Arachidonate 5-Lipoxygenase Promoter Genotype, Dietary Arachidonic Acid, and Atherosclerosis

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Abstract

BACKGROUND
Leukotrienes are inflammatory mediators generated from arachidonic acid (polyunsaturated n–6 fatty acid) by the enzyme 5-lipoxygenase. Since atherosclerosis involves arterial inflammation, we hypothesized that a polymorphism in the 5-lipoxygenase gene promoter could relate to atherosclerosis in humans and that this effect could interact with the dietary intake of competing 5-lipoxygenase substrates.

METHODS
We determined 5-lipoxygenase genotypes, carotid-artery intima–media thickness, and markers of inflammation in a randomly sampled cohort of 470 healthy, middle-aged women and men from the Los Angeles Atherosclerosis Study. Dietary arachidonic acid and marine n–3 fatty acids (including a competing 5-lipoxygenase substrate that reduces the production of inflammatory leukotrienes) were measured with the use of six 24-hour recalls of food intake.

RESULTS
Variant 5-lipoxygenase genotypes (lacking the common allele) were found in 6.0 percent of the cohort. Mean (±SE) intima–media thickness adjusted for age, sex, height, and racial or ethnic group was increased by 80±19 µm (95 percent confidence interval, 43 to 116; P<0.001) among carriers of two variant alleles, as compared with carriers of the common (wild-type) allele. In multivariate analysis, the increase in intima–media thickness among carriers of two variant alleles (62 µm, P<0.001) was similar in this cohort to that associated with diabetes (64 µm, P=0.01), the strongest common cardiovascular risk factor. Increased dietary arachidonic acid significantly enhanced the apparent atherogenic effect of genotype, whereas increased dietary intake of n–3 fatty acids blunted the effect. Finally, the plasma level of C-reactive protein, a marker of inflammation, was increased by a factor of 2 among carriers of two variant alleles as compared with that among carriers of the common allele.

CONCLUSIONS
Variant 5-lipoxygenase genotypes identify a subpopulation with increased atherosclerosis. The observed diet–gene interactions further suggest that dietary n–6 polyunsaturated fatty acids promote, whereas marine n–3 fatty acids inhibit, leukotriene-mediated inflammation that leads to atherosclerosis in this subpopulation.

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Eicosanoids are lipid mediators of inflammation and hypersensitivity reactions, and arachidonate 5-lipoxygenase is the key enzyme in the oxidative biosynthesis of a class of paracrine and autocrine eicosanoids known as leukotrienes. The dihydroxy leukotriene Β₄ is a potent leukocyte chemoattractant, whereas the cysteinyl leukotrienes increase vascular permeability and promote contraction of vascular smooth muscle. The cysteinyl leukotrienes have been linked to asthma, and 5-lipoxygenase promoter genotypes have been identified that interacted strongly with the effects of 5-lipoxygenase inhibition in patients with asthma. This drug–gene interaction suggested a functional difference in the 5-lipoxygenase pathway between carriers of at least one common (wild-type) allele and carriers of two variant alleles.

Atherosclerosis is a chronic inflammatory process involving the recruitment and accumulation of monocytes, macrophages, and dendritic cells in artery walls, where they become loaded with modified and aggregated low-density lipoproteins (LDLs). Molecular determinants of the pathologic chronicity of this process are unknown.

The 5-lipoxygenase pathway has been linked to atherosclerosis in mouse models and in a histologic study in humans. These findings suggested the hypothesis that variation in the 5-lipoxygenase promoter could alter eicosanoid-mediated inflammatory circuits in the artery wall and promote atherogenesis. Since the intake of arachidonic acid increases and the intake of marine n–3 fatty acids reduces the production of leukotriene Β₄ in human monocytes, we further hypothesized that dietary intake of competing 5-lipoxygenase substrates would interact with an atherogenic effect of genotype. We investigated these hypotheses by relating carotid-artery intima–media thickness to 5-lipoxygenase promoter genotypes and dietary intake in the Los Angeles Atherosclerosis Study. Intima–media thickness is an indicator of systemic atherosclerosis that strongly predicts atherothrombotic events.

**Methods**

**Cohort**

The cohort of 573 women (age, 45 to 60 years) and men (age, 40 to 60 years) was free of diagnosed cardiovascular disease when randomly sampled from an employee population. Hispanic subjects and smokers were oversampled, and the participation rate was 85 percent. Base-line examinations in 1995–1996 were followed by two examinations at 1.5-year intervals, in which buffy coat was collected (from 500 subjects) for DNA extraction.

The study protocol was approved by the institutional review board of the Keck School of Medicine in Los Angeles. All subjects provided written informed consent.

**Intima–Media Thickness**

The degree of atherosclerosis in the posterior wall of the common carotid arteries was estimated bilaterally in the base-line examination as intima–media thickness by high-resolution B-mode ultrasonography, as described previously. The coefficient of variation was 2.8 percent for repeated scans by different sonographers.

**Genotyping**

DNA was isolated from 500 subjects, and the number of tandem Sp1 binding motifs (5′GGCGGG3′) in the 5-lipoxygenase (ALOX5) promoter was determined in 470 subjects according to previously described methods. This group included non-Hispanic whites (55.1 percent), Hispanic subjects (29.6 percent), Asian or Pacific Islanders (7.7 percent), blacks (5.3 percent), and other groups (2.3 percent). The genotype of 30 specimens could not be determined owing to polymerase-chain-reaction failure. The resulting six alleles had relative frequencies of 2.9 percent, 13.1 percent, 80.5 percent, 2.8 percent, 0.5 percent, and 0.2 percent for three, four, five, six, seven, or eight tandem Sp1 motifs, respectively. Variant alleles involved deletions (one or two) or additions (one, two, or three) of Sp1 motifs to the five tandem motifs in the common allele. The distribution of the genotypes did not significantly deviate from that expected by random combination of variant and common alleles within any of the racial or ethnic groups (P≥0.05 according to Hardy–Weinberg equilibrium χ²).

**Laboratory Measurements**

Data on serum lipid levels were available for all 470 genotyped subjects. Plasma levels of C-reactive protein, interleukin-6, interleukin-8, and tumor necrosis factor α were measured at base line in 27 of the 28 carriers of two variant alleles and in 38 matched carriers of the common allele, with the use of commercially available, high-sensitivity kits. Fatty acids
in frozen plasma were also measured in this subgroup (at the Heber Laboratory at UCLA) for validation of dietary measurements.

**Dietary Intake**

Six 24-hour recalls of food intake were obtained from the subjects over a period of 1.5 years with the use of the Nutrition Data System. Fatty-acid intake (expressed as grams per 1000 kcal) was averaged over the six dietary recalls. Intakes of long-chain n–3 polyunsaturated fatty acids (eicosapentaenoic and docosahexaenoic acids) were summed, owing to the high correlation between the intake of these two fatty acids (rank-order correlation, 0.85). The intakes of arachidonic acid and its metabolic precursor (linoleic acid) were only weakly correlated (r=0.15) and were estimated separately. Plasma and tissue levels of linoleic acid and long-chain n–3 polyunsaturated fatty acids correlate with dietary intake of corresponding fatty acids, and these associations were estimated in a validation subsample of 66 subjects. Spearman correlations between dietary intake (expressed as grams per 1000 kcal) and the corresponding fatty acid in plasma (expressed as a percentage of total fatty acids) were 0.53 (P<0.001) for eicosapentaenoic plus docosahexaenoic acids and 0.32 (P=0.01) for linoleic acid.

**Statistical Analysis**

Adjusted means and P values for differences between genotype groups were estimated at the mean value of covariates by least-squares regression. The relative odds of an elevated intima–media thickness were estimated by ordinal logistic regression with the use of deciles of intima–media thickness as the ordinal outcome. For figures depicting relations according to three ordinally ranked groups, reported P values are from models with continuous ordinal variables. The covariates in statistical models relating intima–media thickness to genotype were age, interaction of genotype with age (centered at 50 years of age), sex, height, and racial or ethnic group (model 1); all factors in model 1 plus cigarette-smoking status (currently, formerly, or never), level of physical activity, dietary intake of saturated fat (expressed as a percentage of energy intake), and intake of alcohol (model 2, behavioral risk factors); and all factors in model 2 plus serum cholesterol level, serum high-density lipoprotein (HDL) cholesterol level, systolic blood pressure, body-mass index (the weight in kilograms divided by the square of the height in meters), presence or absence of diabetes (type 1 or 2), use of antihypertensive medication, and use of lipid-lowering medication (model 3, biologic risk factors and preventive treatments).

The primary analyses compared carriers of at least one common allele with carriers of two variant alleles. This categorization was derived from a pharmacogenetic interaction involving these two genotype groups. Some a posteriori comparisons for additional 5-lipoxygenase genotype subgroups are also presented for the purpose of hypothesis generation.

**Results**

Genotyping yielded 442 carriers of the 5-lipoxygenase common allele (94.0 percent) and 28 carriers of two variant alleles (6.0 percent). Major cardiovascular risk factors are presented for the cohort in Table 1, according to promoter genotype. No significant differences between carriers of the common allele and carriers of two variant alleles were apparent. However, the prevalence of variant genotypes did differ across racial and ethnic groups (P<0.001 by the chi-square test), with higher prevalences among Asians or Pacific Islanders (19.4 percent), blacks (24.0 percent), and other racial or ethnic groups (18.2 percent) than among Hispanic subjects (3.6 percent) and non-Hispanic whites (3.1 percent).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Carriers of the Common Allele (N=442)</th>
<th>Carriers of Two Variant Alleles (N=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>50.0±4.6</td>
<td>49.3±4.8</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>128±16</td>
<td>132±14</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/liter)</td>
<td>5.6±0.7</td>
<td>5.4±1.0</td>
</tr>
<tr>
<td>Total HDL</td>
<td>1.5±0.3</td>
<td>1.4±0.4</td>
</tr>
<tr>
<td>Female sex (%)</td>
<td>46.8</td>
<td>42.9</td>
</tr>
<tr>
<td>Current smoking (%)</td>
<td>24.2</td>
<td>21.4</td>
</tr>
<tr>
<td>Former smoking (%)</td>
<td>26.5</td>
<td>25.0</td>
</tr>
<tr>
<td>Diabetes (type 1 or 2) (%)</td>
<td>2.3</td>
<td>3.6</td>
</tr>
</tbody>
</table>

*Plus–minus values are means ±SD. There were no significant differences between groups with the use of a t-test for continuous variables and the chi-square test for categorical variables. The common (wild-type) 5-lipoxygenase allele has five tandem Sp1 motifs. To convert values for cholesterol to milligrams per deciliter, divide by 0.02586.
Table 2. Carotid Intima–Media Thickness According to 5-Lipoxygenase Genotype.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intima–Media Thickness</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carriers of the Common Allele (N=442)</td>
<td>Carriers of Two Variant Alleles (N=28)</td>
</tr>
<tr>
<td>No covariates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>661±95</td>
<td>736±41</td>
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<td>Bootstrap P value</td>
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<tr>
<td>Median</td>
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<td>725</td>
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<tr>
<td>Minimum</td>
<td>428</td>
<td>526</td>
</tr>
<tr>
<td>Maximum</td>
<td>1096</td>
<td>1076</td>
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<tr>
<td>Multivariate analysis</td>
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<tr>
<td>Model 1 (mean ±SE)</td>
<td>661±4</td>
<td>740±18</td>
</tr>
<tr>
<td>Model 2 (mean ±SE)</td>
<td>661±4</td>
<td>739±18</td>
</tr>
<tr>
<td>Model 3 (mean ±SE)</td>
<td>662±4</td>
<td>724±16</td>
</tr>
</tbody>
</table>

† P values for differences between means were computed by regression analysis. The P value for the difference in medians was calculated with the use of the Kruskal–Wallis test. The bootstrap P value was estimated with 1 million samplings.

**5-Lipoxygenase Polymorphism and Atherosclerosis**

Means and medians of carotid intima–media thickness according to 5-lipoxygenase genotype are presented in Table 2. The significance level of the unadjusted elevation of intima–media thickness in the group with two variant alleles was confirmed by nonparametric bootstrap analysis. After adjustment for age, sex, height, and racial or ethnic group, the mean (±SE) intima–media thickness was elevated by 80±19 µm among carriers of two variant alleles, as compared with carriers of the common allele (95 percent confidence interval, 43 to 116; P<0.001) (Table 2). This elevation remained significant after adjustment for behavioral risk factors (78±19 µm, P<0.001) and biologic confounders or mediators and preventive treatments (62±17 µm, P<0.001) (Table 2). The magnitude of the apparent genotype effect in this last model is similar to that associated with diabetes in this cohort (64±26 µm, P=0.01) and larger than that associated with current smoking (45±11 µm, P<0.001).

This apparent atherogenic effect did not significantly interact with sex or smoking status, but it did increase with age (P for interaction=0.04). The intima–media thickness was greater in the group with two variant alleles within all five racial and ethnic categories. These differences between genotypes were greatest among blacks and smallest among non-Hispanic whites, with the differences in other groups being intermediate (data not shown). However, these differences were not statistically significant (P=0.26).

The relative magnitude of this 5-lipoxygenase genotype association was estimated with the use of ordinal logistic regression. After adjustment for age, sex, height, and racial or ethnic group, the odds of increased wall thickness were elevated by a factor of 4 among carriers of two variant alleles as compared with carriers of the common allele (odds ratio, 4.1; 95 percent confidence interval, 2.1 to 8.2; P<0.001). Adjustment for numerous potential confounders did not markedly attenuate this relation (odds ratio, 3.7; P<0.001).

The association between genotype and intima–media thickness was further investigated in five 5-lipoxygenase genotype groups derived from combinations of common (W), deletion (D), and addition (A) alleles: DD (18 subjects), DA (9), WD (105), WA (22), and WW (315); the AA genotype was observed in only 1 subject (intima–media thickness, 661 µm). The differences among the five genotype groups confirmed the presence of a recessive pattern of effects (Fig. 1).

**Diet–Gene Interactions**

If the observed increase in the intima–media thickness in the 5-lipoxygenase variants was due to increased production of leukotrienes (e.g., leukotriene B4), then increased availability of the 5-lipoxygenase substrate arachidonic acid and its metabolic precursor (linoleic acid) could amplify the atherogenic effect of the variant genotypes. Similarly, increased intake of eicosapentaenoic and docosahexaenoic acids could reduce the production of inflammatory leukotrienes and inhibit this effect.4

Increased intima–media thickness was significantly associated with intake of both arachidonic acid (P for trend <0.001) and linoleic acid (P for trend=0.03) among carriers of two variant alleles (Fig. 2A and 2B) but not among carriers of the common allele (P values for interaction are listed in Fig. 2). In contrast, the intake of marine n–3 fatty acids was significantly and inversely associated with intima–media thickness only among carriers of two variant alleles.
variant alleles (P for trend=0.007) (Fig. 2C). Diet–gene interactions were specific to these fatty acids and were not observed for dietary intake of mono-unsaturated fat (Fig. 2D), saturated fat (Fig. 2E), or other measured fatty acids (data not shown).

**LDL–GENE INTERACTION**

LDL cholesterol levels in plasma did not differ significantly between 5-lipoxygenase carriers of two variant alleles and carriers of the common allele (P=0.33), nor were there significant differences across the five genotype groups (P=0.21 by analysis of variance) (Fig. 3A). However, the LDL cholesterol level was a more potent atherogenic factor among carriers of two variant alleles than among carriers of the common allele (Fig. 3B).

**5-LIPOXYGENASE POLYMORPHISM AND INFLAMMATION**

The level of C-reactive protein was increased by a factor of 2 among carriers of two variant alleles, as compared with carriers of the common allele (mean, 2.6 vs. 1.3 mg per liter; P=0.007), and levels of interleukin-6 and tumor necrosis factor α were marginally increased by 32 percent (P=0.07) and 17 percent (P=0.11), respectively. The level of interleukin-8 was decreased by 3 percent among carriers of two variant alleles (P=0.77). These analyses of logarithmically transformed values included the covariates age, sex, height, smoking status, and racial or ethnic group.

We found a large increase in carotid intima–media thickness among carriers of two variant 5-lipoxygenase promoter alleles as compared with carriers of the common allele. After multivariate adjustment, the apparent atherogenic effect remained as large as that associated with diabetes. This association was also robust across racial and ethnic groups that differed in the prevalence of variant genotypes. This strong association contrasts with weak associations between polymorphic variation in other inflammatory-pathway genes and cardiovascular disease outcomes.

The diet–gene interactions we observed suggest an effect of genotype on atherosclerosis mediated by the 5-lipoxygenase pathway. Increased dietary intake of n−6 fatty acids (arachidonic acid and its metabolic precursor, linoleic acid) was associated with increased severity of atherosclerosis only among carriers of two variant alleles. Such an interaction would be expected if, for example, the production of leukotrienes in the artery wall was increased and triggered atherogenesis in the variant group. Arachidonic acid is the primary substrate for 5-lipoxygenase, and increased intakes of linoleic acid and arachidonic acid enhance the production of leukotrienes. This increase could induce an atherogenic chronicity of inflammatory circuits in the artery wall.

We also found that increased dietary intake of marine n−3 fatty acids blunted the apparent atherogenic effect of the variant genotypes. This interaction was also suggestive of a leukotriene-mediated effect, since eicosapentaenoic acid is a competing substrate for 5-lipoxygenase. Feeding eicosapentaenoic acid and docosapentaenoic acid to humans reduces the production of leukotriene B4 by activated monocytes. The intake of marine n−3 fatty acids shifts the production of leukotrienes from the more active B4 form to the less active B5 form and may also induce the production of other anti-inflammatory mediators. Involvement of the 5-lipoxygenase pathway in these diet–gene interactions was further implicated by the lack of such interactions with other dietary fatty acids.

There is considerable evidence that fish-oil intake protects against sudden death from cardiac causes—a antiarrhythmic effect that could
Figure 2. Diet–Gene Interactions between Dietary Intake of Arachidonic Acid (Panel A), Linoleic Acid (Panel B), Eicosapentaenoic Acid (EPA) plus Docosahexaenoic Acid (DHA) (Panel C), Monounsaturated Fatty Acids (Panel D), or Saturated Fatty Acids (Panel E) and the Effect of 5-Lipoxygenase Promoter Genotype on Carotid Intima–Media Thickness (IMT).

All interactions were adjusted for age, sex, height, smoking status, and racial or ethnic group; the interactions shown in Panels A, B, and C were adjusted for one another. Arachidonic acid is 5,8,11,14-eicosatetraenoic acid (20:4n–6); linoleic acid is 9,12-octadecadienoic acid (18:2n–6); eicosapentaenoic acid is 5,8,11,14,17-eicosapentaenoic acid (20:5n–3); and docosahexaenoic acid is 4,7,10,13,16,19-docosahexaenoic acid (22:6n–3). Means (±SE) are shown. Because of rounding, categories appear to overlap.
be mediated by cysteinyl leukotrienes.\textsuperscript{33} Taken together, our findings suggest an antiatherosclerotic effect of fish oils among carriers of two 5-lipoxygenase variant alleles and are consistent with the occurrence of increased leukotriene production in this group. The finding that LDL cholesterol was more atherogenic among carriers of two variant alleles is consistent with enhanced LDL-mediated atherosclerosis in that group,\textsuperscript{34} and among these subjects the C-reactive protein levels, which increased by a factor of two, were consistent with the presence of markedly greater chronic arterial inflammation in this group.\textsuperscript{35}

Although data on the 5-lipoxygenase pathway and atherosclerosis are limited, available evidence from two studies in animals and a histologic study in humans is consistent with the hypothesis that increased leukotriene production has an atherogenic effect. The extent of atherosclerosis in the aortic arch was greatly reduced in susceptible mice carrying one null 5-lipoxygenase allele, as compared with the extent among carriers of two functional alleles,\textsuperscript{9} suggesting that this inflammatory pathway is important in atherosclerosis. In a second study, foam-cell formation was reduced in three strains of atherosclerosis-susceptible mice treated with a leukotriene-B\textsubscript{4}–receptor antagonist.\textsuperscript{10} Third, a recent histologic study in humans found an abundance of 5-lipoxygenase (but not 15-lipoxygenase) in macrophages and foam cells, dendritic cells, and artery-wall cells from atherosclerotic lesions.\textsuperscript{11}

Combining these findings with those of recent studies of leukotriene receptors expressed by endothelial cells and macrophages,\textsuperscript{25,36} Habenicht’s group has proposed a model of leukotriene-mediated vascular inflammation in atherosclerosis.\textsuperscript{11,25} In this model, leukotrienes produced by macrophages and dendritic cells in the artery wall have autocrine effects and paracrine effects on endothelial cells, lymphocytes, smooth-muscle cells, and other macrophages or dendritic cells. Up-regulation of this “inflammatory circuit” by environmental or genetic factors would promote atherosclerosis by enhancing the known effects of leukotrienes on the recruitment of leukocytes, endothelial-cell dysfunction, intimal edema, the proliferation of smooth-muscle cells, and immune reactivity.\textsuperscript{11} This model suggests a mechanism whereby increased expression of the 5-lipoxygenase gene or activity of the enzyme in carriers of variant genotypes leads to increased carotid intima–media thickness.

Although a previously reported drug–gene interaction suggested that variant 5-lipoxygenase genotypes have a strong effect on function,\textsuperscript{6} and the gene–diet interactions found in our study are consistent with a hypothesis of increased leukotriene production among promoter variants, findings in vitro studies of gene expression do not provide support for such a hypothesis.\textsuperscript{5,37} Experiments with drosophila SL2 (Schneider) cells found increased reporter construct activity for an addition allele (as compared with the common allele), but activity was
reduced with transfection of deletion alleles.\textsuperscript{37} Reporter constructs in HeLa cells showed a different pattern: gene expression was reduced for both addition and deletion alleles.\textsuperscript{5} However, these promoter–reporter construct studies in nonhuman cells (Schneider) and in tumor cells that do not express 5-lipoxygenase (HeLa) may not reflect the in vivo process of vascular inflammation. Only certain types of cells express the 5-lipoxygenase gene, apparently as a result of different patterns of methylation,\textsuperscript{38} and expression of the gene is regulated by activating factors. Moreover, Serio and colleagues found that the expression of the leukotriene C\textsubscript{4} synthase gene (a leukotriene-pathway gene) in HeLa cells was not regulated in the same manner as that observed in a monocyte-like cell line.\textsuperscript{39} Another investigation of the effect of this polymorphism on leukotriene production by human eosinophils found that variant promoter genotypes only showed increased leukotriene production when cells were stimulated by a calcium ionophore and cyclooxygenase pathways were inhibited.\textsuperscript{40} These findings suggest that the effect of variation in the 5-lipoxygenase promoter sequence of tandem Sp1 motifs on leukotriene production in the artery wall may only be detectable in human macrophages and dendritic cells under conditions that mimic the intimal microenvironment.

If the 5-lipoxygenase pathway in cells involved in atherosclerosis is down-regulated among carriers of two variant alleles, as suggested by the reduction in reporter construct activity in HeLa cells, then our observation of increased atherosclerosis among such subjects appears paradoxical. However, inflammatory pathways are redundant and interacting,\textsuperscript{41} and down-regulation of the 5-lipoxygenase pathway can result in up-regulation of other eicosanoid pathways.\textsuperscript{42} Other candidate eicosanoid pathways for the promotion of atherosclerosis include the 15-lipoxygenase and cyclooxygenase 2 pathways,\textsuperscript{43,44} which are also differentially affected by dietary intake of n–6 and marine n–3 polyunsaturated fatty acids.\textsuperscript{29,45} However, evidence of the atherogenic effects of these other eicosanoid pathways is mixed,\textsuperscript{11,46,47} and n–3 polyunsaturated fatty acids have a much stronger effect on leukotriene production than on other eicosanoids.\textsuperscript{45}

Another possible mechanism linking down-regulation of 5-lipoxygenase to atherosclerosis in carriers of variant genotypes would involve reduced transcellular biosynthesis of antiinflammatory 5-lipoxygenase products such as lipoxins.\textsuperscript{48} However, such a model is inconsistent with the diet–gene interactions we observed and with the substantial reduction of atherosclerosis in 5-lipoxygenase–deficient mice.\textsuperscript{9}

Weaknesses of our study stem from its observational design. The 5-lipoxygenase polymorphic variation could be confounded by unmeasured environmental or genetic factors. The large magnitude of the apparent effect of the genotype relative to other risk factors for atherosclerosis makes confounding with known risk factors unlikely. Although the possibility of linkage disequilibrium with other polymorphisms clearly cannot be ruled out, the confounding polymorphism must have a profound effect on atherosclerosis. Furthermore, the observed diet–gene interactions provide a clear link between the apparent atherogenic polymorphic effect and pathways affected by the intake of n–3 and n–6 polyunsaturated fatty acids.

If replicated, our findings would constitute clear evidence that genetic variation in an inflammatory pathway,\textsuperscript{7} and the leukotriene pathway in particular,\textsuperscript{49} can trigger atherogenesis in humans. These findings could lead to new dietary and targeted molecular approaches to the prevention and treatment of cardiovascular disease according to genotype, particularly in populations of non-European descent.

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\textbf{References}


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Patency and the Pump — The Risks and Benefits of Off-Pump CABG

Eicosanoids are lipid mediators of inflammation; they include a variety of compounds (prostaglandins, thromboxanes, leukotrienes, hydroxy- and epoxy-fatty acids, lipoxins, and isoprostanes) that are derived from the ubiquitous 20-carbon atom arachidonate (20 in Greek is “eicosa”) and a few sim-
ilar polyunsaturated fatty acids. These fatty acids are esterified in the phospholipids of biologic membranes and then released in response to a variety of stimuli to become substrates for metabolizing enzymes. More than 20 years ago, Samuelsson and coworkers identified leukotrienes as a class of eicosanoids that are derived through the action of 5-lipoxygenase. This enzyme, which is selectively expressed in bone marrow–derived cells such as neutrophils, monocytes, macrophages, dendritic cells, and mast cells, catalyzes the transformation of arachidonic acid into leukotriene A$_4$. Leukotriene A$_4$, in turn, is either converted by enzymatic hydrolysis into leukotriene B$_4$ or conjugated with glutathione to form leukotriene C$_4$ (see Figure). Leukotriene C$_4$ and its metabolites leukotrienes D$_4$ and E$_4$ are together referred to as cysteinyl leukotrienes. Leukotriene B$_4$ is a potent chemoattractant for neutrophils, macrophages, and other inflammatory cells and induces chemokinesis and adhesion of these cells to the vascular endothelium. In contrast, cysteinyl leukotrienes increase vascular permeability and contract smooth-muscle cells, causing bronchoconstriction and vasoconstriction.

Leukotrienes have been identified as mediators of a variety of inflammatory and allergic conditions, including rheumatoid arthritis, inflammatory bowel disease, psoriasis, and allergic rhinitis, but their most relevant pathophysiological implication so far has been a link to bronchial asthma. Blockers of the cysteinyl leukotriene receptor CysLT$_1$, or of 5-lipoxygenase are now being marketed worldwide as effective antiasthmatic medications.

Despite the fact that antiasthmatic drugs have proved to be effective in most patients with many types of asthma, their efficacy varies considerably among patients. It has been suggested that genetic variations in 5-lipoxygenase contribute to the variable response to antiasthmatic drugs. The transcription rate of the gene encoding 5-lipoxygenase (located on chromosome 10) is controlled by its promoter, and particularly by a region, termed the core promoter, that contains a sequence of GC-rich tandem repeats, which are binding sites for two transcription factors, Sp-1 and Egr-1. Genetic variants in the core promoter region may change the binding of these transcription factors and the rate of 5-lipoxygenase transcriptional activation under inflammatory conditions. Indeed, a family of mutations in the GC-rich region, consisting of the deletion of one such binding site, the deletion of two of them, or the addition of one, was associated with altered (reduced) transcription of the 5-lipoxygenase gene, as compared with the common allele. It was then found that asthma in carriers of these genetic variants had a diminished response to treatment with antileukotriene drugs, indicating a pharmacogenetic effect of the promoter sequences on responses to treatment.

Originally confined to asthma, the implications of these findings have recently been extended unexpectedly to atherosclerosis. In a search for candidate genes that might contribute to the susceptibility to atherosclerosis in two widely used mouse models of atherosclerosis, the apolipoprotein E$^{-/-}$ mouse and the low-density lipoprotein (LDL)–receptor$^{-/-}$ mouse, a locus on mouse chromosome 6 was found to confer almost total resistance to atherogenesis. The 5-lipoxygenase gene at this locus in the mouse turned out to account for the entirety of this effect, since even LDL-receptor$^{-/-}$ mice that were missing only one of the two allelic copies of the 5-lipoxygenase gene had a dramatic decrease (by a factor of about 26) in the extent and severity of lesions. Also, the transplantation of bone marrow (supplying circulating blood cells) from 5-lipoxygenase$^{-/-}$ mice in LDL-receptor$^{-/-}$ mice conferred substantial protection from atherosclerosis, suggesting that 5-lipoxygenase from white cells (most likely monocyte–macrophages) was necessary for atherogenesis.

5-Lipoxygenase may make an important contribution to atherosclerosis because its leukotriene products, which are produced primarily by monocyte–macrophages or dendritic cells in the arterial intima, foster the chemotraction of monocytes, T-cells, or other types of circulating cells within the vessel wall, increase vascular permeability, or both (see Figure). These mechanisms may create a vicious circle in which inflammatory cells, by producing these lipid mediators, cause local vascular inflammation, perpetuating the recruitment of inflammatory cells and the production of mediators. 5-Lipoxygenase might also contribute by oxidizing LDL or producing natural ligands for nuclear receptors, such as peroxisome-proliferator–activated receptor $\alpha$ (PPAR$\alpha$).

Could it be that genetic variants of the 5-lipoxygenase promoter, which have previously been found to be associated with variable sensitivity to antiasthmatic medications, also influence atherosclerosis? In this issue of the Journal, Doyer and coworkers (pages 29–37) report that this is apparently the case. Variant genotypes of the 5-lipoxygenase gene were found in 6 percent of the 470 healthy, middle-aged women and men in their cohort. Carotid intima–media thickness, used as a marker of the ath-
Erosclerotic burden, was significantly increased, by 80 µm, in the group with variant alleles, as compared with carriers of the common allele. The direction of the change in intima–media thickness in the group with variant alleles is contrary to what one would have expected on the basis of previous in vitro findings and is consistent with increased, rather than decreased, activity of the 5-lipoxygenase promoter associated with the mutant alleles. This issue clearly warrants further investigation through measurements of 5-lipoxygenase expression and of leukotrienes in these genetic variants; the interaction with recently identified mutations in other genes related to leukotriene production, such as that encoding 5-lipoxygenase–activating protein, should also be evaluated.

Dwyer et al. also report on a diet–gene interaction. Dietary arachidonic acid intake significantly enhanced the proatherogenic effect of the 5-lipoxygenase gene variants, whereas intake of n–3 polyunsaturated fatty acids (eicosapentaenoic and docosahexaenoic acids) blunted this effect. Since eicosapentaenoic and docosahexaenoic acids may reduce the formation of leukotrienes by competing with arachidonic acid as substrates for 5-lipoxygenase and also generate the weaker leukotrienes of the 5 series (leukotrienes with five double bonds, in contrast to the leukotrienes derived from arachidonic acid, which have four), these findings suggest that the antiatherogenic effects of eicosapentaenoic and docosahexaenoic acid derived from fish might be more prominent in (or perhaps limited to) persons with genotypes favoring increased 5-lipoxygenase activity.
Overall, these findings contribute to the untangling of the intricate connections between inflammation and atherosclerosis in humans and might actually provide the first proof of a causal link between the two. In addition to indicating potential new targets for therapy in a multifaceted and multifactorial process such as atherosclerosis, they illustrate the complexity of the interactions between nutrients and our genes.

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Advance Care Planning

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One hundred years ago, the odds that a visit to the doctor would result in a measurable improvement in a patient’s condition were slim. But the mere fact that modern physicians are far more likely to be able to influence the course of illness in a particular way does not mean that patients necessarily want them to do so. Patients who are near the end of life often prefer treatment that is focused exclusively on comfort; frail elderly patients may choose to trade longevity for quality of life. Although patients have long been able to refuse burdensome treatment, the U.S. Supreme Court first explicitly stated that patients may accept or reject any proposed treatment, regardless of their underlying medical condition, in the 1990 decision in

Cruzan v. Director, Missouri Department of Health.

In that case, the Court addressed the question of whether artificial nutrition could be withdrawn from a young woman who was in a persistent vegetative state; this is precisely the situation of Terri Schiavo in Florida, whose feeding tube was recently withdrawn at the request of her husband and then reinserted at the order of Governor Jeb Bush, after the Florida legislature granted him the necessary authority.

In Cruzan, while upholding the prerogative of individual states to establish standards for the type of evidence required for the limitation of treatment, the Court supported the right of patients to self-determination. In the words of Justice Sandra Day O’Connor, “The liberty guaranteed [by the Fourteenth Amendment] must protect, if it protects anything, an individual’s deeply personal decision to reject medical treatment, including the artificial delivery of food and water.”1 The implications are far-reaching: only if patients know the benefits and burdens of treatment alternatives can they effectively exercise their autonomy, and only if patients engage in advance care planning can they hope to maintain their autonomy, should they become incapable of making decisions.

Advance directives are widely viewed as the cornerstone of advance planning. They encompass both instructions about what kind of care should be provided (living wills) and who should make the decisions if the patient cannot do so (proxy designations). Though helpful as a means of expressing a general philosophy of end-of-life care, living wills are often vague, using phrases such as “heroic measures,” and most apply only to “terminally ill” patients facing “imminent death.” Some living wills provide concrete instructions for care in a variety of clinical situations, such as the use of feeding tubes or ventilators in the event of permanent unconsciousness. Even detailed instructional directives, however, cannot describe all the situations in which patients may find themselves. Their precision also makes them inflexible: whether or not a given procedure is acceptable may depend on what the intervention is expected to achieve and on what alternatives are available.

Another strategy for advance care planning is to focus on who will make the decisions rather than on what those decisions should be. The naming of a friend or family member to serve as a health care proxy (also referred to as assigning a durable power of attorney for health care) gives physicians someone to talk to who can apply the patient’s preferences to the existing clinical situation. Surrogates are expected to make decisions on the basis of “substituted judgment,” or what the patient would want. If the patient’s views are unknown, surrogates must resort to best interests, or what most people in the same situation would want. Combination directives clarify both who should make decisions and what