Interactions between 5-Lipoxygenase Polymorphisms and Adipose Tissue Contents of Arachidonic and Eicosapentaenoic Acids Do Not Affect Risk of Myocardial Infarction in Middle-Aged Men and Women in a Danish Case-Cohort Study

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Abstract

Background: The 5-lipoxygenase pathway has been linked to atherothrombotic disease, and a functional tandem repeat polymorphism in the arachidonate lipoxygenase-5 (ALOX-5) gene has been associated with the risk of myocardial infarction (MI). Interestingly, 2 studies have reported an interaction between dietary intakes of the ALOX-5 substrates, arachidonic acid (AA) and eicosapentaenoic acid (EPA), and genotype.

Objective: We investigated whether the interactions between the ALOX-5 tandem repeat polymorphism (rs59439148) and adipose tissue AA and EPA were associated with incident MI.

Methods: In the Danish Diet, Cancer and Health study, we conducted a case-cohort study including 3089 participants with incident MI identified from national registries and a randomly selected subcohort of 3000 participants. Participants were men and women with a median age of 56 y at baseline and no previous history of cancer. Adipose tissue and blood samples were collected at baseline along with comprehensive questionnaires on lifestyle and demographic data. The ALOX-5 tandem repeat polymorphism was genotyped by multititer plate sequencing. Associations were analyzed by using Cox proportional hazards models.

Results: We observed a higher risk of MI for homozygous carriers of the variant alleles in the fifth quintile of AA content than for the reference group with the lowest quintile of AA content and carrying the wild-type allele (HR: 3.02; 95% CI: 1.41, 6.44). In contrast, homozygotes for the variant alleles tended to have a higher risk of MI when comparing the lowest quintile of EPA content with the reference group with the highest quintile of EPA and carrying the wild-type allele (HR: 2.15; 95% CI: 0.91, 5.09; P = 0.08). Although our results suggested interactions between the polymorphism and adipose tissue AA and EPA, a quantitative evaluation of interaction by calculating the relative excess risk due to interactions was not significant.

Conclusions: Adipose tissue EPA and AA and the ALOX-5 tandem repeat polymorphism did not significantly interact to affect the risk of MI. However, the results should be replicated in larger, heterogeneous populations.

Keywords: 5-lipoxygenase, cohort study, fatty acids, interaction, myocardial infarction

Introduction

Atherosclerosis is a multifactorial disorder involving complex inflammatory processes in the vessel wall (1, 2). In this context, the 5-lipoxygenase (5-LOX) pathway has received attention and has been linked to atherothrombotic disease in both animal and human studies (3, 4). The 4 key enzymes of the pathway, including arachidonate lipoxygenase-5 (ALOX-5), 5-LOX activating protein, leukotriene B4 hydrolase, and leukotriene C4 synthase, metabolize arachidonic acid (AA) and EPA, leading to the formation of pro-inflammatory leukotrienes (Supplemental Figure 1). Importantly, the 5-series leukotrienes derived from EPA are much less proinflammatory than the 4-series leukotrienes derived from AA (5), and studies have shown that the consumption of marine n-3 PUFA (including EPA) can increase the production of the 5-series leukotrienes at the expense of the more pro-inflammatory 4-series leukotrienes (6).
In line with this, epidemiological studies have generally supported an inverse association between fish intake and the risk of myocardial infarction (MI) (7, 8). In contrast, some studies have reported the intake of AA to be positively associated with the risk of MI (9). We recently tested these findings in a Danish cohort study, the Diet, Cancer and Health study, confirming an inverse association of both dietary fatty fish intake (10) and adipose tissue content of EPA (11) with the risk of MI, whereas AA content in adipose tissue was positively associated with MI (12). As a new approach, we used adipose tissue content of the respective FAs, which is considered a good long-term marker of dietary intake of these FAs, indicating the endogenous exposure to these FAs (13, 14).

A number of studies have examined genetic polymorphisms related to key enzymes in the 5-LOX pathway. Most attention has been focused on the rate-limiting step, catalyzed by ALOX-5. Thus, Dwyer et al. (13) examined a tandem repeat polymorphism in the promoter region of ALOX-5 (rs95439148), containing a varying number of specificity protein 1 transcription factor-binding motifs (5′-GGGGCGG-3′), and found variant alleles associated with higher intima-media thickness of the carotid arteries compared with carriers of 2 wild-type alleles. Since the initial study, this polymorphism has been investigated in a number of studies with different endpoints, including ischemic stroke and MI (16–20), but the results have been conflicting. Interestingly, some studies have suggested an interaction between genotype and intake of marine n-3 PUFAs and/or AA (15, 16), the substrates of the 5-LOX pathway. Thus, a high intake of marine n-3 PUFAs seemed to blunt the effect of the variant genotype, whereas a high intake of AA tended to exacerbate the effect of the variant genotype. Other studies have examined polymorphisms in the ALOX-5 gene, but no polymorphisms other than the tandem repeat have consistently been associated with CVD endpoints. Interestingly, the single nucleotide polymorphism (SNP) rs12762303 was reported to be in close linkage disequilibrium (LD) with the variant and wild-type alleles of the tandem repeat (21), which could provide an easy and more cost-effective means of analyzing the tandem repeat polymorphism.

In this study, we examined the association of this ALOX-5 tandem repeat polymorphism with incident MI, taking into account the relative amounts of AA and EPA in adipose tissue to investigate possible diet-gene interaction. Furthermore, we genotyped the SNP rs12762303 that may be in high LD with the tandem repeat.

**Methods**

**Study design and population.** The Danish Diet, Cancer and Health study is a prospective cohort study, which has been described in detail previously (22). Briefly, 160,725 persons aged 50–64 y were invited to participate between December 1993 and May 1997. Eligible participants were born in Denmark, living in the urban areas of Copenhagen and Aarhus, and not registered with a cancer diagnosis in the Danish Cancer Registry at the time of invitation. A total of 57,053 persons accepted the invitation and were enrolled in the study. Participants registered with a previous MI or cardiac arrest were excluded.

If a cancer diagnosis was reported that was not already recorded in the Cancer Registry at the time of invitation, participants were excluded in line with the intention-to-include criteria. At baseline, each participant filled in a detailed questionnaire on diet, lifestyle, socioeconomic status, and medical history. Blood and adipose tissue samples were collected.

For the present study we used a nested case-cohort design, including all participants with incident MI and a randomly selected subcohort (n = 3000). The subcohort was selected within the Diet, Cancer and Health cohort to represent a subsample of the entire cohort. The principle of randomness was applied by a computer by using the current version of STATA (StataCorp).

The study was conducted in accordance with the Helsinki Declaration and approved by the regional ethics committees.

**DNA extraction.** DNA was extracted from whole blood by using Kleargene XL DNA extraction kit (LG Genomics). The Kleargene method uses a detergent-driven cell lysis technique, followed by guanidinium isothiocyanate-mediated DNA binding to silica. Next, contaminants were removed by washing, and DNA was subsequently eluted into a low-salt buffer. The final DNA product was stored at −20°C until analysis.

**Genotyping of the ALOX-5 tandem repeat polymorphism.** The tandem repeat polymorphism was analyzed by microtitre plate–sequencing technique with the use of standard 96-well plates. PCR products were prepared from genomic DNA by using MyTag DNA polymerase (Bioline Inc.) along with the following primers: 5′-TCAGGAGAAGCGATGGAAAC-3′ (forward) and 5′-GGGCGGATGGTCCCATC-3′ (reverse). Forty reaction cycles were performed at 55°C. From the PCR products, sequencing was performed by using an ABI 3730XL DNA analyzer (Thermo Fischer Scientific Inc.), and chromatograms were interpreted using the chromatogram analysis software. The chromatograms were rechecked by another technician, and the results were discussed and agreed on.

**Genotyping of rs12762303.** SNP genotyping was performed by LG Genomics by using the commercially available KASP genotyping assay (23). The fluorescent signal from the PCR products was analyzed by using a BMG PHERAstar plate reader (BMG Labtech Ltd.). Analysis was performed according to the protocol provided by LG Genomics (24). SNP alleles correspond to the positive and forward DNA strand according to dbSNP, human assembly GRCh38.p2 (25).

**Adipose tissue biopsies.** At baseline, adipose tissue biopsies were taken from the buttocks of all participants by using a Luer lock system (Terumo; Terumo Corp) consisting of a needle, a venoject multisample Luer adaptor, and an evacuated blood tube, according to the method described by Beynen and Katan (26). Samples were flushed with nitrogen and stored at −150°C until analysis. When analyzed, biopsies were thawed and preheated at 50°C for 10 min. Subsequently, the fat was dissolved in heptane at 50°C, and FAs were transesterified by 2 mol/L potassium hydroxide in methanol at 50°C for 2 min. The FA composition was determined by GC by using a Varian 3900 GC with a CP-8400 autosampler (Varian) equipped with a flame ionization detector. Split-injection mode, a CP-sil 88, 50-m × 0.25-mm inner-diameter capillary column, temperature programming from 90°C to 210°C, and constant flow were used. Helium was used as carrier gas. Commercially available standards (Nu-Chek-Prep, Inc.) were used to identify the individual FAs.

The content of FAs was expressed as the weight percentage of total FAs, and the interassay CVs were 3.2% and 6.4% for AA and EPA, respectively.

**Identification of cases.** From the Danish National Patient Registry and/or the Danish Causes of Death Registry we identified all participants in the cohort who were registered with a first-time diagnosis of MI, according to the International Classification of Diseases (ICD) 8 (410.00–410.99) or ICD-10 (I21.0–I21.9) coding, during the study period (27). Furthermore, all cases of cardiac arrest (ICD-8: 427.27 or
Results

Population characteristics. In total, 57,053 (35%) subjects accepted the invitation and were enrolled in the study. Initially 1506 were excluded because baseline questionnaires were missing (n = 42) or participants were identified with a cancer diagnosis in the Danish National Patient Registry before baseline (n = 564) or registered with an MI or cardiac arrest before inclusion (n = 900). We identified 3089 cases of incident MI during a median follow-up time of 17.0 y. Subjects missing information regarding ≥1 covariates used in the adjusted analyses (n = 346), missing DNA samples (n = 255), and missing adipose tissue data (n = 376) were excluded. In total, 2680 cases were included in the analyses, but not all samples were successfully genotyped, and therefore information regarding genotype was missing for 103 and 86 participants for rs12762303 and rs59439148, respectively.

Table 1 describes the cases and the subcohort with respect to important baseline characteristics. Cases had a higher median age, BMI, and waist circumference and included a higher proportion of smokers, whereas weekly physical activity and educational level were lower than in the subcohort. More cases had hypertension, hypercholesterolemia, and diabetes mellitus (Table 1).

Association of the ALOX-5 tandem repeat polymorphism with MI. We genotyped the tandem repeat rs39439148 and calculated genotype frequencies, according to the number of hexamer-repeats (5’TCCGCC-3’) for the 2 alleles, see Table 2. The 5-repeat allele was, by far, the most common allele (84.4%) and considered the wild-type. Next, the 4-repeat allele was the most frequent variant observed (15.2%), whereas alleles with <4 repeats were rare (<1%). As a consequence of the observed allele frequencies, we analyzed the tandem repeat defining the variant allele as alleles with <5 repeats (2–4 repeats) and the wild-type as alleles with ≥5 repeats (5–6 repeats). Table 3 summarizes the allele frequencies of the polymorphism according to our definition of variant or wild type.

Table 4 shows the cross-tabulation of genotype by quintiles of EPA or AA content in adipose tissue. The table displays HRs for the association with incident MI. Looking at AA, we found a positive association with MI when comparing quintile 1 with quintile 5 for both the reference genotype and homozygotes of the variant. Furthermore, HRs were generally higher for homozygous carriers of the variant genotype than for carriers of the wild-type allele across quintiles of AA and EPA, although most associations were not statistically significant (P = 0.01–0.73). This relation seemed to be augmented for the highest quintile (quintile 5) of AA content, where we found a 3-fold higher risk of MI among homozygotes for the variant allele compared with the reference group [HR: 3.02; 95% CI: 1.41, 6.44 (model B)]. Concerning EPA content, we observed a trend toward a negative association with MI (P = 0.02). Accordingly, we selected the highest quintile (quintile 5) as the reference in Table 4 to allow interpretation of the RERI estimates and found the lowest quintile of EPA content to be associated with a higher risk of MI when compared with the reference for all models, e.g., HR: 1.29 (95% CI: 1.05, 1.58), when comparing quintile 1 with quintile 5 for model B. For the reference group with the highest quintile of EPA content, carrier status for the polymorphism did not affect the HRs, but for lower quintiles of EPA, homozygous carriers of the variant had higher HRs than did carriers of the wild type, which was most pronounced for quintile 1 compared with the reference with an HR of 2.15 (95% CI: 0.91, 5.09, P = 0.015) in model B. The associations differed slightly between models.

We also performed sex-stratified analyses and found some discrepancies between men and women (Supplemental Table 1). For women, we observed substantial variations between models with no evident trend across quintiles and large CIs.

Additive measures of the interaction between EPA or AA and ALOX-5 promoter polymorphism. To evaluate a possible interaction between EPA or AA and genotype quantitatively, we calculated RERI estimates, see Table 5. These values reflected
the measures of association from Table 4, but estimation of robust variance measures by bootstrap revealed no statistically significant interactions between the content of EPA or AA in adipose tissue and the genotype.

**TABLE 1** Baseline characteristics of the subcohort and cases

<table>
<thead>
<tr>
<th></th>
<th>Subcohort (n = 1454)</th>
<th>Cases (n = 1924)</th>
<th>Subcohort (n = 1250)</th>
<th>Cases (n = 756)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>56.3 (51.2, 63.2)</td>
<td>57.7 (51.7, 63.9)</td>
<td>56.3 (51.1, 62.9)</td>
<td>59.2 (52.4, 64.1)</td>
</tr>
<tr>
<td>Physical activity, h/wk</td>
<td>2.5 (0.0, 8.5)</td>
<td>2.0 (0.0, 8.0)</td>
<td>2.5 (0.0, 8.0)</td>
<td>2.0 (0.0, 7.0)</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>26.4 (23.0, 31.2)</td>
<td>27.0 (23.4, 32.3)</td>
<td>24.6 (20.9, 31.1)</td>
<td>26.1 (21.1, 33.5)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>95.5 (86.0, 109.0)</td>
<td>97.0 (87.0, 112.0)</td>
<td>80.0 (69.0, 97.0)</td>
<td>84.0 (70.0, 102.0)</td>
</tr>
<tr>
<td>Alcohol intake, g/d</td>
<td>19.4 (3.3, 61.9)</td>
<td>18.2 (2.5, 62.3)</td>
<td>9.4 (1.2, 34.8)</td>
<td>6.5 (0.5, 32.3)</td>
</tr>
<tr>
<td>Smoking</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never smoker</td>
<td>375 (25.6)¹</td>
<td>353 (18.4)</td>
<td>546 (54.6)</td>
<td>214 (28.3)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>515 (35.4)</td>
<td>567 (29.5)</td>
<td>285 (28.5)</td>
<td>146 (19.3)</td>
</tr>
<tr>
<td>&lt;15 g/d</td>
<td>165 (11.4)</td>
<td>248 (12.9)</td>
<td>201 (16.1)</td>
<td>167 (22.1)</td>
</tr>
<tr>
<td>15–25 g/d</td>
<td>242 (16.0)</td>
<td>453 (23.5)</td>
<td>184 (14.7)</td>
<td>189 (25.0)</td>
</tr>
<tr>
<td>&gt;25 g/d</td>
<td>157 (10.8)</td>
<td>303 (15.8)</td>
<td>34 (2.7)</td>
<td>40 (5.3)</td>
</tr>
<tr>
<td>Educational level</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basic school²</td>
<td>402 (33.9)</td>
<td>835 (43.4)</td>
<td>400 (32.0)</td>
<td>334 (44.2)</td>
</tr>
<tr>
<td>Higher education, 1–3 y</td>
<td>817 (62.4)</td>
<td>713 (37.1)</td>
<td>621 (49.7)</td>
<td>351 (46.4)</td>
</tr>
<tr>
<td>Higher education, &gt;3 y</td>
<td>344 (23.7)</td>
<td>378 (19.5)</td>
<td>229 (18.3)</td>
<td>71 (9.4)</td>
</tr>
<tr>
<td>Menopausal status</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>NA</td>
<td>NA</td>
<td>742 (59.4)</td>
<td>530 (70.1)</td>
</tr>
<tr>
<td>Premenopausal</td>
<td>NA</td>
<td>NA</td>
<td>392 (31.4)</td>
<td>129 (17.1)</td>
</tr>
<tr>
<td>Medical history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>215 (14.8)</td>
<td>434 (22.6)</td>
<td>209 (16.7)</td>
<td>240 (31.8)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>123 (8.5)</td>
<td>236 (12.3)</td>
<td>77 (6.2)</td>
<td>101 (13.4)</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>43 (3.0)</td>
<td>99 (5.2)</td>
<td>17 (1.4)</td>
<td>34 (4.5)</td>
</tr>
<tr>
<td>Adipose tissue, % of total FAs</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EPA</td>
<td>0.1 (0.1, 0.2)</td>
<td>0.1 (0.1, 0.2)</td>
<td>0.1 (0.1, 0.2)</td>
<td>0.1 (0.1, 0.2)</td>
</tr>
<tr>
<td>AA</td>
<td>0.3 (0.2, 0.5)</td>
<td>0.4 (0.2, 0.5)</td>
<td>0.4 (0.3, 0.5)</td>
<td>0.4 (0.3, 0.6)</td>
</tr>
</tbody>
</table>

¹ AA, arachidonic acid; NA, not applicable.
² Median; 10th, 90th percentile in parentheses (all such values).
³ Number; percentage in parentheses (all such values).
⁴ Basic school: the first 9 y of mandatory schooling, sometimes called ground or primary school.

**Linkage between the ALOX-5 tandem repeat polymorphism and rs12762303.** We analyzed the association between the SNP, rs12762303, and incident MI, finding measures of association very similar to those of the tandem repeat polymorphism (Supplemental Table 2). Using the Haploview software (33), we found this SNP to be in almost perfect LD with the tandem repeat polymorphism ($D' = 0.99; R^2 = 0.92$).

**Discussion**

Based on the Danish Diet, Cancer and Health cohort, we conducted a case-cohort study to investigate the interaction between the adipose tissue content of AA and EPA and the ALOX-5 tandem repeat polymorphism on incident MI. Cross-tabulation of genotype by quintiles of AA or EPA content indicated a higher risk of MI for carriers of 2 variant alleles. Although the relation between genotype and MI seemed to be augmented by a higher content of AA compared with the reference group with the lowest quintile of AA content, the opposite was observed for EPA. However, the interactions explored on an additive scale assessed by calculation of RERI estimates were not statistically significant.

**Strengths and limitations.** The Diet, Cancer and Health study is a large prospective cohort study, and although earlier studies on the 5-LOX pathway used a case-control design, this study holds the advantages of the prospective design. This might be of importance, in particular when assessing combined effects of...
genetics and diet, because dietary factors are more sensitive to confounding than genotype information alone. Exposure to EPA and AA was assessed by adipose tissue content of the respective FAs (EPA) and as a biomarker it may represent the endogenous FAs, which reflects the average long-term (1–3 y) exposure to and AA was assessed by adipose tissue content of the respective

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### TABLE 3 Distribution of alleles for rs59439148 and rs12762303, ALOX-5 promoter polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genomic position</th>
<th>Allele</th>
<th>Subcohort Men</th>
<th>Cases</th>
<th>Subcohort Women</th>
<th>Cases</th>
<th>All</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs59439148</td>
<td>10: 4537413 (2–7)</td>
<td>V/W</td>
<td>432 (15.4)</td>
<td>589 (15.6)</td>
<td>408 (16.6)</td>
<td>216 (14.5)</td>
<td>840 (15.8)</td>
<td>805 (15.3)</td>
</tr>
<tr>
<td>rs12762303</td>
<td>10: 45373723</td>
<td>C/T</td>
<td>402 (14.1)</td>
<td>549 (14.6)</td>
<td>388 (15.8)</td>
<td>205 (13.9)</td>
<td>790 (14.9)</td>
<td>754 (14.4)</td>
</tr>
</tbody>
</table>

1 Values are allele frequencies for the minor allele (underlined). rs59439148 alleles were defined as wild type if the number of hexamer-repeats (5'-GGGCGG-3') was ≥5 (5–6 repeats) and as variant if <5 repeats (2–4 repeats). Alleles correspond to the positive DNA strand, and genomic positions are obtained from dbSNP, human assembly (25). ALOX-5, arachidonate 5-lipoxygenase; C, cytosine; T, thymine; V, variant allele; W, wild-type allele.

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### TABLE 4 Cross-tabulation of adipose tissue content of EPA or AA and genotype of tandem repeat rs59439148 for the association with MI in middle-aged men and women

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Model A²</th>
<th>Model B²</th>
<th>Model C⁴</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EPA, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Q5 (0.16)</td>
<td>1 (ref)</td>
<td>0.85 (0.39, 1.82)</td>
</tr>
<tr>
<td></td>
<td>Q4 (0.12)</td>
<td>1.18 (0.98, 1.41)</td>
<td>1.02 (0.89, 1.26)</td>
</tr>
<tr>
<td></td>
<td>Q3 (0.10)</td>
<td>1.09 (0.90, 1.32)</td>
<td>1.50 (0.52, 2.23)</td>
</tr>
<tr>
<td></td>
<td>Q2 (0.08)</td>
<td>1.26 (1.06, 1.51)</td>
<td>1.51 (1.00, 2.35)</td>
</tr>
<tr>
<td></td>
<td>Q1 (0.05)</td>
<td>1.30 (1.09, 1.55)</td>
<td>1.52 (0.80, 2.26)</td>
</tr>
</tbody>
</table>

|          | AA, %     |          |          |
|          | Q5 (0.26) | 1 (ref)  | 1.26 (0.52, 3.07) | 1.17 (0.60, 2.32) |
|          | Q4 (0.32) | 1.08 (0.90, 1.29) | 1.30 (0.86, 2.69) | 1.05 (0.87, 1.27) | 1.37 (0.66, 2.86) | 1.31 (0.61, 2.66) |
|          | Q3 (0.36) | 1.26 (1.05, 1.50) | 1.18 (0.80, 2.80) | 1.16 (0.95, 1.42) | 1.15 (0.55, 2.42) | 1.20 (0.98, 1.46) | 1.11 (0.53, 2.35) |
|          | Q2 (0.42) | 1.46 (1.21, 1.75) | 1.39 (0.64, 3.02) | 1.16 (0.93, 1.43) | 1.50 (0.66, 3.50) | 1.24 (1.00, 1.53) | 1.66 (0.74, 3.72) |
|          | Q1 (0.51) | 1.61 (1.35, 1.93) | 2.91 (1.40, 6.03) | 1.17 (0.94, 1.46) | 3.02 (1.41, 6.44) | 1.32 (1.05, 1.65) | 2.50 (1.14, 5.52) |

1 Values are HRs (95% CIs) from Cox proportional hazard models cross-tabulated by quintiles of EPA or AA in adipose tissue (median content in parentheses) and genotype of rs59439148. The reference group was either the lowest quintile of FAs (AA) or the highest quintile of FAs (EPA) and carriers of 1 or 2 wild-type alleles. AA, arachidonic acid; MI, myocardial infarction; Q, quintile; ref, reference; V, variant allele; W, wild-type allele.

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1 Model A: crude analyses adjusted for sex.
2 Model B: adjusted for lifestyle and demographic measures, including sex, smoking status, educational level, physical activity, BMI, waist circumference, and alcohol consumption with additional medical history variables, including history of diabetes mellitus, hypertension, and hypercholesterolemia.
3 Model C: adjusted for lifestyle and demographic measures, including sex, smoking status, educational level, physical activity, BMI, waist circumference, and alcohol consumption with additional dietary variables, including adipose tissue content of total saturated, monounsaturated, and trans FAs and dietary fiber.
outcome events, but subjects might change their lifestyle and habits over time. Also, public awareness of disease prevention and changes in standard medical care might influence the participants’ risk profile and limit our ability to address confounding.

**General discussion.** Since the hallmark study by Dwyer et al. (15) reported that the ALOX-5 tandem repeat polymorphism (rs59439148) is associated with higher intima-media thickness, a number of studies have investigated this polymorphism on other relevant endpoints, e.g., patients with MI and coronary artery disease verified by angiography. Notably, 2 independent case-control studies in MI patients with Northern European origin (Caucasians), did not find an association between MI and variants of the polymorphism (17, 18). In a mixed population of Caucasians and African Americans, Harti et al. (19) demonstrated a positive association, but only in African Americans. In general, the previous studies had a limited size, and none of the studies examined the influence of diet. A recent study by our group (36) investigated the overall association between rs59439148 genotypes and MI and found a positive association when comparing homozygotes for the wild type with homozygotes for the variant. However, results were statistically significant only in men (HR: 1.63; 95% CI: 1.06, 2.52), whereas the pooled analyses were borderline significant (HR: 1.35; 95% CI: 0.96, 1.90).

In the present study, our results indicated a positive association between carriers of 2 variant alleles and MI when compared with the reference genotype, but the association was statistically significant only when comparing homozygotes for the variant genotype in the highest quintile of AA content with the reference group. Furthermore, the measures of associations differed, depending on the content of EPA and AA in adipose tissue, where a high content of EPA seemed to blunt the effect of the polymorphism, whereas a high content of AA seemed to exacerbate the effect. However, measures of interaction on an additive scale were not statistically significant. The initial study by Dwyer et al. (15) was also the first study to suggest interaction between dietary intake of 5-LOX substrates and the tandem repeat polymorphism, well in line with our findings. Later, the same group performed a large study on Costa Rican nationals, including 1885 cases of MI (16), with no overall effect from the variant genotype, but the group with a high intake of AA had a significantly higher risk of MI when the variant genotype was present, indicating an interaction between AA intake and genotype. No interaction could be detected for marine n-3 PUFAs. Both studies used dietary questionnaires to assess the intake of FAs, whereas our study used adipose tissue content as a biomarker reflecting long-term dietary intake or, more precisely, the endogenous exposure to the respective FAs. Notably, the present and previous studies of the tandem repeat polymorphism suggest that more heterogeneous populations be evaluated in future studies investigating diet-gene interaction, because the variant frequency is lower among Caucasian populations than in those of African or Asian descent, limiting the ability to conclusively identify potential interactions in populations with low variant frequencies. In addition to the observational studies mentioned above, a few intervention studies have examined the effect of fish oil supplementation for the tandem repeat polymorphism and found the variant genotypes to be associated with a lower change in n-3 PUFA metabolites compared with carriers of the wild type, indicating that the response to n-3 PUFA varied by genotype (37, 38) which further supports the hypothesis of an interaction between marine n-3 PUFA and the polymorphism.

In addition to the tandem repeat polymorphism, we genotyped the SNP rs12762303 that is located only 409 base pairs upstream from the tandem repeat and was previously
described to be in close LD with rs59439148 (21), confirming that these 2 polymorphisms were in almost perfect LD. In future studies, it may be relevant to genotype the more cost-efficient SNP, rs12762303, instead of the tandem repeat polymorphism.

Studies have examined other polymorphisms in ALOX-5 (19, 21), but none of these, other than rs59439148 and SNPs linked to this polymorphism, has been associated with atherosclerosis traits. Furthermore, a number of studies have supported a role for other candidate genes in the 5-LOX pathway (39–41), but replication studies have been inconclusive, and despite great efforts, researchers have not been able to agree on functional polymorphisms in any of the other candidate genes except ALOX-5.

One of the key mechanisms linking the 5-LOX pathway to the development of atherosclerosis may be attributed to the formation of leukotrienes and their pro-inflammatory properties. Leukotrienes increase vascular permeability, act as potent chemoattractants, and have other proinflammatory functions (4, 42). Furthermore, a high expression of ALOX-5 and 5-LOX activating protein enzymes has been found in human atherosclerotic plaques and linked to the stage of plaque development (43, 44). Genetic association studies have also provided some evidence of a link between the 5-LOX pathway and atherosclerotic disease, and in this regard, our study adds to the body of evidence investigating the tandem repeat polymorphism in ALOX-5 (rs59439148) as a functional polymorphism. Although our results could not confirm a statistically significant interaction between FA substrates for the 5-LOX enzymes and rs59439148, the measures of interaction were well in line with the previously suggested hypothesis that the differential roles of marine n–3 PUFA and AA in atherosclerotic disease states may, at least partly, be explained by the formation of less inflammatory leukotrienes derived from EPA compared with AA. The present study also adds mechanistic evidence for the role of marine n–3 PUFA and AA in MI through the 5-LOX system.

In conclusion, the present study indicated that homozygote carriers of the variant genotype had a higher risk of incident MI than did carriers of the wild type. Although the results suggested that the relation was augmented by adipose tissue content of AA and attenuated by EPA, no significant interaction was detected when evaluated by calculation of RERI estimates. The study, however, indicates the need for replication studies in larger and more heterogeneous populations.

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