Interaction between a common variant in \textit{FADS1} and erythrocyte polyunsaturated fatty acids on lipid profile in Chinese Hans\footnote{To whom correspondence should be addressed.}

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\section*{Abstract}
Little is known about the associations of \textit{FADS1} genetic variants with circulating levels of PUFA and lipids in Asian populations who have a different dietary pattern and dyslipidemia prevalence compared with Western populations. In a population-based sample of 3,210 unrelated Han Chinese living in Beijing and Shanghai, we examined a \textit{FADS1} genetic variant, rs174550, in relation to blood PUFA and lipid levels. C-allele of rs174550 was significantly associated with levels of erythrocyte PUFAs in upstream and downstream pathways of delta-5 desaturase (D5D) \((P = 0.003)\). Moreover, rs174550 C-allele was associated with a lower HDL cholesterol level \((P = 0.02)\) in total population and a higher triglyceride level \((P = 0.0002)\) in Beijing residents. Interestingly, erythrocyte levels of 18:2n-6 and 18:3n-3 modified the effect of rs174550 on HDL cholesterol level: stronger associations between rs174550 C-allele and lower HDL cholesterol levels were exhibited when erythrocyte 18:2n-6 or 18:3n-3 level was low \((P \text{ for interaction } = 0.02 \text{ and } 0.03, \text{ respectively)}\). These data suggested that \textit{FADS1} genetic variant was associated with circulating PUFA and lipid levels and that its effect on HDL cholesterol might depend on PUFA status in the Han Chinese population.—Zhu, J., Q. Sun, G. Zong, Y. Si, C. Liu, Q. Qi, X. Ye, L. Sun, H. Sheng, H. Li, and X. Lin. \textbf{Interaction between a common variant in \textit{FADS1} and erythrocyte polyunsaturated fatty acids on lipid profile in Chinese Hans.} \textit{J. Lipid Res.} 2013, 54: 1477–1483.

Supplementary key words fatty acid desaturase \textbullet{} delta-5 desaturase \textbullet{} high density lipoprotein cholesterol \textbullet{} triglycerides

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\textbf{Abbreviations} BMI, body mass index; CHB, Chinese Han in Beijing; D5D, delta-5 desaturase; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FADS1, fatty acid desaturase 1; FFQ, food frequency questionnaire; GLM, general linear regression; GWAS, genome-wide association studies; LD, linkage disequilibrium; MAF, minor allele frequency; PPARs, peroxisome proliferator activating receptor alpha; SNP, single nucleotide polymorphism.

\footnote{The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of one figure and four tables.}
these common variants are also associated with blood lipids and PUFAs in the Chinese population.

In recent decades, China has been experiencing rapid nutrition transition, characterized mainly by increased consumption of fat and animal foods (23). Based upon 1992–2002 China health and nutrition surveys, energy intake from fat and animal foods increased from 22.0% to 29.8% and from 9.3% to 13.7%, respectively (24). Meanwhile, unlike in Western countries where prevalence of hypercholesterolemia is higher than hypertriglyceridemia (54.9% versus 31.2%), hypertriglyceridemia is more prevalent than hypercholesterolemia (11.9% versus 2.9%) in the Chinese population (25, 26). Previously, findings from three studies conducted among peoples with European ancestry suggested that dietary PUFA intake may modify the associations between FADS1 genetic variant and blood lipids (27–29). One of these studies found that rs174547 was associated with lower LDL cholesterol only if dietary long-chain n-3 PUFA was low (29). Thus, it is of interest to examine whether such an interaction may also exist in the Chinese population.

In the current investigation, we used rs174550 as a tag SNP of FADS1 variants to investigate i) the associations of this variant with plasma lipid profiles and erythrocyte PUFAs, objective markers of dietary intakes (30–32), and ii) interactions between rs174550 and erythrocyte PUFA contents on circulating lipid profiles in a Han Chinese population.

MATERIALS AND METHODS

Study population

The study sample consisted of 3,210 unrelated Han Chinese from the Nutrition and Health of Aging Population in China Study. The total population and study design and protocol have been described in detail previously (33). Briefly, a home interview was conducted to collect information, including sociodemographic data, medical history, and physical activity, using a standardized questionnaire. All participants underwent a physical examination, including standardized anthropometric measurements and overnight fasting blood sample collection. Body mass index (BMI) was calculated as [weight/height² (kg/m²)]. All blood samples collected from different study sites in Beijing and Shanghai were shipped in dry ice to the laboratory at the Institute for Nutritional Sciences and analyzed in the same batch with a random sequence. Total, LDL, and HDL cholesterol and triglyceride concentrations were measured enzymatically on an automatic analyzer (Hitachi 7080, Japan) with reagents purchased from Wako Pure Chemical Industries (Osaka, Japan). Study protocol was approved by the Institutional Review Board of the Institute for Nutritional Sciences and analyzed in the same batch with a random sequence. Generalized linear regression (GLM) was applied to examine associations of rs174550 with erythrocyte PUFAs and plasma lipid concentrations. The gene-PUFA interaction on blood lipid parameters was examined by using GLM, where erythrocyte PUFA concentrations (homozygous for the minor T-allele, and heterozygous and homozygous for the minor C-allele, respectively), and interaction terms (erythrocyte PUFAs × genotype) were entered as independent variables; plasma HDL cholesterol, triglycerides, HDL cholesterol-to-triglycerides ratio, and PUFAs were used as dependent variables. To preserve statistical power, the PUFAs were classified into low and high groups according to the region-specific medians of erythrocyte concentrations [i.e., 14.3% for 18:2n-6, 0.21% for 18:3n-3, 0.32% for EPA, and 3.95% for 22:6n-3 (docosahexaenoic acid, DHA) in Beijing participants; and 13.4% for 18:2n-6, 0.29% for 18:3n-3, 0.54% for EPA, and 4.84% for DHA in Shanghai participants, respectively]. Multivariate analyses were adjusted for age, sex, BMI, and residence (urban/rural). Participants were considered having dyslipidemia if they had one of following conditions: i) triglycerides greater than 1.70 mmol/l; ii) total cholesterol greater than 5.72 mmol/l; or iii) HDL cholesterol less than 0.91 mmol/l. We excluded participants who were self-reported having hyperlipidemia or using anti-hyperlipidemia medications (n = 479) prior to the lipid-related analyses.

Results from Beijing and Shanghai participants were meta-analyzed using a random-effects model, and Cochran’s Q test was performed to assess the heterogeneity of effect sizes between Beijing and Shanghai participants. Due to the nonnormality of distribution, plasma triglycerides and erythrocyte 18:3n-3 levels were log-transformed before analyses and presented as geometric means whenever necessary. Data analyses were performed with SAS version 9.1 (SAS Institute, Cary, NC) and Stata version 9.2 (Stata, College Station, TX). All P values were two-sided, and P < 0.05 was considered to be statistically significant.

RESULTS

Characteristics of the study population were presented in Table 1. In general, Beijing residents had higher BMI, total cholesterol, HDL cholesterol, LDL cholesterol, and erythrocyte fatty acid measurement

The method for erythrocyte fatty acid measurement has been previously described (35). After being extracted by hexane and isopropanol, erythrocyte fatty acids were incubated with mixture of methanol and sulfuric acid for fatty acid methyl esters (FAME). FAMEs were then separated by gas chromatography (Agilent 6890 GC). Individual FAME was identified by positive chemical ionization using methane as reagent gas (Agilent 5975B). The relative amount of each fatty acid was calculated as a percentage of total fatty acids.

Assessment of dietary intakes

At home interviews, the information of dietary intake was collected by trained local medical professionals using a 74-item food frequency questionnaire (FFQ), which was a modified version of that used in the China health and nutrition surveys (36). Food composition values were obtained from the Chinese food composition table (37). All nutrient intakes were energy-adjusted utilizing the residual method (38).

Statistical analysis

Hardy-Weinberg equilibrium and differences of genotypic distributions between genders and geographical regions were tested using a likelihood ratio test. Because of an observed difference in the minor allele frequency (MAF) of rs174550 between Shanghai and Beijing participants (P < 0.0001), analyses were performed separately according to the geographic locations. Generalized linear regression (GLM) was applied to examine associations of rs174550 with erythrocyte PUFAs and plasma lipid concentrations. The gene-PUFA interaction on blood lipid parameters was examined by using GLM, where erythrocyte PUFA concentrations (homozygous for the minor T-allele, and heterozygous and homozygous for the minor C-allele, respectively), and interaction terms (erythrocyte PUFAs × genotype) were entered as independent variables; plasma HDL cholesterol, triglycerides, HDL cholesterol-to-triglycerides ratio, and PUFAs were used as dependent variables. To preserve statistical power, the PUFAs were classified into low and high groups according to the region-specific medians of erythrocyte concentrations [i.e., 14.3% for 18:2n-6, 0.21% for 18:3n-3, 0.32% for EPA, and 3.95% for 22:6n-3 (docosahexaenoic acid, DHA) in Beijing participants; and 13.4% for 18:2n-6, 0.29% for 18:3n-3, 0.54% for EPA, and 4.84% for DHA in Shanghai participants, respectively]. Multivariate analyses were adjusted for age, sex, BMI, and residence (urban/rural). Participants were considered having dyslipidemia if they had one of following conditions: i) triglycerides greater than 1.70 mmol/l; ii) total cholesterol greater than 5.72 mmol/l; or iii) HDL cholesterol less than 0.91 mmol/l. We excluded participants who were self-reported having hyperlipidemia or using anti-hyperlipidemia medications (n = 479) prior to the lipid-related analyses.

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triglycerides than their Shanghai counterparts ($P \leq 0.006$). Levels of total and individual n-6 PUFAs (except 22:6n-6) were higher, whereas total and individual n-3 PUFAs were lower comparing Beijing with Shanghai residents ($P < 0.0001$). The MAF of rs174550 was 28.6% for Beijing residents and 41.8% for Shanghai residents (supplementary Table I). Analysis of pairwise LD among the SNPs within the **FADS1** gene showed that LD is perfect ($r^2 > 0.9$) in the HapMap CHB sample and in the GWAS data of our population (supplementary Fig. I).

The rs174550 C-allele was significantly associated with higher levels (%) of erythrocyte 18:2n-6, 20:2n-6, 22:2n-6, 20:3n-6, and 18:3n-3, and lower levels of 18:3n-6, 20:4n-6, 22:5n-3, and PUFA 41.4 ± 3.0 38.8 ± 4.3 40.1 ± 3.9 <0.0001 18:2 n-6 14.6 ± 2.3 13.6 ± 3.1 14.1 ± 2.8 <0.0001 20:2 n-6 0.40 ± 0.06 0.38 ± 0.06 0.39 ± 0.06 <0.0001 22:2 n-6 0.077 ± 0.019 0.079 ± 0.019 0.078 ± 0.019 0.02 18:3 n-6 0.12 ± 0.07 0.11 ± 0.07 0.12 ± 0.07 <0.0001 20:3 n-6 1.40 ± 0.31 1.27 ± 0.27 1.33 ± 0.30 <0.0001 20:4 n-6 13.8 ± 1.7 12.0 ± 1.7 12.9 ± 1.9 <0.0001 22:4 n-6 3.04 ± 0.55 2.24 ± 0.48 2.63 ± 0.65 <0.0001 22:5 n-6 1.71 ± 0.34 1.33 ± 0.36 1.52 ± 0.40 <0.0001 Total n-6 PUFA 35.1 ± 2.7 31.1 ± 3.9 33.1 ± 3.9 <0.0001 18:3 n-3 0.21 (0.17–0.27) 0.29 (0.23–0.37) 0.25 (0.19–0.33) <0.0001 EPA 0.34 ± 0.13 0.57 ± 0.22 0.46 ± 0.21 <0.0001 22:5 n-3 1.67 ± 0.28 1.80 ± 0.30 1.73 ± 0.30 <0.0001 DHA 3.97 ± 0.85 4.85 ± 0.98 4.41 ± 1.02 <0.0001 Total n-3 PUFA 6.29 ± 1.39 7.71 ± 2.05 7.01 ± 1.89 <0.0001

Data are mean ± SD, median (inter-quartile range), or n (%), unless otherwise indicated. $P$ values represent significance of the differences between individuals from Beijing and Shanghai. Fatty acids are erythrocyte concentration (% wt total fatty acids). PUFA is calculated by summing up the percentages of n-3 and n-6 PUFAs.

To examine whether EPA or 20:4n-6, two downstream products of D5D, could explain the associations of **FADS1** rs174550 with lipid profiles, we repeated the associations between **FADS1** rs174550 and plasma lipids by further adjusting for EPA (Table 3, model 2) and 20:4n-6 (Table 3, model 3), respectively. Notably, adjustment for EPA attenuated the associations of **FADS1** rs174550 with HDL cholesterol ($P = 0.08$), but the association with triglycerides remained significant ($P = 0.0001$). The associations with plasma HDL cholesterol and triglycerides levels were abolished after controlling for 20:4n-6 ($P = 0.82$ and 0.88, respectively) (Table 3).

The interactions between rs174550 and erythrocyte levels of 18:2n-6, 18:3n-3, EPA, and DHA on lipid profiles are presented in Table 4. In the total population, the association between rs174550 C-allele and lower HDL cholesterol was significant only when erythrocyte 18:2n-6 was low ($P = 0.001$) but not when this fatty acid was high ($P = 0.98$; $P$ for interaction $= 0.02$). Similarly, rs174550 Callele was also associated with lower HDL cholesterol ($P = 0.01$ and HDL/triglycerides ratio ($P = 0.03$) with low erythrocyte 18:3n-3 status ($P$ for interaction $= 0.03$ and 0.04, respectively) (Table 4). Moreover, no interaction was found between rs174550 and essential PUFAs on downstream PUFA, such as 20:4n-6, EPA, or DHA (Table 5). Results for participants in different geographic locations are presented in supplementary Table II.

**Dietary fish intake** was moderately correlated with erythrocyte EPA ($r = 0.13$, $P < 0.0001$), DHA ($r = 0.21$, $P < 0.0001$), and EPA/DHA ($r = 0.22$, $P < 0.0001$), while vegetable oil intake was associated with erythrocyte 18:2n-6 ($r = 0.12$, $P < 0.0001$) (supplementary Table III). There was only marginally significant interaction between rs174550

| Characteristic | Beijing | Shanghai | Total | $P$ |
|----------------|---------|----------|-------|-----|
| n (% male)     | 1574 (42.2%) | 1636 (45.3%) | 3210 (44.3%) | 0.35 |
| Urban (%)      | 770 (49.0%) | 799 (48.8%) | 1569 (48.9%) | 0.96 |
| Age (years)    | 58 ± 5 | 58.9 ± 6 | 58 ± 6 | 0.01 |
| BMI (kg/m²)    | 25.2 ± 3.7 | 23.6 ± 3.3 | 24.4 ± 3.6 | <0.0001 |
| Lipid-lowering medication (%) | 141 (9.0%) | 85 (5.2%) | 226 (7.0%) | <0.0001 |
| Hyperlipidemia (%) | 249 (15.8%) | 172 (10.5%) | 421 (13.1%) | <0.0001 |
| Total cholesterol (mmol/l) | 4.92 ± 0.97 | 4.46 ± 0.92 | 4.69 ± 0.97 | <0.0001 |
| HDL cholesterol (mmol/l) | 1.29 ± 0.34 | 1.26 ± 0.33 | 1.28 ± 0.33 | 0.006 |
| LDL cholesterol (mmol/l) | 3.49 ± 0.98 | 3.03 ± 0.90 | 3.26 ± 0.97 | <0.0001 |
| Triglyceride (mmol/l) | 1.13 (0.78–1.77) | 1.03 (0.73–1.59) | 1.09 (0.75–1.67) | 0.0002 |
| Fatty acids (% weight of total FA) | 41.4 ± 3.0 | 38.8 ± 4.3 | 40.1 ± 3.9 | <0.0001 |

TABLE 1. Characteristics of the study population

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**FADS1 interacts with PUFA on lipids**

1479
TABLE 2. Associations of FADS1 rs174550 with erythrocyte PUFAs

| Fatty Acid (% wt of total FA) | Beijing (β (SE)) | Shanghai (β (SE)) | Total (β (SE)) | P for Heterogeneity |
|------------------------------|-----------------|-----------------|----------------|-------------------|
| 18:2n-6                     | 0.81 (0.08)     | 0.77 (0.08)     | 0.79 (0.06)   | 5.11 × 10⁻⁶       |
| 20:2n-6                     | 0.028 (0.002)   | 0.026 (0.002)   | 0.027 (0.001) | 7.91 × 10⁻²⁹      |
| 22:2n-6                     | 0.004 (0.0007)  | 0.005 (0.0006)  | 0.006 (0.0005)| 4.77 × 10⁻²³      |
| 18:3n-6                     | -0.022 (0.003)  | -0.035 (0.002)  | -0.039 (0.006)| 2.98 × 10⁻⁶       |
| 20:3n-6                     | 0.25 (0.010)    | 0.17 (0.009)    | 0.21 (0.04)   | 8.30 × 10⁻⁷       |
| 20:4n-6                     | -0.97 (0.06)    | -0.87 (0.06)    | -0.92 (0.05)  | 1.01 × 10⁻⁴       |
| 22:4n-6                     | -0.21 (0.02)    | -0.16 (0.02)    | -0.19 (0.03)  | 8.97 × 10⁻⁷       |
| 22:5n-6                     | -0.002 (0.012)  | 0.021 (0.012)   | 0.010 (0.008) | 0.26              |
| 18:3n-3                     | 0.007 (0.002)   | 0.10 (0.02)     | 0.08 (0.02)   | 3.02 × 10⁻⁷       |
| EPA                         | -0.025 (0.005)  | -0.050 (0.007)  | -0.04 (0.01)  | 0.003             |
| 22:5n-3                     | -0.06 (0.01)    | -0.07 (0.01)    | -0.07 (0.01)  | 1.86 × 10⁻¹⁵      |
| DHA                         | -0.025 (0.03)   | 0.092 (0.03)    | -0.01 (0.02)  | 0.60              |
| 20:4n-6/20:3n-6             | -2.39 (0.08)    | -1.98 (0.07)    | -2.18 (0.20)  | 1.89 × 10⁻²⁶      |

18:3n-3 was log-transformed before analyses. βs (SE) are expressed as changes in the levels of PUFA or their ratio for increasing copy of FADS1 rs174550 allele in the total population. P values are adjusted for age, sex, BMI, and residence (urban/rural) using an additive model. Random-effect model was used in the meta-analysis.

and fish intake on HDL cholesterol (P for interaction = 0.08) (supplementary Table IV).

DISCUSSION

In this study, all erythrocyte PUFAs examined were associated with rs174550, with the exception of 22:5n-6 and DHA. This genetic variant was also associated with unfavorable plasma HDL cholesterol and triglycerides profiles, and the associations can be largely ascribed to erythrocyte 20:4n-6 level. Moreover, the association between FADS1 rs174550 and plasma HDL cholesterol was modified by erythrocyte 18:2n-6 and 18:3n-3 status, particularly with low 18:2n-6 or 18:3n-3 level.

FADS1 gene plays a crucial role in LC-PUFAs biosynthesis (2). FADS1 genetic variants may lead to lower D5D enzyme activity and subsequently cause accumulation of desaturase substrates and reduced yield of downstream products (39). This notion has been supported by evidence from multiple human studies. For example, Schaeffer et al. reported that haplotypes of 11 SNPs in FADS1 and FADS2 genes were significantly associated with 20:4n-6 (β = -1.48) and EPA (β = -0.18) and that these haplotypes explained 28.5% variability for 20:4n-6 and 6.9% for EPA levels (8). Similarly, our current study and previous investigations in Western populations (3, 5, 8, 10, 40) consistently demonstrated that genetic variants in FADS1 gene were most significantly associated with lower levels of 20:4n-6 (β = -0.92) and EPA (β = -0.04), but not with downstream fatty acids, such as DHA and 22:5n-6, in general populations. On the other hand, FADS1 rs174561 was associated with DHA in plasma phospholipids and human milk among 309 pregnant women (7), whereas significant associations between erythrocyte DHA and FADS1 rs174548, rs174556, and rs174561 were also observed in 4,457 pregnant women (4). The discrepancy between our study and

TABLE 3. Associations of FADS1 rs174550 with lipids

|                | Beijing (β (SE)) | Shanghai (β (SE)) | Total (β (SE)) | P for Heterogeneity |
|----------------|-----------------|-----------------|----------------|-------------------|
| **Total cholesterol (mmol/l)** |                |                  |                |                  |
| Model 1        | 0.003 (0.041)   | -0.061 (0.032)  | -0.037 (0.025) | 0.14              |
| Model 2        | 0.021 (0.041)   | -0.048 (0.033)  | -0.021 (0.026) | 0.42              |
| Model 3        | -0.018 (0.044)  | -0.078 (0.035)  | -0.055 (0.027) | 0.05              |
| **HDL cholesterol (mmol/l)** |                |                  |                |                  |
| Model 1        | -0.009 (0.014)  | -0.030 (0.012)  | -0.021 (0.009) | 0.02              |
| Model 2        | -0.006 (0.014)  | -0.025 (0.012)  | -0.016 (0.009) | 0.08              |
| Model 3        | 0.020 (0.015)   | -0.019 (0.013)  | -0.002 (0.010) | 0.82              |
| **LDL cholesterol (mmol/l)** |                |                  |                |                  |
| Model 1        | 0.008 (0.041)   | -0.029 (0.031)  | -0.016 (0.025) | 0.53              |
| Model 2        | 0.023 (0.041)   | -0.020 (0.032)  | -0.004 (0.025) | 0.88              |
| Model 3        | 0.015 (0.044)   | -0.032 (0.034)  | -0.014 (0.027) | 0.59              |
| **Triglycerides (mmol/l)** |                |                  |                |                  |
| Model 1        | 0.092 (0.025)   | 0.024 (0.020)   | 0.056 (0.034)  | 0.10              |
| Model 2        | 0.096 (0.025)   | 0.025 (0.020)   | 0.059 (0.035)  | 0.10              |
| Model 3        | -0.004 (0.025)  | -0.022 (0.021)  | -0.015 (0.016) | 0.37              |

βs (SE) were expressed as changes in blood lipids for increasing copy of FADS1 rs174550 allele. Triglyceride was log-transformed before analyses. βs (SE) and corresponding P values were adjusted for covariates using an additive model. Random-effect model was used in the meta-analysis.

A Adjusted for age, sex, BMI, and residence (urban/rural).
B Adjusted for EPA.
C Adjusted for DHA.

TABLE 4. Interactions of erythrocyte PUFAs and FADS1 rs174550 genotypes on lipid profiles

| Erythrocyte PUFAs (%) | Low (n = 1335) | High (n = 1,337) | P | P for Interaction |
|-----------------------|---------------|-----------------|---|-----------------|
| 18:2n-6 (%)           |               |                 |   |                 |
| Sample size (n)       | 662           | 566             |   |                 |
| HDL cholesterol (mmol/l) | 1.34 ± 0.01  | 1.29 ± 0.01     | 1.27 ± 0.03 | 0.001 |
| Triglycerides (mmol/l) | 0.95 ± 0.02  | 0.99 ± 0.02     | 1.03 ± 0.05 | 0.07 |
| LDL cholesterol/triglycerides | 1.72 ± 0.04 | 1.54 ± 0.04     | 1.46 ± 0.09 | 0.001 |
| LDI cholesterol (mmol/l) | 3.07 ± 0.03  | 3.04 ± 0.03     | 2.96 ± 0.08 | 0.22 |
| 18:3n-3 (%)           |               |                 |   |                 |
| Sample size (n)       | 582           | 582             |   |                 |
| HDL cholesterol (mmol/l) | 1.30 ± 0.01  | 1.33 ± 0.01     | 1.32 ± 0.03 | 0.01 |
| Triglycerides (mmol/l) | 0.91 ± 0.02  | 0.94 ± 0.02     | 0.89 ± 0.04 | 0.43 |
| HDL cholesterol/triglycerides | 1.79 ± 0.04 | 1.64 ± 0.04     | 1.74 ± 0.10 | 0.03 |
| LDI cholesterol (mmol/l) | 3.16 ± 0.03  | 3.19 ± 0.04     | 3.14 ± 0.08 | 0.92 |
| 18:3n-3/18:2n-6 (%)   |               |                 |   |                 |
| Sample size (n)       | 587           | 587             |   |                 |
| HDL cholesterol (mmol/l) | 1.37 ± 0.01  | 1.33 ± 0.01     | 1.30 ± 0.03 | 0.008 |
| Triglycerides (mmol/l) | 0.90 ± 0.02  | 0.92 ± 0.02     | 0.88 ± 0.03 | 0.96 |
| HDL cholesterol/triglycerides | 1.80 ± 0.04 | 1.66 ± 0.04     | 1.77 ± 0.08 | 0.21 |
| LDI cholesterol (mmol/l) | 3.28 ± 0.04  | 3.24 ± 0.04     | 3.23 ± 0.07 | 0.45 |
| EPA (%)               |               |                 |   |                 |
| Sample size (n)       | 488           | 488             |   |                 |
| HDL cholesterol (mmol/l) | 1.30 ± 0.01  | 1.29 ± 0.01     | 1.28 ± 0.02 | 0.38 |
| Triglycerides (mmol/l) | 1.02 ± 0.02  | 1.07 ± 0.03     | 1.19 ± 0.05 | 0.0002 |
| HDL cholesterol/triglycerides | 1.60 ± 0.04 | 1.48 ± 0.04     | 1.34 ± 0.07 | 0.0004 |
| LDI cholesterol (mmol/l) | 3.13 ± 0.04  | 3.17 ± 0.03     | 3.11 ± 0.06 | 0.95 |
| DHA (%)               |               |                 |   |                 |
| Sample size (n)       | 542           | 542             |   |                 |
| HDL cholesterol (mmol/l) | 1.32 ± 0.01  | 1.29 ± 0.01     | 1.30 ± 0.02 | 0.23 |
| Triglycerides (mmol/l) | 1.05 ± 0.02  | 1.12 ± 0.03     | 1.20 ± 0.06 | 0.01 |
| HDL cholesterol/triglycerides | 1.63 ± 0.04 | 1.44 ± 0.04     | 1.37 ± 0.08 | 0.003 |
| LDI cholesterol (mmol/l) | 3.11 ± 0.04  | 3.17 ± 0.04     | 3.11 ± 0.06 | 0.68 |
| EPA-DHA (%)           |               |                 |   |                 |
| Sample size (n)       | 537           | 537             |   |                 |
| HDL cholesterol (mmol/l) | 1.32 ± 0.01  | 1.29 ± 0.01     | 1.30 ± 0.03 | 0.21 |
| Triglycerides (mmol/l) | 1.04 ± 0.02  | 1.12 ± 0.03     | 1.20 ± 0.06 | 0.003 |
| HDL cholesterol/triglycerides | 1.64 ± 0.04 | 1.44 ± 0.04     | 1.35 ± 0.08 | 0.001 |
| LDI cholesterol (mmol/l) | 3.11 ± 0.04  | 3.15 ± 0.04     | 3.12 ± 0.06 | 0.68 |

Data are means ± SE or n. *P* values were adjusted for age, sex, BMI, residence (urban/rural), and region (Beijing/Shanghai) using an additive model. Low and high groups are stratified by medians of erythrocyte 18:2n-6, 18:3n-3, 18:3n-3/18:2n-6, EPA, DHA, and EPA-DHA, respectively.

these studies might be explained by higher conversion rate of 22:5n-3 to DHA in pregnant women but not in general populations (10).

The associations between FADS1 rs174550 and lipids in the current study were in line with results from a meta-analysis of 122,743 nondiabetic participants (15), although significant associations were also detected between the genetic variant and lower total and LDL cholesterol in the meta-analysis. It is possible that the limited sample size and different dyslipidemia pattern may explain the null findings for total and LDL cholesterol in the current study. Furthermore, distinctive diet and lifestyle may also modify the effects of FADS1 on lipid metabolism, even in genetically similar populations (12), which might serve as another plausible explanation for
the heterogeneity of associations between rs174550 and triglycerides among Beijing and Shanghai participants. It was noteworthy that adjustment of 20:4n-6 abolished the associations between rs174550 and HDL cholesterol and triglycerides, suggesting that 20:4n-6 might mediate the effect of FADS1 genetic variant on lipid metabolism (41). Of note, erythrocyte EPA level (0.46%) in our study population was much lower than that in Western populations (1.15%) (32), which may explain the lack of contribution of EPA to the associations between rs174550 and HDL cholesterol and triglycerides. Apparently, more mechanistic studies are needed to provide insights on whether and how 20:4n-6 mediates the associations between FADS1 variants and blood lipids.

Interestingly, our data showed that rs174550 Callele carriers appeared to be more vulnerable to insufficient essential fatty acid status than noncarriers, as it was shown that the association between FADS1 rs174550 Callele and HDL cholesterol was stronger when erythrocyte 18:2n-6 or 18:3n-3 level was lower. So far, only limited studies have evaluated interactions of fatty acids with FADS1 genetic variants on lipid traits even in populations of European ancestry (27–29). Similar to our finding, Hellstrand et al. reported that rs174547 C-allele was associated with lower LDL or HDL cholesterol when dietary long-chain n-3 PUFA or 18:3n-3/18:2n-6 was low (29). Moreover, Lu et al. and Domont et al. observed that dietary fatty acids could interact with FADS1 genetic variants on lipid traits among Western populations (27, 28). Although biological mechanisms of how dietary PUFA levels modify the effect of FADS1 rs174550 on HDL remain unclear, it is possible that people with minor allele of FADS1 rs174550 might have impaired capacity to produce long-chain n-6 and n-3 PUFA, such as 20:4n-6 and EPA, from those of essential fatty acids. Low yields of LC-PUFAs may lead to lower activity of PPARα, which subsequently reduces HDL cholesterol by suppressing expression of Apo A-I, A-II, and lipoprotein lipase, while inducing expression of Apo C-III (41). Indeed, animal studies demonstrated that FADS1 gene expression in liver was regulated by dietary LC-PUFA intake, which might support the interaction between variant of FADS1 and dietary PUFA intake (42).

To the best of our knowledge, this is the first study to investigate the associations of FADS1 rs174550, as well as its interactions, with erythrocyte PUFA levels and blood lipids in Han Chinese with a distinct dietary pattern from Western populations. The main strength of our study is that erythrocyte PUFAs were measured as objective markers of dietary essential fatty acid intake, levels of which do not depend on the accuracy of participant recall (43, 44). Owing to the cross-sectional nature of our study, possible reverse causation between dietary fat intake and blood lipids cannot be excluded. However, this scenario is unlikely, as participants with diagnosed hyperlipidemia and using anti-hyperlipidemia medications were excluded from analyses. Although there were some heterogeneities in terms of genetic variant distribution and erythrocyte fatty acid levels between Beijing and Shanghai residents, Xu et al. also reported that MAF of SNPs in FADS1 and FADS2 regions were significantly different between northern and southern Han Chinese (45). Thus, we performed statistical analyses separately, and the results were largely consistent between the participants from two geographic regions. Finally, we cannot entirely exclude the possibility that the observed associations were partially due to unmeasured confounding. Certainly, more studies are merited to confirm our findings.

In conclusion, we found significant associations of FADS1 rs174550 with blood PUFA, HDL cholesterol, and triglycerides levels in Han Chinese. The association between rs174550 and HDL cholesterol was modified by erythrocyte PUFA levels (18:2n-6 and 18:3n-3), indicating the importance of essential PUFA status in modulating effects of genes on lipid profiles.

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### TABLE 5. Interactions of 18:2n-6 and 18:3n-3 with FADS1 rs174550 genotypes on erythrocyte PUFA

| Erythrocyte PUFA (%) | TT     | TC     | CC     | P      | Low          | High          | P for Interaction |
|----------------------|--------|--------|--------|--------|--------------|---------------|------------------|
| 18:2 n-6             | 11.7 ± 0.1 | 12.1 ± 0.1 | 12.2 ± 0.1 |        | 16.0 ± 0.1 | 16.3 ± 0.1 | 16.8 ± 0.1 |
| 20:4n-6              | 13.58 ± 0.06 | 12.82 ± 0.07 | 11.91 ± 0.15 | 7.38 × 10⁻⁵¹ | 13.41 ± 0.06 | 12.50 ± 0.05 | 11.42 ± 0.08 | 1.14 × 10⁻⁷³ |
| 18:3 n-3             | 0.18 ± 0.001 | 0.19 ± 0.002 | 0.20 ± 0.003 |        | 0.35 ± 0.01 | 0.36 ± 0.01 | 0.36 ± 0.01 |
| EPA                  | 0.46 ± 0.01 | 0.43 ± 0.01 | 0.38 ± 0.02 | 5.17 × 10⁻⁷ | 0.45 ± 0.01 | 0.45 ± 0.01 | 0.40 ± 0.01 | 8.07 × 10⁻¹⁵ |
| DHA                  | 4.54 ± 0.03 | 4.49 ± 0.04 | 4.49 ± 0.08 | 0.38 |

Data are means ± SE. P values were adjusted for age, sex, BMI, residence (urban/rural), and region (Beijing/Shanghai) using an additive model. Low and high groups are stratified by medians of erythrocyte 18:2n-6 and 18:3n-3 (in bold), respectively.
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