.3±24.1	0.042	8.3±12.5	8.4±12.4	0.635
Coronary calcification (%)	76.8	61.5	0.065	40.9	39.6	0.879
Women	(*n*=110)	(*n*=61)		(*n*=90)	(*n*=33)	
Age	59.8±8.1	58.8±7.2	0.410	44.8±2.8	45.5±2.8	0.250
Systolic blood pressure	121.9±15.3	122.9±17.6	0.699	113.0±13.6	116.3±11.6	0.193
Total:HDL cholesterol	4.4±1.7	4.2±1.3	0.425	3.9±1.7	3.6±1.1	0.184
Body mass index	28.0±5.9	25.9±4.4	0.009	25.8±4.8	27.7±5.8	0.089
Pack years	7.7±15.4	6.5±12.7	0.716	5.3±9.4	4.5±10.5	0.188
Coronary calcification (%)	39.1	37.7	0.858	6.7	27.3	0.002

[a] Significance levels from *t* tests for equality of means between genotypes (continuous variables) or Pearson χ^2 tests for equality of proportions between genotypes (categorical variables). For pack years, significance levels reflect *t* tests for equality of means between genotypes based on ln(pack years+1)

ticipants with (*n*=608) and those participants without (*n*=160) E-selectin genotype data among participants eligible for this study. Few participants had a documented history of myocardial infarction (*n*=6), stroke (*n*=4), or diabetes mellitus (*n*=12). Approximately 10% of study participants (29 men and 33 women) had a history of hypertension. Most CAD risk factors did not differ significantly between participants with the SR or RR genotypes relative to those with SS (Table 2). Among men over age 50, cigarette consumption was significantly greater in those carrying the SS genotype relative to those with SR or RR (Table 2). Similarly, BMI was significantly greater among women over age 50 with the SS genotype versus those with SR or RR (Table 2).

Coronary artery calcification in men

In neither age group did the prevalence of CAC differ significantly between participants with the SS genotype and those with the SR or RR genotypes (Table 2). In logistic regression models for men over the age of 50 years, age and ln(pack years+1) were significantly associated with presence of CAC when all other risk factors and the E-selectin polymorphism were included in the model (Table 3). In men 50 years of age or younger, age, total cholesterol/HDL cholesterol ratio, and BMI were significantly associated with presence of CAC when all other risk factors and the E-selectin polymorphism were considered (Table 3). The E-selectin S128R polymorphism was not a significant predictor of the presence of CAC in men of either age cohort (Table 3).

Table 3 Adjusted associations between risk factors and coronary artery calcification in younger (≤50 years) and older (>50 years) men and women from the Epidemiology of Coronary Artery Calcification Study

Variable	Adjusted odds ratio	95% CI	*P*[a]
Men			
Older (*n*=151)			
Age	2.8	1.5–5.2	<0.001
Systolic blood pressure	0.9	0.5–1.4	0.555
Total:HDL cholesterol	1.2	0.8–1.9	0.435
Body mass index	1.4	0.8–2.3	0.235
Ln(pack years+1)	2.0	1.2–3.1	0.003
E-selectin S128R[b]	0.6	0.2–1.3	0.190
Younger (*n*=163)			
Age	1.5	1.0–2.2	0.031
Systolic blood pressure	1.2	0.9–1.8	0.243
Total:HDL cholesterol	1.7	1.2–2.5	0.004
Body mass index	1.5	1.1–2.2	0.020
Ln(pack years+1)	1.1	0.7–1.5	0.754
E-selectin S128R[b]	0.6	0.3–1.4	0.265
Women			
Older (*n*=171)			
Age	2.3	1.6–3.4	<0.001
Systolic blood pressure	1.5	1.0–2.2	0.062
Total:HDL cholesterol	1.1	0.7–1.5	0.783
Body mass index	1.6	1.1–2.3	0.023
Ln(pack years+1)	1.3	0.9–1.8	0.173
E-selectin S128R[b]	1.3	0.6–2.7	0.568
Younger (*n*=123)			
Age	3.2	1.4–7.1	0.001
Systolic blood pressure	2.9	1.4–6.1	0.002
Total:HDL cholesterol	1.4	0.7–2.6	0.368
Body mass index	1.6	0.8–3.0	0.158
Ln(pack years+1)	1.8	0.9–3.6	0.110
E-selectin S128R[b]	6.5	1.5–28.0	0.009

[a] Test statistic for likelihood ratio tests comparing a full model to a reduced model

[b] Participants considered exposed carry the SR or RR genotype

Figure 1 shows the predicted probability and 95% confidence interval of having detectable CAC by genotype for men in each age group. The predictions are based on the parameter estimates from the full logistic regression model with mean age, BMI, systolic blood pressure, total cholesterol/HDL cholesterol, and ln(pack years+1). There were no significant interaction terms between the E-selectin S128R polymorphism and any risk factor.

Coronary artery calcification in women

In women over the age of 50 years the prevalence of CAC was not significantly different between those with the SS genotype and those with the SR or RR genotypes (Table 2). In women 50 years of age or younger, however, prevalence of CAC did differ significantly between those with the SS genotype (6.7%) and those with the SR or RR genotypes (27.3%; Table 2). In the logistic regression models among women over the age of 50 years, age and BMI were significantly associated with presence of CAC when other CAD risk factors and the E-selectin polymorphism were included in the model (Table 3). The S128R polymorphism was not significantly associated with presence of CAC in women over the age of 50 years when the CAD risk factors were considered (Table 3). Among women aged 50 years or younger, age, systolic blood pressure, and the S128R polymorphism were significantly associated with presence of CAC when all other predictors were included in the model (Table 3). The adjusted odds ratio (95% confidence interval) for the SR or RR genotypes versus the SS genotype was 6.5 (1.5, 28.0) (*P*=0.009; Table 3). The area under the receiver operating curve for the full model was 0.889. The value of the Hosmer-Lemeshow

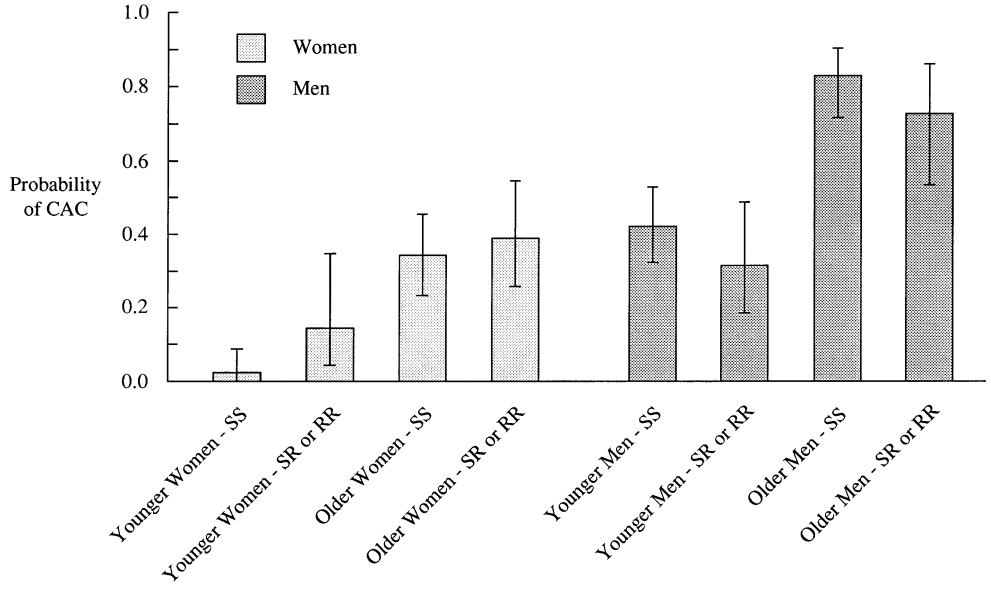

Fig. 1 Predicted probability (95% confidence interval) of coronary artery calcification by age, gender, and E-selectin genotype based on multivariable logistic regression models in 294 asymptomatic women and 314 asymptomatic men from the Epidemiology of Coronary Artery Calcification Study

χ^2 goodness-of-fit statistic with 8 degrees of freedom was 2.092 (P=0.978), indicating that the model fits the data.

Figure 1 shows the predicted probability and 95% confidence interval of having detectable CAC by genotype for women in each age group. The predictions are based on the parameter estimates from the full logistic regression model with mean age, BMI, systolic blood pressure, total cholesterol/HDL cholesterol, and ln(pack years+1). There were no significant interaction terms between the E-selectin S128R polymorphism and any risk factor.

Discussion

Deviations from Hardy-Weinberg equilibrium

Genotype frequencies in the subsample of 500 unrelated men and women did not conform to Hardy-Weinberg expectations, largely as a result of observing fewer RR homozygotes than expected. Others also have observed deviations from Hardy-Weinberg equilibrium for the E-selectin polymorphism [31]. The allele and genotype frequencies, however, did not differ significantly from frequencies in the study group examined by Wenzel et al. [16], and genotype frequencies for other polymorphisms conform to Hardy-Weinberg in the Rochester Family Heart Study from which participants in this study were selected [32]. While having fewer than expected homozygotes for a putative allele should result in a conservative test statistic [33], our strategy of pooling RR homozygotes with SR heterozygotes should have the added effect of diminishing any bias associated with deviations from Hardy-Weinberg equilibrium.

Gender differences in prevalence of CAC

The incidence of cardiovascular disease in premenopausal women is approximately half that in men of equivalent age [34]. This is reflected in the lower prevalence of CAC in women 50 years of age or younger than men in the same age range in this study (12.2% versus 40.5%) as well as in other studies [35]. Such differences between younger men and women in susceptibility to heart disease may be partially attributable to gender differences in risk factors such as serum lipid levels and smoking. Lipid profiles and cigarette consumption among participants in this study were consistent with these expectations; the total cholesterol/HDL cholesterol ratio and ln(pack years+1) were significantly higher in men 50 years of age or younger than in women in the same age group.

Androgens are commonly believed to induce atherogenic changes in lipoprotein concentrations that may predispose toward cardiovascular disease in men; whereas estrogens appear to be associated with beneficial influences (such as higher HDL and lower low-density lipoprotein cholesterol levels) on lipoprotein profiles and cardioprotective effects in women [34]. Estrogens also appear to protect against smoking-induced changes in vascular structure and function by modulating smooth muscle cells and improving arterial tone and reactivity [36].

Association between the S128R polymorphism and CAC in women 50 years of age or younger

In this study the significant association between the E-selectin S128R polymorphism and presence of CAC in women 50 years of age or younger may have been influenced by: (a) endogenous hormones and/or (b) lower levels of risk factors known to influence atherosclerotic development. As the majority of women aged 50 years or younger were premenopausal (83%), while most women over the age of 50 years were not (17%), the age strata we used corresponded well with menopausal status in women. Additionally, among all women in the current study, menopausal status was not significantly different between women with the SR or RR genotypes relative to those with the SS genotype (P=0.374).

Estrogens have been shown to inhibit transcription of adhesion molecule genes in endothelial cells [37, 38], and estrogen replacement therapy has been associated with a reduction in circulating levels of adhesion molecules [22, 39, 40], which may be an indication of reduced levels of adhesion molecule expression in tissues [41]. In the Postmenopausal Estrogen/Progestin Interventions Trial, postmenopausal hormone therapy resulted in a reduction in the concentration of soluble E-selectin [42]. One mechanism by which estrogens inhibit the development of atherosclerosis may be by modulating the expression of cellular adhesion molecules. The E-selectin S128R polymorphism, however, decreases specificity and increases the binding affinity of E-selectin for other ligands [13]. Altered E-selectin molecules may have a more pronounced effect in younger women, who typically have lower levels of adhesion molecules due to higher levels of endogenous hormones.

Levels of circulating adhesion molecules are positively associated with several risk factors for cardiovascular disease [43]. For example, cigarette smoking and plasma lipid levels have been associated with increased expression of certain adhesion molecules and with leukocyte adhesion to the vascular endothelium [44, 45]. Higher CAD risk factor levels and increased adhesion molecule expression, which appears to be characteristic of men and older women, may tend to obscure the effects of the E-selectin S128R polymorphism on presence of CAC.

Role of E-selectin in disease

Observational studies have implicated cellular adhesion molecules in endothelial dysfunction and cardiovascular

disease because they appear to be consistently expressed in atherosclerotic plaques [46] and elevated levels of circulating adhesion molecules have been observed in atherosclerosis [47], ischemic heart disease [48], myocardial infarction [49], and restenosis following peripheral arterial angioplasty [50]. More direct evidence for the involvement of adhesion molecules in the pathophysiology of inflammatory disease stems from population-based studies of relationships between genetic polymorphisms in genes coding for adhesion molecules and susceptibility to endothelial dysfunction, atherosclerosis, and coronary heart disease. For example, recent studies have reported associations between genetic variation in genes encoding selectin molecules and differential risk of early-onset atherosclerosis [15, 16, 17] and myocardial infarction [51].

The relationship between the E-selectin S128R polymorphism and atherosclerotic disease defined by angiography or CAC may reflect an amplified inflammatory response resulting from the action of altered selectin molecules containing the serine→arginine mutation. The substitution of arginine for serine has been shown to dramatically decrease binding specificity while increasing affinity for additional ligands, resulting in an increase in cellular adhesion of two- to threefold. The E-selectin 128R allele may thus increase leukocyte adherence to activated endothelium in areas susceptible to atherosclerotic plaque formation, thereby contributing to the progression of atherosclerosis and calcification [13].

Study limitations

We recognize that participants in this study may have had coronary atherosclerosis that went undetected because not all atherosclerotic plaque is calcified and not all calcified plaque may be readily detected with EBCT [52]. In addition, our findings may be limited to whites and those with milder coronary atherosclerosis because we excluded participants with a history of heart surgery and few participants had a history of myocardial infarction. We were unable to examine relationships between the E-selection S128R polymorphism and CAC in men under the age of 30 years and women under the age of 40 years because there were insufficient numbers of both men and women with CAC in these younger age groups.

We investigated the association between the E-selection S128R polymorphism and the presence of CAC; however, quantity of CAC may be measured with EBCT. We repeated the analyses using Tobit regression models, which can be used when the dependent variable is censored below a threshold value and thus is a mixture of discrete and continuous parts [53]. In the present study participants without detectable CAC were considered as censored observations. Inferences from the full Tobit models were similar to those from the logistic regression models for both genders and in each age group.

Conclusions

The association between the E-selection S128R polymorphism and presence of CAC in women 50 years of age or younger suggests that the 128R allele may be a risk factor for subclinical coronary atherosclerosis in younger women. These findings suggest that women carrying the E-selectin 128R allele may be at increased risk of developing CAC at an early age and point out the need to assess potential genetic influences on early-onset CAC in younger women. Genetic contributions to disease may be important for early-onset disease, but other CAD risk factors may play a more important role in atherosclerotic development at older ages. The contribution of the E-selectin polymorphism to early-onset CAC in young adults from a variety of racial and ethnic groups, the influence of genetic variation in other adhesion molecule genes on risk of CAC, and interactions between genes and environmental risk factors are areas for future research.

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