FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease[1–3]

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ABSTRACT

Background: The δ-5 and δ-6 desaturases, encoded by *FADS1* and *FADS2* genes, are key enzymes in polyunsaturated fatty acid (PUFA) metabolism that catalyze the conversion of linoleic acid (LA) into arachidonic acid (AA) and that of α-linolenic acid (ALA) into eicosapentaenoic acid (EPA). Single-nucleotide polymorphisms (SNPs) in FADS1 and FADS2 have been associated with different concentrations of AA and LA, and those associations have possible functional consequences for desaturase activity.

Objective: We aimed to evaluate the possible association among *FADS* genotypes, desaturase activity, inflammation, and coronary artery disease (CAD).

Design: Thirteen FADS SNPs and the ratio of AA to LA (AA/LA) on red blood cell (RBC) membranes, a marker of desaturase activity, were evaluated in 876 subjects with ($n = 610$) or without ($n = 266$) angiographically documented CAD.

Results: Both AA/LA and the ratio of EPA to ALA (EPA/ALA) were higher in patients with CAD than in those without CAD, but, in a multiple logistic regression model, only a higher AA/LA resulted an independent risk factor for CAD (odds ratio: 2.55; 95% CI: 1.61, 4.05 for higher compared with lower ratio tertile; P for trend < 0.001). Furthermore, concentrations of high-sensitivity C-reactive protein increased progressively across tertiles of AA/LA. Graded increases in high-sensitivity C-reactive protein concentrations and CAD risk were related to the carriership of FADS haplotypes, including the alleles associated with a higher ratio.

Conclusion: In populations following a Western diet, subjects carrying FADS haplotypes that are associated with higher desaturase activity may be prone to a proinflammatory response favoring atherosclerotic vascular damage. *Am J Clin Nutr* 2008;88:941–9.

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are important components of cell membranes, and they influence cellular functions by regulating several metabolic pathways, such as the synthesis of inflammatory mediators (1, 2). Arachidonic acid (AA; 20:4n−6) is the precursor of important molecules involved in inflammation, eg, eicosanoids, and is thought to play a role in the atherosclerotic process (3). High concentrations of AA in adipose tissue have been associated with a greater risk of myocardial infarction, which suggests a proatherosclerotic role of excess AA (4, 5).

The fatty acid (FA) composition of the cell membrane is influenced by both dietary intake and metabolic pathways. For example, AA originates from both diet and the elongation-desaturation process of its precursor, linoleic acid (LA; 18:2n−6). The δ-5 (D5D) and δ-6 (D6D) desaturases are key enzymes of this pathway (**Figure 1**): D6D catalyzes the conversion from LA to γ-linolenic acid (18:3n−6), which is elongated to dihomo-γ-linolenic acid (20:3n−6), which is in turn desaturated to AA by D5D. D5D and D6D are also involved in the n−3 FA pathway (Figure 1), which favors the conversion of α-linolenic acid (ALA) into eicosapentaenoic acid (EPA). D5D and D6D are encoded by *FADS1* and *FADS2* genes, respectively. The *FADS* gene cluster is located on chromosome 11 (11q12–13.1) and includes a third gene, *FADS3*, that shares 52–62% sequence identity with the *FADS1* and *FADS2* genes and encodes for an as-yet-unidentified protein (6). Recently, several polymorphisms and haplotypes of the *FADS1-FADS2* gene cluster have been shown to be associated with serum phospholipid PUFA concentrations in German and Italian populations (7, 8). Various *FADS* alleles were associated with high concentrations of AA and low concentrations of LA, an association that provided evidence for a functional effect on desaturase activity. However, these genetic variants may merely be in linkage with as-yet-unidentified causal variants.

Desaturase activity is assayed in vitro or in animals by measurement of the rate of conversion of radiolabeled precursor FAs to their respective products (9), but ethical and practical reasons prevent this possibility in humans. Nonetheless, the use of a

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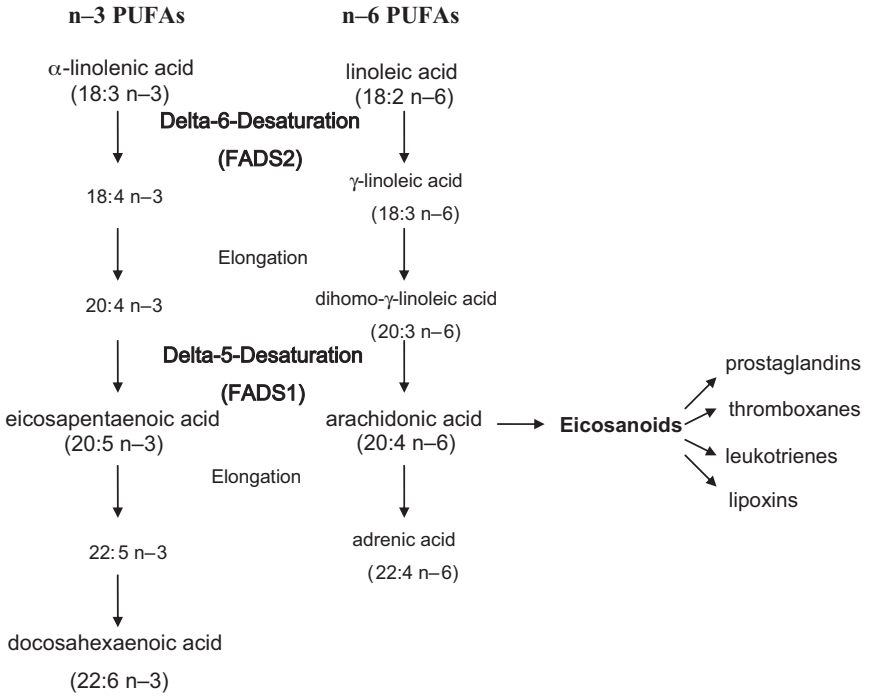


FIGURE 1. The n−3 and n−6 fatty acid metabolism pathways.

product-to-precursor ratio (eg, AA/LA or EPA/ALA) as a surrogate measure to estimate desaturase activity is well established (10). Thus, indirect information can be gathered from the analysis of membrane lipid composition in red blood cells (RBCs) that provides a simple, suitable model for studying FA metabolism (11). The present study was aimed to establish whether differences in desaturase activity may affect the availability of eicosanoid precursor, which may, in turn, condition the vascular responses to an inflammatory damage. Given that the inflammatory response of the vascular wall predisposes to atherosclerotic damage, subjects with greater desaturase activity may have a greater risk of vascular disease. Thus, in the framework of an ongoing case-control study in an Italian population with or without angiographically confirmed coronary artery disease (CAD), we analyzed RBC membrane FA composition and genotyped 13 single-nucleotide polymorphisms (SNPs) in the FADS region to unravel possible relations between desaturase activity [estimated as the ratio of RBC-AA to RBC-LA (RBC-AA/RBC-LA)], a highly sensitive inflammatory marker such as high-sensitivity C-reactive protein (hs-CRP), and CAD risk.

SUBJECTS AND METHODS

Study population

This study was performed within the framework of the Verona Heart Study, a regional survey aimed at searching for new risk factors for CAD in subjects with angiographic documentation of their coronary vessels. Details of the enrollment criteria were given elsewhere (12, 13). A total of 876 subjects, for whom FADS polymorphisms and RBC FA composition were available, were included in the present study. Two hundred sixty-six subjects had completely normal coronary arteries, having undergone coronary angiography for reasons other than CAD, mainly valvular heart disease (CAD-free group), and they served as controls. These subjects were also required to have neither a history of nor clinical or instrumental evidence of atherosclerosis in vascular districts beyond the coronary bed. Six hundred ten subjects had angiographically proven CAD (most of them were candidates for coronary artery bypass grafting). Disease severity was determined by counting the number of major epicardial coronary arteries (ie, left anterior descending, circumflex, and right) affected with ≥1 significant stenosis (eg, ≥50% lumen reduction). According to the hypothesis to be tested, subjects with nonadvanced CAD (ie, coronary stenosis < 50%) were not included in the study. The angiograms were assessed by cardiologists who were unaware that the patients were to be included in the present study.

All participants came from the same geographical area (Northern Italy) and were of similar socioeconomic status. At the time of blood sampling, a complete clinical history, including the assessment of cardiovascular risk factors such as obesity, smoking, hypertension, and diabetes, was collected.

Written informed consent was obtained from all participants after a full explanation of the study. The study was approved by the Ethics Committee of the Azienda Ospedaliera (Verona, Italy).

Biochemical analysis

Samples of venous blood were drawn from each subject after an overnight fast. Serum lipids and other CAD risk factors, including hs-CRP, were measured as described previously (14).

Measurement of serum phospholipids and red blood cell membrane fatty acid concentrations

Blood samples were transported to the laboratory within 1 h of collection and processed immediately. Analysis of plasma (100

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μL) and erythrocyte membrane (250 μL packed erythrocytes hemolyzed in an equal volume of double-distilled water) FAs was performed on total lipids extracted by using 4.5 mL of a ratio of isopropanol to chloroform (11:7, by vol) containing 0.45 mmol 2,6-di-ter-*p*-cresol/L as antioxidant. A gas-chromatographic method (Hewlett-Packard 5980 chromatograph, equipped with an HP-FFAP 25-m, 0.2-mm internal diameter, 0.3-μm phase column; Hewlett-Packard, Palo Alto, CA) based on a direct FA transesterification technique was used as described previously (13). Analyses were performed in duplicate on each sample. Peak identification and quantification were done with commercially available reference FAs (Sigma, St Louis, MO). We used 17:0 as the internal standard. The areas of the peaks were measured, and the quantification was performed with the aid of a Vectra QS/16S PC equipped with HP-3365 CHEM STATION software (version 3.0; Hewlett-Packard). FAs were expressed as g/100 g FA methyl ester (% by wt). FAs from 12:0 to 26:0 were measured [saturated FAs: 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, 24:0, and 26:0; monounsaturated FAs: 16:1, 18:1n−9, and 20:1; and PUFAs: 18:2n−6, 18:3n−3, 20:2n−6, 20:4n−6, 20:5n−3, and 22:6n−3); unidentified peaks accounted for <0.5% of the total. Only the most important FAs for this analysis (ie, the PUFAs involved along the desaturase pathway) were specified in the results. All CVs were <5%.

Single-nucleotide polymorphisms and genotyping

Thirteen SNPs tagging the greatest variability of the *FADS* gene region were selected from the European Community Respiratory Health Survey (7, 8) and the HapMap project (Internet: www.hapmap.org): rs174545, rs174556, rs174561, rs3834458, rs174570, rs2524299, rs174583, rs174589, rs498793, rs174611, rs17831757, rs174627, and rs1000778. For further information, see Figure S1 under "Supplemental data" in the current online issue.

Genotyping was performed as described previously (7). Briefly, genotyping was performed by using matrix-assisted laser desorption–ionization time-of-flight–mass spectrometry (MALDI-TOF-MS) to detect allele-specific primer extension products (Mass Array; Sequenom, San Diego, CA). Polymerase chain reaction primers were designed by using the Mass Array–Assay Design program (Sequenom).

Statistical analysis

Calculations were performed with SPSS software (version 13.0; SPSS Inc, Chicago, IL). Distributions of continuous variables in groups were expressed as means ± SDs. Skewed variables, such as hs-CRP, were logarithmically transformed, and the geometric means (and 95% CIs) were reported. Quantitative data were assessed by using Student's *t* test or analysis of variance (ANOVA), with polynomial contrast for linear trend and Tukey's post hoc comparison of the means when indicated. Correlations between quantitative variables were assessed by using Pearson's correlation test. Qualitative data were analyzed with the chi-square test and with chi-square for linear trend analysis when indicated. $P < 0.05$ was considered statistically significant.

Within each group examined, the frequencies of the *FADS* genotypes associated with each of the polymorphisms were compared by using the chi-square test, and the values were predicted on the basis of the Hardy-Weinberg equilibrium. Pairwise linkage disequilibrium was examined as described by Devlin and Risch (15).

To assess possible genetic predictors of AA/LA variability, FADS polymorphisms were included in a linear regression model according to a forward-stepwise variables selection. Such analysis was replicated by means of a different, nonlinear statistical approach based on the estimation of variable importance by using random forest plots (16, 17). Four SNPs were independent contributors to AA/LA variability and were included into the final model. After excluding significant interactions among SNPs, we focused on an additive model by calculating a score reflecting the sum of risk alleles (ie, alleles associated with a greater AA/LA) for each patient.

Haplotype frequencies were estimated by using R software with the haplo.stats package (R Foundation for Statistical Computing, Vienna, Austria; ISBN 3–900051-07–0; Internet: http://www.R-project.org). Haplotypes present in <10 subjects were excluded from the analyses. The relations between haplotypes and laboratory and clinical outcomes were examined by using generalized linear model regression of a trait on haplotype effects, with adjustment for ambiguous haplotypes (haplo.glm function) (18). The statistical significance of associations was ascertained by random permutation of the disease status of the sample subjects in 1000 replicates with the use of the Monte Carlo method.

To assess associations with CAD, odds ratios (ORs) (and 95% CIs) were estimated by logistic regression models adjusted for both classic risk factors (eg, sex, age, smoking, hypertension, diabetes, body mass index, creatinine, cholesterol, triglycerides, and hs-CRP) and FAs differently distributed between subjects with and without CAD. Statistical power was calculated by using the Altmann nomogram.

RESULTS

The ratio of red blood cell arachidonic acid to red blood cell linoleic acid, high-sensitivity C-reactive protein, and coronary artery disease

The general characteristics of the study population, divided into CAD-free and CAD subgroups, are summarized in **Table 1**. As expected, the classic risk factors for atherosclerosis were more likely to appear in the subjects with CAD than in those without CAD.

CAD patients had lower RBC-LA concentrations than did CAD-free subjects, but the RBC-AA concentrations were similar in the 2 groups. As a consequence, RBC-AA/RBC-LA in CAD patients was highly significant ($P = 2.5 \times 10^{-10}$, *t* test). CAD subjects had lower concentrations of ALA than did CAD-free subjects, and both groups had similar concentrations of RBC-EPA. Thus, the ratio of RBC-EPA to RBC-ALA (RBC-EPA/RBC-ALA) was higher in CAD patients than in CAD-free subjects ($P = 5.1 \times 10^{-5}$, *t* test). RBC-AA/RBC-LA and RBC-EPA/RBC-ALA were significantly correlated (Pearson's coefficient: 0.265; $P < 0.001$).

Substantially analogous results were obtained by comparing CAD patients and CAD-free subjects for FA composition of serum phospholipids (See Table S1 under "Supplemental data" in the current online issue.). In particular, serum AA/LA was

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TABLE 1
Characteristics of the study population, with or without coronary artery disease (CAD)[1]

Characteristics	CAD-free (n = 266)	CAD (n = 610)	P
Age (y)	58.2 ± 12.8[2]	60.7 ± 9.4	0.001[3]
Male (%)	66.9	82.1	<0.001[4]
BMI (kg/m^2)	25.3 ± 3.4	26.6 ± 3.3	<0.001[3]
Hypertension (%)	32.0	62.5	<0.001[4]
Smoking (%)	42.5	66.7	<0.001[4]
Diabetes (%)	6.4	16.2	<0.001[4]
Creatinine (mmol/L)	92 ± 18	98 ± 26	<0.01[3]
Total cholesterol (mmol/L)	5.50 ± 1.07	5.81 ± 1.11	<0.001[3]
LDL cholesterol (mmol/L)	3.53 ± 0.93	3.93 ± 0.98	<0.001[3]
HDL cholesterol (mmol/L)	1.44 ± 0.42	1.21 ± 0.32	<0.001[3]
Triglycerides (mmol/L)	1.49 ± 0.70	1.98 ± 1.04	<0.001[3]
hs-CRP (mg/L)	1.94 (1.67–2.25)[5]	3.12 (2.84–3.43)	<0.001[3]
RBC-SFA (g/100 g)	48.8 ± 1.4	49.1 ± 1.3	<0.01[3]
RBC-MUFA (g/100 g)	14.8 ± 1.3	14.9 ± 1.4	NS[3]
RBC-PUFA (g/100 g)	36.4 ± 1.5	36.1 ± 1.4	<0.01[3]
RBC-18:2n–6, LA (g/100 g)	9.77 ± 1.38	9.05 ± 1.40	<0.001[3]
RBC-20:4n–6, AA (g/100 g)	19.0 ± 1.45	19.1 ± 1.45	NS[3]
RBC-20:4n–6/RBC-18:2n–6	1.99 ± 0.36	2.17 ± 0.41	<0.001[3]
RBC-18:3n–3, ALA (g/100 g)	0.10 ± 0.03	0.09 ± 0.04	0.001[3]
RBC-20:5n–3, EPA (g/100 g)	0.68 ± 0.26	0.70 ± 0.29	NS[3]
RBC-20:5n–3/RBC-18:3n–3	7.12 ± 2.91	8.09 ± 3.82	<0.001[3]
RBC-22:6n–3, DHA (g/100 g)	6.14 ± 1.20	6.46 ± 1.38	0.001[3]

[1] hs-CRP, high-sensitivity C-reactive protein; RBC, red blood cell; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LA, linoleic acid; AA, arachidonic acid, ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
[2] $\bar{x}$ ± SD (all such values).
[3] t Test.
[4] Chi-square test.
[5] $\bar{x}$; range in parentheses (all such values).

higher in CAD patients than in CAD-free subjects ($P < 0.001$, t test).

According to the tertile distribution of RBC-AA/RBC-LA in the whole population, the proportion of CAD patients increased significantly ($P < 0.001$, chi-square test for trend analysis) from the lowest to the highest tertile (**Figure 2**A). It is noteworthy that hs-CRP concentrations also increased progressively across the ratio tertiles ($P < 0.001$, ANOVA with polynomial contrast for linear trend; Figure 2B). Considering the subjects with a ratio in the lowest tertile as the reference group, a progressively greater risk of CAD was observed in the other groups after adjustment for all the classic risk factors—ie, sex, age, smoking, hypertension, diabetes, body mass index, creatinine, cholesterol, and triglycerides (Figure 2C). Such associations with CAD remained significant after the inclusion in the logistic model of FAs differently distributed between CAD patients and CAD-free subjects—ie, RBC-saturated FAs, RBC-ALA, and RBC-DHA. When both n−3 and n−6 ratios (AA/LA and EPA/ALA) were included in the regression model, only RBC-AA/RBC-LA remained significantly associated with hs-CRP and CAD (data not shown).

FADS genotypes, ratio of red blood cell arachidonic acid to red blood cell linoleic acid, and coronary artery disease

Analysis of single-nucleotide polymorphisms

The genotype distribution for each SNP was consistent with Hardy-Weinberg equilibrium. In general, most of SNPs tested were in pairwise linkage disequilibrium (LD) (for further information, see Table S2 under "Supplemental data" in the current online issue). In the gene cluster, 3 LD blocks were observed: block 1 from rs174545 to rs3834458, block 2 from rs2524299 to rs174589, and block 3 from rs174611 to rs174627 (see Figure S1 under "Supplemental data" in the current online issue) (19).

All SNPS in the *FADS1-FADS2-FADS3* cluster were associated with the concentrations of RBC-LA or RBC-AA or with RBC-AA/RBC-LA (See Table S3 under "Supplemental data" in the current online issue). Some SNPs were also associated with the concentration of RBC-ALA or with RBC-EPA/RBC-ALA (See Table S4 under "Supplemental data" in the current online issue), but no single variant was differently distributed between the CAD and CAD-free groups (See Table S5 under "Supplemental data" in the current online issue).

An additive model and haplotype analysis

When we looked for significant associations between FADS polymorphisms and RBC-AA/RBC-LA, all SNPs were included in a linear regression model with RBC-AA/RBC-LA as the dependent variable. With the use of a stepwise selection method, 4 significant associations were found: RS174545 G→C (β-coefficient = −0.30), RS174570 C→T (β-coefficient = −0.07), RS174583 C→T (β-coefficient = 0.14), and RS1000778 C→T (β-coefficient = 0.04) with G, C, T, and T alleles, respectively, associated with a high ratio. These results were also confirmed by means of a different statistical approach based on the estimation of variable importance by using random forest plots. It is noteworthy that none of these 4 SNPs were

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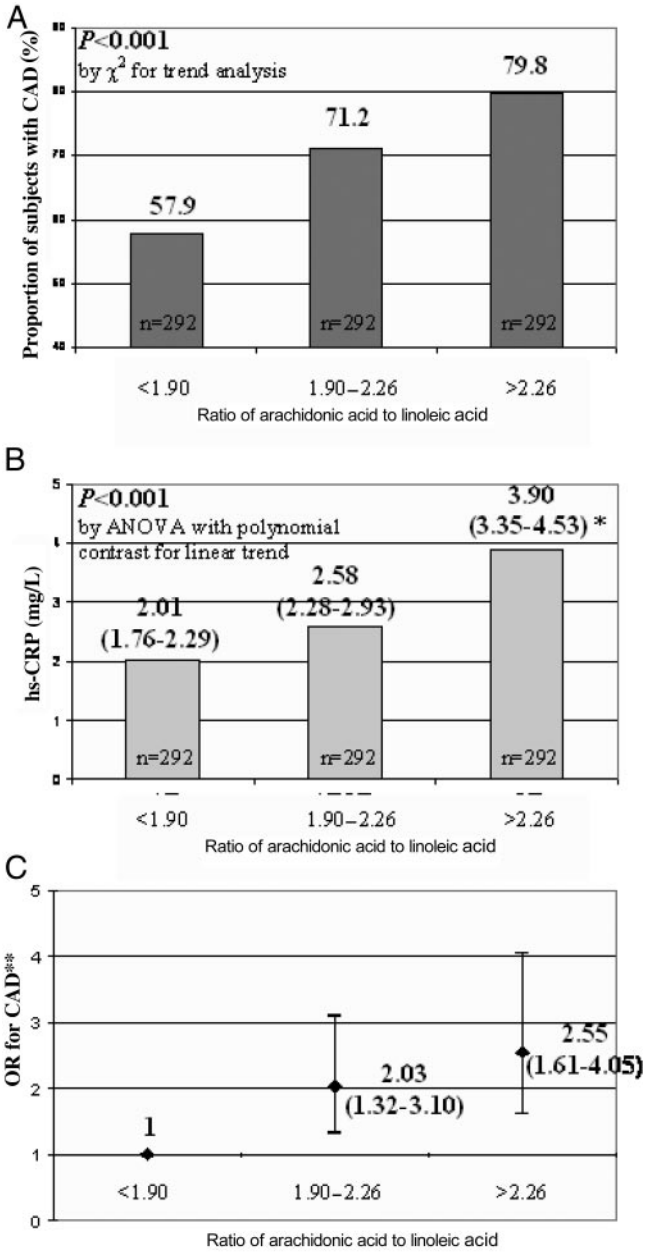


FIGURE 2. Proportion of coronary artery disease (CAD) patients (A), high-sensitivity C-reactive protein (hs-CRP) concentrations (B), and CAD risk (C, with the lowest tertile as the reference group) for each tertile of the ratio of red blood cell (RBC) arachidonic acid to RBC linoleic acid in the population as a whole (n = 876). *Significantly different from the lowest tertile, $P < 0.001$ (ANOVA with Tukey post hoc comparison). **Values obtained by multiple logistic regression after adjustment for sex, age, smoking, hypertension, diabetes, BMI, and creatinine, cholesterol, and triglyceride concentrations.

synonymous, because each was located in a different LD block (see Figure S1 under "Supplemental data in the current online issue).

Based on the number of alleles associated with a higher RBC-AA/RBC-LA, an additive model predicted AA/LA variability. Given that very few subjects carried 2 ($n = 2$) or 7 ($n = 3$) alleles, those subjects were jointly considered with those who carried 3 or 6 alleles, respectively. As expected, subjects with a higher number of such alleles had a higher RBC-AA/RBC-LA (2.14 ± 0.43 for carriers of 6–7 alleles and 1.94 ± 0.33 for carriers of 2–3 alleles; $P < 0.05$, ANOVA with Tukey post hoc comparison). As is consistent with the previously observed association of RBC-AA/RBC-LA with hs-CRP concentrations and CAD risk, subjects carrying more risk alleles also had progressively higher concentrations of hs-CRP ($P < 0.05$, ANOVA with polynomial contrast for linear trend; **Figure 3**A); they also were more frequently represented in the CAD group (P for trend = 0.006, chi-square test; Figure 3B). Subjects with a higher number of risk alleles (ie, 6 or 7) have a greater CAD risk than do those with 2 or 3 alleles (OR: 2.75; 95% CI: 1.27, 5.98). This association remained significant after adjustment for all of the classic risk factors—sex, age, smoking, hypertension, diabetes, body mass index, creatinine, cholesterol, and triglycerides (OR: 2.68; 95% CI: 1.08, 6.66)—and also for RBC-LA and RBC-ALA concentrations (OR: 2.69; 95% CI: 1.02, 7.13).

Finally, we performed an haplotype analysis in which we considered the 4 polymorphisms of interest (RS174545, RS174570, RS174583, and RS1000778). The most frequent haplotype was G-C-C-C. RBC-AA/RBC-LA and RBC-EPA/RBC-ALA and concentrations of hs-CRP differed significantly among FADS haplotypes; the highest values were for haplotypes with a higher number of risk alleles (**Table 2**). As is consistent with such results, haplotypes with a higher number of risk alleles were represented more frequently in patients with CAD than in CAD-free subjects. As a consequence, subjects with haplotypes with 3

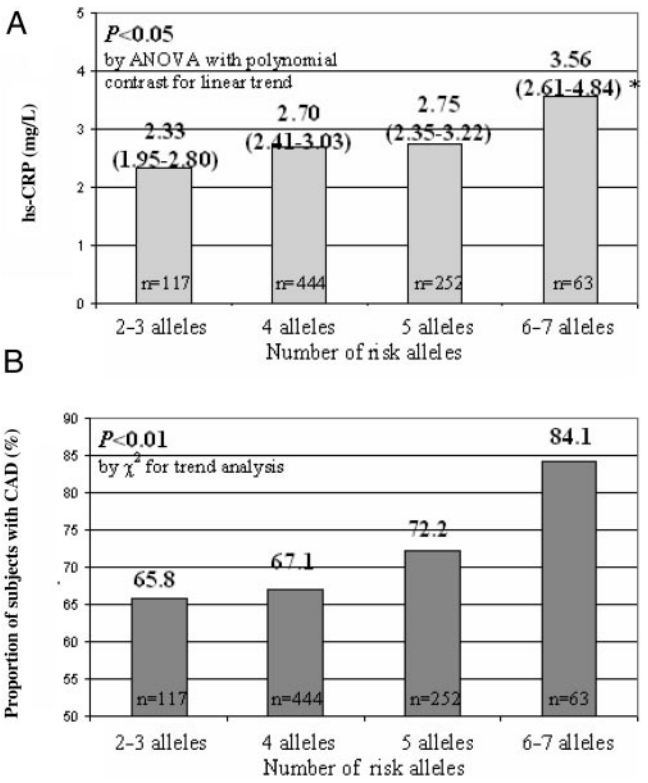


FIGURE 3. Association of the number of risk alleles with high-sensitivity C-reactive protein (hs-CRP) concentrations (A) or coronary artery disease (CAD) prevalence (B). The *FADS* alleles associated with a greater ratio of red blood cell arachidonic acid to red blood cell linoleic acid are considered risk alleles. *Significantly different from the group with 2–3 risk alleles, $P < 0.05$ (ANOVA with Tukey post hoc comparison).

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TABLE 2
Associations of FADS haplotypes (on the basis of RS174545, RS174570, RS174583, RS1000778 single-nucleotide polymorphisms) with the ratios of red blood cell (RBC) arachidonic acid to linoleic acid (RBC-AA/RBC-LA) and of RBC eicosapentaenoic acid to α-linolenic acid (RBC-EPA/RBC-ALA) and concentrations of high-sensitivity C-reactive protein (hs-CRP)

	FADS genotype							
	RS174545	RS174570	RS174583	RS1000778	Risk alleles	RBC-AA/RBC-LA[1]	RBC-EPA/RBC-ALA[1]	Concentrations of hs-CRP[1]
FADS haplotype								*mg/L*
	C	T	T[2]	C	1	1.97 ± 0.44[3]	7.58 ± 2.91	2.38 (1.66-3.42)[4]
	G[2]	C[2]	C	C	2	2.19 ± 0.66	8.23 ± 4.32	2.64 (1.55-4.48)
	C	C[2]	T[2]	C	2	2.07 ± 0.45	7.79 ± 2.90	2.88 (2.02-4.11)
	C	T	T[2]	T[2]	2	1.97 ± 0.42	7.32 ± 2.73	2.07 (1.45-2.95)
	C	C[2]	T[2]	T[2]	3	2.05 ± 0.42	7.30 ± 2.76	2.55 (1.82-3.57)
	G[2]	C[2]	C	T[2]	3	2.28 ± 0.47	8.27 ± 3.00	3.49 (2.39-5.09)
	G[2]	C[2]	T[2]	T[2]	4	2.35 ± 0.42	8.57 ± 2.71	4.12 (2.96-5.72)

[1] $P < 0.001$ by generalized linear model regression among FADS haplotypes.
[2] Risk alleles associated with a higher AA/LA ratio.
[3] $\bar{x} \pm$ SD (all such values).
[4] $\bar{x}$; range in parentheses (all such values).

or 4 risk alleles carried a greater risk of CAD than did subjects with haplotypes with 1 or 2 risk alleles (OR: 1.36; 95% CI: 1.02, 1.81; $P = 0.020$ after adjustment for traditional cardiovascular risk factors and $P = 0.040$ after 1000 permutations by means of Monte Carlo method; **Table 3**).

DISCUSSION

The first major result of the present study is the finding that a greater proportion of the C20-chain FAs per unit of precursor essential FA (18:0) is present in RBC membranes and serum phospholipids of patients with CAD. This finding applied to FAs of both the n−3 and n−6 families, which strongly suggested that elongation and the D5D-D6D desaturation process are accelerated in these patients.

The second significant result is that a continuous gradient of both higher hs-CRP and a greater risk of CAD was observed across AA/LA tertiles in the whole study population (Figure 2B and C). Thus, it seems reasonable to suppose that inappropriately high elongase or desaturase activity (as reflected by the higher ratio of 20:0 FA to precursor) may indicate a peculiar susceptibility to the inflammatory stimuli involving the arterial wall during the atherosclerotic process. Whereas the idea of a relation between a sensitive marker of subclinical inflammation such as hs-CRP and CAD is long-lasting (20–22), a link of both of those variables with n−6 AA/LA has been unrecognized to date, and it represents an element of novelty in the present work.

EPA/ALA was also higher in CAD patients than in CAD-free subjects; in addition, EPA/ALA and AA/LA were significantly correlated. Theoretically, higher ratios of n−3 metabolite to precursor are associated with higher EPA or DHA concentrations, and they have protective rather than harmful effects. Thus, the central point is that a greater activity of the same enzymatic

TABLE 3
Distribution of FADS haplotypes (on the basis of RS174545, RS174570, RS174583, RS1000778 single-nucleotide polymorphisms) in the whole population and in subjects with or without coronary artery disease (CAD)

	FADS genotype				Risk	Whole	CAD-free	CAD	CAD-free	CAD			
	RS174545	RS174570	RS174583	RS1000778	alleles	population	subjects	patients	subjects	patients	OR[1]	P^2	P^3
						%	%	%					
Haplotypes with 1 or 2 risk alleles									81.7	76.3	1	0.020	0.040
	C	T	T[4]	C	1	8.3	9.9	7.7					
	G[4]	C[4]	C	C	2	59.2	61.8	58.7					
	C	C[4]	T[4]	C	2	6.9	6.6	7.0					
	C	T	T[4]	T[4]	2	3.0	3.4	2.9					
Haplotypes with 3 or 4 risk alleles									18.0	22.6	1.36 (1.02, 1.81)		
	C	C[4]	T[4]	T[4]	3	11.8	9.7	12.2					
	G[4]	C[4]	C	T[4]	3	7.8	7.0	8.2					
	G[4]	C[4]	T[4]	T[4]	4	2.0	1.3	2.2					

[1] Odds ratio (OR) was calculated by comparing haplotypes with 3 or 4 risk alleles with haplotypes with 1 or 2 risk alleles (reference group).
[2] Values obtained by generalized linear model regression after adjustment for traditional cardiovascular disease risk factors.
[3] Values obtained by using the Monte Carlo method after 1000 permutations.
[4] Risk alleles associated with a greater ratio of red blood cell (RBC) arachidonic acid to RBC linoleic acid.

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complex should have both pro-inflammatory (as a result of greater bioavailability of AA) and anti-inflammatory (as a result of an increased bioavailability of long-chain n−3 FAs) consequences. This apparent inconsistency may be explained by considering n−3 and n−6 PUFAs from a quantitative point of view. In the present study and in many other studies, LA was almost 100 times as abundant as ALA, and AA was almost 30 times as abundant as EPA. The prevalence of n−6 FAs in Western diets may account for the proinflammatory effect we observed, and the detrimental effects of increased desaturase activity may be exaggerated by diets abundant in n−6 and relatively deficient in n−3 FAs (23). It is consistent with this point of view that, when both EPA/ALA and AA/LA were included in a regression model with hs-CRP or CAD as dependent variables, only AA/LA remained as a significant predictor.

A third major result, consistent with the previous ones, is the finding that haplotypes of the *FADS* gene cluster, including variants associated with an elevated AA/LA, were also related to both a higher hs-CRP concentration and greater risk of CAD (Figure 3 and Tables 2 and 3). As a consequence, apparently distinct phenotypic features (elevated AA/LA, increase in hs-CRP, and greater CAD risk) linked together functionally and with specific variants on the *FADS* gene complex, which in turn affected desaturase activity. Given the relatively small effect of any single gene variant and the observed functional effect in terms of CAD risk, the involved mechanism seems to be of considerable relevance. It may be hypothesized as follows: *1*) carriers of specific FADS haplotypes are constitutively able to form more AA from dietary LA; *2*) greater AA availability at the membrane and cellular levels facilitates the synthesis of AA-derived inflammatory mediators such as leukotrienes and prostaglandins; *3*) vascular inflammatory responses involved in the atherosclerotic process are greatly amplified by the cascade (with multiple effects) of AA-derived mediators; and *4*) carriers of unfavorable haplotypes are at a greater risk of developing clinically significant CAD. Although this hypothesis implies an oversimplification of the complex eicosanoid metabolism—eg, inhibition of this pathway by Cox-2 inhibitors may promote CAD risk by reducing the concentration of the protective prostacyclin—the consistent association among AA/LA, hs-CRP concentration, and CAD risk was impressive.

In the population of the present study, patients in the highest tertile of AA/LA had concentrations of hs-CRP nearly double those in patients in the lowest tertile. This difference implies that a difference of ≈20% in desaturase activity (the cutoff was 1.90 and 2.26 for the lowest and highest tertile, respectively) accounted for a 100% increase in the concentration of the inflammatory marker. Given that AA is the substrate of 5-lipoxygenase and cycloxygenase, key enzymes in the biosynthetic pathway of leukotrienes and prostaglandins, it is reasonable that a modest increase in the substrate availability produces larger effects on the subsequent cascade of mediators. Accordingly, selective inhibitors of D5D and D6D have shown effective antiinflammatory properties in mice (24).

We have measured a suitable inflammatory marker of cardiovascular risk, hs-CRP, because it has been clinically validated in large, prospective population studies (20–22). However, it is plausible that a similar association may be observed with other molecules that are more subtly linked to the vascular atherosclerotic damage, such as interleukins and chemokines.

In the literature, few and conflicting results on desaturase activity and atherosclerotic risk have been reported so far. Some reports suggested a possible causality link between vascular disease and lower desaturase activity (25). Other studies did not shown any significant difference in AA concentration between CAD patients and CAD-free subjects (26, 27). Several years ago, our group showed that patients with essential hypertension have a higher RBC-AA/LA than do normotensive persons, and patients with "white coat" hypertension (that is not confirmed by ambulatory 24-h blood pressure monitoring) have intermediate values (28). Similarly, a higher ratio was observed in persons with insulin resistance and metabolic syndrome (29–32), conditions in which hs-CRP often is abnormally elevated. Thus, those studies support the view that exaggerated desaturase activity may characterize patients carrying well-recognized, major risk factors for CAD. The present study is the first to show an association between desaturase activity and angiographically confirmed CAD.

In the population of the present study, CAD patients had lower LA concentrations than did CAD-free subjects. Given that LA is an essential FA derived exclusively from the dietary source, a deficiency of LA intake may also have contributed to the vascular disease of the patients in the present study. Several studies have reported an inverse relation between LA concentration and risk of atherosclerosis (5, 26, 33–35), and LA deficiency seems to promote mitochondrial respiratory uncoupling with resulting excessive oxidative stress (36).

To the best of our knowledge, this is the first study showing an association between *FADS* genotypes and CAD. In the only other study reporting on the issue, Baylin et al (37) showed that the rs3834458 FADS2 promoter polymorphism was associated with lower EPA concentrations in adipose tissue and higher triglyceride concentrations in serum, but not with CAD risk, in a population from Costa Rica. Those authors hypothesized that impaired desaturase activity may induce an unbalanced proportion of n−3 and n−6 20:0 FAs, which would have both favorable and unfavorable effects of lower AA and EPA, respectively; this possibility underscores the potentially Janus-faced (n−3- or n−6-related) effects of desaturase activity. The present results show that the proinflammatory, detrimental effects are predominant in a population assumed to be following a Western diet that is abundant in n−6 FAs and somewhat deficient in n−3 FAs (23).

In the present study, *FADS* gene variants had little or no effect on CAD risk when considered individually, and this result was consistent with that reported by Baylin et al (37). However, a more complex approach that took into consideration an additive model of FADS SNPs, FADS haplotypes, or both showed a significant effect on a person's susceptibility to CAD. FADS polymorphisms are relatively frequent in the general population, so that only a mild effect on a multifaceted phenotype such as CAD could theoretically be expected. Nevertheless, an additive model of unfavorable alleles was shown to fit well in explaining an increase in the inflammatory marker hs-CRP and a greater probability of CAD. This complex genotype × phenotype interaction appears biologically plausible after adjustment for the potential effects of *FADS* gene variants on desaturase activity and AA availability. Schaeffer et al (7) showed that *FADS1* and *FADS2* gene polymorphisms and haplotypes are correlated with PUFA membrane content. As is consistent with the role of AA

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concentration as an important determinant of the inflammatory response, the prevalence of inflammatory disorders such as allergic rhinitis and atopic eczema seems to be lower in carriers of *FADS* gene variants associated with lower AA concentrations (7).

The present study has some limitations, the first of which is the retrospective case-control design. Furthermore, a somewhat lower number of controls than of CAD patients was enrolled. However, in consideration of the genotype frequency previously reported for *FADS* gene polymorphisms in the general population and a genotype-associated near-doubling of CAD risk, the statistical power of the analysis was >95% by Altmann nomogram at an 0.05 significance level after adjustment for unequal sample size. Another limitation of the present study was related to the lack of data for and di-homo-γ-linolenic acid FAs—intermediate FAs of the metabolic conversion of linoleic or linolenic acids by D5D and D6D—which could provide a more specific estimation of both of those enzymatic activities. In contrast, a relative strength of the study is represented by the angiographic evaluation of coronary arteries in both cases and controls, which allowed for a clear-cut definition of the clinical phenotype, a crucial requirement in any genotype-phenotype association study.

In conclusion, the present study is the first to show an association between greater desaturase activity, *FADS* genotypes, an increase in inflammatory markers, and a greater risk of CAD. Our data support a role of PUFA metabolism in CVD and suggest that a greater desaturase activity, in the context of a Western diet characterized by an unbalanced ratio of n−6 to n−3, may contribute to the atherosclerotic process by favoring a proinflammatory vascular environment. Clearly, these findings have to be confirmed by larger prospective studies; however, they seem to indicate that therapeutic strategies aimed at reducing cell AA availability (eg, by n−3 PUFA supplementation) would be particularly advisable in patients with a genetically determined increase in desaturase activity.

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