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FADS gene polymorphisms in Koreans: Association with ω6 polyunsaturated fatty acids in serum phospholipids, lipid peroxides, and coronary artery disease

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ABSTRACT

Objective: We investigated the association of polymorphisms in *FADS* genes with polyunsaturated fatty acids (PUFAs) in serum phospholipids, lipid peroxides, and coronary artery disease (CAD) in Koreans.
Methods: In this case–control study, CAD patients ($n = 756$, 40–79 years) and healthy controls ($n = 890$) were genotyped for rs174537 near *FADS1* (*FEN1* rs174537G > T), *FADS2* (rs174575, rs2727270), and *FADS3* (rs1000778). We calculated the odds ratios (ORs) for CAD risk and measured serum PUFA composition and lipid peroxide.
Results: Among four SNPs, only rs174537G > T differed in allele frequencies between controls and CAD patients after adjustment for age, BMI, cigarette smoking, alcohol consumption, hypertension, diabetes mellitus, and hyperlipidemia ($P = 0.017$). The minor T allele was associated with a lower risk of CAD [OR 0.75 (95%CI 0.61–0.92), $P = 0.006$] after adjustment. rs174537T carriers had a significantly higher proportion of linoleic acid (LA, 18:2ω6), lower arachidonic acid (AA, 20:4ω6), and lower ratios of AA/dihomo-γ-linolenic acid (DGLA, 20:3ω6) and AA/LA than G/G subjects in both control and CAD groups. In the control group, 174537T carriers had significantly lower levels of total- and LDL-cholesterol, malondialdehyde, and ox-LDL. In CAD patients, rs174537T carriers showed a larger LDL particle size than G/G subjects. The proportion of AA in serum phospholipids positively correlated with LDL-cholesterol, ox-LDL, and malondialdehyde in controls and with 8-epi-prostaglandin $F_{2\alpha}$ in both control and CAD groups.
Conclusion: The rs174537T is associated with a lower proportion of AA in serum phospholipids and reduced CAD risk, in association with reduced total- and LDL-cholesterol and lipid peroxides.

1. Introduction

Polyunsaturated fatty acid (PUFA) composition in phospholipids has been associated with the outcome of several complex human diseases including coronary artery disease (CAD) [1]. PUFAs can affect membrane fluidity and cholesterol content, and influence the generation of signaling molecules [2]. They are also processed to powerful promoters of inflammation called eicosanoids [3]. PUFA levels in phospholipids are determined by both nutrition [4,5] and metabolism. The key enzymes in PUFA metabolism are Δ5 desaturase (D5D) and D6D, which are encoded by fatty acid desaturase 1 and 2 (*FADS1* and *FADS2*) genes, respectively [6–8]. These two genes are located in the desaturase gene cluster on chromosome 11q12-13.1. This cluster also includes *FADS3*, a gene sharing 52 and 62% sequence of the *FADS1* and *FADS2* genes respectively and encodes another desaturase of unknown activity [6].

A recent genome-wide association study for plasma PUFAs showed strong evidence for association with the region of chromosome 11 that encodes *FADS1*, *FADS2*, and *FADS3* [9]. The most significant association was between the single nucleotide polymorphism (SNP) rs174537 (*FEN1*, flapstructure specific endonuclease) near *FADS1* and arachidonic acid (AA, 20:4ω6). Minor allele homozygotes of rs174537G > T had lower plasma concentrations of AA and higher LDL-cholesterol and total-cholesterol compared to major allele homozygotes [9]. The *FADS* locus is now considered

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the major contributor to circulating PUFA concentration, thus may have implications for CAD.

Therefore, we examined the association of *FADS* polymorphisms, including rs174537, with PUFAs in serum phospholipids and CAD related biomarkers in Koreans through the case–control (CAD patients vs. healthy subjects) study design. We also determined the effects of these SNPs on lipid peroxides, i.e. oxidized LDL (ox-LDL), malondialdehyde (MDA), and 8-epi-prostaglandin $F_{2\alpha}$ (8-epi-$PGF_{2\alpha}$).

2. Materials and methods

2.1. Study population

Study participants were enrolled in a case–control designed clinical study (project#: 2010-0015017 and M10642120002-06N4212-00210) in Yonsei University, Seoul, Korea. Control participants were recruited from the Health Service Center during routine check-up visits or through advertisements briefly describing the study design. Exclusion criteria were orthopedic limitations, about 10% of weight loss/gain over the previous 6 months, or 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. None of them was taking any drugs or supplementations. CAD patients (case) were recruited from the Cardiovascular Genome Center, Yonsei University Severance Hospital, Seoul, Korea. The inclusion criteria were as follows: (a) angiographically confirmed CAD with ≥50% occlusion of one or more major coronary arteries, (b) myocardial infarction confirmed according to World Health Organization (WHO) criteria for symptoms, enzyme elevation, or electrocardiographic changes, (c) absence of non-atherogenic occlusion, i.e. osteal stenosis and spasm, (d) neither orthopedic limitations nor any diagnosis of the diseases mentioned above. Finally, 1646 genetically unrelated Koreans (40–79 years) were included in this study (n = 756 CAD, n = 890 controls). Participation rates in case and control subjects from the recruitment were 50% and 70%, respectively. Written informed consent was obtained from all participants, and the study protocol was approved by the Institutional Review Board of Yonsei University.

2.2. Anthropometric parameters and blood collection

Body mass index (BMI) was calculated as body weight in kilograms divided by height in square meters (kg/m^2). Systolic and diastolic blood pressure (SBP, DBP) were obtained from the left arm of seated patients with an automatic blood pressure monitor (TM-2654, A&D, Tokyo, Japan) after 20 min of rest. After overnight fasting, venous blood specimens were collected in EDTA-treated or plain tubes, and separated into plasma or serum, then stored at −70 °C until analysis.

2.3. Genotyping of FADS gene polymorphisms

Genomic DNA was extracted from 5 mL whole blood using a commercially available DNA isolation kit (WIZARD® Genomic DNA purification kit, Promega, Madison, WI) according to the manufacturer's protocol. Based on previous reports and public databases on the *FADS* gene cluster [9–11] and HapMap project (http://www.hapmap.org), eight relevant *FADS* SNPs were prescreened and finally 4 SNPs (*FEN*1-10154 rs174537G > T, *FADS*2 rs174575C > G, *FADS*2 rs2727270C > T, *FADS*3 1000778C > T) were selected for further analysis (Supplementation 1).

2.4. Serum lipid profile and fasting glucose

Serum total cholesterol, HDL-cholesterol and triglyceride (TG) were measured using commercially available kits (Choongwae, Seoul, Korea and Roche, Basel, Switzerland) on a Hitachi 7150 Autoanalyzer (Hitachi Ltd., Tokyo, Japan). LDL-cholesterol was estimated indirectly using the Friedewald formula for subjects with serum TG < 400 mg/dL. The intra-assay and inter-assay coefficients of variance of TG were 1.50% and 1.80%, respectively and those of total cholesterol were 0.80% and 1.70%, respectively. Fasting glucose was measured by a glucose oxidase method using the Glucose Analyzer (Beckman Instruments, Irvine, CA).

2.5. LDL particle size, ox-LDL and serum high-sensitivity C-reactive protein

Particle size distribution of LDL (d1.019–1.063 g/mL) isolated by sequential flotation ultracentrifugation was examined by a pore-gradient lipoprotein system (CBS Scientific, CA). LDL particle size was calculated with reference to the relative migration value of the standards. The intra-assay and inter-assay coefficients of variance were 0.73% and 1.46%, respectively. Plasma ox-LDL was measured using an enzyme immunoassay (Mercodia, Uppsala, Sweden). The resulting color reaction was read at 450 nm with a Wallac Victor^2 multilabel counter (Perkin Elmer Life Sciences, Turku, Finland). The intra-assay and inter-assay coefficients of variance were 6.30% and 8.20%, respectively. Serum high sensitivity C-reactive protein (hs-CRP) were measured with an ADVIA 1650 (Bayer, Tarrytown, NY) using a commercially available, high-sensitivity CRP-Latex(II) X2 kit (Seiken Laboratories Ltd., Tokyo, Japan) that allowed detection of CRP in the range of 0.001–31 mg/dL. The intra-assay and inter-assay coefficients of variance were 1.87% and 1.89%, respectively.

2.6. Urinary 8-epi-prostaglandin $F_{2\alpha}$ and plasma malondialdehyde

Urine was collected in polyethylene tubes containing 1% butylated hydroxytoluene after a 12-h fast. The tubes were immediately covered with aluminum foil and stored at −70 °C until analysis. 8-epi-$PGF_{2\alpha}$ was measured using an enzyme immunoassay (BIOXYTECH Urinary 8-epi-$PGF_{2\alpha}$™ Assay Kit, OXIS International Inc., Portland, OR) and the resulting color reaction was read at 650 nm using a Wallac Victor^2 multilabel counter. Urinary creatinine was determined by the alkaline picrated (Jaffe) reaction. 8-epi-$PGF_{2\alpha}$ concentrations were expressed as pg/mg creatinine. The intra-assay and inter-assay coefficients of variance were 2.26% and 2.51%, respectively. Plasma MDA was assayed according to the fluorometric method described by Buckingum [12]. The intra-assay and inter-assay coefficients of variance were 1.15% and 1.98%, respectively.

2.7. Fatty acid composition in serum phospholipids

Serum phospholipid fatty acid (FA) composition was analyzed by gas chromatography (Hewlett Packard 5890A, CA, USA) using the modification of previous methods [13,14]. Individual FAs were calculated as a relative percentage with the elevated 26 kinds of FAs set at 100% using Chemstation software. The inter-assay coefficients of variance were 4.15%. D5D activity was estimated as the ratio of AA to dihomo-γ-linolenic acid (DGLA, 20:3ω6) and D6D activity was estimated as the ratio of γ-linolenic acid (GLA, 18:3ω6) to linoleic acid (LA, 18:2ω6).

Table 1
Anthropometric and biochemical parameters of controls and CAD patients.

	Controls (n = 890)	CAD patients (n = 756)
Male/female (%)	81.2/18.8	83.5/16.5
Age (yr)	56.5 ± 6.40	57.3 ± 8.74*
Body mass index (kg/m^2)	23.7 ± 2.09	25.2 ± 2.92***
Cigarette smoker (%)	24.4	20.6
Alcohol drinker (%)	63.9	55.4***
Antihypertensive therapy (%)	–	89.8
ACE inhibitor	–	27.5
β-Blocker	–	36.8
α-Blocker	–	22.1
Ca-antagonist	–	37.7
Anti-hypertensive complex	–	52.0
Antidyslipidemic therapy (%)	–	64.4
Statin	–	94.9
Fibrate	–	5.1
Antiplatelet therapy (%)	–	91.7
Blood pressure (mm Hg)		
Systolic	123.5 ± 15.3	127.4 ± 17.0***
Diastolic	78.3 ± 10.6	77.4 ± 10.0
Fasting glucose (mg/dL)[a]	86.7 ± 7.91	89.7 ± 15.4***
Triglyceride (mg/dL)[a]	128.1 ± 75.6	152.6 ± 91.7***
Total-cholesterol (mg/dL)	194.9 ± 32.9	166.6 ± 39.0***
LDL-cholesterol (mg/dL)	118.3 ± 30.6	91.2 ± 35.1***
HDL-cholesterol (mg/dL)[a]	51.5 ± 14.2	45.5 ± 11.1***
LDL particle size (nm)	23.78 ± 0.66	23.45 ± 0.55***
Malondialdehyde (nmol/mL)[a]	9.19 ± 2.83	10.7 ± 4.95***
hs-CRP (mg/dL)[a]	1.30 ± 3.27	2.12 ± 4.27***
8-epi-PGF$_{2\alpha}$ (pg/mg creatinine)[a]	1266.6 ± 616.1	1414.7 ± 752.8**
Oxidized LDL (U/L)[a]	64.2 ± 23.0	59.5 ± 22.3***
FA composition (%) in serum PL		
Total saturated FA	54.6 ± 5.43	53.8 ± 4.60*
Total monounsaturated FA[a]	11.2 ± 2.16	11.2 ± 1.82
Total polyunsaturated ω6 FA	20.3 ± 4.60	20.7 ± 4.00
18:2(ω6)	12.9 ± 3.10	12.2 ± 2.66**
18:3(ω6)[a]	0.29 ± 0.30	0.26 ± 0.21*
20:2(ω6)[a]	0.60 ± 1.22	0.36 ± 0.15***
20:3(ω6)	1.54 ± 0.59	1.87 ± 0.62***
20:4(ω6)	4.61 ± 1.70	5.74 ± 1.97***
Total polyunsaturated ω3FA[a]	5.26 ± 2.34	5.55 ± 2.40
18:3(ω3)[a]	0.15 ± 0.10	0.18 ± 0.12**
20:3(ω3)[a]	0.09 ± 0.16	0.09 ± 0.10
Eicosapentaenoic acid[a]	1.26 ± 0.77	1.46 ± 0.92**
Docosapentaenoic acid[a]	0.58 ± 0.28	0.60 ± 0.27
Docosahexaenoic acid[a]	3.18 ± 1.57	3.23 ± 1.38
Total energy expenditure (kcal)	2197.8 ± 261.2	2235.9 ± 305.1**
Estimates of daily nutrient intakes[b]		
Total energy intake (kcal)	2278.8 ± 270.2	2376.9 ± 342.2***
Carbohydrate (% of energy)	62.1 ± 2.62	62.6 ± 3.24**
Protein (% of energy)	17.0 ± 1.52	17.1 ± 1.90
Fat (% of energy)	21.1 ± 2.37	20.6 ± 2.98***

Mean ± S.D.
[a] Tested by logarithmic transformation.
[b] Nutrient intakes, obtained from weighed food records and calculated using the database of the computerized Korean food code.
* P-values derived from independent t-test. $P < 0.05$.
** P-values derived from independent t-test. $P < 0.01$.
*** P-values derived from independent t-test. $P < 0.001$.

2.8. Assessment of dietary intake and physical activity level

Information about the subjects' usual diet was obtained using both a 24-h recall method and a semi-quantitative food frequency questionnaire, the validity of which had been previously tested [15]. We used the former to carry out analyses and the latter to check whether the data collected by the 24-h recall methods was representative of the usual dietary pattern. All subjects received

Table 2
Adjusted odds ratios (ORs) for CAD patients according to *FEN1* rs174537 G > T.

	Unadjusted OR (95%CI[a])	Unadjusted P-value	Adjusted OR (95%CI[a])	Adjusted P-value
FEN1 rs174537 (G > T)				
G[b] vs. T9	0.868 (0.75–1.01)	0.063	0.825 (0.71–0.97)	0.017
G/G + G/T[b] vs. T/T	0.954 (0.69–1.33)	0.781	0.900 (0.63–1.28)	0.558
G/G[b] vs. G/T + T/T	0.799 (0.66–0.97)	0.023	0.746 (0.61–0.92)	0.006

Odds ratios were calculated by logistic regression model with adjustment for age, BMI, smoking, drinking, hypertension, diabetes mellitus, hyperlipidemia.
[a] Confidence interval.
[b] Indicates reference.

Table 3
Lipid profiles, lipid peroxides, and fatty acid composition in serum phospholipids according to *FEN1* rs174537G > T.

	FEN1 rs174537G > T			
	Controls		CAD patients	
	GG (n = 401)	GT + TT (n = 489)	GG (n = 383)	GT + TT (n = 373)
Total-cholesterol (mg/dL)	198.2 ± 34.2	192.1 ± 31.4**	164.6 ± 38.0	168.7 ± 40.0
LDL-cholesterol (mg/dL)	121.4 ± 31.0	115.7 ± 30.0**	89.3 ± 34.7	93.1 ± 35.5
LDL particle size (nm)	23.74 ± 0.66	23.82 ± 0.66	23.38 ± 0.50	23.52 ± 0.59*
Malondialdehyde (nmol/mL)[a]	9.55 ± 3.39	8.88 ± 2.20**	10.9 ± 5.60	10.5 ± 4.20
8-epi-$PGF_{2\alpha}$ (pg/mg creatinine)[a]	1325 ± 720.4	1218 ± 507.7	1439 ± 792.0	1390 ± 711.1
Oxidized LDL (U/L)[a]	66.4 ± 24.2	62.3 ± 21.8*	57.9 ± 20.8	61.1 ± 23.6
FA composition (%) in serum PL				
18:2(ω6)	12.4 ± 2.67	13.3 ± 3.31**	11.6 ± 2.66	12.8 ± 2.51***
18:3(ω6)[a]	0.27 ± 0.29	0.30 ± 0.30	0.25 ± 0.21	0.26 ± 0.20
20:2(ω6)[a]	0.61 ± 1.37	0.59 ± 1.11	0.35 ± 0.16	0.37 ± 0.15
20:3(ω6)	1.61 ± 0.61	1.49 ± 0.57	1.81 ± 0.59	1.93 ± 0.65
20:4(ω6)	5.10 ± 1.76	4.27 ± 1.58***	6.11 ± 2.10	5.33 ± 1.72***
18:3(ω3)[a]	0.15 ± 0.11	0.14 ± 0.08	0.16 ± 0.11	0.19 ± 0.13*
20:3(ω3)[a]	0.11 ± 0.21	0.08 ± 0.11	0.08 ± 0.09	0.09 ± 0.10
Eicosapentaenoic acid[a]	1.29 ± 0.76	1.24 ± 0.78	1.44 ± 0.90	1.48 ± 0.94
Docosapentaenoic acid[a]	0.61 ± 0.32	0.56 ± 0.24	0.62 ± 0.30	0.59 ± 0.24
Docosahexaenoic acid[a]	3.35 ± 1.69	3.06 ± 1.46	3.15 ± 1.42	3.32 ± 1.33
20:4(ω6)/20:3(ω6)	3.41 ± 1.96	3.01 ± 1.02*	3.86 ± 5.44	2.95 ± 1.08*
20:4(ω6)/18:2(ω6)	0.41 ± 0.12	0.32 ± 0.10***	0.54 ± 0.21	0.42 ± 0.14***
20:5(ω3)/18:3(ω3)	10.9 ± 7.90	11.4 ± 11.7	10.6 ± 7.14	10.0 ± 7.71

Mean ± S.D.
[a] Tested by logarithmic transformation.
* P-values derived from independent t-test. $P < 0.05$.
** P-values derived from independent t-test. $P < 0.01$.
*** P-values derived from independent t-test. $P < 0.001$.

written and verbal instructions from a registered dietitian on completion of a 3-day (2 weekdays and 1 weekend day) dietary record and their all day long physical activities for the same 3 days. Dietary energy values and nutrient content from 3-day food records were calculated using the Computer Aided Nutritional analysis program (CAN-pro 2.0, Korean Nutrition Society, Seoul, Korea). Total energy expenditure (TEE) (kcal/day) was calculated from activity patterns including basal metabolic rate, physical activity over 24-h, and specific dynamic action of food. Basal metabolic rate for each subject was calculated with the Harris–Benedict equation.

2.9. Statistical analysis

Statistical analyses were performed with SPSS ver12.0 (Statistical Package for the Social Science, SPSS Ins., Chicago, IL). The Hardy Weinberg Equilibrium (HWE) and the Linkage Disequilibrium (LD) tests were examined using the Haploviewer 4.1 (Broad Inst., MA). The association of CAD with genotype was calculated using the odds ratio (OR) [95% confidence intervals (CIs)] of a logistic regression model with adjustment for confounding factors. Student's t-test was used for the comparison of parameters between the two groups. One-way analysis of variance followed by Bonferroni method was used to compare the difference among the genotype groups. Skewed variables in distribution were log-transformed and then tested. Frequency was tested by chi-square test. Pearson's correlation coefficients were used to examine the relationships between variables. For descriptive purposes, mean values are presented using untransformed and unadjusted values. Results are expressed as mean ± S.D. or %. A two-tailed value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of controls and CAD patients

General characteristics of control subjects and CAD patients are shown in Table 1. CAD patients had lower concentrations of total-, LDL- and HDL-cholesterol, and ox-LDL; higher concentrations of glucose, TG, MDA, 8-epi-$PGF_{2\alpha}$, and hs-CRP; and smaller LDL particle size than controls before and after adjustment for age, BMI, cigarette smoking, alcohol consumption, and BP. CAD patients had a lower proportion of LA and eicosadienoic acid (20:2ω6) and a higher proportion of DGLA, AA, α-linolenic acid (ALA, 18:3ω3), and eicosapentaenoic acid (EPA, 20:5ω3) in serum phospholipids than controls after the adjustment (Table 1). In CAD patients, lipid-lowering drug (LLD) group (n = 487) showed lower concentrations of total cholesterol (157.4 ± 36 vs. 183.3 ± 39 mg/dL; $P < 0.001$) and LDL-cholesterol (82.4 ± 32 vs. 107.2 ± 36 mg/dL; $P < 0.001$) than non-LLD group (n = 269).

3.2. Genotype distribution of four selected SNPs

Genotype distributions were in HWE in the entire population as well as in cases and controls separately. Among four SNPs, only rs174537G > T differed in genotype distribution and the minor T allele frequencies between controls and CAD patients (Supplementation 2). rs174537T allele was associated with lower risk of CAD before [OR 0.80 (95%CI 0.66–0.97), P = 0.023] and after adjusted for age, BMI, cigarette smoking, alcohol consumption, hypertension, DM, and hyperlipidemia [OR 0.75 (95%CI 0.61–0.92), P = 0.006] (Table 2). As the rs1000778C > T genotype-related phenotype including serum phospholipid ω6FA was not significantly different in control and CAD groups (data not shown), we did not perform further analysis on rs1000778C > T. The haplotype distribution of rs174537–rs174575–rs2727270 also tended to be different between controls and CAD patients (Supplementation 2). Since haplotype analysis did not provide information beyond that revealed by each SNP (data not shown), we presented only the results of individual SNPs.

3.3. Lipid profiles, LDL particle size, and lipid peroxides according to the genotypes

In the control group, rs174537T carriers had significantly lower levels of total- and LDL-cholesterol, MDA, and ox-LDL than G/G subjects (Table 3). Although none of these associations was observed

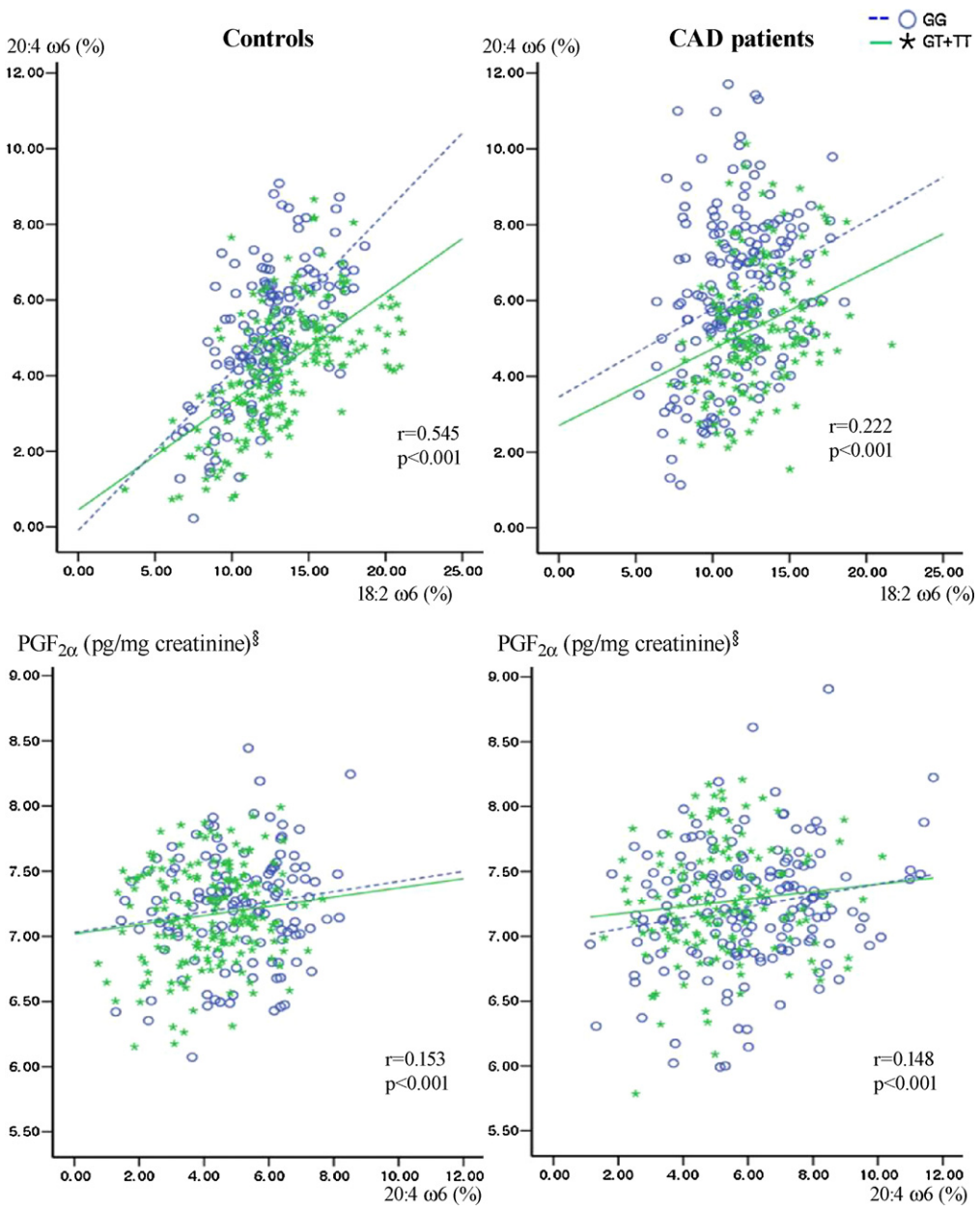

Fig. 1. Relationship of the proportion of arachidonic acid (20:4ω6) in serum phospholipids with the proportion of linoleic acid (18:2ω6) and urinary excretion of 8-epi-$PGF_{2\alpha}$ in controls and CAD patients tested by Pearson correlation analysis *r*: correlation coefficient.

in CAD group, CAD patients with rs174537T allele showed a larger LDL particle size than G/G subjects. Similarly, rs174575G carriers in controls showed lower concentrations of total- and LDL-cholesterol and ox-LDL than C/C subjects, and T carriers of rs2727270C > T showed lower levels of MDA in the control group and larger LDL particle size in the CAD group compared with C/C subjects (Supplementation 3).

3.4. Serum phospholipid FA composition according to the genotypes

In the control group, rs174537T carriers had a significantly higher proportion of LA and a lower proportion of AA in serum phospholipids than G/G subjects (Table 3). Similarly, CAD patients with rs174537T allele had higher LA and ALA and lower AA. Consequently, rs174537T carriers showed higher ratios of AA/DGLA and AA/LA in both control and CAD groups. G carriers of rs174575C > G compared with C/C subjects showed lower AA and AA/LA in control group and higher LA and ALA and lower AA and AA/LA in CAD group (Supplementation 3). Additionally, T carriers of rs2727270C > T showed higher LA and lower AA, AA/DGLA and AA/LA in controls, and higher LA and DGLA and lower AA and AA/LA in CAD patients (Supplementation 3).

3.5. Correlation of AA proportion with LDL-cholesterol, lipid peroxides, and LA

Serum phospholipid AA positively correlated with LDL-cholesterol ($r = 0.189$, $P < 0.001$), ox-LDL ($r = 0.256$, $P < 0.001$), MDA ($r = 0.175$, $P < 0.01$), and 8-epi-$PGF_{2\alpha}$ ($r = 0.153$, $P < 0.01$) in control subjects. In CAD patients, however, AA proportion negatively correlated with LDL-cholesterol ($r = -0.212$, $P < 0.001$) and ox-LDL ($r = -0.133$, $P < 0.05$) and positively with 8-epi-$PGF_{2\alpha}$ ($r = 0.148$, $P < 0.01$). 8-epi-$PGF_{2\alpha}$ positively correlated with hs-CRP in both

control ($r = 0.154$, $P < 0.001$) and CAD ($r = 0.100$, $P < 0.01$) groups. Additionally, AA proportion positively correlated with LA in both control ($r = 0.545$, $P < 0.001$) and CAD ($r = 0.222$, $P < 0.001$) groups (Fig. 1). Among the FA proportions in serum phospholipids, LA positively correlated with PUFA intake in both control ($r = 0.152$, $P < 0.01$) and CAD ($r = 0.139$, $P < 0.01$) groups.

4. Discussion

The major finding of this study was that minor T allele frequency of rs174537G > T was significantly lower in CAD patients than in controls. The rs174537T allele was associated with a lower proportion of AA, but a higher proportion of LA in the serum phospholipids. This result is in agreement with a recent report of association between the *FADS* gene cluster and CAD [11]. Since there was no significant difference in proportions of energy intake derived from fat and PUFA intake according to genotypes in either control subjects or CAD patients, our results suggest that rs174537T allele may reduce the elongation–desaturation process of LA by the D5D and D6D enzymes. This association with FAs is consistent with previous studies [9] showing accumulation of substrates of the PUFA metabolic pathway in rs174537 variants. Recently, rs174546 allele in LD with rs174537 allele ($r^2 = 0.99$) has been reported to be associated with expression of *FADS1*, but not *FADS2*, in lymphoblastoid cells [16]. In this study, T carriers of rs174537 near *FADS1* showed association with decreased activity of D5D, but not D6D.

Martinelli et al. [11] demonstrated that a higher AA/LA ratio was an independent risk factor for CAD in a multiple logistic regression model. Additionally, the endogenous pools of AA are associated with an increased risk of myocardial infarction [17]. In this study, CAD patients with a higher frequency of wild type homozygotes of rs174537G > T also showed a higher ratio of AA/LA than controls. This result supports previous reports that exaggerated desaturation and elongation might characterize CAD patients and subjects carrying major CAD risk factors [11,18]. However, some studies did not show any significant difference in AA concentration between CAD patients and CAD-free subjects [19,20]. Furthermore, a possible causality link between vascular disease and lower desaturase activity has been also suggested [21]. These inconsistencies might be partly due to different methods for assessing PUFA status or to genetic differences in phospholipid metabolism.

Although the minor variants of all three SNPs were associated with higher LA and lower AA, rs174537 showed the most significant association with AA or AA/LA in both subject groups. This result supports the previous report of a genome-wide association study [9] showing the most significant association of rs174537 with AA. However, rs174537 is located in an intron and in LD with rs174546 ($r^2 = 0.99$) and rs3834458 ($r^2 = 0.98$), which are candidates for a direct influence on gene expression [10,16]. Therefore, it is possible that this variant is the marker of other functional polymorphisms or is in linkage with currently unidentified causal variants affecting FA concentrations, and consequently CAD.

rs174537T allele also showed significant associations with decreased total and LDL-cholesterol, consistent with previous findings of Tanaka et al. [9]. A possible mechanism has been suggested that a higher concentration of LA in T carriers results in increased membrane fluidity, thus increasing LDL-receptor recycling resulting in decreased LDL-cholesterol [9]. Additionally, the present findings of a direct relationship between serum phospholipid AA and LDL-cholesterol, ox-LDL, and MDA could also suggest a possible role of AA in lipid peroxidation.

MDA, an index of lipid peroxidation is generated during LDL oxidation [22] and estimates the circulating levels of lipoperoxidation aldehydes secondary to lipid peroxidation [23]. In fact, serum phospholipid AA has been identified as a predictor of MDA during long-term intervention with high-dose PUFAs [23]. Peroxidation of lipids and lipoproteins are related to small LDL particle size and to play an important role in the development of atherosclerotic plaques. Evidences have suggested that circulating lipid peroxides play a pivotal role in atherogenesis leading to coronary heart disease [22]. In contrast to control subjects, the association of rs174537G > T with LDL-cholesterol and lipid peroxides was not observed in CAD patients. Widespread use of statin therapy in CAD patients may partly account for the lack of rs174537 genotypic effects on these variables. This difference may also reflect that CAD progression is related to lipid peroxidation and inflammation regardless of rs174537 genotype, since we found that MDA, 8-epi-$PGF_{2\alpha}$, and hs-CRP were higher in CAD patients than in controls. This result is consistent with previous findings that peroxidative potential of PUFAs was not modified by statin treatment [23,24].

Differently from MDA, 8-epi-$PGF_{2\alpha}$, one of the radical peroxides of AA, is a sensitive and independent risk marker of CAD [25] probably released into biological fluids through a phospholipase-mediated pathway and consequently excreted in urine. We found a positive correlation between serum phospholipid AA and urinary excretion of 8-epi-$PGF_{2\alpha}$ in both controls and CAD patients. Interestingly, 8-epi-$PGF_{2\alpha}$ positively correlated with CRP in the absence of correlation between AA and CRP in both subject groups, consistent with the previous finding of Schewedhelm et al. [25].

Several points should be considered when interpreting our findings. First, PUFA levels were expressed as a percent of total FAs in serum phospholipids not as an absolute concentration. Therefore, we were able to detect relative differences in PUFA levels and D5D/D6D activities but unable to decipher the mechanisms, which depend on the absolute values. Second, our study was case–control design, not designed for assessing the time sequential associations because the exposure and outcomes are collected at one point in time. Third, the frequency of rs174537T/T homozygote was similar between controls (9.7%) and CAD patients (9.3%), whereas the frequency of heterozygote was significantly lower in CAD patients (40.1%) than controls (45.3%). Finally, we specifically focused on a representative group of Korean adults aged 40–69 years. This selection was made based on the knowledge that CAD is greatly influenced by genetic factors at younger ages [26]. Our controls had normal fasting glucose (<100 mg/dL) and were not taking any medications/functional foods. Therefore, our data cannot be generalized to other ethnic groups or other populations. Despite these limitations, our data confirmed that rs174537T variant was related to lower proportions of AA and higher proportions of LA in serum phospholipids, as shown in the genome-wide association study of plasma PUFAs [9], and demonstrated an interesting association between rs174537T allele and a reduced risk for CAD as well as reduced concentrations of total and LDL-cholesterol, ox-LDL, and MDA.

Conflicts of interest

There is no potential conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2010.10.004.

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