Association Between A Leukotriene C₄ Synthase Gene Promoter Polymorphism and Coronary Artery Calcium in Young Women

The Muscatine Study

David M. Iovannisci, Edward J. Lammer, Lori Steiner, Suzanne Cheng, Larry T. Mahoney, Patricia H. Davis, Ronald M. Lauer, Trudy L. Burns

Objective—A majority of the recognized risk factors for atherosclerosis and the development of cardiovascular disease have been derived from the study of older populations who have already manifested clinical symptoms. If risk factors can be identified earlier in life, such as genetic variation, preventive measures may be taken before overt symptoms of pathology have manifested, and when treatments may be most effective.

Methods and Results—In an effort to identify individuals at increased risk for cardiovascular disease, we genotyped 732 members of the Muscatine Study Longitudinal Adult Cohort for candidate genetic markers associated with several pathogenetic processes. We identified age-adjusted increased risks for coronary artery calcium (OR 4.29; 95% CI 1.78, 10.31) and increased mean carotid artery intimal-medial thickness associated with the (−444)A>C promoter polymorphism of Leukotriene C₄ Synthase (LTC₄S) in women. There were no similar associations in men.

Conclusions—LTC₄S plays a key role in the process of inflammation as the rate limiting enzyme in the conversion of arachidonic acid to cysteinyl-leukotrienes, important mediators of inflammatory responses. The (−444)C variant upregulates LTC₄S mRNA expression, increasing the synthesis of proinflammatory leukotrienes. Our results support genetic variation modifying inflammatory pathways as an important mechanism in the development of atherosclerosis.

Key Words: atherosclerosis • epidemiology • coronary artery calcification • calcification • leukotriene

Most of the risk factors for atherosclerosis and the development of cardiovascular disease (CVD) have been derived from the study of older populations who have already manifested clinical symptoms. Therefore, there is a need to identify younger subjects at risk for CVD so that preventive measures may be instituted before occlusive vascular disease occurs. A current goal of The Muscatine Study, initiated in 1970,¹ is to identify early predictors of coronary artery calcium (CAC) and carotid artery intimal-medial thickness (IMT). A representative subset (Muscatine Study Longitudinal Adult Cohort; n=866) of the 11 377 children who participated in the Muscatine Study school surveys conducted between 1971 and 1981 is currently being followed with cardiovascular risk factors measured during childhood and at several stages during adulthood, along with measures of subclinical CVD (CAC and IMT). Risk factors include anthropometric and blood pressure measurements, serum lipid profiles and other biochemical indices, dietary surveys, and behavioral questionnaires.²⁻⁵

Underlying genetic susceptibilities, as well as environmental factors, are involved in the initiation and progression of complex diseases such as atherosclerosis. Therefore it is important to add an assessment of the genetic contribution to the CVD risk for the Muscatine Study Longitudinal Adult Cohort. We have augmented the Muscatine Study data set by incorporating information on genetic polymorphisms within candidate genes associated with a variety of pathogenetic processes that include oxidative stress, lipid metabolism, thrombosis/coagulopathies, blood pressure regulation, inflammation, and cell adhesion,⁶⁻⁸ to investigate the association of these candidate gene polymorphisms with assessments of subclinical CVD. Here we report on the associations between CAC, IMT, and a promoter polymorphism of Leukotriene C₄ Synthase (LTC₄S), the rate limiting enzyme in the production of the potent proinflammatory cysteinyl-leukotriene metabolites of arachidonic acid. Because CAC and IMT are strongly associated with the amount of coronary atherosclerotic plaque,⁹⁻¹¹ either the amount or presence of CAC or...
the increase in mean IMT can be used as an early measure of coronary atherosclerosis.

Methods

The University of Iowa Institutional Review Board approved the Muscatine Study protocol and informed consent was obtained from all subjects. Both the University of Iowa, and Children’s Hospital Oakland Institutional Review Boards approved protocols for genotyping subjects.

Study Population

Between 1971 and 1981, 11,377 school children 8 to 18 years of age from Muscatine, Iowa underwent 26,919 total examinations in 6 biennial school surveys. Between 1981 and 1991, 2,547 of the school survey participants (67% of those eligible) who were then 20 to 37 years of age were examined (anthropometric, blood pressure, lipid measurements, and health history questionnaires) 1 or 2 times during the Young Adult Follow-Up phase of The Muscatine Study. Between 1992 and 1996, a representative subset of the Young Adult Follow-Up participants was invited to undergo a clinic examination and electron beam computed tomographic (EBCT) scanning for the measurement of coronary artery calcium (CAC). More than 95% of those invited became members of the Longitudinal Adult Cohort (n = 866, 29 to 43 years of age; 98% non-Hispanic white). Childhood risk factor levels (adjusted for age, gender, and survey year) of the 866 cohort members did not differ significantly from the entire group of childhood participants with respect to height, weight, blood pressure, triglycerides, triceps skinfold thickness, total cholesterol, or triglycerides at the time of their childhood examinations (data not shown).

Beginning in 1996, Cohort members were invited to return for a clinic examination and ultrasound examination for measurement of IMT. Data from the first EBCT examination for CAC and the first ultrasound examination for IMT, along with risk factor measurements obtained throughout the period of their participation in The Muscatine Study, are reported herein. At the time of the CAC examination, 2 men and 1 woman had been diagnosed with diabetes, and 1 man and 3 women were taking lipid-lowering medications. None of these individuals had evidence of coronary artery calcium.

Measurement of Coronary Artery Calcium

Calcium examinations for the 866 cohort members were performed using an Imatron C-150 ultrafast electron beam computed tomographic (EBCT) scanner (Imatron) as previously described. Briefly, 40 contiguous 3-mm slices were acquired during a single breath hold. Total radiation dose to the skin was estimated to be 10 mGy (1.0 rad). For this analysis, CAC was defined as a focus located within 5 mm of the arterial midlines, with at least 3 contiguous pixels (area 1.03 mm²) having a density of at least 130 Hounsfield units. Image-processing software computed the area of all pixels meeting these criteria within the regions of interest. For the analyses reported here, only the presence or absence of CAC was evaluated, because of the relatively low prevalence of CAC in this age group. Subjects with duplicate scans (approximately 50% of the cohort members) were classified as having CAC if either scan was positive.

Measurement of Carotid Artery Intimal-Medial Thickness

Carotid artery ultrasound examination was performed as previously reported. For each participant, the maximum carotid IMT was measured for the near and far wall of each common carotid artery, carotid bifurcation, and internal carotid artery. The mean of the maximum carotid IMT measured at the 12 locations (3 sites × 2 sides × 2 walls) was the variable used for analysis. The mean of non-missing walls was used for participants with missing data for one or more of the 12 locations. Approximately 55% of the participants had a maximum IMT for each of the 12 locations; 35% had a maximum IMT for 9 to 11 of the 12 locations; 98.5% had a maximum IMT for at least 6 of the 12 locations. The minimum number of measurements was 3.

Genotyping

DNA was available for 756 of the 866 cohort members. We used a multilocus allele-specific hybridization assay as previously described for genotyping. Briefly, the first step consisted of a multiplex polymerase chain reaction amplification using a primer blend containing a biotinylated primer pair for each polymorphic site. Next, the biotin-tagged amplification products were hybridized to a linear array of immobilized oligonucleotide probes specific for the alternate alleles of each polymorphic site. After a stringent wash, chromogenic substrates were used to visualize the biotin-tagged amplicons that remained hybridized. After color development, arrays were scanned for archiving, and genotypes were interpreted by two independent observers. All genotyping was performed blinded to participant’s identity and CAC status. The (-444)A>C polymorphism of LTC₄S corresponds to rs730012 (www.ncbi.nlm.nih.gov). LTC₄S genotyping was successful for 725 (96%) of the cohort members.

Statistical Analysis

Hardy-Weinberg equilibrium was tested using the chi-square goodness-of-fit test with exact probability values determined by a Monte Carlo permutation procedure. The risk factor data collected since childhood were standardized by age, gender, and survey year to obtain z scores. These z scores were used to calculate a “risk factor load” which is a time-weighted average z score that uses all of the longitudinal measurements available for a given risk factor for each participant. For example, pack years of smoking can be considered to be the smoking “load.”

Student t test and the nonparametric Wilcoxon rank-sum test were used to compare mean risk factor levels between men and women with (CAC+) and without (CAC−) coronary artery calcium. Pearson chi-square statistics were computed to compare the genotype distributions among participants with and without CAC.

To investigate the 100 candidate genetic markers, we conducted an association analysis using the recursive partitioning technique represented by the nonparametric classification and regression tree (CART) approach. The goal of the tree-based analysis was to partition the cohort into a number of more homogeneous subgroups based on the presence or absence of coronary artery calcium, by considering each of the 100 candidate genotypes along with age and gender. The results presented here only relate to the analysis of the LTC₄S (-444)A>C genetic polymorphism which showed the strongest association with CAC in women.

Logistic regression analysis was used to further investigate LTC₄S (-444)A>C, with adjustment for possible confounding effects attributable to age and recognized cardiovascular risk factors (CRFs). CRF predictor variables considered for inclusion in prediction models were risk factor loads: total cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, Apo A1, Apo B, triglycerides, total cholesterol/high-density lipoprotein cholesterol, systolic blood pressure, diastolic blood pressure, waist/hip circumference, and pack years of smoking. LTC₄S (-444)A>C was evaluated for its association with age-risk factor-adjusted odds of CAC. The presence or absence of CAC was assigned as the dependent variable. Receiver operating characteristic (ROC) curves were computed and the area (c) under the ROC curve is reported for each logistic model. A general linear model was fitted to compare age-risk factor–adjusted least squares genotype-specific mean carotid IMT. All analyses were conducted using procedures from the Statistical Analysis System (SAS, version 9.1).

Results

At the time of the EBCT examination to assess CAC, the 341 men with genotype data ranged in age from 29 to 37 years; the 391 women ranged in age from 29 to 43 years. The wider age range for women was by design because the prevalence of calcium among women 29 to 37 years of age is considerably lower than in men. One hundred one (13.8%) of the 732 cohort members with genotype data had evidence of CAC.
(20.5% of men and 7.9% of women). Among the women, 6% of those 29 to 37 years of age had CAC, compared with 16% of those 40 to 43 years of age.

The distribution of \( \text{LTC4S} \) \((\sim 444)A>C\) genotypes was consistent with Hardy–Weinberg expectations; \( \chi^2 = 0.55 \) \((P > 0.40)\) for men and women combined \((P > 0.10\) for women; \(P > 0.60\) for men). Table 1 shows the \( \text{LTC4S} \) genotype distribution by CAC status for men and women. \( \text{LTC4S} \) shows a strong association with CAC status for women, with 77% of the women with CAC having at least one copy of the variant allele compared with 46% of the women without CAC. In contrast, there is no association between CAC status and \( \text{LTC4S} \) genotype for men.

Comparing risk factor levels for men and women by their CAC status, we found that those with CAC tend to be slightly older, with lower HDL-cholesterol, higher systolic and diastolic blood pressures, and higher body mass indices (data not shown). Table 2 shows several logistic regression models for women that include an indicator variable for \( \text{LTC4S} \) genotype. The age-risk factor-adjusted odds of CAC relative to those with the AA genotype of 4.29 (95% CI 1.78, 10.31). Three of the load variables were also consistently significant in the logistic models: body mass index, diastolic blood pressure, and Apolipoprotein A1. Models 2 through 5 show the results from inclusion of various combinations of these three risk factor loads. The genotype-specific odds ratios are greater than those of Model 1, indicating that the association identified between the \( \text{LTC4S} \) genotype and CAC in women is not explained by an association between \( \text{LTC4S} \) and any of these recognized cardiovascular risk factors. The odds of CAC increase with increasing BMI and DBP loads and decrease with increasing Apolipoprotein A1 load. The age-BMI load-DBP load-Apolipoprotein A1 load-adjusted odds of CAC are almost 7-fold higher for women who carry at least one \( \text{LTC4S} \) C allele relative to women who are AA homozygotes.

Carotid IMT was available for these women from an examination 4 to 5 years after the EBCT examination, which allowed a further investigation of the association between the \( \text{LTC4S} \) and subclinical cardiovascular disease in a different vascular bed. Table 3 displays the results from including CRF loads in a general linear model along with an indicator variable for \( \text{LTC4S} \) genotype. The age-risk factor-adjusted odds of CAC relative to those with the AA genotype of 3.84 (95% CI 1.41, 10.21). Among the women, the odds of CAC were higher for those with \( \text{LTC4S} \) AC genotype compared with those with AA genotype.

### Table 1. Distribution of \( \text{LTC4S} \) Genotypes in Women and Men With (CAC+) and Without (CAC−) Coronary Artery Calcium

<table>
<thead>
<tr>
<th></th>
<th>CAC− (n/%)</th>
<th>CAC+ (n/%)</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA AC CC</td>
<td>AA AC CC</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>191 144 21</td>
<td>7 22 2</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td></td>
<td>54% 40% 6%</td>
<td>23% 71% 6%</td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>133 110 26</td>
<td>30 31 8</td>
<td>&gt;0.60</td>
</tr>
<tr>
<td></td>
<td>49% 41% 10%</td>
<td>43% 45% 12%</td>
<td></td>
</tr>
</tbody>
</table>

\( \text{LTC4S} \) indicates leukotriene C4 synthase.

### Table 2. Logistic Regression Models of the Association Between Coronary Artery Calcium Status and Risk Factor Loads, and \( \text{LTC4S} \) Genotypes in Women

<table>
<thead>
<tr>
<th>Model</th>
<th>V1 CAC Age</th>
<th>BMI Load</th>
<th>DBP Load</th>
<th>Apo A1 Load</th>
<th>( \text{LTC4S} )</th>
<th>n</th>
<th>c</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.16 (1.05, 1.28)*</td>
<td>2.64 (1.73, 4.04)</td>
<td>1.90 (1.02, 3.53)</td>
<td>—</td>
<td>4.29 (1.78, 10.31)</td>
<td>387</td>
<td>0.716</td>
</tr>
<tr>
<td>2</td>
<td>1.18 (1.07, 1.31)</td>
<td>2.26 (1.44, 3.55)</td>
<td>—</td>
<td>—</td>
<td>5.21 (2.05, 13.26)</td>
<td>387</td>
<td>0.795</td>
</tr>
<tr>
<td>3</td>
<td>1.19 (1.06, 1.31)</td>
<td>2.53 (1.64, 3.92)</td>
<td>2.10 (1.15, 4.06)</td>
<td>0.57 (0.34, 0.97)</td>
<td>6.64 (2.45, 18.02)</td>
<td>387</td>
<td>0.818</td>
</tr>
<tr>
<td>4</td>
<td>1.20 (1.08, 1.33)</td>
<td>2.11 (1.33, 3.34)</td>
<td>2.16 (1.15, 4.06)</td>
<td>0.51 (0.29, 0.88)</td>
<td>5.04 (1.98, 12.82)</td>
<td>386</td>
<td>0.851</td>
</tr>
<tr>
<td>5</td>
<td>&lt;0.005</td>
<td>&lt;0.0025</td>
<td>&lt;0.005</td>
<td>&lt;0.025</td>
<td>6.81 (2.49, 18.68)</td>
<td>386</td>
<td></td>
</tr>
</tbody>
</table>

*Odds Ratio (95% Confidence Interval), \( P \) value.

— indicates that the variable was not included in the model; DBP, diastolic blood pressure; BMI, body mass index; c, the area under the receiver operating characteristic (ROC) curve.

The odds ratios associated with BMI Load, DBP Load, and Apo A1 Load represent an \( z \)-score unit, eg, participants whose BMI Load was 1.0, ie, one standard deviation above the gender-specific-survey specific-mean throughout their lifetime, relative to participants whose BMI load was 0.0, ie, at the age-gender-specific survey specific-mean throughout their lifetime. Apo A1 was not measured until the Young Adult Follow-up examination.
LTC₄S genotype-specific least squares means are also included. For this LTC₄S polymorphism, women with at least one copy of the C allele have significantly higher \( P < 0.0025 \) adjusted mean carotid IMT when compared with women who are AA homozygotes. There was no difference in the genotype-specific age-risk factor–adjusted mean carotid IMT for the men \( (0.788 \pm 0.009 \text{ for AA homozygotes, } 0.788 \pm 0.009 \text{ for C allele carriers; } P > 0.90) \).

**Discussion**

Based on measurements that assess the early atherosclerotic process in two different vascular beds, we found evidence for a significant association between LTC₄S and subclinical disease for women, but not for men in The Muscatine Study. In women, the age-adjusted odds ratio for CAC+ associated with the \((-444)A>C\) promoter region polymorphism of LTC₄S was 4.29 (95% CI 1.78, 10.31). This increased risk is higher than that associated with elevated levels of established CVD risk factors in the Muscatine Study.² We also identified an association between the LTC₄S polymorphism and carotid artery IMT measured in the same women 4 to 5 years later. At the time of the carotid examination, 136 women had a concurrent repeat CAC examination. Davis et al³ identified a strong age-adjusted association between tertile of IMT and prevalence of CAC in this subset of women from the Muscatine Study Longitudinal Adult Cohort.

Our study represents one of the first attempts to assess the risk of CVD in a cohort of young subjects having no clinical symptoms of overt disease, by combining genetic, physical, and biochemical risk factor measurements with noninvasive indicators of subclinical atherosclerotic CVD. Previously, studies of cysteinyl-leukotrienes have focused on their role in asthma where it has been firmly established that both LTD₄ and LTE₄ can induce bronchospasm when taken by inhaler;²⁷,²⁸ In addition, a substantial body of evidence demonstrates that the cysteinyl-leukotrienes are increased in the sputum,²⁹,³⁰ lung lining fluid,³¹ blood, and urine³² of asthmatics compared with healthy individuals, thus associating these compounds with the pathophysiology of bronchial asthma. The \((-444)A>C\) SNP within the LTC₄S gene has been found to be associated with an increased risk for aspirin intolerant asthma.³³ The \((-444)C\) promoter region sequence alteration results in upregulation of LTC₄S mRNA expression attributable to the generation of a putative H4TF-2 transcription factor binding site, and subsequently increased levels of the inflammatory molecules LTC₄, LTD₄, and LTE₄.³⁴ Increased bronchial expression of LTC₄S, resulting in marked overproduction of cysteinyl-leukotrienes and subse-

---

**TABLE 3. Association Between LTC₄S and Carotid Artery Intimal–Medial Thickness (IMT) in Women (n=371)**

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>( P ) Value‡</th>
<th>LTC₄S Genotype</th>
<th>Mean IMT±SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMT age</td>
<td>&lt;0.0025</td>
<td>AA</td>
<td>0.702±0.007</td>
</tr>
<tr>
<td>Systolic BP load</td>
<td>&lt;0.01</td>
<td>AC and CC</td>
<td>0.733±0.007</td>
</tr>
<tr>
<td>Cholesterol load</td>
<td>&lt;0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chol/HDL load</td>
<td>&lt;0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI load</td>
<td>&lt;0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LTC₄S C allele</td>
<td>&lt;0.0025</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

‡ Adjusted for age, systolic blood pressure load, cholesterol load, cholesterol/HDL cholesterol load, and BMI load differences between genotype groups.

---

Metabolism of arachidonic acid. Arachidonic acid is released from membrane phospholipids by the action of Phospholipase A2. Once generated, arachidonic acid may be metabolized by either the lipoxygenase or the cyclooxygenase pathways. Through the coordinated activity of Arachidonate 5-Lipoxygenase and Arachidonate 5-Lipoxygenase Activating Protein, arachidonic acid is converted to leukotriene \( A_{\text{LT}} \). Leukotriene \( C_{\text{LT}} \) synthase (bold arrow), the rate limiting step along the cysteinyl-leukotriene pathway branch, converts leukotriene \( A_{\text{LT}} \) to leukotriene \( C_{\text{LT}} \), which can subsequently be converted to the additional cysteinyl-leukotrienes, leukotriene \( D_{\text{LT}} \) and leukotriene \( E_{\text{LT}} \). Leukotriene \( C_{\text{LT}} \), leukotriene \( D_{\text{LT}} \), and leukotriene \( E_{\text{LT}} \) each possess proinflammatory activities. ALOX5 indicates Arachidonate 5-Lipoxygenase; ALOX5AP, Arachidonate 5-Lipoxygenase Activating Protein; COX, cyclooxygenase; LT, leukotriene; PG, prostaglandin.

Arachidonic acid is first converted to the epoxide intermediate leukotriene \( A_{\text{LT}} \) by the coordinated activity of Arachidonate 5-Lipoxygenase and Arachidonate 5-Lipoxygenase Activating Protein.²⁵,²⁶ Additional leukotrienes are subsequently generated through the specific conjugation of leukotriene \( A_{\text{LT}} \) with glutathione by \( LTC_{\text{LT}} \), leading to the formation of leukotriene \( C_{\text{LT}} \). In themachal asthma. The \((-444)A>C\) SNP within the LTC₄S gene has been found to be associated with an increased risk for aspirin intolerant asthma. Arachidonic acid is released from membrane phospholipids by the action of Phospholipase A2. Once generated, arachidonic acid may be metabolized by either the lipoxygenase or the cyclooxygenase pathways. Through the coordinated activity of Arachidonate 5-Lipoxygenase and Arachidonate 5-Lipoxygenase Activating Protein, arachidonic acid is converted to leukotriene \( A_{\text{LT}} \). Leukotriene \( C_{\text{LT}} \) synthase (bold arrow), the rate limiting step along the cysteinyl-leukotriene pathway branch, converts leukotriene \( A_{\text{LT}} \) to leukotriene \( C_{\text{LT}} \), which can subsequently be converted to the additional cysteinyl-leukotrienes, leukotriene \( D_{\text{LT}} \) and leukotriene \( E_{\text{LT}} \). Leukotriene \( C_{\text{LT}} \), leukotriene \( D_{\text{LT}} \), and leukotriene \( E_{\text{LT}} \) each possess proinflammatory activities. ALOX5 indicates Arachidonate 5-Lipoxygenase; ALOX5AP, Arachidonate 5-Lipoxygenase Activating Protein; COX, cyclooxygenase; LT, leukotriene; PG, prostaglandin.

Arachidonic acid is released from membrane phospholipids by the action of Phospholipase A2. Once generated, arachidonic acid may be metabolized by either the lipoxygenase or the cyclooxygenase pathways. Through the coordinated activity of Arachidonate 5-Lipoxygenase and Arachidonate 5-Lipoxygenase Activating Protein, arachidonic acid is converted to leukotriene \( A_{\text{LT}} \). Leukotriene \( C_{\text{LT}} \) synthase (bold arrow), the rate limiting step along the cysteinyl-leukotriene pathway branch, converts leukotriene \( A_{\text{LT}} \) to leukotriene \( C_{\text{LT}} \), which can subsequently be converted to the additional cysteinyl-leukotrienes, leukotriene \( D_{\text{LT}} \) and leukotriene \( E_{\text{LT}} \). Leukotriene \( C_{\text{LT}} \), leukotriene \( D_{\text{LT}} \), and leukotriene \( E_{\text{LT}} \) each possess proinflammatory activities. ALOX5 indicates Arachidonate 5-Lipoxygenase; ALOX5AP, Arachidonate 5-Lipoxygenase Activating Protein; COX, cyclooxygenase; LT, leukotriene; PG, prostaglandin.

Our study represents one of the first attempts to assess the risk of CVD in a cohort of young subjects having no clinical symptoms of overt disease, by combining genetic, physical, and biochemical risk factor measurements with noninvasive indicators of subclinical atherosclerotic CVD. Previously thought to be a passive accumulation of lipids within artery walls, atherosclerosis is now understood to be a disease characterized by endothelial injury with active recruitment of inflammatory cells, the accumulation of lipids, and the formation of a fibrous cap to form complex plaque structures on the vessel walls.¹⁶⁻¹⁹ The resulting chronic inflammatory process involving the arterial endothelium may be caused by a response to lipid free radicals, lipid hydroperoxides, lysophospholipids, carbonyl compounds, and other biologically active moieties.²⁰ LTC₄S plays a key role in the process of inflammation as the rate limiting enzyme in the conversion of arachidonic acid to cysteinyl-leukotrienes, important mediators of the inflammatory response.²¹ In this pathway (Figure),
quent bronchoconstriction, has been confirmed in aspirin intolerant asthmatics relative to aspirin tolerant asthmatics, or when compared with normal individuals.35 Thus the role of the LTC4S (−444)C allele in the inflammatory process of asthma, likely through enhanced synthesis of inflammatory leukotrienes, has clearly been established. However, to our knowledge no previous reports have provided direct evidence for a role of LTC4S in the development of atherosclerosis.

Recently several studies have linked atherosclerosis risk with genetic variants of two other proteins participating in the leukotriene B arm of the 5-Lipoxygenase metabolic pathway: Arachidonate 5-Lipoxygenase Activating Protein and Leukotriene A4 Hydrolase (Figure).36–38 Several of the associations, risk factors. Recently, Dwyer et al39 has correlated ALOX5 with evidence of recent Caucasian admixture. In addition, these studies did not account for the contributions of other disease that included at least one cardiovascular event such as stroke or myocardial infarction. Furthermore, the association with Leukotriene A4 Hydrolase was for African Americans with evidence of recent Caucasian admixture. In addition, the study did not account for the contributions of other risk factors. Recently, Dwyer et al40 has correlated ALOX5 promoter sequence polymorphisms with risk for atherosclerosis employing IMT as an end point measurement. Although this work supports our observation that alterations in the genes encoding the arachidonate metabolic pathway correlate this work supports our observation that alterations in the genes encoding the arachidonate metabolic pathway correlate with early manifestations of atherosclerosis in subclinical individuals, to our knowledge our data represent the first report of an association with a LTC4S DNA sequence alteration and two early measurements of cardiovascular disease development, CAC, and carotid IMT.

There is no universally accepted definition for the presence of CAC. However, the more widely used definition is at least one focus with three contiguous pixels, each with a pixel density ≥130 Hounsfield units (HU). Several reports have demonstrated that foci with three or four pixels are not reproducible on duplicate scans.40,41 This lack of reproducibility has been attributed to patient positioning differences between the duplicate scans, partial volume effect, breath holding, and artifact, among other possible explanations, and it was observed among the duplicate scans in the present study. Therefore, we classified women as having CAC only if they had at least one focus with five or more contiguous pixels ≥130 HU; 19 of the women were classified with CAC using this more conservative definition. The distribution of LTC4S genotypes among these 19 women was compared with the remaining 368 women (those with no evidence of CAC or with foci smaller than five contiguous pixels) and the association remained (79% of those with CAC versus 47% of those without CAC have at least one copy of the variant C allele P<0.025). The age-adjusted odds of CAC for women with at least one copy of the variant C allele were 4.66 times higher than the odds of CAC for women with the AA genotype (OR=4.66; 95% CI 1.49, 14.55). Therefore the association is slightly stronger when a more conservative definition of coronary artery calcium is applied.

Whether the definition of a focus is three or five contiguous pixels, the number of women with CAC in the present study is small because the prevalence of coronary artery calcium is this age group is low. Ideally, an observed genetic association such as this, especially one based on a small sample size, should be replicated in another population. Because we did not have access to a second population of women in this age group who had been examined for CAC, we chose to replicate the observed CAC association by investigating the association in a different vascular bed (carotid artery IMT) in the same group of women.

Whereas women tend to develop atherosclerosis about a decade later than men, cardiovascular disease remains the leading cause of death for women, claiming more lives than the next six causes of death combined and totaling approximately 500 000 women’s lives each year. The effect of genetic predisposition to develop early atherosclerosis, which LTC4S may contribute to, may be more apparent earlier in the course of the disease before the overwhelming effects of environmental and pathophysiologic influences (CRFs) obscure it. Whether this explains our observed association in women remains to be investigated. Therefore improved methods are needed to identify those women most at risk for the development of CVD early in life, before overt symptoms of pathology have manifested and at a time when preventive interventions may be most effective.

Acknowledgments
We thank Christa Haun, Eric Lloyd, and Pouлина Uddin (Children’s Hospital and Research Center Oakland) for technical assistance with the genotyping; John Witt, Rick Paulos, and Elena Letuchy (The University of Iowa) for their help with data management and data analysis; and the Muscatine Study Clinic personnel and participants for their long-term commitment to this landmark investigation.

Sources of Funding
This research was supported by funds from the California Tobacco-Related Disease Research Program of the University of California, grant #1IRT-0208, by a grant from the American Heart Association, award number #0265155Y, and by National Institutes of Health Muscatine Study RO1s HL01357, HL48050, and HL54730.

Disclosures
L.S. and S.C. are employed by Roche Molecular Systems, Alameda Calif, which provided some research reagents under a materials transfer agreement with Children’s Hospital and Research Center Oakland.

References
Iovannisci et al


Association Between A Leukotriene C4 Synthase Gene Promoter Polymorphism and Coronary Artery Calcium in Young Women: The Muscatine Study
David M. Iovannisci, Edward J. Lammer, Lori Steiner, Suzanne Cheng, Larry T. Mahoney, Patricia H. Davis, Ronald M. Lauer and Trudy L. Burns

Arterioscler Thromb Vasc Biol. 2007;27:394-399; originally published online November 16, 2006;
doi: 10.1161/01.ATV.0000252680.72734.10
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2006 American Heart Association, Inc. All rights reserved.
Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://atvb.ahajournals.org/content/27/2/394

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

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

Subscriptions: Information about subscribing to Arteriosclerosis, Thrombosis, and Vascular Biology is online at:
http://atvb.ahajournals.org//subscriptions/