Promotor Polymorphisms in Leukotriene C₄ Synthase and Risk of Ischemic Cerebrovascular Disease

Jacob J. Freiberg, Anne Tybjærg-Hansen, Henrik Sillesen, Gorm B. Jensen, Børge G. Nordestgaard

Objective—Cysteinyl leukotrienes are involved in inflammation and possibly in early carotid atherosclerosis. We tested the hypothesis that the −444 A/C and −1072 G/A polymorphisms of the leukotriene C₄ synthase associate with risk of ischemic cerebrovascular disease.

Methods and Results—We genotyped 10,592 individuals from the Danish general population, the Copenhagen City Heart Study. During 24 years of follow-up, 557 individuals developed ischemic cerebrovascular disease. The allele frequency was 0.07 for −1072 A and 0.29 for −444 C. Cumulative incidence for ischemic cerebrovascular disease was higher for −1072 AA versus GG genotype (log-rank: P=0.002), and lower for −444 CC versus AA genotype (log-rank: P=0.008). Combined genotypes showed corresponding cumulative incidence differences (log-rank: P=0.003). Multifactorially adjusted hazard ratios for ischemic cerebrovascular disease were 2.8 (1.4 to 5.7) for −1072 AA versus GG genotype, 0.6 (0.4 to 0.9) for −444 CC versus AA genotype, 2.5 (1.2 to 5.4) for combined AA-AA versus GG-AA genotype, and 0.6 (0.4 to 0.9) for combined GG-CC versus GG-AA genotype. Genotype did not associate with risk of deep venous thrombosis or severe carotid atherosclerosis, or with levels of platelets and coagulation factors.


Key Words: 5-lipoxygenase pathway ■ cysteinyl leukotrienes ■ LTC₄ synthase ■ ischemic cerebrovascular disease ■ atherosclerosis

Atherosclerosis is an inflammatory disease involving several proinflammatory mediators.¹² This inflammatory process involves products of the 5-lipoxygenase pathway, including leukotriene B₄ (LTB₄) and the cysteinyl leukotrienes LTC₄, LTD₄, and LTE₄.³ Both LTB₄ and the cysteinyl leukotrienes have been implicated in acute and chronic cardiovascular disease as well as in asthma and cancer.³⁴ In atherosclerotic coronary arteries, cysteinyl leukotrienes act as potent vasoconstrictors and reduce vascular blood flow,⁵ and after an ischemic event increased urinary leukotriene excretion is observed.⁶ Furthermore, in vitro studies indicate that cysteinyl leukotrienes induce vascular smooth muscle cell (VSMC) proliferation.⁷

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Cysteinyl leukotrienes are synthesized in the final steps of the 5-lipoxygenase pathway from membrane-derived arachidonic acid by the action of the human LTC₄ synthase gene LTC₄ synthase.⁸ Two polymorphisms in the promoter region of LTC₄ synthase may be involved in the level of transcription. The −444 A/C polymorphism has been predicted to influence recognition sites for several transcription factors, including AP-2, H4TF-2, GRE, NF-Elc, and NF-E, whereas the −1072 G/A polymorphism has been predicted to influence binding of the transcription factors LF-A1 and α-IFN-2.⁹,¹⁰ For the −444 C allele an additional responsive element to H4TF-2 increases the transcription rate of LTC₄ synthase.¹¹ In a recent study, the LTC₄ synthase −444 A/C polymorphism associated with increased risk of early carotid atherosclerosis.¹² However, it is unknown whether this and the −1072 G/A polymorphism influence the risk of ischemic cerebrovascular disease.

We tested the hypothesis that the −444 A/C and −1072 G/A polymorphisms of LTC₄ synthase associate with risk of ischemic cerebrovascular disease. We also tested whether these polymorphisms associate with risk of deep venous thrombosis and severe carotid atherosclerosis. Finally, we examined whether these polymorphisms influence levels of platelets and coagulation factors. To do so, we conducted 3 studies: (1) a prospective study of 10,576 participants from the general population with 24 to 28 years of follow-up, (2) a case-control study including 864 patients with severe carotid atherosclerosis and 3102 controls, and (3) a cross-sectional study of 5,951 participants from the general population.
Methods

Study Design
The studies were approved by the Danish ethics committees for the City of Copenhagen and Frederiksberg and Copenhagen County, and by Herlev Hospital. Participants gave written informed consent.

Prospective Study
Within the Copenhagen City Heart Study, we studied risk of ischemic cerebrovascular disease (ICVD) and of deep venous thrombosis (DVT). The participants in these studies were individuals who had no stroke or hemorrhage within the preceding 5 years.

Case-Control Study
We matched patients from the Copenhagen University Hospital diagnosed with severe carotid atherosclerosis with controls from the Copenhagen City Heart Study without known atherosclerosis.

Cross-Sectional Study
On participants from the Copenhagen City Heart Study examined in 2001 to 2003, we studied levels of platelets and coagulation factors.

Participants

The Copenhagen City Heart Study
This is a prospective cardiovascular study of the Danish general population initiated in 1976. We invited women and stratified into 5-year age groups from 20 to 80 years and above, and drawn randomly from the Copenhagen Central Person Register. Participants were followed using their unique Central Person Register number from baseline at the 1976 to 1978 examination onwards. Follow-up was 100% complete, and roughly 99% were white and of Danish descent. Participants were examined in 1976 to 1978, 1981 to 1983, 1991 to 1994, and in 2001 to 2003; at each of the follow-up examinations, the cohort was supplemented with individuals in the younger age groups. All 4 examinations included a self-administered questionnaire, a physical examination, and blood samples; whole blood for DNA isolation was available at the 1991 to 1994 and 2001 to 2003 examinations. Of the 17,600 individuals invited to these 2 examinations, 10,994 (65%) gave blood for DNA isolation. Of these, 10,576 individuals were genotyped for both the LTC4 synthase -444 A/C and -1072 G/A polymorphisms.

End points used in the study were ICVD and DVT. Information on diagnoses of ICVD (World Health Organization International Classification of Diseases, 8th and 10th revisions: codes 431 to 438 and I61-I69+G45) and DVT (ICD-8 codes 451.00, 451.08, 451.09, 451.90, 451.92, 451.91, 01 to 671.03, 671.08, 671.09 and ICD-10 codes D801-D803, D0223, D0871) was gathered until the end of 2000 (ICVD) or the beginning of 2004 (DVT), from the national Danish Patient Registry and the national Danish Causes of Death Registry. For each person registered with ICVD, hospital records were requested. To also include nonfatal nonhospitalized ischemic stroke patients, the participants were asked at the study examinations whether they previously had a stroke. If a person answered “yes,” further information was obtained from that person’s general practitioner. Experienced neurologists reviewed all potential cases. Possible stroke events (hospitalized as well as nonhospitalized) were validated using the World Health Organization definition of stroke: an acute disturbance of focal or global cerebral function with symptoms lasting longer than 24 hours or leading to death with presumably no other reasons than of vascular origin. To distinguish among infarction, intracerebral hemorrhages, and subarachnoid hemorrhages, either CT or MRI scan, autopsy, spinal fluid examination, or surgical description was necessary. If the scan did not visualize an infarction or hemorrhage, but the person had symptoms that met the criteria of the stroke definition, then the event was diagnosed as ischemic infarction. The diagnosis of stroke was not applied in cases where a scan revealed signs of prior cerebrovascular disease, but without history of any symptoms. The diagnostic criteria for a diagnosis of ICVD were ischemic stroke, transient ischemic attack (focal neurological symptoms lasting less than 24 hours), or amaurosis fugax (transient blindness on one eye only). During 24 years of follow-up, 680 individuals were recorded with cerebrovascular disease. Of these, 123 events were excluded (43 events were diagnosed before study entry and 80 events were hemorrhagic strokes or subarachnoid hemorrhages). Thus, the study on ICVD comprised 9198 individuals: 557 individuals with ICVD and 8641 individuals free from ICVD; those only attending the 2001 to 2003 examination were not included in this study because follow-up for ICVD terminated at the end of 2000. During 28 years follow-up, 374 individuals were diagnosed with DVT; the diagnostic criteria used were ultrasonography or venography. Of these, 16 participants were diagnosed before study entry and excluded from analysis. Thus, the study on DVT comprised 10,576 individuals: 358 individuals diagnosed with DVT and 10,218 individuals without DVT.

Copenhagen University Hospital
From 1994 through 2005, patients were referred for ultrasonography of the carotid artery at Copenhagen University Hospital because of relevant symptoms. Experienced vascular technicians performed the duplex scans under the supervision of vascular surgeons. The degree of stenosis was separated into the following groups: 0% to 49% (peak systolic velocity <120 cm/s), 50% to 99% (peak systolic velocity >120 cm/s or end diastolic velocity >100 cm/s), and total occlusion, determined by generally accepted Doppler criteria. The cutoff point of 50% stenosis was determined in relation to the distal internal carotid diameter. Patients with carotid artery stenosis of at least 50% diameter reduction on the most stenotic side were included, whereas those with <50% carotid stenosis were excluded. We genotyped 864 cases with >50% carotid stenosis. Roughly 99% were white and of Danish descent. The 864 patients were age- and gender-matched with 3102 controls from the general population of the Copenhagen City Heart Study, all free from any cerebrovascular disease. A complete match was not possible because of the lack of controls within some one year groups for each gender used for matching.

Cardiovascular Risk Factors
Body mass index was calculated as weight in kilograms divided by height in meters squared. Alcohol drinkers consumed alcohol at least twice weekly. Smokers were active smokers. Hypertension was use of antihypertensive medication, a systolic blood pressure ≥140 mm Hg, or a diastolic blood pressure ≥90 mm Hg. Physical inactivity was leisure time activity less than 4 hours weekly and predominantly sedentary work. Diabetes mellitus was self-reported disease, use of insulin or oral hypoglycemic agents, or nonfasting plasma glucose >11 mmol/L. Atrial fibrillation was diagnosed from electrocardiographic recordings obtained at all 4 study examinations and confirmed by 2 independent reviewers. Furthermore, information on atrial fibrillation (ICD-8 codes 424.00 to 424.99, 427.93, and 427.94 and ICD-10 codes E05.0 to 05.9 and I48.9) was gathered until the beginning of 2004 from the Danish National Hospital Discharge Registry and the Danish National Register of Causes of Death. Women reported menopausal status and use of oral contraceptives and hormone replacement therapy.

Analyses
Participants and patients were genotyped for the LTC4 synthase -444 A/C and -1072 G/A polymorphisms using TaqMan assays (Applied Biosystems, Stockholm, Sweden). Because we performed 2 rounds of reruns, the call rate for genotyping was >99.9%. Enzymatic methods (Boehringer Mannheim, Mannheim, Germany) were used on fresh samples to measure plasma levels of nonfasting triglycerides, total cholesterol, and high density lipoprotein cholesterol, the latter under precipitation of apolipoprotein B containing lipoproteins. Low-density lipoprotein was calculated using the Friedewald equation if triglycerides were below 4 mmol/L, and measured directly at higher triglyceride levels (Thermo). Standard hospital assays were used to measure platelets (Siemens) and coagulation factor II + VII + X, fibrinogen, and activated partial thromboplastin time (APTT; Instrumentation Laboratory).

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Statistical Analysis

Data were analyzed using Stata 9.2. For power calculations NCCS-PASS was used. A 2-sided probability value \( P < 0.05 \) was considered significant. Student \( t \) test or Pearson \( \chi^2 \) test were used in 2-group comparisons; high-density lipoprotein cholesterol, plasma triglycerides, and fibrinogen were logarithmically transformed to approach normal distribution. Kruskall-Wallis analysis of variance was used to test for differences between 3 or more genotypes.

Cumulative incidences were plotted using Kaplan-Meier curves and differences between genotypes determined using log-rank tests. Cox proportional hazards regression models estimated hazard ratios for ICVD and DVT. Proportionality of hazards over time for different genotypes were assessed by plotting -\( \ln(-\ln(survival)) \) versus \( \ln(\text{analysis time}) \). Suspicion of nonparallel lines was further tested using Schoenfeld residuals. No major violations of the proportional hazard assumption were detected. For all survival statistics, age was the time scale using left truncation (or delayed entry), which implies that age is automatically adjusted for. Hazard ratios were adjusted for age alone, or multifactorially. For ICVD hazard ratios were multifactorially adjusted for age, gender, body mass index, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, lipid lowering medication, antirheumatic medication, fibrinogen, alcohol intake, smoking status, hypertension, physical inactivity, diabetes mellitus, and atrial fibrillation. Women were furthermore adjusted for oral contraceptives, hormone replacement therapy, and postmenopausal status.

Information on baseline covariates was >99% complete; individuals with incomplete information on covariates were excluded from multifactorial analysis. Data from the 1976 to 1978, 1981 to 1983, 1991 to 1994, and 2001 to 2003 examinations were used as time-dependent covariates for multifactorial adjustments; this implies that initially baseline covariate values are used for the following years until that person is examined again at a later examination, after which the new value is used in the analyses. If only baseline values are available, these are used for adjustment during the entire follow-up period.

In a 1:4 age- and gender-matched case-control design, a conditional logistic regression model was used to estimate odds ratios for

### Table. Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Prospective Study</th>
<th>Ischemic Cerebrovascular Disease</th>
<th>Prospective Study</th>
<th>Deep Venous Thrombosis</th>
<th>Case-Control Study</th>
<th>Carotid Atherosclerosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Observations, n</strong></td>
<td>9198</td>
<td>557</td>
<td>10218</td>
<td>358</td>
<td>3102</td>
<td>864</td>
</tr>
<tr>
<td><strong>Women, n (%)</strong></td>
<td>5139 (56)</td>
<td>269 (48)</td>
<td>5682 (56)</td>
<td>189 (53)</td>
<td>1312 (42)</td>
<td>328 (38)</td>
</tr>
<tr>
<td><strong>Age, years</strong></td>
<td>45 (37–54)</td>
<td>55 (48–60)*</td>
<td>45 (36–54)</td>
<td>52 (45–57)*</td>
<td>49 (43–55)</td>
<td>65 (58–70)*</td>
</tr>
<tr>
<td><strong>Body mass index, kg/m²</strong></td>
<td>24 (22–27)</td>
<td>25 (23–28)*</td>
<td>24 (22–26)</td>
<td>26 (23–28)*</td>
<td>25 (23–27)</td>
<td>25 (23–28)*</td>
</tr>
<tr>
<td><strong>Cholesterol, mmol/L</strong></td>
<td>5.7 (4.9–6.5)</td>
<td>6.2 (5.5–7.1)*</td>
<td>NR</td>
<td>NR</td>
<td>5.9 (5.2–6.6)</td>
<td>5.9 (5.2–6.7)*</td>
</tr>
<tr>
<td><strong>HDL cholesterol, mmol/L</strong></td>
<td>1.5 (1.2–1.8)</td>
<td>1.4 (1.1–1.7)*</td>
<td>NR</td>
<td>NR</td>
<td>1.4 (1.2–1.7)</td>
<td>1.4 (1.1–1.7)*</td>
</tr>
<tr>
<td><strong>LDL cholesterol, mmol/L</strong></td>
<td>3.6 (2.9–4.4)</td>
<td>3.9 (3.2–4.7)*</td>
<td>NR</td>
<td>NR</td>
<td>3.8 (3.1–4.5)</td>
<td>3.7 (2.9–4.5)*</td>
</tr>
<tr>
<td><strong>Triglycerides, mmol/L</strong></td>
<td>1.3 (0.9–1.9)</td>
<td>1.7 (1.2–2.4)*</td>
<td>NR</td>
<td>NR</td>
<td>1.4 (1.0–2.1)</td>
<td>1.6 (1.1–2.3)*</td>
</tr>
<tr>
<td><strong>Lipid lowering medication, n (%)</strong></td>
<td>8 (0)</td>
<td>0 (0)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Antirheumatic medication, n (%)</strong></td>
<td>47 (1)</td>
<td>1 (0)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Fibrinogen, μmol/L</strong></td>
<td>8.9 (7.3–10.7)</td>
<td>9.9 (8.3–11.7)*</td>
<td>8.9 (7.4–10.8)</td>
<td>9.6 (8.1–11.6)*</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Alcohol drinker, n (%)</strong></td>
<td>5362 (58)</td>
<td>321 (58)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Smoker, n (%)</strong></td>
<td>5280 (57)</td>
<td>353 (63)†</td>
<td>5651 (55)</td>
<td>232 (65)*</td>
<td>1807 (58)</td>
<td>425 (50)*</td>
</tr>
<tr>
<td><strong>Hypertension, n (%)</strong></td>
<td>3163 (34)</td>
<td>327 (59)*</td>
<td>NR</td>
<td>NR</td>
<td>1230 (40)</td>
<td>397 (50)*</td>
</tr>
<tr>
<td><strong>Physical inactivity, n (%)</strong></td>
<td>1878 (21)</td>
<td>114 (21)</td>
<td>2053 (21)</td>
<td>82 (23)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Diabetes mellitus, n (%)</strong></td>
<td>94 (1)</td>
<td>17 (3)*</td>
<td>NR</td>
<td>NR</td>
<td>45 (1)</td>
<td>120 (14)*</td>
</tr>
<tr>
<td><strong>Atrial fibrillation, n (%)</strong></td>
<td>53 (1)</td>
<td>6 (1)</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Oral contraceptives, n (%) (women)</strong></td>
<td>514 (10)</td>
<td>12 (4)†</td>
<td>706 (12)</td>
<td>11 (6)†</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Hormone replacement therapy, n (%) (women)</strong></td>
<td>755 (15)</td>
<td>67 (25)*</td>
<td>822 (15)</td>
<td>35 (19)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td><strong>Postmenopausal, n (%) (women)</strong></td>
<td>2241 (44)</td>
<td>215 (80)*</td>
<td>2402 (42)</td>
<td>130 (69)*</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

*Individuals in prospective studies are shown by diagnostic status at the end of follow-up. Continuous variables are shown as median (interquartile range). HDL indicates high-density lipoproteins; LDL, low-density lipoproteins; NR, not relevant; NA, not available; *\( P < 0.001 \), †\( P < 0.01 \), and ‡\( P < 0.05 \) by Student \( t \) test or Pearson chi-square test comparing individuals with the event in question and control.
severe carotid atherosclerosis. Risk factor status for controls was based on information from the 1991 to 1994 or 2001 to 2003 examination, where DNA was isolated. Box-Tidwell transformation was used to test for linearity in the logit among continuous covariates; logarithmic transformation of triglycerides was used. No major violations of the linearity in the logit assumption were observed. Odds ratios for severe carotid atherosclerosis were multi-factorially adjusted for age, gender, body mass index, cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, smoking status, hypertension, and diabetes mellitus.

Finally, with 90% power and 2-sided probability values, we calculated the minimal/maximal hazard or odds ratio that could be excluded in our study.

Results

Characteristics of participants are shown by diagnostic status in the Table. The -1072 A allele had a frequency of 0.07 whereas the -444 C allele had a frequency of 0.29; these 2 alleles were in linkage disequilibrium ($r^2 = 0.03$; $D' = 0.97$). Combined genotype frequencies for the general population were: -1072 / -444 GG-CC 0.08, GG-AC 0.38, GG-AA 0.40, GA-CC 0.0004, GA-AC 0.04, GA-CC 0.09, AA-AC 0.0002, AA-AA 0.006. Genotype distributions for both polymorphisms were in Hardy-Weinberg equilibrium ($\chi^2$: $P > 0.05$).

Ischemic Cerebrovascular Disease

Cumulative incidence for ICVD was higher for -1072 AA versus GG genotype (log-rank: $P = 0.002$), and lower for -444 CC versus AA genotype (log-rank: $P = 0.008$) (Figure 1). Combined genotypes showed corresponding cumulative incidence differences (log-rank: $P = 0.003$). Multifactorially adjusted hazard ratios were 2.8 (1.4 to 5.7) for -1072 AA versus GG genotype, 0.6 (0.4 to 0.9) for -444 CC versus AA genotype, 2.5 (1.2 to 5.4) for combined AA-AA versus GG-AA genotype, and 0.6 (0.4 to 0.9) for combined GG-CC versus GG-AA genotype (Figure 2). The hazard ratio was 16 (2.2 to 114) for the combined AA-AC versus GG-AA genotype; however, this was only based on a single event among 2 participants with this genotype.

In posthoc analyses, multifactorially adjusted hazard ratios did not reveal any changed risk for ICVD in participants ever diagnosed with atrial fibrillation versus participants never diagnosed with atrial fibrillation (Figure 3). Interaction tests between -444 A/C and -1072 G/A genotypes, and atrial fibrillation on ICVD risk, were not significant ($P = 0.79$ and $P = 0.07$, respectively).

Deep Venous Thrombosis

Multifactorially adjusted hazard ratios for DVT were insignificant for all genotypes except for the combined AA-AC versus GG-AA genotype with a hazard ratio of 68 (16 to 284). However, only 2 participants had this genotype and both had DVT, whereas none of the 70 participants with the AA-AA genotype had DVT. Multifactorially adjusted hazard ratios were 0.9 (0.2 to 3.8) for -1072 AA versus GG genotype, and 1.2 (0.9 to 1.8) for -444 CC versus AA genotype (Figure 4). We had 90% power to exclude a hazard ratio of 3.0 or larger for -1072 AA versus GG genotype, and a hazard ratio of 0.6 or less for -444 CC versus AA genotype.

Severe Carotid Atherosclerosis

Multifactorially adjusted odds ratios were insignificant for all genotypes except for the -444 AC versus AA genotype.

**Table:**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Participant</th>
<th>Events</th>
<th>Ischemic cerebrovascular disease</th>
<th>90% Power</th>
</tr>
</thead>
<tbody>
<tr>
<td>-1072</td>
<td>A/G</td>
<td>A/C</td>
<td>Age adjusted</td>
<td>Multifactorially adjusted</td>
</tr>
<tr>
<td>GG</td>
<td>8381</td>
<td>409</td>
<td>Ref.</td>
<td>Ref.</td>
</tr>
<tr>
<td>GA</td>
<td>1308</td>
<td>30</td>
<td>1.5</td>
<td>2.7</td>
</tr>
<tr>
<td>AA</td>
<td>66</td>
<td>8</td>
<td>Ref.</td>
<td>0.8</td>
</tr>
<tr>
<td>-444</td>
<td>A/C</td>
<td>CC</td>
<td>Ref.</td>
<td>0.7</td>
</tr>
<tr>
<td>GC</td>
<td>811</td>
<td>36</td>
<td>Ref.</td>
<td>0.6</td>
</tr>
<tr>
<td>GA</td>
<td>418</td>
<td>128</td>
<td>Ref.</td>
<td>0.5</td>
</tr>
<tr>
<td>AA</td>
<td>3709</td>
<td>199</td>
<td>Ref.</td>
<td>0.5</td>
</tr>
<tr>
<td>-1072</td>
<td>AA</td>
<td>A/A</td>
<td>Ref.</td>
<td>2.7</td>
</tr>
<tr>
<td>GA</td>
<td>884</td>
<td>62</td>
<td>Ref.</td>
<td>1.4</td>
</tr>
<tr>
<td>A/A</td>
<td>64</td>
<td>7</td>
<td>Ref.</td>
<td>2.7</td>
</tr>
</tbody>
</table>

![Figure 1](http://atvb.ahajournals.org/Downloaded from) Cumulative incidences of ischemic cerebrovascular disease for the LTC4 synthase -1072 AA versus GG, -444 AA versus CC, and combined AA-AA versus GG-AA versus GG-CC genotypes. Values are from the Copenhagen City Heart Study with 24 years follow-up. Probability values for log-rank tests examine whether the Kaplan-Meyer curves differ.

![Figure 2](http://atvb.ahajournals.org/Downloaded from) Hazard ratios for ischemic cerebrovascular disease for the LTC4 synthase -1072 A/G, -444 A/C, and common combined genotypes. Values are from the Copenhagen City Heart Study with 24 years follow-up. Ref indicates reference genotype.
0.8 (0.7 to 1.0), and for the combined GG-AC versus GG-AA genotype 0.8 (0.7 to 1.0) (Figure 5). We had 90% power to exclude an odds ratio of 2.8 or larger for 

\[ H11002 \]

1072 AA versus GG genotype, and an odds ratio of 0.7 or less for 

\[ H11002 \]

444 CC versus AA genotype.

### Platelets and Coagulation Factors

For the \( \text{LTC}_4 \) synthase \( -1072 \) A/G, \( -444 \) A/C, and the combined genotype, platelet counts did not differ between genotypes. Likewise, levels of coagulation factors \( \text{II} + \text{VII} + \text{X} \), fibrinogen, and aPTT did not differ between genotypes.

### Discussion

We found that \( \text{leukotriene} \ C_4 \text{ synthase} \ -1072 \) AA genotype predicts increased risk, whereas the \( -444 \) CC genotype predicts decreased risk of ICVD. Genotype did not associate with risk of deep venous thrombosis or severe carotid atherosclerosis, or with levels of platelets and coagulation factors.

A connection between the 5-lipoxygenase pathway and atherosclerotic disease has been established by several studies. Expression of the 5-lipoxygenase pathway is increased in advanced atherosclerotic lesions. Genetic variants in the \( \text{ALOX5AP} \) gene coding for the 5-lipoxygenase activating protein involved in the initial steps of leukotriene synthesis has been associated with ischemic stroke. A hexanucleotide repeat polymorphism in the promoter region of \( \text{ALOX5} \) coding for 5-lipoxygenase has been associated with carotid intima thickness. Furthermore, \( \text{LTB}_4 \), a chemoattractant, has in mice models been associated with atherosclerotic disease.

Finally, in a rat model, the degree of focal cerebral ischemia has been associated with cysteinyl leukotriene formation.

Recently, at least one copy of the \( C \) allele versus AA homozygosity of the \( -444 \) A/C polymorphism has also been associated with early carotid atherosclerosis and a 4-fold risk of coronary artery calcium in women, but not in men. Our study apparently contradicts this finding by demonstrating a decreased risk for ICVD for CC versus AA homozygotes, and no changed risk for severe carotid atherosclerosis. However, the former study examined 732 women and men aged 29 to 43 years for 2 preclinical end points, carotid artery intimal-medial thickness and coronary artery calcium, whereas we studied clinical identified disease, that is, ICVD and >50% carotid artery stenosis, in a total of >11 000 20- to 80-year-old women and men. Therefore, we cannot exclude that findings in both studies represent real phenomena. Nevertheless, because of limited sample sizes findings in either of the 2 studies could also be attributable to chance alone. Importantly, different components of the inflammatory cascade may predominate in different stages of the atherosclerotic disease. Thus, some factors may promote atherogenesis, others plaque progression, and others still symptomatic conversion into ICVD.

Mechanistically, the explanation for our findings possibly can be sought in the contribution of cysteinyl leukotrienes to inflammation and atherosclerotic disease. Alternatively, because the change in risk was found for ICVD, and not for severe carotid atherosclerosis, the mechanism could be via changed risk of thrombi formed from platelet aggregation at atherosclerotic lesions, thrombi that detach from the intima to cause ICVD. Because levels of platelets and coagulation factors...
Genotype   | Participant No. | Events | Severe carotid atherosclerosis |
---|---|---|---|
| -1072 GA | 2641 | 745 | | |
| -444 AC | 440 | 116 | | |
| AA | 21 | 3 | | |

| -1072 GA | 1484 | 447 | | |
| -444 AC | 1354 | 345 | | |
| CC | 264 | 72 | | |

| -1072 GA | 261 | 71 | | |
| -444 AC | 144 | 36 | | |
| GG | 1210 | 309 | | |
| GA | 1170 | 305 | | |
| AA | 295 | 79 | | |

Factors as well as risk of deep venous thrombosis were not affected by these polymorphisms, a possible explanation could be that a changed level of cysteinyll leukotrienes could influence the level of platelet activating factor, and therefore risk of ICVD. Indeed, the leukotriene C₄ synthase genotype may result in a changed basal or stimulated transcript level of the LTC₄ synthase protein, and thus in a more or less pronounced synthesis of cysteinyll leukotrienes. Though 2 studies did not identify a role for the LTC₄ synthase −1072 G/A, −444 A/C, and common combined genotypes, the LTC₄ synthase −1072 G/A and −444 A/C genotypes predict increased risk, whereas the −444 CC genotype predict decreased risk of ICVD, as demonstrated in the present study. Limitations include that DNA samples were not obtained before the 1991 to 1994 examination; however, genotypes are present at birth even if only determined later in life. If mortality was higher among individuals with the combined AA-AA versus GG-AA versus GG-CC genotype, our study mortality was higher among individuals with the combined AA genotype even if only determined later in life. If this indeed were true, it would mean that an increase in LTC₄ levels correlates with a 40% decrease in risk of ICVD, as demonstrated in the present study.

In conclusion, we found that leukotriene C₄ synthase −1072 AA genotype predict increased risk, whereas the −444 CC genotype predict decreased risk of ICVD.

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Disclosures

None.

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In conclusion, we found that leukotriene C₄ synthase −1072 AA genotype predict increased risk, whereas the −444 CC genotype predict decreased risk of ICVD.
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