Int J Clin Exp Pathol 2015;8(6):7318-7331
www.ijcep.com /ISSN:1936-2625/IJCEP0008375

Original Article

Association of two polymorphisms in the FADS1/FADS2 gene cluster and the risk of coronary artery disease and ischemic stroke

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Received March 24, 2015; Accepted May 22, 2015; Epub June 1, 2015; Published June 15, 2015

Abstract: Little is known about the association of the *FADS1/FADS2* SNPs and serum lipid levels and the risk of coronary artery disease (CAD) and ischemic stroke (IS) in the Chinese southern population. The present study aimed to determine such association in the Chinese southern population. A total of 1,669 unrelated subjects (CAD, 534; IS, 553; and healthy controls, 582) were recruited in the study. Genotypes of the *FADS1* rs174546 SNP and the *FADS2* rs174601 SNP were determined by the SNaPshot Multiplex Kit. The T allele and TT genotype frequencies of the two SNPs were predominant in our study population. The T alleles were associated with increased risk of CAD and IS. Correspondingly, the C alleles were associated with reduced risk of CAD and IS. Haplotype analyses showed that the haplotype of T-T (rs174546-rs174601) was associated with an increased risk for IS, and the haplotype of C-C (rs174546-rs174601) was associated with a reduced risk for CAD and IS. The two SNPs were likely to influence serum lipid levels. The T allele carriers of the two SNPs and rs174601 TT genotype were associated with decreased serum HDL-C and ApoAI levels in the patient groups and with an increased risk of CAD and IS. The present study suggests that the *FADS1* rs174546 SNP and the *FADS2* rs174601 SNP are associated with the risk of CAD and IS, and are likely to influence serum lipid levels. However, further functional studies are needed to clarify how the two SNPs actually affect serum lipid levels and the risk of CAD and IS.

Keywords: The FADS1/FADS2 gene cluster, single nucleotide polymorphism, ischemic stroke, coronary artery disease, lipids

Introduction

Both coronary artery disease (CAD) and ischemic stroke (IS) are the most prevalent geriatric diseases and the major determinant of mortality and morbidity worldwide [1-3]. As multifactorial diseases, both CAD and IS share common risk factors, including both lipid and nonlipid variables, such as metabolic factors, thrombogenic/hemostatic factors, and inflammatory markers [4]. These risk factors are involved in the initiation, progression and rupture of atherosclerotic plaque.

Long-chain polyunsaturated fatty acids (LC-PUFAs) are important components of cell membranes, serve as substrates for the synthesis of inflammatory eicosanoids (leukotrienes and prostaglandins), act as signaling molecules, and regulate gene expression [5]. LC-PUFAs composition of plasma and body tissues has been associated with numerous health outcomes including cardiovascular health, allergies, mental health, and cognitive development [6, 7]. LC-PUFAs could reduce hepatic very low-density lipoprotein synthesis contributes to lower plasma triglycerides [8, 9] and reduce plasma and urine levels of eicosanoids such as leukotriene E4 and alter several inflammatory pathways [10]. Additionally, in several trials, LC-PUFAs are commonly considered to have anti-thrombotic effects and improve endothelial function and health [11, 12]. The roles of each of these molecular pathways could partly mediate protective effects of LC-PUFAs against cardiovascular disease.

Tissue LC-PUFAs levels are determined by both dietary intake and endogenous synthesis via the successive elongation and desaturation of

dietary fatty acids precursors. Delta-5 desaturase (D5D) and delta-6 desaturase (D6D), the key enzymes required for the synthesis of LC-PUFAs [13], are respectively encoded by the FADS1 and FADS2 genes, which is clustered with family members at 11q12-q13.1. Many studies have reported associations between genetic variants in the FADS1/FADS2 gene cluster and LC-PUFAs levels [14]. Bokor et al. [14] indicated that the minor alleles of SNPs including rs174546 were associated with lower D5D desaturase activity. Stoffel et al. [15] found that Loss of FADS2 expression in the FADS2-null mouse, abolished the synthesis of LC-PUFAs. Thus, genetic variations in the FADS gene cluster correlated with a decrease of desaturase reaction products and an accumulation of substrates, which might have implications for cardiovascular disease [6, 16-23]. Recently, several studies have showed strong associations between single nucleotide polymorphisms (SNPs) in the FADS1/FADS2 gene cluster and the risk of cardiovascular disease [6, 16-23]. Other studies have also reported relationships between several *FADS1/FADS2* variants and plasma lipid concentrations [21, 22, 24, 25]. However, the mechanisms underlying these associations have not been clearly established. At present, the associations of the SNPs in the FADS1/FADS2 gene cluster with serum lipid levels and risk of cardiovascular disease have been more reported in the European populations, relatively little is known about such association in the Chinese populations, especially in Chinese south population. Considering the differences in genetic background and living habits among different population, and searching for novel pathway in lipid regulation and new therapeutic or preventive methods for cardiovascular disease, it is of great interest to evaluate these associations. Therefore, the aim of the present study was to investigate the possible association between the SNPs in the FADS1/FADS2 gene cluster and serum lipid levels, as well as the risk of CAD and IS in Chinese south population through the case-control study.

Materials and methods

Study population

A total of 1,087 unrelated patients with CAD (*n* = 534) and IS (*n* = 553) were recruited from hospitalized patients in the First Affiliated Hospital, Guangxi Medical University from September 2009 to October 2011. The diagnosis of CAD was based on typical clinical symptoms and electrocardiographic changes, as well as increases in the serum markers including creatinine kinase-MB and troponin T. Coronary angiography was performed in patients with CAD. The selected CAD patients were subject to significant coronary stenosis (≥ 50%) in at least either one of the three main coronary arteries or their major branches (branch diameter ≥ 2 mm). Additionally, angiographic severity of disease was defined as single or multi-vessel disease based on the number of involved artery (luminal narrowing ≥ 50%) in the three major coronary arteries [26, 27]. The classification of IS was made according to the TOAST (Trial of Org 10172 in Acute Stroke Treatment) criteria [28]. The selected IS patients in the study included individuals who were eligible for one of the two subtypes of TOAST criteria: Large-artery atherosclerosis and Small-vessel occlusion. Subjects with a history of hematologic, neoplastic, renal, liver, thyroid, autoimmune diseases and type I diabetes mellitus were excluded. The selected IS patients who had a past history of CAD were excluded, while the selected CAD patients who had a past history of IS were excluded from the study.

A total of 582 healthy subjects matched by age, gender, and ethnic group (Han Chinese) were consecutively recruited from Physical Examination Center of the First Affiliated Hospital, Guangxi Medical University during the same period when CAD and IS patients were recruited. The controls were free of CAD and IS by questionnaires, history taking and clinical examination. All enrolled individuals were Han Chinese from Guangxi, the People's Republic of China. A standard questionnaire was used to ascertain the general information and medical history for all participants. The study protocol was approved by the Ethics Committee of the First Affiliated Hospital, Guangxi Medical University. Informed consent was obtained from all subjects after they received a full explanation of the study.

Biochemical measurements

Venous blood sample was drawn from all subjects after at least 12 hours of fasting. The levels of serum total cholesterol (TC), triglyceride

Table 1. General characteristics and serum lipid levels between the controls and patients

Characteristic	Controls (n = 582)	patients		*P*		
		CAD (n = 534)	IS (n = 553)	P_1	P_2	P_3
Male/female	418/164	403/131	399/154	0.095	0.477	0.214
Age, years	61.40 ± 10.54	61.93 ± 10.69	62.54 ± 12.11	0.405	0.090	0.374
Body mass index, kg/m^2	22.28 ± 2.82	23.64 ± 4.10	24.71 ± 2.08	< 0.001	0.011	0.270
Systolic blood pressure, mmHg	130.45 ± 2.18	132.80 ± 23.11	147.71 ± 22.18	0.072	< 0.001	< 0.001
Diastolic blood pressure, mmHg	82.63 ± 13.43	79.05 ± 14.08	83.63 ± 13.43	< 0.001	0.144	< 0.001
Pulse pressure, mmHg	50.10 ± 14.87	53.65 ± 18.80	63.66 ± 18.49	< 0.001	< 0.001	< 0.001
Cigarette smoking, n (%)	248 (42.6)	240 (45.0)	231 (41.8)	0.469	0.810	0.299
Alcohol consumption, n (%)	259 (44.5)	211 (39.5)	203 (36.7)	0.101	0.008	0.349
Total cholesterol, mmol/L	4.93 ± 1.06	4.52 ± 1.23	4.52 ± 1.15	< 0.001	< 0.001	0.952
Triglyceride, mmol/L	1.00 (0.65)	1.36 (0.96)	1.36 (0.92)	< 0.001	< 0.001	0.432
HDL-C, mmol/L	1.90 ± 0.49	1.14 ± 0.34	1.23 ± 0.40	< 0.001	< 0.001	< 0.001
LDL-C, mmol/L	2.76 ± 0.77	2.73 ± 1.00	2.68 ± 0.90	0.567	0.108	0.390
Apolipoprotein (Apo) AI, g/L	1.40 ± 0.27	1.00 ± 0.27	1.02 ± 0.22	< 0.001	< 0.001	0.288
ApoB, g/L	0.91 ± 0.21	0.91 ± 0.27	0.89 ± 0.25	0.726	0.325	0.242
ApoAI/ApoB	1.62 ± 0.48	1.27 ± 1.88	1.17 ± 0.61	< 0.001	< 0.001	0.233
Type 2 diabetes mellitus, n (%)	25 (4.3)	124 (23.2)	95 (17.2)	< 0.001	< 0.001	0.013
Hypertension, n (%)	180 (30.9)	272 (50.9)	298 (53.9)	< 0.001	< 0.001	0.330
Hyperlipidemia, n (%)	198 (34.0)	254 (47.6)	267 (48.3)	< 0.001	< 0.001	0.813

CAD, coronary artery disease; IS, ischemic stroke; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol. P_1: comparison of CAD and controls; P_2: comparison of IS and controls; P_3: comparison of CAD and IS.

(TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in samples were determined by enzymatic methods with commercially available kits. Serum apolipoprotein (Apo) AI and ApoB levels were detected by the immunoturbidimetric immunoassay. The normal values of serum TC, TG, HDL-C, LDL-C, ApoAI, ApoB levels, and the ratio of ApoAI to ApoB in our Clinical Science Experiment Center were 3.10-5.17, 0.56-1.70, 0.91-1.81, 2.70-3.20 mmol/L, 1.00-1.78, 0.63-1.14 g/L, and 1.00-2.50; respectively [29-31]. The individuals with TC > 5.17 mmol/L, and/or TG > 1.70 mmol/L were defined as hyperlipidemic [32]. Hypertension was diagnosed according to the criteria of 1999 World Health Organization International Society of Hypertension Guidelines for the management of hypertension [33]. Uncontrolled hypertension was defined as a systolic blood pressure of 140 mmHg or higher and a diastolic blood pressure of 90 mmHg or higher. The subjects with systolic blood pressure of only 140 mmHg or higher but a diastolic blood pressure of < 90 mmHg were diagnosed as isolated systolic hypertension. Normal weight, overweight and obesity were defined as a body mass index (BMI) < 24, 24-28, and > 28 kg/m^2; respectively [34].

SNP selection and genotyping

The SNPs of rs174546 and rs174601 were selected as genetic markers. rs174561 (C/T) is located in 3'-untranslated regions (UTR) of *FADS1* and rs174601 (C/T) is located in intron 1 of *FADS2*. The two SNPs were selected on the basis of the following assumptions: (1) Selected SNPs were established by Haploview (Broad Institute of MIT and Harvard, USA, version 4.2); (2) SNPs information was obtained from NCBI dbSNP Build 132 (http://www.Ncbi.nlm.nih.gov/SNP/); (3) SNPs were restricted to minor allele frequency (MAF) > 1%. (4) SNPs might be associated with the plasma lipids levels in a recent GWAS [24, 25]; and (5) SNPs might be either functional or LD with other functional SNPs [35].

Genomic deoxyribonucleic acid (DNA) was extracted from leucocytes of venous blood using the phenol-chloroform method. The two SNPs (rs174546, rs174601) were determined using SNaPshot Multiplex Kit (Applied Biosystems Co., USA). The restriction enzymes for loci rs174546, rs174601 were *SAP* and *EXO I* (Promega, Epicentre), respectively. The sense and antisense primers were as follows:

Table 2. Genotypic and allelic frequencies and the risk of coronary artery disease (CAD) and ischemic stroke (IS)

Genotype or allele	Controls (%)	CAD (%)	IS (%)	CAD		IS	
	N = 582	N = 534	N = 553	OR (95% CI)	*P*	OR (95% CI)	*P*
rs174546							
CC	60 (10.3)	51 (9.6)	49 (8.9)	1		1	
CT	257 (44.2)	200 (37.4)	209 (37.8)	0.92 (0.58-1.47)	0.729	0.86 (0.54-1.37)	0.528
TT	265 (45.5)	283 (53.0)	295 (53.3)	1.39 (0.88-2.21)	0.161	1.31 (0.83-2.06)	0.255
P		0.041	0.031				
C	377 (32.3)	302 (28.3)	307 (27.8)	1		1	
T	787 (67.7)	766 (71.7)	799 (72.2)	1.30 (1.06-1.59)	0.010	1.29 (1.06-1.57)	0.011
P		0.020	0.009				
HWE (*P*)	0.842	0.076	0.175				
TT	265 (45.5)	283 (53.0)	295 (53.3)	1		1	
CC + TC	317 (54.5)	251 (47.0)	258 (46.7)	0.57 (0.44-0.75)	< 0.001	0.62 (0.48-0.80)	< 0.001
P		0.008	0.005				
CC	60 (10.3)	51 (9.6)	49 (8.9)	1		1	
TT + TC	522 (89.7)	483 (90.4)	504 (91.1)	1.12 (0.72-1.75)	0.603	1.08 (0.70-1.68)	0.730
P		0.374	0.234				
Rs174601							
CC	60 (10.3)	48 (9.0)	44 (8.0)	1		1	
CT	254 (43.6)	194 (36.3)	199 (36.0)	0.92 (0.57-1.48)	0.724	0.97 (0.60-1.56)	0.889
TT	268 (46.1)	292 (54.7)	310 (56.0)	1.51 (0.95-2.40)	0.084	1.58 (0.99-2.53)	0.055
P		0.015	0.003				
C	374 (32.1)	290 (27.2)	287 (25.9)	1		1	
T	790 (67.9)	778 (72.8)	819 (74.1)	1.38 (1.13-1.69)	0.002	1.42 (1.16-1.73)	0.001
P		0.006	0.001				
HWE (*P*)	0.987	0.059	0.135				
TT	268 (46.0)	292 (54.7)	310 (56.1)	1		1	
CC + TC	314 (54.0)	242 (45.3)	243 (43.9)	0.54 (0.41-0.70)	< 0.001	0.61 (0.48-0.79)	< 0.001
P		0.002	< 0.001				
CC	60 (10.3)	48 (9.0)	44 (8.0)	1		1	
TT + TC	522 (89.7)	486 (91.0)	509 (92.0)	1.23 (0.78-1.92)	0.372	1.27 (0.81-2.01)	0.291
P		0.260	0.102				

HWE, Hardy-Weinberg equilibrium. CAD, coronary artery disease; IS, ischemic stroke.

rs174546F: 5'-CAAACCCAACCCCCTCTGAGTA-3', rs174546R: 5'-CCATTTTGTCCCTGCAGCTCA-CTA-3'; rs174601F: 5'-TTCTGGGGGTTCTTCAG-CTGGT-3', rs174601R: 5'-AGGGAAGGGACCTTG-GATGATG-3'.

Statistical analyses

The statistical analyses were carried out using the statistical software package SPSS 21.0 (SPSS Inc., Chicago, Illinois). Quantitative variables were expressed as mean ± standard deviation (serum TG levels were presented as medians and interquartile ranges). Qualitative variables were expressed as percentages. Allele frequency was determined via direct counting, and the standard goodness-of-fit test was used to test the Handy-Weinberg equilibrium. A chi-square analysis was used to evaluate the difference in genotype distribution and sex ratio between the groups. The general characteristics between patients and controls were tested by the Student's unpaired *t*-test. The association of genotypes and serum lipid parameters was tested by analysis of covariance (ANCOVA). Any variants associated with

Table 3. Haplotype and the risk of CAD and IS

Haplotype	Haplotype frequency (%)			CAD		IS	
	Controls (n = 582)	CAD (n = 534)	IS (n = 553)	OR (95% CI)	*P*	OR (95% CI)	*P*
H1 CC	31.8	24.6	25.8	0.72 (0.60-0.87)	0.001	0.76 (0.63-0.91)	0.003
H2 TT	67.3	69.2	72.1	1.18 (0.98-1.41)	0.076	1.32 (1.10-1.59)	0.003

Loci are arranged in the order rs174546, rs174601. Haplotype with frequency less than 3% was pooled and not analyzed. CAD, coronary artery disease; IS, ischemic stroke.

Table 4. Effect of genotypes on angiographic severity of CAD

Genotype	rs174546		rs174601	
	OR (95% CI)	*P*	OR (95% CI)	*P*
CC	1		1	
CT	0.99 (0.47-2.07)	0.972	1.19 (0.56-2.51)	0.658
TT	0.90 (0.44-1.84)	0.766	0.94 (0.46-1.93)	0.869
C	1		1	
T	1.05 (0.71-1.54)	0.822	1.04 (0.63-1.17)	0.875
CC	1		1	
CT + TT	0.92 (0.46-1.84)	0.809	1.17 (0.59-2.33)	0.653
TT	1		1	
CC + TC	1.03 (0.69-1.53)	0.888	1.15 (0.77-1.71)	0.504

CAD, coronary artery disease.

the serum lipid parameter at a value of $P < 0.025$ (corresponding to $P < 0.05$ after adjusting for two independent tests by the Bonferroni correction) were considered statistically significant. Unconditional logistic regression was used to assess the correlation between the risk of CAD and IS and genotypes. Age, gender, BMI, smoking and alcohol consumption were adjusted for the statistical analysis. Odds ratio (OR) and 95% confidence interval (CI) were calculated using unconditional logistic regression. Results were considered to be statistically significant if bilateral *P*-values were less than 0.05. The pattern of pair-wise LD between the selected SNPs was measured by D' and r^2, and haplotype frequencies were calculated using the SHEsis software [36].

Results

Characteristics of the subjects

The clinical characteristics of the patients and healthy controls are shown in **Table 1**. The differences in age, gender, serum LDL-C and ApoB levels, and the percentages of subjects who smoked cigarettes were not significant between controls and CAD or IS patients ($P > 0.05$). Compared with the controls group, more patients in CAD or IS groups had type 2 diabetes mellitus (T2DM), hypertension and hyperlipidemia. The patient groups also had significant higher BMI, pulse pressure, serum TG, and lower levels of serum TC, HDL-C, ApoAI, the ApoAI/ApoB ratio ($P < 0.05$). In addition, compared with the control group, the IS patients had higher systolic blood pressure levels and lower percentage of subjects who consumed alcohol ($P < 0.05$), but there was no difference in diastolic blood pressure ($P > 0.05$). The CAD patients, in contrast, had lower levels of diastolic blood pressure ($P < 0.05$). There was no difference in systolic blood pressure ($P > 0.05$). In comparison with CAD patients, the IS patients had higher levels of blood pressure and HDL-C, and lower the percentages of subjects who suffered from T2DM ($P < 0.05$).

Genotypic and allelic frequencies

The genotypic and allelic frequencies of *FADS1* rs174546 and *FADS2* rs174601 are presented in **Table 2**. The genotype distribution was concordant with the Hardy-Weinberg proportions in both patients and controls. For rs174546 SNP, the frequency of the T and C alleles was 67.7% and 32.3% in the controls, 71.7% and 28.3% in the CAD patients, and 72.2% and 27.8% in the IS patients respectively. The frequency of the TT, TC and CC genotypes was 45.5%, 44.2% and 10.3% in the controls, 53.0%, 37.4% and 9.6% in the CAD patients, and 53.3%, 37.8% and 8.9% in the IS patients respectively. For rs174601 SNP, the frequency of the T and C alleles was 67.9% and 32.1% in the controls, 72.8% and 27.2% in the CAD patients, and 74.1% and 25.9% in the IS patients respectively. The frequency of the TT, TC and CC genotypes was 46.1%, 43.6% and 10.3% in the controls, 54.7%, 36.3% and 9.0% in the CAD patients, and 56.0%, 36.0% and 8.0% in the IS patients respectively. The genotypic and allelic frequencies were different between the con-

Table 5. The relative risk factors for CAD and IS

Relative Factors	CAD		IS	
	OR (95% CI)	*P*	OR (95% CI)	P
Male	1		1	
Female	0.78 (0.56-1.08)	0.130	0.87 (0.63-1.21)	0.415
Aged ≤ 60 year	1		1	
Aged > 60 year	1.05 (0.81-1.37)	0.712	0.96 (0.74-1.25)	0.745
BMI ≤ 24 kg/m^2	1		1	
BMI > 24 kg/m^2	2.45 (1.85-3.23)	< 0.001	1.84 (1.39-2.44)	< 0.001
Nonsmoking	1		1	
Smoking	1.32 (0.99-1.77)	0.058	1.17 (0.88-1.56)	0.284
Nondrinking	1		1	
Drinking	0.71 (0.54-0.94)	0.016	0.64 (0.48-0.86)	0.003
Normotensive	1		1	
Hypertension	1.96 (1.50-2.56)	< 0.001	2.38 (1.83-3.09)	< 0.001
Normal blood lipids	1		1	
Hyperlipidemia	1.57 (1.21-2.05)	0.001	2.38 (1.83-3.09)	< 0.001
Non-diabetes	1		1	
Diabetes	5.60 (3.51-8.94)	< 0.001	3.26 (2.01-5.31)	< 0.001

CAD, coronary artery disease; IS, ischemic stroke.

trols and CAD patients and between the controls and IS patients.

FADS1/FADS2 SNPs and the risk of CAD and IS

For rs174546 SNP, the T allele was associated with an increased risk of CAD (adjusted Odds ratio (OR) = 1.30, 95% confidence interval (CI) = 1.06-1.59) and IS (adjusted OR = 1.29, 95% CI = 1.06-1.57) (**Table 2**). The CC/TC genotypes were also associated with a decreased risk of CAD (adjusted OR = 0.57, 95% CI = 0.44-0.75 for CC/TC vs. TT) and IS (adjusted OR = 0.62, 95% CI = 0.48-0.80 for CC/TC vs. TT). Similarly, for rs174601 SNP, the T allele was associated with an increased risk of CAD (adjusted OR = 1.38, 95% CI = 1.13-1.69) and IS (adjusted OR = 1.42, 95% CI = 1.16-1.73). The CC/TC genotypes were also associated with a decreased risk of CAD (adjusted OR = 0.54, 95% CI = 0.41-0.70 for CC/TC vs. TT) and IS (adjusted OR = 0.61, 95% CI = 0.48-0.79 for CC/TC vs. TT).

Haplotype and the risk of CAD and IS

The SNPs' LD patterns were assessed by using both the D' and r^2 values. The rs174546 SNP was in strong LD with rs174601 (D' = 0.951; r^2 = 0.860). Hence, we performed haplotype analyses with the two SNPs to assess the associations of the SNPs with the risk of CAD and IS. Two common haplotypes (frequency > 3%) derived from the two SNPs accounted for above 90% haplotype variations. The frequency of C-C haplotype (rs174546-rs174601) was 31.8% in the controls, 24.6% in the CAD patients, and 25.8% in the IS patients. The frequency of T-T haplotype (rs174546-rs174601) was 67.3% in the controls, 69.2% in the CAD patients, and 72.1% in the IS patients. The haplotype of C-C was associated with a decreased risk for CAD and IS (adjusted OR = 0.72, 95% CI = 0.60-0.87 for CAD, and adjusted OR = 0.76, 95% CI = 0.63-0.91 for IS; respectively). In contrast, the haplotype of T-T was associated with an increased risk for IS (adjusted OR = 1.32, 95% CI = 1.10-1.59 for IS **Table 3**).

FADS1/FADS2 SNPs and the angiographic severity of CAD

As shown in **Table 4**, there were no significant effects of the rs174546 and rs174601 on angiographic severity of CAD in different genetic models ($P > 0.05$).

Related Risk Factors for CAD and IS

Multivariate logistic analysis showed that the incidence of CAD and IS positively correlated with BMI, diabetes, hypertension and hyperlipidemia and negatively correlated with alcohol consumption (**Table 5**).

FADS1/FADS2 SNPs and serum lipid levels

The serum lipid levels were different between genotypes in the CAD and IS patients, but not in the controls (**Table 6**). After correction for multiple testing, the subjects with rs174601 TT genotype had lower TC, HDL-C and ApoAI levels than the subjects with CC/TC genotypes in CAD patients ($P < 0.025$). Carrying T allele carriers of the rs174546 or rs174601 SNP had lower HDL-C and ApoAI levels than the non- carriers in IS patients ($P < 0.025$).

Table 6. Effect of the genotypes on serum lipid levels

Genotype Controls	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)	ApoAI (g/L)	ApoB (g/L)	ApoAI/ApoB
rs174546							
CC	4.98 ± 0.14	1.06 (0.88)	1.85 ± 0.06	2.70 ± 0.10	1.41 ± 0.03	0.92 ± 0.03	1.56 ± 0.06
CT	4.93 ± 0.07	0.99 (0.62)	1.93 ± 0.03	2.75 ± 0.05	1.40 ± 0.02	0.90 ± 0.01	1.62 ± 0.03
TT	4.91 ± 0.06	1.01 (0.66)	1.89 ± 0.03	2.79 ± 0.05	1.39 ± 0.02	0.90 ± 0.01	1.64 ± 0.03
P	0.898	0.727	0.490	0.593	0.860	0.713	0.520
TT	4.91 ± 0.06	1.01 (0.66)	1.89 ± 0.03	2.79 ± 0.05	1.39 ± 0.02	0.90 ± 0.01	1.64 ± 0.03
CC + TC	4.94 ± 0.06	0.99 (0.63)	1.91 ± 0.03	2.75 ± 0.04	1.41 ± 0.01	0.91 ± 0.01	1.61 ± 0.03
P	0.699	0.474	0.465	0.456	0.463	0.814	0.504
CC	4.98 ± 0.14	1.06 (0.88)	1.85 ± 0.06	2.70 ± 0.10	1.41 ± 0.03	0.92 ± 0.03	1.56 ± 0.06
TT + TC	4.92 ± 0.05	1.00 (0.64)	1.90 ± 0.02	2.77 ± 0.03	1.40 ± 0.01	0.90 ± 0.01	1.63 ± 0.02
P	0.680	0.910	0.423	0.397	0.801	0.490	0.320
rs174601							
CC	5.03 ± 0.14	1.06 (0.88)	1.88 ± 0.06	2.73 ± 0.10	1.42 ± 0.03	0.93 ± 0.03	1.57 ± 0.06
CT	4.90 ± 0.07	0.99 (0.63)	1.92 ± 0.03	2.74 ± 0.05	1.40 ± 0.02	0.90 ± 0.01	1.62 ± 0.03
TT	4.92 ± 0.06	1.01 (0.64)	1.89 ± 0.03	2.80 ± 0.05	1.39 ± 0.02	0.90 ± 0.01	1.64 ± 0.03
P	0.549	0.768	0.669	0.628	0.737	0.567	0.561
TT	4.92 ± 0.06	1.01 (0.64)	1.89 ± 0.03	2.80 ± 0.05	1.39 ± 0.02	0.90 ± 0.01	1.64 ± 0.03
CC + TC	4.93 ± 0.06	0.99 (0.64)	1.91 ± 0.03	2.74 ± 0.04	1.40 ± 0.01	0.91 ± 0.01	1.61 ± 0.03
P	0.884	0.500	0.486	0.335	0.603	0.819	0.427
CC	5.03 ± 0.14	1.06 (0.88)	1.88 ± 0.06	2.73 ± 0.10	1.42 ± 0.03	0.93 ± 0.03	1.57 ± 0.06
TT + TC	4.91 ± 0.05	1.00 (0.65)	1.90 ± 0.02	2.77 ± 0.03	1.40 ± 0.01	0.90 ± 0.01	1.63 ± 0.02
P	0.401	0.612	0.842	0.700	0.448	0.360	0.402
CAD							
rs174546							
CC	4.87 ± 0.17	1.53 (1.31)	1.14 ± 0.05	2.96 ± 0.14	0.99 ± 0.04	0.99 ± 0.04	1.04 ± 0.26
CT	4.56 ± 0.08	1.33 (0.94)	1.18 ± 0.02	2.73 ± 0.07	1.02 ± 0.02	0.91 ± 0.02	1.09 ± 0.13
TT	4.44 ± 0.07	1.35 (0.93)	1.12 ± 0.02	2.70 ± 0.06	1.00 ± 0.02	0.90 ± 0.02	1.37 ± 0.11
P	0.046	0.371	0.079	0.213	0.711	0.091	0.381
TT	4.44 ± 0.07	1.35 (0.93)	1.12 ± 0.02	2.70 ± 0.06	1.00 ± 0.02	0.90 ± 0.02	1.37 ± 0.11
CC + TC	4.64 ± 0.08	1.38 (0.96)	1.17 ± 0.02	2.78 ± 0.06	1.01 ± 0.02	0.93 ± 0.02	1.17 ± 0.12
P	0.052	0.485	0.033	0.358	0.650	0.203	0.202
CC	4.87 ± 0.17	1.53 (1.31)	1.14 ± 0.05	2.96 ± 0.14	0.99 ± 0.04	0.99 ± 0.04	1.04 ± 0.26
TT + TC	4.50 ± 0.05	1.35 (0.91)	1.14 ± 0.02	2.71 ± 0.05	1.01 ± 0.01	0.91 ± 0.01	1.30 ± 0.09
P	0.034	0.169	0.972	0.085	0.624	0.034	0.338
rs174601							
CC	4.69 ± 0.18	1.47 (1.18)	1.15 ± 0.05	2.80 ± 0.14	0.99 ± 0.04	0.94 ± 0.04	1.08 ± 0.27
CT	4.69 ± 0.09	1.34 (0.60)	1.20 ± 0.02	2.76 ± 0.07	1.05 ± 0.02	0.93 ± 0.02	1.22 ± 0.14
TT	4.42 ± 0.07	1.36 (0.90)	1.10 ± 0.02	2.71 ± 0.06	0.99 ± 0.02	0.90 ± 0.02	1.35 ± 0.11
P	0.054	0.657	0.012	0.774	0.027	0.287	0.575
TT	4.42 ± 0.07	1.36 (0.90)	1.10 ± 0.02	2.71 ± 0.06	0.99 ± 0.02	0.90 ± 0.02	1.35 ± 0.11
CC + TC	4.67 ± 0.08	1.37 (1.02)	1.19 ± 0.02	2.77 ± 0.06	1.04 ± 0.02	0.93 ± 0.02	1.20 ± 0.12
P	0.017	0.516	0.005	0.509	0.021	0.124	0.345
CC	4.69 ± 0.18	1.47 (1.18)	1.15 ± 0.05	2.80 ± 0.14	0.99 ± 0.04	0.94 ± 0.04	1.08 ± 0.27
TT + TC	4.52 ± 0.05	1.36 (0.92)	1.14 ± 0.02	2.73 ± 0.05	1.01 ± 0.01	0.91 ± 0.01	1.30 ± 0.09
P	0.353	0.405	0.889	0.625	0.631	0.381	0.446
IS							

rs174546							
CC	4.42 ± 0.17	1.21 (0.87)	1.36 ± 0.06	2.54 ± 0.13	1.13 ± 0.03	0.88 ± 0.04	1.25 ± 0.09
CT	4.50 ± 0.08	1.35 (0.95)	1.23 ± 0.03	2.69 ± 0.06	1.01 ± 0.02	0.89 ± 0.02	1.13 ± 0.04
TT	4.57 ± 0.07	1.36 (0.93)	1.21 ± 0.02	2.71 ± 0.05	1.02 ± 0.02	0.90 ± 0.01	1.21 ± 0.04
P	0.610	0.242	0.054	0.465	0.003	0.883	0.240
TT	4.57 ± 0.07	1.36 (0.93)	1.21 ± 0.02	2.71 ± 0.05	1.02 ± 0.02	0.90 ± 0.01	1.21 ± 0.04
CC + TC	4.48 ± 0.07	1.33 (0.91)	1.26 ± 0.03	2.66 ± 0.07	1.04 ± 0.01	0.89 ± 0.02	1.15 ± 0.04
P	0.361	0.676	0.141	0.488	0.358	0.669	0.256
CC	4.42 ± 0.17	1.21 (0.87)	1.36 ± 0.06	2.54 ± 0.13	1.13 ± 0.03	0.88 ± 0.04	1.25 ± 0.09
TT + TC	4.54 ± 0.05	1.36 (0.92)	1.21 ± 0.02	2.70 ± 0.04	1.02 ± 0.01	0.90 ± 0.01	1.17 ± 0.03
P	0.503	0.162	0.022	0.232	0.001	0.703	0.417
rs174601							
CC	4.39 ± 0.18	1.22 (0.88)	1.37 ± 0.06	2.50 ± 0.14	1.15 ± 0.03	0.87 ± 0.04	1.27 ± 0.09
CT	4.51 ± 0.08	1.35 (0.97)	1.23 ± 0.03	2.70 ± 0.07	1.01 ± 0.02	0.89 ± 0.02	1.13 ± 0.04
TT	4.56 ± 0.07	1.36 (0.92)	1.21 ± 0.02	2.71 ± 0.05	1.02 ± 0.01	0.90 ± 0.01	1.20 ± 0.03
P	0.642	0.410	0.045	0.349	0.001	0.815	0.226
TT	4.56 ± 0.07	1.36 (0.92)	1.21 ± 0.02	2.71 ± 0.05	1.02 ± 0.01	0.90 ± 0.01	1.20 ± 0.03
CC + TC	4.49 ± 0.07	1.32 (0.93)	1.26 ± 0.03	2.66 ± 0.06	1.04 ± 0.01	0.89 ± 0.02	1.15 ± 0.04
P	0.448	0.816	0.145	0.537	0.305	0.686	0.313
CC	4.39 ± 0.18	1.22 (0.88)	1.37 ± 0.06	2.50 ± 0.14	1.15 ± 0.03	0.87 ± 0.04	1.27 ± 0.09
TT + TC	4.54 ± 0.05	1.36 (0.92)	1.22 ± 0.02	2.70 ± 0.04	1.02 ± 0.01	0.90 ± 0.01	1.17 ± 0.03
P	0.407	0.245	0.017	0.149	< 0.001	0.547	0.319

TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; ApoAI, apolipoprotein AI; ApoB, apolipoprotein B. The value of triglyceride was presented as median (interquartile range) and used Nonparametric Test.

Discussion

Serum concentrations of TC, LDL-C, HDL-C, and TG are one of the most important risk factors for atherosclerosis related diseases and are targets for therapeutic intervention [37]. With the rapid progress in genome-wide association (GWA) studies, a number of novel lipid-related loci have been identified [24, 25]. Several SNPs in the FADS1/FADS2 gene cluster have been reported to be associated with lipid metabolism, suggesting that altered desaturase activities may impact serum lipoprotein levels [14, 21, 24, 25]. However, not all researches have consistent findings. In a meta-analysis, Teslovich et al. [24] reported that the T allele of rs174546 was associated with lower concentrations of HDL-C and LDL-C and higher TG concentrations in European ancestry. In the Doetinchem Cohort Study, Lu et al. [38] found that TC concentrations differed significantly according to the rs174546 genotype. The C allele of rs174546 was associated with higher TC. Additionally, Sone et al. [39] reported that the rs174546 TT genotype was found to be associated with lower LDL-C levels and a lower LDL-C/TC ratio, and the TT genotype reduced the risk of high LDL-C level in Japanese men aged 40-60. In the Chinese middle-east populations, Liu et al. [18] reported that the *FADS1* rs174547 SNP, which was completed LD with rs174546 SNP, the subjects with CC/TC genotypes had significantly higher TG and lower HDL-C concentrations in individuals with average age of 40-50 years old than the subjects with TT genotype. Zhang et al. [40] also found that the T allele of rs174546 marginally associated with TG, with same effect direction to that in Europeans. In contrast, in the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) study, Bokor et al. [14] found that some *FADS1/FADS2* variants, including the rs174546 SNP, were not associated with serum lipid and lipoprotein concentrations in European adolescents. In the present study, our study showed that no association was observed between the SNPs of rs174546 and rs174601 and serum lipid levels in controls. However, after correction for multiple testing, the sub-

jects with rs174601 TT genotype had lower TC, HDL-C and ApoAI levels than the subjects with CC/TC genotypes in CAD patients. The subjects with the rs174546 or rs174601 T allele carriers had lower HDL-C and ApoAI levels than the subjects with non-carriers in IS patients, which inferred that the rs174546 SNP and the rs174601 SNP were likely to influence serum lipid levels. Carrying T allele carriers of the two SNPs and rs174601 TT genotype were associated with decreased serum HDL-C and ApoAI and with an increased risk of CAD and IS. Our results were partly consistent with previous studies. Reasons for these diverse findings remain unclear; one of the possible explanations was based on different genetic background. In our study, the T allele and TT genotype frequencies of the two SNPs were more predominant than the C allele and CC genotype frequencies, which somewhat differed with the data from the data of the International HapMap project: the C and T allele frequencies of rs174546 SNP were 65.9% and 34.1% in CEU (Utah residents with ancestry from northern and western Europe); 98.7% and 1.3% in YRI (Yoruba in Ibadan, Nigeria); 69.8% and 30.2% in JPT (Japanese in Tokyo, Japan); 67.3% and 32.7% in CHB (Han Chinese in Beijing, China); 45.9% and 54.1% in CAD (Chinese in Metropolitan Denver, Colorado). In the previous studies, the C allele frequencies of rs174546 SNP was predominant, which was inconsistent with our study. Another possible explanation was dietary intervention. Dietary habits may differ in different regions, especially dietary LC-PUFAs intake, which could confound the association between the SNPs in this gene cluster and serum lipid levels. In agreement with this hypothesis, AlSaleh et al. [41] recently observed that after the 1.8 g/day eicosapentaenoic acid (20: 5n-3, EPA) and docosahexaenoic acid (22: 6n-3, DHA), TG concentration decreased significantly more in rs174546 T allele carriers than in non-carriers. Thirdly, the participants, especially the individuals with CAD and IS, might have received statins therapies to influence the lipid metabolism. Therefore, the results might not truly reflect the situation of lipids in CAD and IS patients.

In the present study, we showed that the rs174546 and rs174601 T alleles were associated with a higher risk of CAD and IS, suggesting a positive association between the T allele of the two SNPs and CAD and IS. Multivariate analysis also showed that well-known risk factors, such as BMI, diabetes, hypertension and hyperlipidemia, were independently associated with CAD and IS. Additionally, the rs174546 and rs174601 C allele carriers had lower risk of CAD and IS than non-carriers. In our study, we found that the rs174546 SNP and the rs174601 SNP existed in strong LD. Haplotypes analyses suggested that the haplotype of T-T (rs174546-rs174601) was associated with an increased risk of IS. Meanwhile, the haplotype of C-C (rs174546-rs174601) was associated with a decreased risk of CAD and IS after adjusting for potential confounding factors. Previous studies showed that the rs174546 T allele is located within a microRNA target site and is associated with lower *FADS1* mRNA levels in human liver [24]. What's more, all haplotypes carrying rs174546 T allele were associated with lower arachidonic acid (ARA; C20: 4n-6) levels and lower D5D activity [14], suggesting that the rs174546 SNP might be either functional or in LD with other functional SNPs and influence *FADS1/FADS2* transcript abundance. Recently published studies reported that the rs965867 SNP in the 5' UTR of *FADS2*, recently demonstrated to be functional, was in strong LD with rs174546 SNP [14, 24, 33] and influenced *FADS2* transcription [42]. The rs3834458 [T/del] in upstream region of *FADS2*, was also reported to be in strong LD with rs174546 SNP, had mostly associations with the level of the direct precursor of inflammatory eicosanoids [35]. The rs174547 SNP in intron region of *FADS1* was in complete LD with rs174546 SNP, and had been found to associate with *FADS1* transcript levels in the human liver [25]. These associated SNPs modulates expression of *FADS1/FADS2* [25], which affect desaturase activity, as a result, leading to lower LC-PUFAs levels that might be prone to a proinflammatory response favoring atherosclerotic vascular damage [14, 18]. However, the effects of SNPs in the FADS1/FADS2 gene cluster on risk of CAD and IS were an extremely complex process. In the Malmö Diet and Cancer (MDC) cohort study, Hellstrand et al. [43] have not observed any statistically significant association between rs174546 and risk of CAD and IS, but a borderline interaction was observed between the a-linolenic acid (ALA) (18: 3n-3)-to-linoleic acid (LA) (18: 2n-6) intake ratio and *FADS1* genotype on CAD incidence. Likewise, they also observed a significant interaction

between ALA and *FADS1* genotype on IS incidence, suggesting the associated effects of SNPs in the FADS gene cluster on incident CAD and IS may be modified by dietary PUFAs intakes, and the health benefit of dietary PUFAs may depend on individual *FADS1/FADS2* genotypes.

In the present study, we further investigated the association between the SNPs of rs174546 and rs174601 and angiographic severity of CAD. Consequently, we failed to found their relationship, which indicated that the pathogenic effects of this gene cluster were unlikely to be a major pathway for lower CAD and IS risk, but subtle effects cannot be excluded.

This study has two potential limitations. First, the drug information was not available in some participants, thus the effect of drugs or treatments could not be analyzed or excluded. The patient groups had lower levels of serum TC, HDL-C than the healthy controls, which was possibly related to taking cholesterol-lowering medications in the patient groups. Second, although we found that the two SNPs were associated with serum lipid levels and risk of CAD and IS, we did not explore an association between the two SNPs and the desaturase activities, which are important for the further functional evaluation.

In conclusion, The present study firstly report that the rs174601SNP is associated with risk of CAD and IS, and confirm that the T allele and TT genotype frequencies of the rs174546 SNP and rs174601 SNP are predominant in Chinese south population. The T allele, haplotype of T-T are associated with increased risk of CAD and IS. Correspondingly, carrying C allele, haplotype of C-C are associated with reduced risk of CAD and IS. The two SNPs are likely to influence serum lipid levels. Carrying T allele carriers and rs174601 TT genotype are associated with decreased serum HDL-C and ApoAI in the patients groups and with an increased risk of CAD and IS. Further functional investigations are needed to clarify whether the two SNPs are functional or not and how they actually affect the serum lipid levels and risk of CAD and IS.

Acknowledgements

This study was supported by the National Natural Science Foundation of China (No. 30960130), the Science Foundation of Guangxi Returned Oversea Scholars (No. 0991004) and the Innovation Project of Guangxi Graduate Education (No. YCBZ2013013).

Disclosure of conflict of interest

None.

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References

[1] Ruff CT and Braunwald E. The evolving epidemiology of acute coronary syndromes. Nat Rev Cardiol 2011; 8: 140-147.

[2] Strong K, Mathers C and Bonita R. Preventing stroke: saving lives around the world. Lancet Neurol 2007; 6: 182-187.

[3] Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, Ezzati M, Shibuya K, Salomon JA, Abdalla S, Aboyans V, Abraham J, Ackerman I, Aggarwal R, Ahn SY, Ali MK, Alvarado M, Anderson HR, Anderson LM, Andrews KG, Atkinson C, Baddour LM, Bahalim AN, Barker-Collo S, Barrero LH, Bartels DH, Basanez MG, Baxter A, Bell ML, Benjamin EJ, Bennett D, Bernabe E, Bhalla K, Bhandari B, Bikbov B, Bin Abdulhak A, Birbeck G, Black JA, Blencowe H, Blore JD, Blyth F, Bolliger I, Bonaventure A, Boufous S, Bourne R, Boussinesq M, Braithwaite T, Brayne C, Bridgett L, Brooker S, Brooks P, Brugha TS, Bryan-Hancock C, Bucello C, Buchbinder R, Buckle G, Budke CM, Burch M, Burney P, Burstein R, Calabria B, Campbell B, Canter CE, Carabin H, Carapetis J, Carmona L, Cella C, Charlson F, Chen H, Cheng AT, Chou D, Chugh SS, Coffeng LE, Colan SD, Colquhoun S, Colson KE, Condon J, Connor MD, Cooper LT, Corriere M, Cortinovis M, de Vaccaro KC, Couser W, Cowie BC, Criqui MH, Cross M, Dabhadkar KC, Dahiya M, Dahodwala N, Damsere-Derry J, Danaei G, Davis A, De Leo D, Degenhardt L, Dellavalle R, Delossantos A, Denenberg J, Derrett S, Des Jarlais DC, Dharmaratne SD, Dherani M, Diaz-Torne C, Dolk H, Dorsey ER, Driscoll T, Duber H, Ebel B, Edmond K, Elbaz A, Ali SE, Erskine H, Erwin PJ, Espindola P, Ewoigbokhan SE, Farzadfar F, Feigin V, Felson DT, Ferrari A, Ferri CP, Fevre EM, Finucane MM, Flaxman S, Flood L, Foreman K, Forouzanfar

MH, Fowkes FG, Fransen M, Freeman MK, Gabbe BJ, Gabriel SE, Gakidou E, Ganatra HA, Garcia B, Gaspari F, Gillum RF, Gmel G, Gonzalez-Medina D, Gosselin R, Grainger R, Grant B, Groeger J, Guillemin F, Gunnell D, Gupta R, Haagsma J, Hagan H, Halasa YA, Hall W, Haring D, Haro JM, Harrison JE, Havmoeller R, Hay RJ, Higashi H, Hill C, Hoen B, Hoffman H, Hotez PJ, Hoy D, Huang JJ, Ibeanusi SE, Jacobsen KH, James SL, Jarvis D, Jasrasaria R, Jayaraman S, Johns N, Jonas JB, Karthikeyan G, Kassebaum N, Kawakami N, Keren A, Khoo JP, King CH, Knowlton LM, Kobusingye O, Koranteng A, Krishnamurthi R, Laden F, Lalloo R, Laslett LL, Lathlean T, Leasher JL, Lee YY, Leigh J, Levinson D, Lim SS, Limb E, Lin JK, Lipnick M, Lipshultz SE, Liu W, Loane M, Ohno SL, Lyons R, Mabweijano J, MacIntyre MF, Malekzadeh R, Mallinger L, Manivannan S, Marcenes W, March L, Margolis DJ, Marks GB, Marks R, Matsumori A, Matzopoulos R, Mayosi BM, McAnulty JH, McDermott MM, McGill N, McGrath J, Medina-Mora ME, Meltzer M, Mensah GA, Merriman TR, Meyer AC, Miglioli V, Miller M, Miller TR, Mitchell PB, Mock C, Mocumbi AO, Moffitt TE, Mokdad AA, Monasta L, Montico M, Moradi-Lakeh M, Moran A, Morawska L, Mori R, Murdoch ME, Mwaniki MK, Naidoo K, Nair MN, Naldi L, Narayan KM, Nelson PK, Nelson RG, Nevitt MC, Newton CR, Nolte S, Norman P, Norman R, O'Donnell M, O'Hanlon S, Olives C, Omer SB, Ortblad K, Osborne R, Ozgediz D, Page A, Pahari B, Pandian JD, Rivero AP, Patten SB, Pearce N, Padilla RP, Perez-Ruiz F, Perico N, Pesudovs K, Phillips D, Phillips MR, Pierce K, Pion S, Polanczyk GV, Polinder S, Pope CA 3rd, Popova S, Porrini E, Pourmalek F, Prince M, Pullan RL, Ramaiah KD, Ranganathan D, Razavi H, Regan M, Rehm JT, Rein DB, Remuzzi G, Richardson K, Rivara FP, Roberts T, Robinson C, De Leon FR, Ronfani L, Room R, Rosenfeld LC, Rushton L, Sacco RL, Saha S, Sampson U, Sanchez-Riera L, Sanman E, Schwebel DC, Scott JG, Segui-Gomez M, Shahraz S, Shepard DS, Shin H, Shivakoti R, Singh D, Singh GM, Singh JA, Singleton J, Sleet DA, Sliwa K, Smith E, Smith JL, Stapelberg NJ, Steer A, Steiner T, Stolk WA, Stovner LJ, Sudfeld C, Syed S, Tamburlini G, Tavakkoli M, Taylor HR, Taylor JA, Taylor WJ, Thomas B, Thomson WM, Thurston GD, Tleyjeh IM, Tonelli M, Towbin JA, Truelsen T, Tsilimbaris MK, Ubeda C, Undurraga EA, van der Werf MJ, van Os J, Vavilala MS, Venketasubramanian N, Wang M, Wang W, Watt K, Weatherall DJ, Weinstock MA, Weintraub R, Weisskopf MG, Weissman MM, White RA, Whiteford H, Wiebe N, Wiersma ST, Wilkinson JD, Williams HC, Williams SR, Witt E, Wolfe F, Woolf AD, Wulf S, Yeh PH, Zaidi AK, Zheng ZJ, Zonies D, Lopez AD, AlMazroa MA and Memish ZA. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012; 380: 2197-2223.

[4] Fruchart JC, Nierman MC, Stroes ES, Kastelein JJ and Duriez P. New risk factors for atherosclerosis and patient risk assessment. Circulation 2004; 109: III15-19.

[5] Sampath H and Ntambi JM. Polyunsaturated fatty acid regulation of genes of lipid metabolism. Annu Rev Nutr 2005; 25: 317-340.

[6] Glaser C, Lattka E, Rzehak P, Steer C and Koletzko B. Genetic variation in polyunsaturated fatty acid metabolism and its potential relevance for human development and health. Matern Child Nutr 2011; 7 Suppl 2: 27-40.

[7] Mozaffarian D and Wu JH. Omega-3 fatty acids and cardiovascular disease: effects on risk factors, molecular pathways, and clinical events. J Am Coll Cardiol 2011; 58: 2047-2067.

[8] Harris WS and Bulchandani D. Why do omega-3 fatty acids lower serum triglycerides? Curr Opin Lipidol 2006; 17: 387-393.

[9] Hein GJ, Bernasconi AM, Montanaro MA, Pellon-Maison M, Finarelli G, Chicco A, Lombardo YB and Brenner RR. Nuclear receptors and hepatic lipidogenic enzyme response to a dyslipidemic sucrose-rich diet and its reversal by fish oil n-3 polyunsaturated fatty acids. Am J Physiol Endocrinol Metab 2010; 298: E429-439.

[10] Nakamura K, Kariyazono H, Komokata T, Hamada N, Sakata R and Yamada K. Influence of preoperative administration of omega-3 fatty acid-enriched supplement on inflammatory and immune responses in patients undergoing major surgery for cancer. Nutrition 2005; 21: 639-649.

[11] Rizza S, Tesauro M, Cardillo C, Galli A, Iantorno M, Gigli F, Sbraccia P, Federici M, Quon MJ and Lauro D. Fish oil supplementation improves endothelial function in normoglycemic offspring of patients with type 2 diabetes. Atherosclerosis 2009; 206: 569-574.

[12] Haberka M, Mizia-Stec K, Mizia M, Janowska J, Gieszczyk K, Chmiel A, Zahorska-Markiewicz B and Gasior Z. N-3 polyunsaturated fatty acids early supplementation improves ultrasound indices of endothelial function, but not through NO inhibitors in patients with acute myocardial infarction: N-3 PUFA supplementation in acute myocardial infarction. Clin Nutr 2011; 30: 79-85.

[13] Nakamura MT and Nara TY. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. Annu Rev Nutr 2004; 24: 345-376.

[14] Bokor S, Dumont J, Spinneker A, Gonzalez-Gross M, Nova E, Widhalm K, Moschonis G, Stehle P, Amouyel P, De Henauw S, Molnar D, Moreno LA, Meirhaeghe A and Dallongeville J. Single nucleotide polymorphisms in the FADS gene cluster are associated with delta-5 and delta-6 desaturase activities estimated by serum fatty acid ratios. J Lipid Res 2010; 51: 2325-2333.

[15] Stoffel W, Holz B, Jenke B, Binczek E, Gunter RH, Kiss C, Karakesisoglou I, Thevis M, Weber AA, Arnhold S and Addicks K. Delta6-desaturase (FADS2) deficiency unveils the role of omega3- and omega6-polyunsaturated fatty acids. EMBO J 2008; 27: 2281-2292.

[16] Lu Y, Vaarhorst A, Merry AH, Dolle ME, Hovenier R, Imholz S, Schouten LJ, Heijmans BT, Muller M, Slagboom PE, van den Brandt PA, Gorgels AP, Boer JM and Feskens EJ. Markers of endogenous desaturase activity and risk of coronary heart disease in the CAREMA cohort study. PLoS One 2012; 7: e41681.

[17] Malerba G, Schaeffer L, Xumerle L, Klopp N, Trabetti E, Biscuola M, Cavallari U, Galavotti R, Martinelli N, Guarini P, Girelli D, Olivieri O, Corrocher R, Heinrich J, Pignatti PF and Illig T. SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. Lipids 2008; 43: 289-299.

[18] Liu SJ, Zhi H, Chen PZ, Chen W, Lu F, Ma GS, Dai JC, Shen C, Liu NF, Hu ZB, Wang H and Shen HB. Fatty acid desaturase 1 polymorphisms are associated with coronary heart disease in a Chinese population. Chin Med J (Engl) 2012; 125: 801-806.

[19] Qin L, Sun L, Ye L, Shi J, Zhou L, Yang J, Du B, Song Z, Yu Y and Xie L. A case-control study between the gene polymorphisms of polyunsaturated fatty acids metabolic rate-limiting enzymes and coronary artery disease in a Chinese Han population. Prostaglandins Leukot Essent Fatty Acids 2011; 85: 329-333.

[20] Martinelli N, Girelli D, Malerba G, Guarini P, Illig T, Trabetti E, Sandri M, Friso S, Pizzolo F, Schaeffer L, Heinrich J, Pignatti PF, Corrocher R and Olivieri O. FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. Am J Clin Nutr 2008; 88: 941-949.

[21] Kwak JH, Paik JK, Kim OY, Jang Y, Lee SH, Ordovas JM and Lee JH. FADS gene polymorphisms in Koreans: association with omega6 polyunsaturated fatty acids in serum phospholipids, lipid peroxides and coronary artery disease. Atherosclerosis 2011; 214: 94-100.

[22] Harris WS, Poston WC and Haddock CK. Tissue n-3 and n-6 fatty acids and risk for coronary heart disease events. Atherosclerosis 2007; 193: 1-10.

[23] Li SW, Lin K, Ma P, Zhang ZL, Zhou YD, Lu SY, Zhou X and Liu SM. FADS gene polymorphisms confer the risk of coronary artery disease in a Chinese Han population through the altered desaturase activities: based on high-resolution melting analysis. PLoS One 2013; 8: e55869.

[24] Teslovich TM, Musunuru K, Smith AV, Edmondson AC, Stylianou IM, Koseki M, Pirruccello JP, Ripatti S, Chasman DI, Willer CJ, Johansen CT, Fouchier SW, Isaacs A, Peloso GM, Barbalic M, Ricketts SL, Bis JC, Aulchenko YS, Thorleifsson G, Feitosa MF, Chambers J, Orho-Melander M, Melander O, Johnson T, Li X, Guo X, Li M, Shin Cho Y, Jin Go M, Jin Kim Y, Lee JY, Park T, Kim K, Sim X, Twee-Hee Ong R, Croteau-Chonka DC, Lange LA, Smith JD, Song K, Hua Zhao J, Yuan X, Luan J, Lamina C, Ziegler A, Zhang W, Zee RY, Wright AF, Witteman JC, Wilson JF, Willemsen G, Wichmann HE, Whitfield JB, Waterworth DM, Wareham NJ, Waeber G, Vollenweider P, Voight BF, Vitart V, Uitterlinden AG, Uda M, Tuomilehto J, Thompson JR, Tanaka T, Surakka I, Stringham HM, Spector TD, Soranzo N, Smit JH, Sinisalo J, Silander K, Sijbrands EJ, Scuteri A, Scott J, Schlessinger D, Sanna S, Salomaa V, Saharinen J, Sabatti C, Ruokonen A, Rudan I, Rose LM, Roberts R, Rieder M, Psaty BM, Pramstaller PP, Pichler I, Perola M, Penninx BW, Pedersen NL, Pattaro C, Parker AN, Pare G, Oostra BA, O'Donnell CJ, Nieminen MS, Nickerson DA, Montgomery GW, Meitinger T, McPherson R, McCarthy MI, McArdle W, Masson D, Martin NG, Marroni F, Mangino M, Magnusson PK, Lucas G, Luben R, Loos RJ, Lokki ML, Lettre G, Langenberg C, Launer LJ, Lakatta EG, Laaksonen R, Kyvik KO, Kronenberg F, Konig IR, Khaw KT, Kaprio J, Kaplan LM, Johansson A, Jarvelin MR, Janssens AC, Ingelsson E, Igl W, Kees Hovingh G, Hottenga JJ, Hofman A, Hicks AA, Hengstenberg C, Heid IM, Hayward C, Havulinna AS, Hastie ND, Harris TB, Haritunians T, Hall AS, Gyllensten U, Guiducci C, Groop LC, Gonzalez E, Gieger C, Freimer NB, Ferrucci L, Erdmann J, Elliott P, Ejebe KG, Doring A, Dominiczak AF, Demissie S, Deloukas P, de Geus EJ, de Faire U, Crawford G, Collins FS, Chen YD, Caulfield MJ, Campbell H, Burtt NP, Bonnycastle LL, Boomsma DI, Boekholdt SM, Bergman RN, Barroso I, Bandinelli S, Ballantyne CM, Assimes TL, Quertermous T, Altshuler D, Seielstad M, Wong TY, Tai ES, Feranil AB, Kuzawa CW, Adair LS, Taylor HA Jr, Borecki IB, Gabriel SB, Wilson JG, Holm H, Thorsteinsdottir U, Gudnason V, Krauss RM, Mohlke KL, Ordovas JM, Munroe PB, Kooner

JS, Tall AR, Hegele RA, Kastelein JJ, Schadt EE, Rotter JI, Boerwinkle E, Strachan DP, Mooser V, Stefansson K, Reilly MP, Samani NJ, Schunkert H, Cupples LA, Sandhu MS, Ridker PM, Rader DJ, van Duijn CM, Peltonen L, Abecasis GR, Boehnke M and Kathiresan S. Biological, clinical and population relevance of 95 loci for blood lipids. Nature 2010; 466: 707-713.

[25] Kathiresan S, Willer CJ, Peloso GM, Demissie S, Musunuru K, Schadt EE, Kaplan L, Bennett D, Li Y, Tanaka T, Voight BF, Bonnycastle LL, Jackson AU, Crawford G, Surti A, Guiducci C, Burtt NP, Parish S, Clarke R, Zelenika D, Kubalanza KA, Morken MA, Scott LJ, Stringham HM, Galan P, Swift AJ, Kuusisto J, Bergman RN, Sundvall J, Laakso M, Ferrucci L, Scheet P, Sanna S, Uda M, Yang Q, Lunetta KL, Dupuis J, de Bakker PI, O'Donnell CJ, Chambers JC, Kooner JS, Hercberg S, Meneton P, Lakatta EG, Scuteri A, Schlessinger D, Tuomilehto J, Collins FS, Groop L, Altshuler D, Collins R, Lathrop GM, Melander O, Salomaa V, Peltonen L, Orho-Melander M, Ordovas JM, Boehnke M, Abecasis GR, Mohlke KL and Cupples LA. Common variants at 30 loci contribute to polygenic dyslipidemia. Nat Genet 2009; 41: 56-65.

[26] Chen Q, Reis SE, Kammerer CM, McNamara DM, Holubkov R, Sharaf BL, Sopko G, Pauly DF, Merz CN and Kamboh MI. Association between the severity of angiographic coronary artery disease and paraoxonase gene polymorphisms in the National Heart, Lung, and Blood Institute-sponsored Women's Ischemia Syndrome Evaluation (WISE) study. Am J Hum Genet 2003; 72: 13-22.

[27] Xu Y, Wang W, Zhang L, Qi LP, Li LY, Chen LF, Fang Q, Dang AM and Yan XW. A polymorphism in the ABCG1 promoter is functionally associated with coronary artery disease in a Chinese Han population. Atherosclerosis 2011; 219: 648-654.

[28] Adams HP Jr, Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL and Marsh EE 3rd. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993; 24: 35-41.

[29] Wu DF, Yin RX, Hu XJ, Aung LH, Cao XL, Miao L, Li Q, Yan TT, Wu JZ and Pan SL. Association of rs5888 SNP in the scavenger receptor class B type 1 gene and serum lipid levels. Lipids Health Dis 2012; 11: 50.

[30] Wu DF, Yin RX, Yan TT, Aung LH, Cao XL, Miao L, Li Q, Hu XJ, Wu JZ and Liu CW. The SCARB1 rs5888 SNP and serum lipid levels in the Guangxi Mulao and Han populations. Int J Med Sci 2012; 9: 715-724.

[31] Wu DF, Yin RX, Cao XL, Chen WX, Aung LH, Wang W, Huang KK, Huang P, Zeng XN and Wu J. Scavenger receptor class B type 1 gene rs5888 single nucleotide polymorphism and the risk of coronary artery disease and ischemic stroke: a case-control study. Int J Med Sci 2013; 10: 1771-1777.

[32] Huang KK, Yin RX, Zeng XN, Huang P, Lin QZ, Wu J, Guo T, Wang W, Yang DZ and Lin WX. Association of the rs7395662 SNP in the MADD-FOLH1 and several environmental factors with serum lipid levels in the Mulao and Han populations. Int J Med Sci 2013; 10: 1537-1546.

[33] Chalmers J, MacMahon S, Mancia G, Whitworth J, Beilin L, Hansson L, Neal B, Rodgers A, Ni Mhurchu C and Clark T. 1999 World Health Organization-International Society of Hypertension Guidelines for the management of hypertension. Guidelines sub-committee of the World Health Organization. Clin Exp Hypertens 1999; 21: 1009-1060.

[34] Zhou BF. Effect of body mass index on all-cause mortality and incidence of cardiovascular diseases--report for meta-analysis of prospective studies open optimal cut-off points of body mass index in Chinese adults. Biomed Environ Sci 2002; 15: 245-252.

[35] Schaeffer L, Gohlke H, Muller M, Heid IM, Palmer LJ, Kompauer I, Demmelmair H, Illig T, Koletzko B and Heinrich J. Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. Hum Mol Genet 2006; 15: 1745-1756.

[36] Shi YY and He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005; 15: 97-98.

[37] Kathiresan S, Manning AK, Demissie S, D'Agostino RB, Surti A, Guiducci C, Gianniny L, Burtt NP, Melander O, Orho-Melander M, Arnett DK, Peloso GM, Ordovas JM and Cupples LA. A genome-wide association study for blood lipid phenotypes in the Framingham Heart Study. BMC Med Genet 2007; 8 Suppl 1: S17.

[38] Lu Y, Feskens EJ, Dolle ME, Imholz S, Verschuren WM, Muller M and Boer JM. Dietary n-3 and n-6 polyunsaturated fatty acid intake interacts with FADS1 genetic variation to affect total and HDL-cholesterol concentrations in the Doetinchem Cohort Study. Am J Clin Nutr 2010; 92: 258-265.

[39] Sone Y, Kido T, Ainuki T, Sonoda M, Ichi I, Kodama S, Sone H, Kondo K, Morita Y, Egawa S, Kawahara K, Otsuka Y and Fujiwara Y. Genetic variants of the fatty acid desaturase gene cluster are associated with plasma LDL cholesterol levels in Japanese males. J Nutr Sci Vitaminol (Tokyo) 2013; 59: 325-335.

[40] Zhang Z, Tao L, Chen Z, Zhou D, Kan M, Zhang D, Li C, He L and Liu Y. Association of genetic loci with blood lipids in the Chinese population. PLoS One 2011; 6: e27305.

[41] AlSaleh A, Maniou Z, Lewis FJ, Hall WL, Sanders TA and O'Dell SD. Genetic predisposition scores for dyslipidaemia influence plasma lipid concentrations at baseline, but not the changes after controlled intake of n-3 polyunsaturated fatty acids. Genes Nutr 2014; 9: 412.

[42] Lattka E, Eggers S, Moeller G, Heim K, Weber M, Mehta D, Prokisch H, Illig T and Adamski J. A common FADS2 promoter polymorphism increases promoter activity and facilitates binding of transcription factor ELK1. J Lipid Res 2010; 51: 182-191.

[43] Hellstrand S, Ericson U, Gullberg B, Hedblad B, Orho-Melander M and Sonestedt E. Genetic variation in FADS1 has little effect on the association between dietary PUFA intake and cardiovascular disease. J Nutr 2014; 144: 1356-1363.