Interactions between the *APOA5* -1131T>C and the *FEN1* 10154G>T polymorphisms on ω6 polyunsaturated fatty acids in serum phospholipids and coronary artery disease

Ju Yeon Park,[1,*,†] Jean Kyung Paik,[1,*,§] Oh Yoen Kim,[*,§] Jey Sook Chae,[*,§] Yangsoo Jang,[§,,††,§§] and Jong Ho Lee[2,*,†,§]**

National Research Laboratory of Clinical Nutrigenetics/Nutrigenomics, Department of Food and Nutrition,* College of Human Ecology, Yonsei University, Seoul, Korea; Department of Food and Nutrition, BK21 Project,† College of Human Ecology, Yonsei University, Seoul, Korea; Yonsei University Research Institute of Science for Aging,§ Yonsei University, Seoul, Korea; and Cardiology Division,** Cardiovascular Genome Center; and Severance Medical Research institute,†† and Severance Biomedical Science Research institute,§§ Yonsei University College of Medicine, Seoul, Korea

Abstract We determined the contribution of the combination of *FEN1* 10154G>T with the most significant association in the analysis of plasma arachidonic acid (AA, 20:4ω6) and the *APOA5*-1131T>C on phospholipid ω6PUFA and coronary artery disease (CAD). Patients with CAD (n = 807, 27–81 years of age) and healthy controls (n = 1123) were genotyped for *FEN1* 10154G>T and *APOA5*-1131T>C. We found a significant interaction between these two genes for CAD risk (P = 0.007) adjusted for confounding factors. *APOA5*-1131C allele carriers had a higher CAD risk [odds ratio (OR):1.484, 95% confidence interval (CI):1.31–1.96; P = 0.005] compared with *APOA5*-1131TT individuals in the *FEN1* 10154GG genotype group but not in the *FEN1* 10154T allele group (OR:1.096, 95%CI:0.84–1.43; P = 0.504). Significant interactions between these two genes were also observed for the AA proportion (P = 0.04) and the ratio of AA/linoleic acid (LA, 18:2ω6) (P = 0.004) in serum phospholipids of controls. The *APOA5*-1131C allele was associated with lower AA (P = 0.027) and AA/LA (P = 0.014) only in controls carrying the *FEN1* 10154T allele. In conclusion, the interaction between these genes suggests that the *FEN1* 10154T variant allele decreases AA and AA/LA in the serum phospholipids of carriers of the *APOA5*-1131C allele, but contributes no significant increase in CAD risk for this population subset despite their increased triglylcerides and decreased apoA5.—Park, J. Y., J. K. Paik, O. Y. Kim, J. S. Chae, Y. Jang, and J. H. Lee. **Interactions between the *APOA5* -1131T>C and the *FEN1* 10154G>T polymorphisms on ω6 polyunsaturated fatty acids in serum phospholipids and coronary artery disease. *J. Lipid Res.* 2010.** 51: **3281–3288.**

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The apolipoprotein A5 gene (*APOA5*) is predominantly expressed in the liver and secreted into the blood where it resides on HDL and VLDL particles (1). Despite its very low levels in plasma, apoA5 appears to facilitate the interaction between circulating lipoprotein particles and proteoglycans on the vascular wall with the subsequent activation of proteoglycan-bound lipoprotein lipase (2–4). The *APOA5* -1131T>C single nucleotide polymorphism (SNP) has been reported to be functional and is associated with reduced apoA5 concentration or activity (5). In particular, the *APOA5* -1131C variant allele modulates the effects of dietary PUFA intake on fasting triglyceride levels, lipoprotein particle size (6), and obesity (7) and PUFA-*APOA5* interactions are specific for dietary ω6 PUFAs. The fatty acid composition of serum phospholipids mirrors dietary fatty acid intake over previous weeks and also reflects

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Abbreviations: 8-epi-$PGF_{2\alpha}$, 8-epi-prostaglandin $F_{2\alpha}$; AA, arachidonic acid; apo, apolipoprotein; BMI, body mass index; BP, blood pressure; CAD, coronary artery idsease; CI, confidence interval; CRP, C-reactive protein; D5D, delta 5 desaturase; DGLA, dihomo-γ-linolenic acid; DM, diabetes mellitus; LA, linoleic acid; LLD, lipid-lowering drug; MDA, malondialdehyde; OR, odds ratio; ox-LDL, oxidized LDL; SNP, single nucleotide polymorphism; TNF, tumor necrosis factor.

[1] These two authors contributed equally to this work.
[2] To whom correspondence should be addressed.
e-mail: jhleeb@yonsei.ac.kr

endogenous fatty acid metabolism (8–11). However, an interaction of the *APOA5* -1131T>C SNP with the gene associated with intrinsic phospholipid PUFA metabolism has not been studied.

The key enzymes in PUFA metabolism are delta 5 desaturase (D5D) and delta 6 desaturase, which are encoded by the fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*) genes, respectively (12–14). These two genes are located in the desaturase gene cluster on chromosome 11 (11q12-13.1). A recent genome-wide association study for plasma PUFAs showed strong evidence for association with this region of chromosome 11 (15). The most significant association was between the SNP rs174537 (flap endonuclease 1, *FEN1* 10154G>T) near *FADS1* and the analysis of arachidonic acid (AA, 20:4ω6). AA is synthesized primarily in the liver and then mobilized to inflammatory cells via blood lipoproteins (16). AA is the precursor of prostaglandins, leukotrienes, and related compounds, all of which have important roles in inflammation (17–19). Thus, the *FEN1* 10154G>T SNP may represent a candidate gene of importance in coronary artery disease (CAD). Therefore, we hypothesized that genetic variation at the *APOA5* -1131 locus has an interactive effect with genetic variation at the *FEN1* 10154 locus on serum phospholipid ω6 PUFA metabolism and CAD. Our aim was to determine the contribution of the combination of the *FEN1* 10154G>T and *APOA5* -1131T>C SNPs on serum phospholipid ω6 PUFA metabolism and CAD.

SUBJECTS AND METHODS

Study participants

Study participants were enrolled in a clinical study conducted by the Laboratory of Clinical Nutrigenetics/Nutrigenomics (project#: 2010-0015017 and M10642120002-06N4212-00210) at Yonsei University. Study participants ranged in age from 27 to 81 years old. Participants were recruited from the Cardiovascular Genome Center, Yonsei University Severance Hospital, Seoul, Korea. Control participant exclusion criteria were any diagnosis of vascular disease, diabetes mellitus (DM), cancer (clinically or by anamnesis), renal disease, liver disease, thyroid disease, and acute or chronic inflammatory disease. No participant was taking any drugs or supplements. Inclusion criteria of patients with CAD were: *a*) angiographically confirmed CAD with ⩾50% occlusion of one or more major coronary arteries, *b*) myocardial infarction confirmed according to the World Health Organization criteria for symptoms, enzyme elevation, or electrocardiographic changes, *c*) absence of nonatherogenic occlusion such as osteal stenosis and spasm, *d*) no orthopedic limitations or any diagnosis of DM, liver disease, renal disease, thyroid, or pituitary disease, and *e*) no acute or chronic inflammatory disease. In total, 1,930 genetically unrelated Koreans were included; 807 CAD patients and 1,123 control participants. Written informed consent was obtained from all participants, and the protocol was approved by the Institutional Review Board of Yonsei University.

Anthropometric parameters, BP, and blood collection

Body weight and height were measured unclothed and without shoes in the morning for the calculation of body mass index (BMI) (kg/m^2). Blood pressure (BP) was measured in the left arm of seated patients with an automatic blood pressure monitor (TM-2654, A and D, Tokyo, Japan) after a 20 min rest. After a 12 h fasting period, venous blood specimens were collected in EDTA-treated and plain tubes, centrifuged to produce plasma or serum, and stored at −70°C until analysis.

Genotyping of *FEN1* 10154G>T and *APOA5* -1131T>C

Genomic DNA was extracted from 5 ml whole blood with a commercially available DNA isolation kit (WIZARDR Genomic DNA purification kit, Promega, Madison, WI). Genotyping of *FEN1* 10154G>T was performed with the Taqman assay (Applied Biosystems, Foster City, CA). Genotyping of *APOA5* -1131T>C was performed with SNP-IT^{TM} assays and single primer extension technology (SNPstream 25K^{TM} System, Orchid BioSciences, NJ). Colorimetric reactions were detected with an enzyme-linked immunosorbent assay (ELISA) reader, and the genotype was determined with QCReview^{TM} software.

Serum lipid profile and fasting glucose

Fasting total cholesterol and triglycerides were measured using commercially available kits on a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). After precipitation of serum chylomicrons with dextran sulfate magnesium, the concentrations of LDL- and HDL-cholesterol in the supernatants were measured enzymatically. LDL cholesterol was indirectly estimated in participants with serum triglyceride concentrations less than 400 mg/ml with the Friedewald formula. Fasting glucose levels were measured with a glucose oxidase method and a Beckman Glucose Analyzer (Beckman Instruments, Irvine, CA).

Plasma apoA5 concentration

Plasma concentrations of apoA5 were measured with an enzyme immunoassay (Human Apolipoprotein A ELISA kit, Millipore, MO). The resulting color reaction was read at 450 nm on a Victor^2 plate reader (Perkin Elmer Life Sciences, Turka, Finland).

Fatty acid composition in serum phospholipids

The fatty acid composition in serum phospholipids was analyzed by gas chromatography (HP 7890A, Hewlett-Packard) with the modified method of Folch et al. (20) and Lepage and Roy (21). Individual fatty acids were identified by comparing their retention times with those of standard fatty acid methyl esters and quantitated according to the peak areas relative to the total area (total fatty acid area was set at 100%).

Plasma ox-LDL and LDL particle size

Plasma oxidized LDL (ox-LDL) was measured using an enzyme immunoassay (Mercodia, Uppsala, Sweden). Particle size distribution of LDL (d1.019–1.063 g/ml) isolated by sequential flotation ultracentrifugation was examined with a pore-gradient lipoprotein system (CBS Scientific, CA) with commercially available nondenaturing polyacrylamide slab gels containing a linear gradient of 2–16% acrylamide (Alamo Gels Inc., San Antonio, TX). Standards of latex beads (34 nm), thyroglobulin (17 nm), apoferritin (12.2 nm), and catalase (10.4 nm) were used to estimate the relative migration rates of each band. Gels were scanned with a GS-800 Calibrated Imaging Densitometer (Bio-Rad, Graz, Austria). LDL particle size was calculated with reference to the relative migration value of the standards.

Urinary 8-epi-PGF$_{2\alpha}$ and plasma MDA

Urine was collected in polyethylene bottles containing 1% butylated hydroxytoluene after 12 h of fasting. The bottles were immediately covered with aluminum foil and stored at –70°C until analysis. The compound 8-epi-prostaglandin F$_{2\alpha}$ (8-epi-PGF$_{2\alpha}$)

was measured with an enzyme immunoassay (BIOXYTECH urinary 8-epi-$PGF_{2\alpha}$™ Assay kit, OXIS International Inc., OR). Urinary creatinine was determined with the alkaline picrated (Jeffe) reaction. Urinary 8-epi-$PGF_{2\alpha}$ concentrations were expressed as pmol/mmol creatinine. Plasma malondialdehyde (MDA) was measured from thiobarbituric acid-reactive substances (TBARS Assay Kit, Zepto Metric Inc.).

Serum hs-CRP and TNF-α concentration

Serum hs-CRP (C-reactive protein) concentrations were measured with an Express+ autoanalyzer (Chiron Diagnostics Co., Walpole, MA) with a commercially available, high-sensitivity CRP-Latex (II) X2 kit (Seiken Laboratories Ltd., Tokyo, Japan). Serum tumor necrosis factor-α (TNF-α) concentrations were measured with a Bio-plex human cytokine panel (Bio-Rad, CA) using a Quantikine ELISA kit (Human TNF-α, R and D Systems, Minneapolis, MN).

Assessment of dietary intake

Dietary intake was assessed with a 24 h recall method and semi-quantitative food frequency questionnaire. Dietary energy values and nutrient content were calculated using the Computer Aided Nutritional analysis program (CAN-pro 2.0. Korean Nutrition Society, Seoul, Korea). Total energy expenditure was calculated from activity patterns including basal metabolic rate, physical activity over a 24-h period, and specific dynamic action of food. The base metabolic rate of each participant was calculated with the Harris-Benedict equation.

Statistical analysis

Statistical analyses were performed with SPSS version 12.0 for Windows (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL). Hardy-Weinberg Equilibrium and linkage disequilibrium tests were examined using the Haploviewver 4.1 (Broad Inst., MA). The association of CAD with genotype was calculated using the odds ratio (OR) [95% Confidence intervals (CIs)] for a chi-square test or logistic regression model after adjusting for confounding factors. The Student's *t*-test was used to compare parameters between the two groups. General linear model analysis was also used to compare groups after adjusting for confounding factors. We determined whether each variable was normally distributed before statistical testing and then logarithmic transformation was performed on skewed variables. Frequency was tested with the chi-square test. Pearson correlation coefficients were used to examine relationships between variables. For descriptive purposes, mean values are presented using untransformed and unadjusted values. Results are the means ± SE or percentage. A two-tailed value of $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of controls and patients with CAD

General characteristics of controls and patients with CAD are shown in **Table 1**. Patients with CAD had a lower prevalence of current smoking (20.1% vs. 24.4%; $P = 0.025$) and alcohol drinking (54.8% vs. 64.1%; $P < 0.001$) than controls. Lipid-lowering drugs (LLD), antihypertensive drugs, and antiplatelet drugs were used more frequently in patients than in controls (64.4% vs. 0%, $P < 0.001$; 88.7% vs. 0%; $P < 0.001$; 91.8% vs. 0%; $P < 0.001$, respectively). After adjusting for sex, age, BMI, cigarette smoking, alcohol consumption, and systolic and diastolic blood pressure, CAD patients had lower concentrations of apoA5, total cholesterol, LDL cholesterol, HDL cholesterol, and ox-LDL; higher concentrations of glucose, triglyceride, CRP, MDA, and 8-epi-$PGF_{2\alpha}$; and smaller LDL particle size than controls. CAD patients had a lower proportion of linoleic acid (LA, 18:2ω6), γ-linolenic acid (20:3ω6), and eicosadienoic acid (20:2ω6), a higher proportion of dihomo-γ-linolenic acid (DGLA, 20:3ω6) and AA (20:4ω6), and a higher ratio of AA/LA in serum phospholipids than controls after adjusting for confounding factors (Table 1).

When we subdivided patients with CAD into two groups, those not treated with LLD (n = 286) and those treated with LLD (n = 521), those not treated with LLD showed higher concentrations of total cholesterol (183.2 ± 2 vs. 156.9 ± 2 mg/dl; $P < 0.001$) and LDL cholesterol (106.8 ± 2 vs. 82.0 ± 1 mg/dl; $P < 0.001$) than patients treated with LLD.

TABLE 1. Anthropometric and biochemical parameters of controls and patients with CAD

	Control (n = 1123)	CAD (n = 807)
Male/Female, (%)	78.4/21.6	81.5/18.5
Age (yr)	56.1 ± 0.23	56.9 ± 0.34
BMI (kg/m^2)	24.1 ± 0.07	25.1 ± 0.10
Blood pressure (mm Hg)		
Systolic	124.1 ± 0.46	127.2 ± 0.60
Diastolic	78.4 ± 0.32	77.3 ± 0.36
Fasting glucose (mg/dl)[a]	86.6 ± 0.24	88.8 ± 0.44*
Triglycerides (mg/dl)[a]	128.9 ± 2.22	147.3 ± 2.80***
ApoA5 (ng/ml)[a]	225.1 ± 3.55	197.4 ± 5.02***
Total-cholesterol (mg/dl)	195.5 ± 0.99	166.2 ± 1.38***
LDL-cholesterol (mg/dl)	117.9 ± 0.91	90.7 ± 1.25***
HDL-cholesterol (mg/dl)[a]	51.7 ± 0.42	45.5 ± 0.39***
LDL particle size (nm)	23.76 ± 0.02	23.47 ± 0.03***
TNF-α (pg/ml)[a]	5.38 ± 0.10	5.65 ± 0.43
hs-CRP (mg/dl)[a]	1.14 ± 0.06	2.08 ± 0.15***
Malondialdehyde (nmol/ml)[a]	9.18 ± 0.09	10.5 ± 0.17***
8-epi-$PGF_{2\alpha}$ (pg/mg creatinine)[a]	1296.4 ± 18.2	1417.5 ± 27.0***
Ox-LDL (μ/l)[a]	63.5 ± 0.75	59.5 ± 0.89***
FA composition (%) in serum PL		
Total polyunsaturated ω-6 FA	20.6 ± 0.19	20.6 ± 0.20
18:2(ω-6)	12.9 ± 0.13	12.1 ± 0.13***
18:3(ω-6)[a]	0.27 ± 0.01	0.24 ± 0.01
20:2(ω-6)[a]	0.63 ± 0.05	0.36 ± 0.01***
20:3(ω-6)	1.61 ± 0.03	1.86 ± 0.03***
20:4(ω-6)	4.84 ± 0.07	5.72 ± 0.10***
Total polyunsaturated ω-3 FA	5.39 ± 0.09	5.51 ± 0.12
18:3(ω-3)[a]	0.16 ± 0.00	0.17 ± 0.01
20:3(ω-3)[a]	0.09 ± 0.01	0.08 ± 0.00*
20:5(ω-3)[a]	1.28 ± 0.03	1.44 ± 0.05
22:5(ω-3)[a]	0.60 ± 0.01	0.60 ± 0.01
22:6(ω-3)[a]	3.25 ± 0.06	3.21 ± 0.07
20:4(ω-6)/20:3(ω-6)	3.17 ± 0.04	3.21 ± 0.06*
AA/LA	0.38 ± 0.01	0.48 ± 0.01***
APOA5 -1131T>C, n(%)		
TT	566 (50.4)	363 (45.0)
TC	455 (40.5)	367 (45.5)
CC	102 (9.1)	77 (9.5)
FEN1 10154 G>T, n(%)		
GG	516 (45.9)	399 (49.4)
GT	494 (44.0)	330 (40.9)
TT	113 (10.1)	78 (9.7)

Mean ± SE. *P*-values were tested with a paired *t*-test. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$; adjusted for sex, age, BMI, cigarette smoking, alcohol consumption, and systolic and diastolic BP.
[a] Logarithmic transformation.

Distribution of *FEN1* 10154G>T and *APOA5* -1131T>C polymorphisms

Genotype frequencies of *FEN1* 10154G>T and *APOA5* -1131T>C SNPs did not deviate from Hardy-Weinberg Equalibrium expectation. The genotype distribution and allele frequency of the *FEN1* 10154G>T SNP between controls and patients were comparable. As shown in Table 1, the genotype distribution of *APOA5* -1131T>C in controls and patients differed ($P = 0.058$), and the -1131C allele was more frequent among CAD patients (55%) than controls (49.6%; $P = 0.019$). Therefore, the presence of the *APOA5* -1131C allele increased the OR of CAD risk after adjustment for confounders (OR = 1.274; CI = 1.052-1.544, $P = 0.013$).

Genotype combinations for the two SNPs were constructed to evaluate interaction between the genes and CAD risk. **Table 2** shows OR estimations of CAD risk for the *APOA5* -1131T>C polymorphism in stratified analyses by *FEN1* 10154G>T genotype. We found a statistically significant interaction between *APOA5* -1131T>C and *FEN1* 10154G>T on CAD risk ($P = 0.007$) after adjusting for confounders. The *APOA5* -1131C minor allele was associated with a 48.4% increase in CAD risk ($P = 0.005$) in participants carrying the *FEN1* 10154GG genotype relative to homozygous carriers of the wild-type alleles for each SNP (*FEN1* 10154GG+*APOA5* -1131TT). In contrast, the *APOA5* -1131C minor allele was not associated with CAD risk ($P = 0.524$) in participants carrying the *FEN1* 10154T allele when compared with the concomitant carrier status for the *FEN1* 10154T allele and the *APOA5* -1131TT genotype (*FEN1* 10154T allele+*APOA5* -1131TT) (Table 2).

ApoA5, lipid profiles, LDL particle size, lipid peroxides, and ω6 PUFA in serum phospholipids according to *APOA5* -1131T>C and *FEN1* 10154G>T polymorphisms

After adjusting for confounders, C allele carriers of *APOA5* -1131T>C showed lower apoA5, higher triglyceride, lower HDL cholesterol, and smaller LDL particle size than TT participants in both the control and CAD group (**Table 3**). Controls with the *APOA5* -1131C allele showed lower DGLA (20:3ω6) in serum phospholipids. No genotype differences in levels of LDL, total cholesterol, lipid peroxides, and ω3 fatty acids in serum phospholipids were observed for *APOA5* -1131T>C (data not shown). Controls with the *FEN1* 10154T allele showed lower total cholesterol, MDA, ox-LDL, and DGLA than those with GG (**Table 4**). CAD patients carrying the *FEN1* 10154T allele showed larger LDL particle size and a trend toward a decrease in 8-epi-$PGF_{2\alpha}$. Minor T allele carriers of *FEN1* 10154G>T showed higher LA (18:2ω6), lower AA (20:4ω6), and lower ratio of AA/DGLA and AA/LA than GG participants in both the control and CAD groups. No genotype differences in apoA5, triglycerides, or ω3 fatty acids in serum phospholipids were observed for *FEN*1 10154G>T (data not shown).

Genotype combinations for the two SNPs were constructed to evaluate interactions between the genes for serum lipid profiles, LDL particle size, lipid peroxides, and ω6 PUFA in serum phospholipids. Except for serum phospholipid AA, no significant interactions were observed between *APOA5* -1131T>C and *FEN1* 10154G>T for these variables (data not shown). A significant interaction between *APOA5* -1131T>C and *FEN1* 10154G>T was observed for AA in serum phospholipids ($P = 0.04$) and the AA/LA ratio ($P = 0.004$) in healthy controls (**Fig. 1**). Significantly lower AA ($P = 0.029$) was observed in controls with the minor C allele for *APOA5* -1131T>C who also carried the *FEN1* 10154 minor T allele. Significantly lower AA/LA ratios ($P = 0.014$) were observed in controls with the *APOA5* -1131C minor allele who also carried the *FEN1* 10154 minor T allele. In contrast, a trend toward an increase in the AA/LA ratio ($P = 0.085$) was observed in controls with the *APOA5* -1131C minor allele who also carried the *FEN1* 10154 GG genotype. Although an interaction between the two genes for the 20:4(ω6)/20:3(ω6) ratio failed to reach statistical significance ($P = 0.128$), the *APOA5* -1131C allele was associated with a higher 20:4(ω6)/20:3(ω6) ratio ($P = 0.016$) only in individuals carrying the *FEN1* 10154T allele. The *FEN1* 10154T allele was associated with lower 20:3(ω6) ($P = 0.005$) only in individuals carrying the *APOA5* -1131C allele (Fig. 1).

Relation of serum phospholipid AA with apoA5, lipid peroxides, and TNF-α

Pearson correlation analysis showed that the AA proportion in serum phospholipids was positively correlated with ox-LDL ($r = 0.177$, $P < 0.001$), MDA ($r = 0.153$, $P = 0.002$), 8-epi-$PGF_{2\alpha}$ ($r = 0.122$, $P = 0.007$), and TNF-α ($r = 0.187$, $P < 0.001$) in healthy controls. In addition, 8-epi-$PGF_{2\alpha}$ was positively correlated with TNF-α ($r = 0.094$, $P = 0.037$) and CRP ($r = 0.175$, $P < 0.001$) in healthy controls. In CAD

TABLE 2. Risk of CAD depending on the *APOA5* –1131 T>C and *FEN1* 10154 G>T polymorphisms

	FEN1 10154 G>T				
	GG		GT+TT		P^b for interaction between *APOA5* and *FEN1*
APOA5−1131 T>C	Adjusted OR (95% CI)	P^a	Adjusted OR (95% CI)	P^a	
TT	1	0.005	1	0.504	0.007
TC+CC	1.484 (1.13–1.96)		1.096 (0.84–1.43)		

CI, Confidence interval, OR, odds ratio.

[a] *P*-value for the genotype obtained from the corresponding logistic regression model after adjusting for age, BMI, sex, cigarette smoking, alcohol consumption, and systolic and diastolic BP.

[b] *P* for the interaction from the corresponding logistic regression model after adjusting for age, BMI, sex, cigarette smoking, alcohol consumption, and systolic and diastolic BP.

TABLE 3. Associations of the *APOA5* -1131T>C genotypes with plasma apoA5, lipid profiles, and ω6 PUFA in serum phospholipids

	Control (n = 1123)		CAD (n = 807)	
	APOA5 TT (n = 566)	APOA5 C allele (n = 557)	APOA5 TT (n = 363)	APOA5 C allele (n = 444)
ApoA5 (ng/ml)[a]	238.2 ± 5.21	213.7 ± 4.78***	213.1 ± 7.70	185.0 ± 6.50***
Triglyceride (mg/dl)[a]	118.3 ± 2.56	139.6 ± 3.58***	136.8 ± 3.51	156.0 ± 4.17***
HDL-cholesterol (mg/dl)[a]	52.8 ± 0.59	50.7 ± 0.59**	46.2 ± 0.58	44.9 ± 0.52*
LDL particle size (nm)	23.8 ± 0.03	23.7 ± 0.03***	23.6 ± 0.04	23.4 ± 0.04***
18:2(ω6)	12.9 ± 0.17	12.8 ± 0.18	12.0 ± 0.18	12.3 ± 0.19
20:3(ω6)	1.69 ± 0.04	1.53 ± 0.03**	1.89 ± 0.05	1.84 ± 0.04
20:4(ω6)	4.95 ± 0.10	4.74 ± 0.10	5.65 ± 0.15	5.77 ± 0.13
20:4(ω6)/20:3(ω6)	3.11 ± 0.06	3.23 ± 0.06	3.11 ± 0.08	3.29 ± 0.08
AA/LA	0.39 ± 0.01	0.37 ± 0.01	0.48 ± 0.01	0.49 ± 0.01

Mean ± SE. *P*-values were tested with a paired *t*-test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001; adjusted for sex, age, BMI, cigarette smoking, alcohol consumption, and systolic and diastolic BP.
[a] Logarithmic transformation.

patients, AA was negatively correlated with apoA5 (r = −0.146, P = 0.030) and positively correlated with 8-epi-$PGF_{2\alpha}$ (r = 0.126, P = 0.018).

DISCUSSION

We found an interaction between *APOA5* -1131T>C and *FEN1* 10154G>T for CAD risk. The interaction effect between these two genes revealed that carriers of the *APOA5* -1131C allele, despite their increased triglycerides and decreased apoA5, were not associated with CAD risk when carrying the *FEN1* 10154T variant allele as compared with participants homozygous for the *APOA5* -1131T allele. This circumstance may mask or reduce the risk estimation of CAD if the interaction between *APOA5* -1131T>C and *FEN1* 10154G>T is not considered. This finding partly explains the highly controversial results regarding the association of the *APOA5* -1131C variant allele with CAD risk despite the general consistency regarding its relationship with higher triglyceride and lower apoA5 concentrations.

ApoA5 is an important regulator of triglyceride-rich lipoprotein metabolism (22). In controls with the *APOA5* -1131C allele, higher triglycerides, smaller LDL particle size, and lower levels of apoA5 and serum phospholipid DGLA (20:3ω6) are consistent with the suggestion of a role for apoA5 in VLDL assembly (3, 23) or triglyceride hydrolysis (24). Previous studies have shown interaction effects between dietary ω6 PUFA and *APOA5* -1131T>C when determining fasting triglyceride, lipoprotein particle size (6), postprandial triglyceride (2, 25, 26), BMI, and obesity risk (7). Although PUFA levels in phospholipids are known to be determined by a combination of dietary intake and metabolic efficiency (15, 27–29), an interaction between *APOA5* -1131T>C and *FEN1* 10154G>T polymorphisms on ω6 PUFA metabolism and CAD has not been previously reported. *FEN1* 10154G>T near *FADS1* was found to be the SNP with the most significant association in the analysis of AA (20:4ω6), the long-chain ω6 derivative of LA (18:2ω6), in a recent genome-wide association study of plasma PUFAs (15).

Martinelli et al. (30) showed that a higher ratio of AA/LA is an independent risk factor for CAD, and a graded increase in CAD risk is related to the carrier status of *FADS* haplotypes associated with a higher desaturase activity. Conversely, a possible causality link between vascular disease and lower desaturase activity also has been suggested (31). However, exaggerated desaturation and elongation generally have been reported to characterize CAD patients and individuals with major risk factors for CAD (30, 32–35). In addition, AA in adipose tissue was

TABLE 4. Associations of the *FEN1* 10154 G>T genotypes with serum lipids, lipid peroxides, and ω6 PUFA in serum phospholipids

	Control (n = 1123)		CAD (n = 807)	
	FEN1 10154 GG (n = 516)	*FEN1* 10154 T allele (n = 607)	*FEN1* 10154 GG n = 399)	*FEN1* 10154 T allele (n = 408)
Total-cholesterol (mg/dl)	197.6 ± 1.48	193.6 ± 1.34*	164.4 ± 1.91	168.0 ± 1.99
LDL particle size (nm)	23.7 ± 0.03	23.8 ± 0.03	23.4 ± 0.03	23.5 ± 0.04**
Malondialdehyde (nmol/ml)[a]	9.41 ± 0.15	8.98 ± 0.11*	10.6 ± 0.27	10.4 ± 0.21
8-epi-$PGF_{2\alpha}$ (pg/mg creatinine)[a]	1316.4 ± 28.3	1279.2 ± 23.6	1458.7 ± 41.2	1377.9 ± 35.0
Ox-LDL (μ/L)[a]	65.0 ± 1.13	62.2 ± 1.01*	58.2 ± 1.17	60.7 ± 1.33
18:2(ω6)	12.4 ± 0.18	13.2 ± 0.17**	11.6 ± 0.18	12.7 ± 0.19***
20:3(ω6)	1.67 ± 0.04	1.56 ± 0.03*	1.82 ± 0.04	1.91 ± 0.05
20:4(ω6)	5.36 ± 0.11	4.46 ± 0.09***	6.17 ± 0.15	5.22 ± 0.12***
20:4(ω6)/20:3(ω6)	3.33 ± 0.06	3.05 ± 0.06***	3.52 ± 0.08	2.87 ± 0.07***
AA/LA	0.43 ± 0.01	0.34 ± 0.01***	0.54 ± 0.01	0.42 ± 0.01***

Mean ± SE. P-values were tested with a paired *t*-test, **P* < 0.05, ***P* < 0.01, ****P* < 0.001 adjusted for sex, age, BMI, cigarette smoking, alcohol consumption, and systolic and diastolic BP.
[a] Logarithmic transformation.

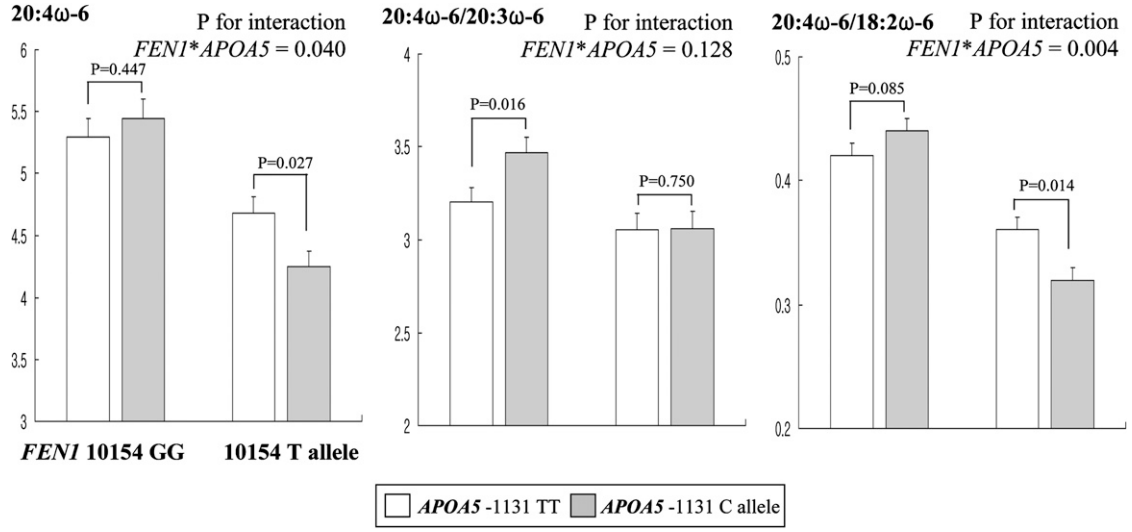

Fig. 1. Serum phospholipid arachidonic acid (AA, 20:4ω6) and the ratio of 20:4ω6/20:3ω6 and 20:4ω6/18:2ω6 by *APOA5* -1131T>C and *FEN1* 10154G>T genotype combination in healthy controls. Mean ± SE. *P* for interaction was tested by multiple logistic regression analysis after adjusting for sex, age, BMI, cigarette smoking, alcohol consumption, and systolic and diastolic BP.

found to be associated with acute myocardial infarction (36, 37), and this association was not related to dietary intake of ω6 PUFA including LA (37). In this study, CAD patients also showed higher AA and AA/LA in serum phospholipids, but there was no significant difference in the proportion of energy intake derived from fat and PUFA intake between control and CAD groups. Furthermore, fat intake did not differ according to genotype combination in either controls or CAD patients. Therefore, the difference in ω6 PUFAs in serum phospholipids between controls and CAD patients was shown not to be derived from dietary fat intake but may be due to intrinsic phospholipid metabolism. In part, not only AA, but also its precursors including LA and DGLA in phospholipids, are known to be genetically determined (15, 29).

An interaction effect between *APOA5* -1131T>C and *FEN1* 10154G>T was also found in serum phospholipid AA and AA/LA in controls. In the *FEN1* 10154GG genotype group, *APOA5* -1131C allele carriers showed higher activity of D5D (AA/DGLA) and a trend toward an increase in AA/LA. In contrast, in the *FEN1* 10154T minor allele group, the *APOA5* -1131C allele was associated with a lower AA/LA ratio. The mechanism of this interaction cannot be determined from our experimental approach. However, the *FEN1* 10154T allele is associated with higher LA and lower AA in plasma PUFA (15), suggesting that this variant allele may reduce D5D expression and the elongation-desaturation process of converting LA to AA. Therefore, the interaction effect between the two genes may be partly due to lower activity of D5D in controls with the *FEN1* 10154T variant allele who also carry the *APOA5* -1131C allele, and the result may be lower DGLA. This may also partly explain our current observation that the lowest AA and AA/LA in serum phospholipids was found in controls carrying minor alleles at both genes, despite their increased serum triglyceride levels.

AA, a precursor of eicosanoids including prostaglandins and leukotrienes, is liberated from the hydrolysis of the *sn*-2 position of glycerophospholipids (38). Radical peroxidation of AA produces a family of prostaglandin F_2-isomers called F_2-isoprostanes (39). One such F_2-isoprostane is 8-epi-$PGF_{2\alpha}$, a sensitive and independent risk marker for CAD (40–42) that is probably released into biological fluids through a phospholipase-mediated pathway and consequently excreted in urine. Here, we found a positive correlation between serum phospholipid AA and urinary excretion of 8-epi-$PGF_{2\alpha}$ in both controls and CAD patients. In addition, serum phospholipid AA was positively correlated with ox-LDL, a strong predictor for CAD (43), as well as MDA and TNF-α in controls. Interestingly, we also found a positive relationship between 8-epi-$PGF_{2\alpha}$, TNF-α, and CRP. These results support the previous suggestion that decreased synthesis and thus, availability of AA, mitigates the inflammatory response by altering AA metabolism, for example, by decreasing eicosanoid levels (11).

Several points should be considered when interpreting our findings. First, our study measured D5D activity rather than concentration. PUFA levels were also expressed as a percent of total FAs in serum phospholipids rather than an absolute concentration. Therefore, we could detect relative differences in PUFA levels and D5D activity but were unable to decipher the mechanisms, which depend on the absolute values. Second, our results share the general limitations of other cross-sectional observational studies in that we evaluated association, not prospective prediction. Finally, we specifically focused on a representative group of Korean adults aged 27–81 years. This selection was based on the knowledge that CAD is greatly influenced by genetic factors at younger ages. Our controls had normal fasting glucose levels (<100 mg/dl) and were not taking any medications or functional foods. Therefore, our data cannot be generalized to other ethnic groups or other populations. Despite these limitations, we found an interaction

between *APOA5* -1131T>C and *FEN1* 10154G>T polymorphisms on AA and AA/LA in serum phospholipids as well as on CAD risk. The observed interaction between these genes suggests that the *FEN1* 10154T variant allele decreases AA and AA/LA in serum phospholipids in carriers of the *APOA5* -1131C allele, contributing to an insignificant increase in CAD risk for this subset of the population, despite their increased triglyceride and decreased apoA5 levels.

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