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Interaction among dietary n-3 and n-6 polyunsaturated fatty acid intake, fatty acid desaturase 2 genetic variants, and low-density lipoprotein cholesterol levels in type 2 diabetes patients

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ABSTRACT

Aims/Introduction: Fatty acid desaturase (FADS) genetic polymorphisms are strongly correlated with the risk of dyslipidemia and cardiovascular disease. In this study, we examined the impact of FADS1 and FADS2 genetic variants on plasma lipid status, and assessed interactions between FADS genetic polymorphisms and plasma n-3/n-6 fatty acids regarding lipid status within a population of 816 Taiwanese patients with type 2 diabetes.

Materials and Methods: Selected tag single-nucleotide polymorphisms (*FADS1* rs174546 [T/C]; *FADS2* rs174602 [A/G] and rs2072114 [A/G]) were genotyped (n = 816).

Results: The distribution of genotypes were compared with reports publicly available in the Genome Aggregation Database for East Asian populations (https://gnomad.broadinstitute.org). In the subgroup of patients not taking lipid-lowering medications (n = 192), we observed that the G allele of *FADS2* rs174602 was statistically significantly correlated with lower low-density lipoprotein cholesterol (LDL-C) concentrations (P = 0.001), whereas the G allele of rs2072114 was marginally associated with LDL-C concentrations (P = 0.091). Using a general linear model adjusted for confounding factors, statistically significant interactions (P = 0.016) between single-nucleotide polymorphisms in rs2072114 and a low alpha-linolenic acid (18:3n-3)/linoleic acid (18:2n-6) ratio; the G allele correlated with lower LDL-C levels among individuals with a low alpha-linolenic acid/linoleic acid ratio. Interaction between rs174602 single-nucleotide polymorphisms and low alpha-linolenic acid/linoleic acid values on LDL-C was only marginally significant (P = 0.063).

Conclusions: Our results show the role of n-3/n-6 dietary polyunsaturated fatty acids in modifying the effects of genetic susceptibility on lipoprotein concentrations in patients with type 2 diabetes. Our findings highlight the potential of interventions with dietary polyunsaturated fatty acids regarding developing individualized prevention strategies for type 2 diabetes presenting with co-occurring dyslipidemia and cardiovascular diseases.

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INTRODUCTION

Type 2 diabetes mellitus is known to be an important risk factor for various cardiovascular diseases, as well as chronic kidney disease, and is frequently accompanied by altered blood lipid status[1,2]. The prevalence of diabetic dyslipidemia was reported as approximately 46.8% and 59.3% in Asians with pre-diabetes and type 2 diabetes mellitus, respectively (these conditions represent the most common metabolic complications/conditions occurring in this population)[3]. Fatty acid desaturase (*FADS1* and *FADS2*) genes encode delta-5 and delta-6 desaturase[4], which are rate-limiting enzymes involved in the desaturation and elongation of α-linolenic acid (ALA; C18:3n-3) and linoleic acid (LA; C18:2n-6) into long-chain polyunsaturated fatty acids (PUFAs), such as arachidonic acid (AA; 20:4n-6), eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)[5]. The effects of PUFAs might differ between individuals as a result of genetic variations in the general population, as well as due to metabolic syndromes and cardiovascular diseases[6–8]. Single-nucleotide polymorphisms (SNPs) in the fatty acid desaturase (*FADS*) gene cluster have been associated with blood PUFA composition in genome-wide association studies[9–11].

Some randomized control trials have found that eicosapentaenoic acid and docosahexaenoic acid intake is derived from fish, and is generally considered to be beneficial for decreasing cardiovascular diseases risk[12,13], especially in patients with diabetes mellitus[14]. Meanwhile, other trials have failed to confirm similar benefits[15,16]. More recently, results from the Reduction of Cardiovascular Events With Icosapent Ethyl–Intervention (REDUCE-IT) trial showed a dramatic 25% reduction in cardiovascular events, as well as an important reduction (−20%) in the risk of cardiovascular death as a consequence of administering high-dose icosapent ethyl (4 g/day) combined with statin treatment in patients with elevated triglyceride levels despite previously having been prescribed statins alone[17].

Both internal and external factors can modulate an individual's plasma lipid levels, as well as interindividual variability in cardiovascular profiles. Recently, common FADS genetic variants were reported to influence blood lipid profiles and/or desaturase activities in studies carried out among healthy adults of Mexican[18], European[19] and Asian origins[20,21]. Furthermore, gene–diet interactions between *FADS* SNPs and dietary PUFAs have also been shown to affect blood lipids in white people[22,23].

To date, very few studies have explored the interrelationships among *FADS* genetic polymorphisms, dietary PUFA intake and dyslipidemia in individuals with diabetes[24,25]. In this study, we hypothesized that we would detect a gene–diet interaction between variations in the *FADS* gene and plasma PUFA composition. SNPs in *FAD1* (rs174546) and *FADS2* (rs2072114) were selected to examine their interactions with n-3/n-6 PUFA regarding effects on blood lipid in Taiwanese patients with type 2 diabetes mellitus.

MATERIALS AND METHODS

Study participants and inclusion criteria

The enrolled participants were patients with type 2 diabetes mellitus who originally participated in diabetes management through an integrated delivery system (DMIDS; ClinicalTrials.gov NCT00288678)[26]. We recruited patients aged between 30 and 70 years who had been diagnosed or newly diagnosed with type 2 diabetes by their primary care physicians at a range of primary healthcare clinics based on the definition established by the American Diabetes Association[27]. Exclusion criteria at baseline recruitment included pregnancy, as well as a history of myocardial infarction, cerebrovascular accident (i.e., stroke), foot amputation and uremia under dialysis. The protocol details of the study have been described previously[26]. Briefly, 1,209 newly diagnosed type 2 diabetes mellitus patients were recruited for a randomized controlled trial to assess the effects of diabetes management on glycemic control (August 2003 to December 2005). Of these patients, a total of 874 participants completed the follow up through the end of 2009. We only included 816 participants with available deoxyribonucleic acid between the year of 2008 and 2009 for the genotyping analysis. Furthermore, 192 patients with complete plasma fatty acid measures who were not using lipid-lowering drugs (statins and fibrates) based on medical records were then selected to examine the joint impact of PUFA intake and FADS polymorphisms on blood lipid levels. The study protocol was approved by the institutional review board of the National Health Research Institute and Kaohsiung Medical University Hospital, Taiwan (approval no.EC0970302 and KMUHIRB-98-02-01 [I]). Each participant provided their written informed consent before participation. The present study was carried out in accordance with the principles of the Declaration of Helsinki and its later amendments.

Clinical data collection and measurements

Trained research nurses and assistants measured height, weight and blood pressure. Venous blood was collected after fasting overnight for at least 8 h for the purpose of carrying out accurate and informative hematological analyses. All blood samples were stored at 2–8°C, and were delivered to a central laboratory certified by the College of American Pathology and the US Commission on Office Laboratory Accreditation for comprehensive evaluations. Glycated hemoglobin A1c was measured using high-performance liquid chromatography (Variant II; Bio-Rad Laboratories, Hercules, CA, USA). Levels of blood glucose, triglycerides, cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein-cholesterol (HDL-C) and serum creatinine were measured using an automatic analyzer (Hitachi 7,060; Hitachi High Technologies, Tokyo, Japan).

Gas chromatography analysis of plasma lipids

The Bligh and Dyer methods have previously been used to extract plasma total lipids[28]. We carried out derivatization of

the extracted lipids into fatty acid methyl esters using boron trifluoride-methanol, and carried out a subsequent analysis by gas chromatography (6,890 N GC system; Agilent Technologies, Santa Clara, CA, USA). An Agilent J&W DB-225 capillary column (30 m × 0.25 mm inner diameter × 0.25 mm film) was selected and equipped with gas chromatography with N_2 as the carrier gas. Fatty acid levels were examined using C17:0 (Sigma, St. Louis, MO, USA) as an internal standard, and the measured fatty acid concentrations were calculated and expressed as the percentage of the total area under the peaks relative to that of C17:0 (weight %)[29].

SNP selection and genotyping

SNPs were identified in 816 type 2 diabetes mellitus patients who participated in the DMIDS cohort study. Three SNPs, including FAD1 SNPs (rs174546, 3′-UTR) and FADS2 SNPs (rs174602 and rs2072114-both intronic), were tag genes selected based on their use in previous studies[30–32]. *FADS* SNP genotyping was carried out using the GenomeLabTM SNPstream® genotyping platform (Beckman Coulter Inc., Fullerton, CA, USA) and the associated SNP stream software suite. Experimental procedures were carried out using multiplex polymerase chain reaction based on previously reported methods[33]. Genotyping call rates for all evaluated SNPs were >95%.

Statistical analysis

All data were analyzed using SPSS statistical software (version 22.0; SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to test the normality of the continuous variables. If the data did not fit a normal distribution, the data were logarithmically transformed before carrying out the statistical analyses. On stratification by low or high PUFA intake status, blood lipid profiles among patients with the *FADS1* and *FADS2* SNP genotypes were compared using analysis of variance (ANOVA), and trends of blood lipids against genotypes were assessed using simple linear regression (*P*-for-trends). Interactions between *FADS* genotypes (rs174546, rs174602 and rs2072114) and various risk factors with regard to total cholesterol and LDL-C levels were explored using a general linear model adjusting for age, sex, diabetes duration, smoking, alcohol intake, exercise, fish oil supplementation, and blood triglycerides and hemoglobin A1c. All statistical results were considered statistically significant at a two-sided *P*-value of <0.05.

RESULTS

Patient characteristics

The clinical characteristics of the 816 enrolled participants, as well as the subsets of those using (n = 624) or not using lipid-lowering drugs (n = 192), are presented in Table 1. The mean age of the enrolled type 2 diabetes patients was 61.5 years; 52.7% of the enrolled participants were women, and the average diabetes duration was 10.9 years. A total of 54.4% (n = 475) of participants were reported to have ≥6 years of education, and the proportions of those with smoking, drinking and exercise habits were 30.0% (n = 256), 12.6% (n = 107) and 65.5% (n = 558), respectively. In total, 796 participants (612 lipid-lowering drug users and 184 non-lipid lowering drug users) responded to the questionnaire item asking them whether they were using fish oil supplementation. A total of 34 of the 612 (5.5%) lipid-lowering drug users and 14 of the 184 (7.6%) no lipid-lowering drug users reported using fish oil supplementation. For blood lipid status, patients using lipid-lowering drugs had a statistically significantly higher mean body mass index (kg/m^2; P = 0.005), as well as statistically significantly higher triglyceride levels (P = 0.003), as compared with non-users; we found marginally significant findings with regard to higher total cholesterol (P = 0.076) and LDL-C (P = 0.110) levels in lipid-lowering medication users. Glycated hemoglobin A1c (%; P = 0.006), and fasting plasma glucose (P = 0.009) levels were statistically significantly lower in lipid-lowering drug users as compared with in non-users.

Genotype and allele distributions

The *FADS1* rs174546 SNP, as well as the *FADS2* rs174602 and rs2072114 SNP, were genotyped in 816 participants (Table 2), and the genotype distributions for each SNP were not found to deviate from the Hardy–Weinberg equilibrium (P > 0.05). Distributions of the TT, TC and CC genotypes within the *FADS1* rs174546 SNP were as follows: 34.8%, 48.0% and 17.2% (T = 0.588, C = 0.412). The proportions of the AA, AG and GG genotypes in the *FADS2* rs174602 SNP were 38.4%, 44.7% and 16.9% (A = 0.607, G = 0.392), respectively, and those of the AA, AG and GG genotypes in the rs2072114 SNP were 29.6%, 51.3% and 19.1% (A = 0.552, G = 0.448), respectively. Allele frequencies for the three polymorphisms were comparable with those reported in the Genome Aggregation Database East Asian population for rs174546 (T = 0.5865 C = 0.4135), rs174602 (T = 0.6131 C = 0.3869) and rs2072114 (A = 0.5635 G = 0.4365). As aforementioned, we classified the participants into lipid-lowering medication users and non-users, and the frequencies of rs174546, rs174602 and rs2072114 distributions were all found to be similar between the two groups (data not shown).

FADS1 and FADS2 genotypes and lipid traits

The effects of genetic variation on blood lipid levels in participants with type 2 diabetes (n = 624), including in those not using lipid-lowering medications (n = 192), are presented in Table 3. There were no statistically significant associations between *FADS* SNPs and plasma lipid levels in participants using lipid-lowering drugs. The evaluated polymorphisms in rs174546 (P for trend = 0.032) and rs174602 (P for trend = 0.001) were significantly and positively correlated with LDL-C concentrations, whereas rs2072114 was only marginally associated with LDL-C (P for trend = 0.091). Similarly, the evaluated rs174602 and rs2072114 SNPs were statistically significantly associated with HDL-C (both P for trends were <0.05), whereas rs174546 was marginally associated (P for trend = 0.099).

Table 1 | Baseline characteristics of type 2 diabetes patients (n = 816) with or without lipid-lowering drug use[†]

Characteristics	All type 2 diabetes patients (n = 816)	Lipid-lowering drug use (n = 624)	No lipid-lowering drug use (n = 192)[‡]	P-value[§]
Demographic characteristics				
Age (years)	61.5 ± 8.5	61.8 ± 8.5	60.4 ± 8.7	0.046
Female (%)	461 (52.7)	326 (52.2)	110 (57.3)	0.247
Diabetes duration (year)	10.9 ± 5.7	11.0 ± 5.7	10.7 ± 5.2	0.542
Education ≥6 years (%)	475 (54.4)	277 (44.5)	91 (47.4)	0.507
Smoking (%)	256 (30.0)	183 (30.0)	49 (26.6)	0.406
Drinking (%)	107 (12.6)	74 (12.61)	24 (13.0)	0.703
Exercise (%)	558 (65.5)	399 (65.3)	119 (64.7)	0.930
Use of fish oil supplementation (n = 796)[¶]	48 (6.0)	34 (5.5)	14 (7.6)	0.305
Clinical measures				
BMI (kg/m^2)	21.6 ± 3.8	26.3 ± 3.8	25.4 ± 3.9	0.005
Cholesterol (mmol/L)	4.94 ± 0.93	4.98 ± 0.94	4.85 ± 0.90	0.076
LDL-C (mmol/L)	3.07 ± 0.83	3.10 ± 0.84	2.98 ± 0.81	0.110
HDL-C (mmol/L)	1.00 ± 0.28	0.99 ± 0.28	1.02 ± 0.28	0.175
Triglycerides (mmol/L)	1.80 ± 1.14	1.88 ± 1.16	1.60 ± 1.10	0.003
Fasting plasma glucose (mmol/L)	8.31 ± 2.77	8.18 ± 2.72	8.78 ± 3.05	0.009
Hemoglobin A1c (%)	7.99 ± 1.48	7.92 ± 1.45	8.26 ± 1.57	0.006
Serum creatinine (μmol/L)	86.0 ± 66.5	86.9 ± 69.6	79.3 ± 61.1	0.099
PUFAs (%)				
C18:3 n-3 (ALA, alpha-linolenic acid)			0.92 ± 0.27	
C20:5n-3 (EPA, eicosapentaenoic acid)			0.94 ± 0.72	
n-3 PUFA			6.90 ± 2.17	
C18:2n-6 (LA, linoleic acid)			24.14 ± 3.99	
C20:3n-6			1.67 ± 0.53	
C20:4n-6 (AA, arachidonic acid)			6.58 ± 1.67	
n-6 PUFA			33.61 ± 3.51	
C18:3 n-3/ C18:2n-6 (ALA/LA)			0.04 ± 0.11	
n-3/n-6 PUFA			0.21 ± 0.08	

[†]Data are presented as the mean ± standard deviation or n (%). [‡]A total of 192 patients with type 2 diabetes who were not taking lipid-lowering drugs (statins and fibrates) and with complete fatty acid measures were selected for this subgroup. type="InLatin-1_Supplement">[§]T-tests or χ^2-tests were carried out to test the differences between type 2 diabetes patients with or without lipid-lowering drug use. [¶]Of 816 participants, 12 and eight participants were found to have missing data related to their use of fish oil supplementation, leaving us with 796 participants (612 lipid-lowering drug uses and 184 non-users of this drug). Of those taking lipid-lowering drugs and with complete data on fish oil use, 34 reported using fish oil supplementation (34 / 612 × 100% = 5.5%). Of those without taking lipid-lowering drugs and with complete data on fish oil use, 14 reported using fish oil supplementation (14/184*100% = 7.6%). BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PUFA, polyunsaturated fatty acid.

Table 2 | Genotype frequencies of *FADS1* and *FADS2* single-nucleotide polymorphisms in type 2 diabetes patients (n = 816)[†]

Gene	SNP	Allele (major [M]/minor [m])	Genotype (MM/Mm/mm) (%)	Allele frequency (M/m)	GnomAD[‡] Allele frequency (M/m)[‡]	H-W equilibrium (P-value)[§]
FADS1	rs174546	T/C	284/391/140 (34.8/48/17.2)	959/671 (0.588/0.412)	0.5865/0.4135	0.942
FADS2	rs174602	A/G	309/360/136 (38.4/44.7/16.9)	978/632 (0.607/0.392)	0.6131/0.3869	0.207
FADS2	rs2072114	A/G	240/416/155 (29.6/51.3/19.1)	896/726 (0.552/0.448)	0.5635/0.4365	0.179

[†]There were 1, 11 and 4 participants with missing data in the genotyping of rs174550, rs174575 and rs2727270, respectively. [‡]The Genome Aggregation Database (GnomAD) (https://gnomad.broadinstitute.org) is a resource developed by an international coalition of investigators seeking to aggregate and harmonize exome and genome sequencing data from a variety of large-scale sequencing projects and to make summary data publicly available for the wider scientific community. The allele frequencies of rs174550, rs174575 and rs2727270 were adopted from those of the East Asian population, as reported by GnomAD, and compared with those of our diabetes participants. [§]The Hardy–Weinberg equilibrium for genotype frequencies was analyzed using χ^2-tests.

Impacts of genetic polymorphisms of *FADS* on desaturase activities and n-6 or n-3 pathway (Table 4) were examined using product/precursor fatty acids ratios[32,34]. Activities of delta-6 and delta-5 desaturase (D6D and D5D) were defined as follows: D6D activity = [18:3 (n-6) / 18:2 (n-6)] and D5D activity = [20:4 (n-6) / 20:3 (n-6)][32]. Activities for n-6 and n-3 pathway were as follows: n-3 pathway = [20:5 (n-3) / 18:3 (n-3)] and n-6 pathway = [20:4 (n-6) / 18:2 (n-6)][34]. The present results showed that *FADS1* rs174546 (TT, TC and CC), *FADS2* rs174602 (GG, GA and AA) and rs2072114 (GG, GA and AA) were significantly associated with increased of D6D activities (all *P* for trend <0.001) and n-6 pathways (all *P* for trend <0.001), but not those of D5D and n-3 pathways. Even after excluding participants who reported using fish oil or those with missing fish oil use data (n = 22), we found the results to remain similar (n = 170, data not shown).

FADS1 and FADS2 genotypes and lipid traits in type 2 diabetes patients

Plasma PUFA have been found to be suitable biomarkers for estimating dietary PUFA intake[35]. In the present study, we stratified our participants into low (<50th percentile) and high (≥50th percentile) n-3/n-6 PUFA status groups based on ratios of ALA/LA or of n-3/n-6 PUFA to examine the interactions of these biomarkers with *FADS* polymorphisms with regard to blood lipid profiles in patients not using lipid-lowering medications (n = 192; Table 5, Figure 1a,b). Using general linear models adjusted for confounders, we noted statistically significant interactions between rs2072114 *FADS2* genotypes and the ALA/LA ratio regarding their effects on LDL-C concentrations (*P*-interaction = 0.016) and total cholesterol (*P*-interaction = 0.003). Furthermore, we only observed marginally significant interactions for rs174602 and their effect on plasma LDL-C (*P*-interaction = 0.063) and total cholesterol concentrations (*P*-interaction = 0.082) in those having low ALA/LA levels. We noted no interactive effects of *FADS2* SNPs (*FADS2* rs174546, rs174602, rs2072114) and plasma n-3/n-6 PUFA on total cholesterol and LDL-C levels. Furthermore, no statistically significant interactions were observed between *FADS* genotype categories and PUFA intake levels with regard to HDL-C levels as well. Overall, for diabetes patients using lipid-lowering drugs, all lipid profiles were similar among patients with *FADS1* rs174546, *FADS2* rs174602 and *FADS2* rs2072114 polymorphisms regardless of their plasma n-3/n-6 fatty acid status (data not shown).

DISCUSSION

In the present study, we found that *FADS* genetic polymorphisms modulated plasma cholesterol and LDL-C concentrations in patients with type 2 diabetes mellitus who were not using lipid-lowering medications. The G allele of the *FADS*2 rs174602 SNP was statistically significantly (*P* = 0.001) correlated with lower LDL-C concentrations, whereas the G allele of rs2072114 was only marginally (*P* = 0.091) associated. We observed significant interactions between SNPs in rs2072114 and a low ALA/LA ratio; the G allele was associated with lower

Table 3 | Profiles of plasma lipid concentrations according to *FADS* single-nucleotide polymorphisms in type 2 diabetes patients (n = 816) with (n = 624) or without the use of lipid-lowering drugs (n = 192)[†‡]

	Total cholesterol (mmol/L)		LDL-cholesterol (mmol/L)		HDL-cholesterol (mmol/L)		Triglycerides (mmol/L)	
	Lipid-lowering drugs		Lipid-lowering drugs		Lipid-lowering drugs		Lipid-lowering drugs	
	Non-user	User	Non-user	User	Non-user	User	Non-user	User
rs174546								
TT	4.77 ± 1.03	4.98 ± 0.88	2.86 ± 0.91	3.06 ± 0.81	0.99 ± 0.31	0.98 ± 0.28	1.66 ± 1.10	1.94 ± 1.15
TC	4.84 ± 0.82	4.99 ± 1.02	2.98 ± 0.71	3.12 ± 0.85	1.03 ± 0.25	1.00 ± 0.29	1.62 ± 1.19	1.83 ± 1.07
CC	5.08 ± 0.84	4.95 ± 0.84	3.30 ± 0.87	3.11 ± 0.89	1.09 ± 0.31	0.98 ± 0.23	1.38 ± 0.57	1.89 ± 1.39
P for trend	0.191	0.788	0.032	0.351	0.099	0.758	0.471	0.268
rs174602								
GG	4.50 ± 0.79	5.01 ± 0.90	2.66 ± 0.75	3.11 ± 0.81	0.91 ± 0.25	1.02 ± 0.28	1.76 ± 0.82	1.84 ± 1.09
GA	4.72 ± 0.88	5.01 ± 0.99	2.90 ± 0.81	3.12 ± 0.83	1.03 ± 0.29	0.98 ± 0.30	1.61 ± 1.36	1.88 ± 1.09
AA	5.11 ± 0.88	4.93 ± 0.90	3.23 ± 0.77	3.06 ± 0.87	1.06 ± 0.28	0.99 ± 0.25	1.50 ± 0.82	1.91 ± 1.28
P for trend	<0.001	0.399	0.001	0.522	0.016	0.743	0.399	0.833
rs2072114								
GG	4.67 ± 1.06	5.05 ± 0.87	2.83 ± 0.94	3.11 ± 0.82	0.96 ± 0.29	0.97 ± 0.24	1.58 ± 1.28	1.99 ± 1.19
GA	4.87 ± 0.85	4.98 ± 0.99	2.98 ± 0.69	3.10 ± 0.85	1.02 ± 0.27	0.99 ± 0.29	1.65 ± 0.94	1.86 ± 1.07
AA	4.95 ± 0.87	4.96 ± 0.90	3.13 ± 0.92	3.09 ± 0.86	1.09 ± 0.29	1.01 ± 0.29	1.50 ± 1.29	1.86 ± 1.28
P for trend	0.170	0.470	0.091	0.827	0.028	0.378	0.575	0.152

[†]Data are presented as the mean ± standard deviation, and simple linear regression analysis was performed to test trends against *FADS* single-nucleotide polymorphism genotypes. [‡]If the data were not normally distributed, parameters were transformed logarithmically before conducting analyses. HDL, high-density lipoprotein; LDL, low density lipoprotein.

Table 4 | Profiles of fatty acid desaturase activities according to *FADS* single nucleotide polymorphisms in type 2 diabetes patients (n = 192) without the use of lipid-lowering drugs[†,‡]

	D5D activity[§]	D6D activity[¶]	n-3 pathway[§]	n-6 pathway[¶]
rs174546				
TT	4.03 ± 1.79	0.006 ± 0.005	1.18 ± 2.89	0.23 ± 0.07
TC	4.30 ± 1.43	0.011 ± 0.006	1.25 ± 1.03	0.29 ± 0.09
CC	4.68 ± 1.18	0.019 ± 0.009	1.15 ± 0.73	0.39 ± 0.08
P for trend	0.071	<0.001	0.982	<0.001
rs174602				
GG	3.86 ± 1.67	0.006 ± 0.004	1.51 ± 4.24	0.23 ± 0.08
GA	4.30 ± 1.63	0.009 ± 0.007	1.16 ± 0.99	0.28 ± 0.09
AA	4.41 ± 1.37	0.013 ± 0.008	1.18 ± 0.89	0.32 ± 0.10
P for trend	0.145	<0.001	0.515	<0.001
rs2072114				
GG	4.15 ± 1.93	0.005 ± 0.002	1.50 ± 3.71	0.24 ± 0.07
GA	4.19 ± 1.45	0.011 ± 0.007	1.06 ± 0.87	0.27 ± 0.09
AA	4.51 ± 1.38	0.015 ± 0.009	1.36 ± 1.07	0.35 ± 0.10
P for trend	0.273	<0.001	0.807	<0.001

[†]Data are presented as the mean ± standard deviation, and simple linear regression analysis was performed to test trends against *FADS* SNP genotypes. [‡]If the data was not normally distributed, parameters were transformed logarithmically prior to conducting analyses. [¶]n-3 pathway = [20:5 (n-3) / 18:3 (n-3)] and n-6 pathway = [20:4 (n-6) / 18:2 (n-6)]. §delta-6 desaturase (D6D) activity = [18:3 (n-6) / 18:2 (n-6)] and delta-5 desaturase (D5D) activity = [20:4 (n-6) / 20:3 (n-6)].

plasma LDL-C and cholesterol levels in individuals who had low ALA/LA ratios. We only observed marginally significant interactions between rs174602 and low ALA/LA values and their effect on plasma LDL-C (P = 0.063) and cholesterol (P = 0.082). The present study shows that dietary intake of varying n-3/n-6 ratios might modify the associations of genetic variation in *FADS* with LDL-C and cholesterol metabolism in patients with type 2 diabetes.

The gene-lifestyle interactions and complex traits involved in elevated disease risk study, carried out in a healthy population in Sweden (n = 5,160), explored the interactive effects of rs174602, along with five other candidate genes (rs74771917, rs3168072, rs12577276, rs7115739 and rs174570) in the *FADS1-FADS2-FADS3* gene cluster, across multiple obesity-related traits and dietary PUFA status with regard to blood lipid levels. Among these genetic variations, n-3 PUFA intake derived from the frequency questionnaire was found to modify the associated effects of the minor C allele of rs174602 with regard to blood cholesterol levels. More specifically, the minor C allele of rs174602 was statistically significantly correlated with reduced blood cholesterol among participants with low n-3 PUFA intake (P = 0.02), but not in those with high n-3 PUFA intake. Similarly, in our study of patients with type 2 diabetes carried out in Taiwan, we also found modifying effects of the ALA/LA ratio on the association between the rs174602 SNP and blood cholesterol levels. It should be noted that the G allele represents the minor variant of rs174602 in most Asian populations, including in studies carried out in China[36,37], whereas the C allele represents the minor variant in white people[32]. It has been reported that n-3 PUFAs might prolong quality-adjusted life years, owing to a reduction in the risk of cardiac death in patients with type 2 diabetes mellitus[38]. The results of the present study suggest that supplementation with n-3 PUFAs might compensate for genetic impacts on blood lipid levels, especially in individuals with a low ALA/LA intake ratio and without the protective minor G allele within either rs174602 or rs2072114.

In the present study, the minor C allele of *FADS1* rs174546 was correlated with high LDL-C concentrations in patients with type 2 diabetes mellitus who were not using lipid-lowering drugs. However, we did not find that the n-6/n-3 PUFA ratio modified the association between patients' *FADS1* rs174546 genotype and blood lipid levels.

Quantitative and qualitative alteration in PUFAs, due to changes in activities of desaturase conversions, are known to correlate with dysmetabolic phenotypes[39]. A subsample cross-sectional analysis of European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study showed that D6D activities correlated with elevated LDL-C[40]. A recent prospective study found D5D and D6D activities were favorably and unfavorably correlated with stroke risk factors[41]. Furthermore, four prospective investigations consistently observed negative and positive relationships with D5D and D6D activities, respectively, to diabetes risk after adjustment of confounders[42]. It appears that accelerated D6D activities are linked to unfavorable metabolic outcomes. In the present type 2 diabetes patients, we observed correlations in patients carrying *FADS1* rs174546 (P = 0.032), *FADS2* rs174602 (P = 0.001) and rs2072114 (P = 0.091) SNPs with increased LDL-C, D6D activity (all P for trend <0.001) and n-6 pathway activity (all P for trend <0.001). D6D and D5D are highly expressed in the

Table 5 | Associations between *FADS* gene single-nucleotide polymorphisms and blood lipids according to strata of plasma fatty acid ratio values among type 2 diabetes patients (n = 192) with no use of lipid-lowering drugs (n = 192)

FADS genotype	Total cholesterol (mmol/L)				LDL-C (mmol/L)			HDL-C (mmol/L)		
	ALA/LA ratio	Effect size†	*P* for trend‡	*P*-interaction§	Effect size†	*P* for trend‡	*P*-interaction§	Effect size†	*P* for trend‡	*P*-interaction§
rs174546	Low	0.326	0.029	0.127	0.370	0.008	0.268	0.033	0.078	0.264
	High	−0.012	0.934		0.099	0.438		0.015	0.352	
rs174602	Low	0.442	0.003	0.082	0.349	0.016	0.063	0.043	0.026	0.429
	High	0.259	0.033		0.275	0.015		0.017	0.227	
rs2072114	Low	0.377	0.005	0.003	0.380	0.002	0.016	0.044	0.009	0.238
	High	−0.235	0.130		−0.138	0.338		0.001	0.970	
	n-3/n-6 PUFA ratio									
rs174546	Low	0.152	0.325	0.914	0.278	0.064	0.610	0.025	0.175	0.550
	High	0.163	0.196		0.189	0.096		0.011	0.501	
rs174602	Low	0.438	0.003	0.560	0.412	0.005	0.636	0.040	0.027	0.346
	High	0.250	0.031		0.232	0.027		0.008	0.593	
rs2072114	Low	0.234	0.128	0.627	0.263	0.078	0.512	0.035	0.050	0.157
	High	0.007	0.954		0.051	0.649		0.004	0.806	

†Effect size (β) = difference in blood lipid concentration for each additional allele using general linear modeling. ‡Adjusted for age, sex and fish oil supplementation. §Adjusted for age, sex, diabetes duration, body mass index, smoking, drinking, exercise, education, hemoglobin A1c (%), triglyceride concentrations and fish oil supplementation use. ALA, alpha-linolenic acid; HDL, high-density lipoprotein cholesterol; LA, alpha-linolenic acid, LDL, low-density lipoprotein cholesterol; PUFA, polyunsaturated fatty acid.

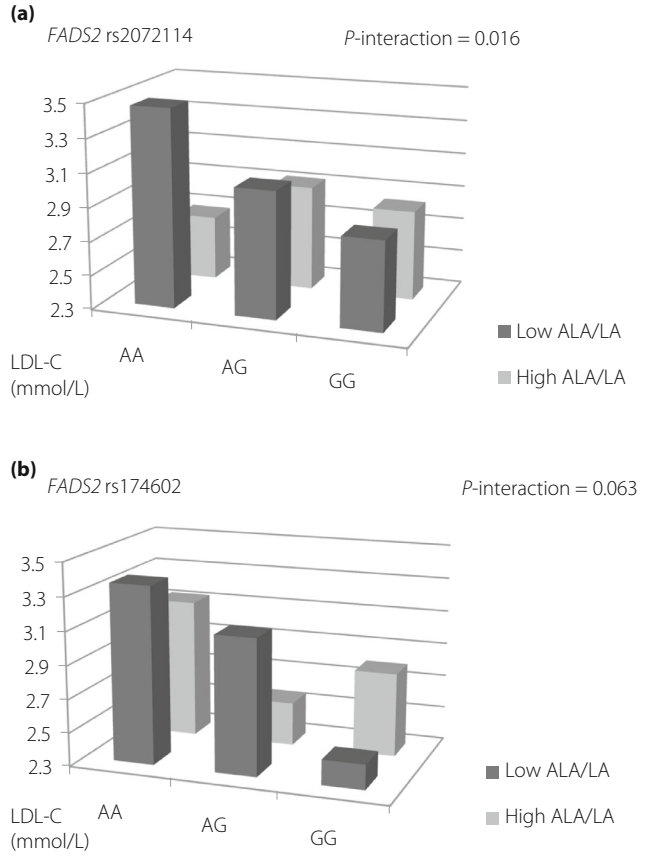

Figure 1 | (a) Fatty acid desaturase (*FADS*)2 rs2072114, (b) FADS2 rs174602, interaction between FADS2 single-nucleotide polymorphisms (SNPs) and plasma n-3/n-6 fatty acid ratio on low-density lipoprotein-cholesterol (LDL-C; mmol/L) among type 2 diabetes patients (n = 192) without use of lipid-lowering drugs. Levels of LDL-C concentration against FADS2 SNPs presented were stratified by low (<50th percentile) or high (≥50th percentile) plasma alpha-linolenic acid/linoleic acid (ALA/LA) ratios. Interactions between FADS genotypes (rs2072114 and rs174602) and LDL-C levels were determined based on a general linear model adjusting for age, sex, diabetes duration, smoking, alcohol intake, exercise, fish oil supplementation, and blood levels of triglycerides and hemoglobin A1c.

liver[43,44], suggesting that the liver desaturase–elongase enzyme system might play important roles in the relationships between genetic variations in the *FADS* genes and plasma cholesterol metabolism.

Among our diabetes patients, interactions were observed between FADS2 rs174602 (P = 0.063) and rs2072114 (P = 0.016) SNPs and low ALA/LA ratio, and further leading to increased LDL-C. The mechanistic explanation regarding how *FADS* genetic variations interplay with desaturase activities or PUFAs on lipoproteins (i.e., LDL-C or HDL-C) metabolism remains largely unknown[39,45], very few functional studies have been completed in this aspect. In the present study, we speculated that carriers of major A alleles of *FADS2* rs174602 and rs2072114 might have augmented D6D and n-6 pathway activities, leading to increased n-6 PUFAs production. It has been postulated that *FADS* genotype inclining to high desaturase activity might be more vulnerable to marked vascular inflammatory damage in those consuming diets with ample n-6 fatty acids, such as the Western dietary pattern, yet might have benefits from increasing dietary n-3 unsaturates[45]. Furthermore, FADs SNPs and desaturase activities on metabolic alterations are possibly mediated by changes in cell-membrane fatty acid composition. n-3/n-6 long-chain PUFAs also serve as ligands for nuclear factor kB, peroxisome proliferators activating receptors and sterol regulatory element-binding protein, which are all involved in regulating lipid or lipoprotein metabolism, such as lipogenesis and FA oxidation[46].

A few earlier studies have reported on the impact of the *FADS1* rs174546 genetic polymorphism with regard to dietary interactions. In a Dutch population, carriers of the T allele of rs174546 were found to have lower levels of total and non-HDL-C. Furthermore, T allele carriers were found only in those with a high intake of n-3 PUFAs (vs in the low-intake group)[47]. Similarly, similar findings were observed in the high ALA-intake group, but not in the low ALA-intake group in a study of European adolescents[31]. Additionally, *FADS1* rs174546 was previously found to interact with dietary PUFA with regard to modulating desaturase activities, including D-5 or D-6 desaturases, in a study carried out among participants of Canadian ethnicity (i.e., primarily white people of Western European descent)[48] and in a study evaluating blood n-3 LCPUFA (eicosapentaenoic acid or docosahexaenoic acid) concentrations conducted in the USA[49]. Thus, various PUFA appear to modify associations among *FADS* polymorphisms, desaturase activities and blood lipids within different ethnicities[18,20,47], thus supporting the known roles of these dietary components in affecting cardiovascular-associated outcomes.

The Genome Aggregation Database (GnomAD) is a resource developed by an international coalition of investigators that has aggregated 15,708 whole genomes and 125,748 exomes from 2,504 unrelated individuals within 26 populations. Allele frequencies of SNPs in the present study, including rs174546 (T = 0.588, C = 0.412), rs174602 (A = 0.607, G = 0.392) and s2072114 (A = 0.552, G = 0.448), were similar to those reported in GnomAD (rs174546 [T = 0.5865, C = 0.4135], rs174602 [T = 0.6131, 0.3869] and rs2072114 [A = 0.5635, G = 0.4365]). In addition, the frequency of the rs174546 C allele in the present study was 0.412, which was comparable with reported ranges of 0.30–0.56 in Japanese[20], Mexican[18] and white[32] study populations. Because the population in the present study exclusively comprised type 2 diabetes patients, future studies enrolling larger sample sizes and recruiting healthy control populations are required to further confirm the differences in allele frequencies detected within the current investigation.

For rs174546, TT, TC and CC carriers showed statistically significantly increased LDL-C levels with a linear trend (P = 0.032), as well as a marginal increase in HDL-C, thus suggesting a modifying role of the minor C allele with regard to

cholesterol metabolism. Several earlier studies showed that healthy individuals carrying the rs174546 C allele had elevated HDL-C concentrations in studies carried out in Japanese and Koreans populations[50,51], and have also found similar associations with regard to the rs174547 C allele, as well as the rs174550 T allele of *FADS*1 in people of Han Chinese descent[52]. The *FADS* rs174546 SNP has been reported to be in linkage disequilibrium with several other SNPs in various ethnic groups. These findings show that minor alleles might modify cholesterol levels[18,20]. Furthermore, the rs174546 polymorphism has also been shown to be in high linkage disequilibrium with SNPs that were recently reported to be associated with plasma or erythrocyte membrane PUFA concentrations[30,53]. More specifically, haplotypes including the C allele were observed to correlate with an increase in n-3 desaturase activity, suggesting the role of this allele in facilitating the efficiency of the fatty acid desaturase reaction. The putative mechanism mediating this association can be attributed to the correlation between high desaturase activity and high total or LDL-C concentrations[54]. As the expression of desaturases is highest in the liver, a major contribution of the liver desaturase–elongase enzyme system to the observed associations between genetic variation in the *FADS*1 and *FADS*2 genes and plasma cholesterol metabolism seems likely[21].

The main strength of the present study was that the plasma ALA/LA ratio was measured as an objective marker of dietary fatty acid intake, instead of using questionnaires or dietary records. However, we note several limitations of this work as well. For example, we did not implement multiple testing corrections to adjust the effective statistical significance of our findings. Although it would have been ideal to use multiple testing corrections to ensure the robustness of our analysis, this methodology was not implemented in this study because the hypotheses tested in this study were tested in *a priori* analyses (vs post-hoc analyses). However, we had many comparisons to carry out. Together, these considerations render an ideal *P*-value indication of statistical significance too small to have been realistically achieved. Namely, after excluding individuals taking lipid-lowering medications, just 192 participants were evaluated. Thus, the sample size of the present study was modest. Furthermore, our findings might not be generalizable to diabetes populations of other ethnicities. Future studies should enroll larger sample sizes of patients with diabetes and should include a healthy control group.

In conclusion, the distributions of genotypes of the *FADS* rs174602 and rs2072114 SNPs in patients with type 2 diabetes were comparable with those reported in the Genome Aggregation Database for East Asian populations. Among patients not taking lipid-lowering medications, the minor alleles of the rs174602 SNP were statistically significantly associated with lower LDL-C levels, whereas the associations between the rs2072114 polymorphism and LDL-C levels was only marginally significant. We observed a statistically significant interaction between rs2072114 polymorphism and the plasma ALA/LA ratio with regard to LDL-C levels (both <0.05), the G allele was related to lower LDL-C levels only among individuals with a low ALA/LA ratio. Marginal significant interaction were observed between rs174602 and low ALA/LA on LDL-C. Notably, the present results also highlight the role of dietary PUFAs with regard to mediating the effects of *FADS*1/*FADS*2 polymorphisms on plasma lipid profiles. The present findings might facilitate preventive strategies for public health genomics, as well as approaches in precision medicine. Future research within larger-scale investigations and with the inclusion of a control group are required to confirm the present findings more definitively.

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DISCLOSURE

The authors declare no conflict of interest.
Approval of the research protocol: The protocol of this study was approved by the Ethical Committee of National Health Research Institute (EC0970302) and Kaohsiung Medical University Hospital, Taiwan (KMUHIRB-98-02-01 (I)).
Informed consent: Each participant provided their written informed consent before participation.
Registry and the registration no. of the study/trial: N/A.
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