Genetic variation at the FADS1-FADS2 gene locus influences delta-5 desaturase activity and LC-PUFA proportions after fish oil supplement

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Abstract Delta-5 and delta-6 desaturases (D5D and D6D) are key enzymes in endogenous synthesis of long-chain PUFAs. In this sample of healthy subjects (n = 310), genotypes of single nucleotide polymorphisms (SNPs) rs174537, rs174561, and rs3834458 in the FADS1-FADS2 gene cluster were strongly associated with proportions of LC-PUFAs and desaturase activities estimated in plasma and erythrocytes. Minor allele carriage associated with decreased activities of D5D (FADS1) (5.84 × 10⁻¹⁸ ≤ P ≤ 4.5 × 10⁻¹⁸) and D6D (FADS2) (6.05 × 10⁻⁸ ≤ P ≤ 4.20 × 10⁻⁷) was accompanied by increased substrate and decreased product proportions (0.05 ≤ P ≤ 2.49 × 10⁻¹⁶). The significance of haplotype association with D5D activity (P = 2.19 × 10⁻¹⁷) was comparable to that of single SNPs, but haplotype association with D6D activity (P = 3.39 × 10⁻⁷) was much stronger. In a randomized controlled dietary intervention, increasing eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) intake significantly increased D5D (P = 4.0 × 10⁻⁸) and decreased D6D activity (P = 9.16 × 10⁻⁸) after doses of 0.45, 0.9, and 1.8 g/day for six months. Interaction of rs174537 genotype with treatment was a determinant of D5D activity estimated in plasma (P = 0.05). In conclusion, different sites at the FADS1-FADS2 locus appear to influence D5D and D6D activity, and rs174537 genotype interacts with dietary EPA+DHA to modulate D5D.—Al-Hilal, M., A. AlSaleh, Z. Maniou, F. J. Lewis, W. L. Hall, T. A. B. Sanders, and S. D. O’Dell on behalf of the MARINA study team. Genetic variation at the FADS1-FADS2 gene locus influences delta-5 desaturase activity and LC-PUFA proportions after fish oil supplement. J. Lipid Res. 2013. 54: 542–551.

Supplementary key words single nucleotide polymorphism • haplotype • eicosapentaenoic acid • docosahexaenoic acid • gene-nutrient interaction • long-chain polyunsaturated fatty acids

Long-chain polyunsaturated fatty acids PUFA (LC-PUFA) are components of cell membranes and precursors of inflammatory eicosanoids (1). The major dietary LC-PUFA is linoleic acid (LA, 18:2n-6), with lower amounts of n-3 fatty acids, chiefly α-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3). The composition of fatty acids in tissues reflects both dietary fat (2) and the efficiency of elongation and desaturation of their dietary precursors, the two essential fatty acids LA and ALA (1).

Delta-5 and delta-6 desaturases (D5D and D6D) are the key enzymes in endogenous desaturation (3, 4), expressed at high levels in liver, brain, heart, and lung (5, 6). D5D and D6D are encoded, respectively, by the FADS1 and FADS2 genes, which lie in a region of strong linkage disequilibrium (LD) on chromosome 11. Several single nucleotide polymorphisms (SNP) in the FADS1-FADS2 cluster have been shown to be associated with plasma or erythrocyte phosphoglyceride LC-PUFA concentrations in genome-wide association (GWA) studies (7, 8). Associations of SNPs and haplotypes in this region have been shown with fatty acids in plasma (4, 9–14), erythrocyte membranes (4, 8, 10–12, 15), and adipose tissue (16). Surrogate estimates of desaturase activity based on ratios of product:substrate (15, 17) suggest lower levels are associated with the minor alleles of SNPs in the FADS1-FADS2 LD block. GWA studies have also identified several loci in the FADS gene cluster, at which minor alleles were associated with lower total cholesterol (18), low-density lipoprotein cholesterol (LDL-C) (18–20), high-density lipoprotein cholesterol (HDL-C) (19), and body mass index (20).LDL-C in this sample of healthy subjects (n = 310), genotypes of single nucleotide polymorphisms (SNPs) rs174537, rs174561, and rs3834458 in the FADS1-FADS2 gene cluster were strongly associated with proportions of LC-PUFAs and desaturase activities estimated in plasma and erythrocytes. Minor allele carriage associated with decreased activities of D5D (FADS1) (5.84 × 10⁻¹⁸ ≤ P ≤ 4.5 × 10⁻¹⁸) and D6D (FADS2) (6.05 × 10⁻⁸ ≤ P ≤ 4.20 × 10⁻⁷) was accompanied by increased substrate and decreased product proportions (0.05 ≤ P ≤ 2.49 × 10⁻¹⁶). The significance of haplotype association with D5D activity (P = 2.19 × 10⁻¹⁷) was comparable to that of single SNPs, but haplotype association with D6D activity (P = 3.39 × 10⁻⁷) was much stronger. In a randomized controlled dietary intervention, increasing eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) intake significantly increased D5D (P = 4.0 × 10⁻⁸) and decreased D6D activity (P = 9.16 × 10⁻⁸) after doses of 0.45, 0.9, and 1.8 g/day for six months. Interaction of rs174537 genotype with treatment was a determinant of D5D activity estimated in plasma (P = 0.05). In conclusion, different sites at the FADS1-FADS2 locus appear to influence D5D and D6D activity, and rs174537 genotype interacts with dietary EPA+DHA to modulate D5D.—Al-Hilal, M., A. AlSaleh, Z. Maniou, F. J. Lewis, W. L. Hall, T. A. B. Sanders, and S. D. O’Dell on behalf of the MARINA study team. Genetic variation at the FADS1-FADS2 gene locus influences delta-5 desaturase activity and LC-PUFA proportions after fish oil supplement. J. Lipid Res. 2013. 54: 542–551.

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Abbreviations: AA, arachidonic acid; ALA, α-linolenic acid; BMI, body mass index; CI, confidence interval; D5D, Delta-5 desaturase; D6D, Delta-6 desaturase; DGLA, dihomo-γ-linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; EPA, eicosapentaenoic acid; FADS, fatty acid desaturase; GLA, γ-linolenic acid; GLC, gas-liquid chromatography; GWA, genome-wide association; LA, linoleic acid; LD, linkage disequilibrium; MARINA, modulation of atherosclerosis risk by increasing doses of n-3 fatty acids trial; NCBI, National Center for Biotechnology Information; SNP, single nucleotide polymorphism.

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The online version of this article (available at http://www.jlr.org) contains supplementary data in the form of six tables.
The study was approved by the St Thomas Hospital NHS Research Ethics Committee (NREC 08/H0802/3). Written, informed consent was given by participants, who were healthy nonsmoking men and women between 45 and 70 years of age, recruited through media advertisements and screened as described previously (23). Three hundred sixty-seven participants were randomized to treatment by computer-generated sequence, using the process of minimization to balance age, gender, and ethnicity between treatment groups. During an initial run-in period of four weeks, participants took olive oil (BP specification) placebo capsules while restricting oily fish intake, after which baseline measurements of outcome variables were made. The dietary intervention phase involved supplementation with encapsulated EPA and DHA at three doses (0.45, 0.9, and 1.8 g/day), compared with placebo. The present investigation is based on measurements made at baseline and after 6 months. Compliance was determined by assessing the DHA and EPA content in erythrocyte phosphoglycerides at baseline, 6 months, and 12 months. The participants were supplied with capsules at regular intervals; any unused capsules were returned and their numbers were recorded. The oil blends were supplied by Croda Chemicals Europe Ltd. (Hull, UK) and encapsulated in gelatin by Powerhealth (Pocklington, UK) as described previously (23). Quality control analysis was performed by Croda Chemicals Europe Ltd.

**SUBJECTS AND METHODS**

**The MARINA Study**

The MARINA (modulation of atherosclerosis risk by increasing doses of n-3 fatty acids) trial was a single-center dietary intervention study of randomized double-blind parallel design to test the effects of three daily doses of DHA and EPA on endothelial function and established cardiovascular disease risk factors (25). The study was approved by the St Thomas Hospital NHS Research Ethics Committee (NREC 08/H0802/3). Written, informed consent was given by participants, who were healthy nonsmoking men and women between 45 and 70 years of age, recruited through media advertisements and screened as described previously (23). Three hundred sixty-seven participants were randomized to treatment by computer-generated sequence, using the process of minimization to balance age, gender, and ethnicity between treatment groups. During an initial run-in period of four weeks, participants took olive oil (BP specification) placebo capsules while restricting oily fish intake, after which baseline measurements of outcome variables were made. The dietary intervention phase involved supplementation with encapsulated EPA and DHA at three doses (0.45, 0.9, and 1.8 g/day), compared with placebo. The present investigation is based on measurements made at baseline and after 6 months. Compliance was determined by assessing the DHA and EPA content in erythrocyte phosphoglycerides at baseline, 6 months, and 12 months. The participants were supplied with capsules at regular intervals; any unused capsules were returned and their numbers were recorded. The oil blends were supplied by Croda Chemicals Europe Ltd. (Hull, UK) and encapsulated in gelatin by Powerhealth (Pocklington, UK) as described previously (23). Quality control analysis was performed by Croda Chemicals Europe Ltd.

**Blood sampling and analysis**

Erythrocyte lipids were extracted from washed cells within three days of blood collection as described elsewhere (24). Extracted lipids were stored at −20°C until analyzed. Blood samples for analysis were drawn after a minimum 8 h overnight fast.

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 cholesterol (HDL-C) (20, 21), and higher triglyceride concentrations (21), suggesting that altered desaturase activities may impact plasma lipoprotein levels. Previous studies have suggested that dietary intake of different PUFAs interact with FADS1 variation to affect blood lipids (13, 22).

Here we first investigated associations of three FADS1-FADS2 SNPs and reconstructed haplotypes with proportions of LC-PUFAs in plasma and erythrocytes, estimates of D5D and D6D activities, and plasma lipid concentrations at baseline in healthy subjects recruited to a randomized controlled trial. We then investigated the influence on genetic associations of supplements of EPA and DHA equivalent to 1–4 portions of fish per week for six months. The aim was to discover whether interaction between SNP genotypes and n-3 LC-PUFA dosage was a significant determinant of plasma or erythrocyte LC-PUFA proportions, estimated desaturase activities, or plasma lipids.
preceded by a low-fat evening meal (<10 g fat, 3 MJ), and serum was stored at −45°C until analyzed. Plasma total fatty acids were determined by capillary gas-liquid chromatography (GLC) as previously described (23), substituting toluene for benzene and using pentadecanoic acid as an internal standard (25). Measurements of plasma lipid concentrations were as previously described (26), and interassay coefficients of variation were as previously reported (23). We based surrogate estimates of desaturase activity on product:substrate ratios of n-6 LC-PUFA measures available. We used AA:DGLA (20:4n-6:20:3n-6) to estimate D5D activity (FADS1) and GLA:LA (18:3n-6:18:2n-6) for D6D (FADS2).

Selection of SNPs

Selected SNPs were sited within FADS1 and FADS2 genes or potential 5′ or 3′ regulatory regions and in strong LD to enable haplotype analysis (r² > 0.8). High minor allele frequencies (>10%) were required for investigation of diet-gene interaction. rs174537 (G/T) located 14 kb upstream of the FADS1 gene is associated with the strongest GWA signal and accounts for up to 19% of the variation in plasma arachidonic acid (AA, 20:4n-6) (8). rs174561 (T/C) is located in intron 1 of FADS1 and rs3834458 (T/del) is located in the promoter region of FADS2.

DNA extraction and SNP genotyping

Buff coats removed from blood samples were stored in EDTA at −20°C. Genomic DNA was extracted from 200 µL using an illustra blood genomic prep mini spin kit (GE Healthcare, Amersham, UK) according to manufacturer’s instructions. Genotyping was performed on the 310 participants for whom DNA was available by Kbiosciences (Hoddesdon, UK), using the KASPPar system. Genotype accuracy, as assessed by inclusion of duplicates in the array, was 98%, and negative controls (water blanks) were included on each plate. The mean genotyping success rate was 97.7% (95.8–99.7%).

Haplotype analysis

Haplotype analysis was performed using the graphical Java interface of the THESIAS software package (27) (available online at http://egene.net/genecanvas). This program is based on the maximum likelihood model linked to the SEM algorithm (28) and used to statistically reconstruct haplotypes in unrelated individuals and perform haplotype-based association analysis of phenotypes. Covariate-adjusted haplotype effects as well as interactions between haplotypes and covariates can be investigated.

Statistical analysis

All genotype distributions were tested for deviation from Hardy-Weinberg equilibrium using a χ² test with 1 df (P > 0.05). Interlocus linkage disequilibrium based on observed numbers of sample size, especially after stratification for dietary intake, SNP of the normal distribution prior to analysis. Due to the limited variables were log-transformed to obtain better approximations of the normal distribution prior to analysis. Due to the limited sample size, especially after stratification for dietary intake, SNP genotype association analyses were based on a dominant inheritance model. The presence of any significant difference in three SNP genotype frequencies between the four treatment groups was ascertained by χ² test with 6 df (P > 0.05). Linear regression was used to assess independent SNP and haplotype associations with phenotypes and interaction with dietary treatment. All data presented in text and tables are expressed as means or geometric means ± SD or mean change with respect to baseline value ± SE. Ethnicity, sex, age, and body mass index (BMI) were added to the models as covariates to adjust for possible confounding effects. Interaction between FADSSNP genotype and dosage of EPA+DHA was explored by adding interaction terms to the multiple linear regression models. Association between proportions of LC-PUFAs in plasma and erythrocyte phosphoglycerides was evaluated by Spearman correlation coefficient (r) with significance set at P < 0.01 (two-tailed test). Multivariate ANOVA was used to allow for multiple testing of genotype associations with proportions of nine fatty acids and univariate ANOVA to test genotype associations with surrogate measures of desaturase activities without adjustment for multiple comparisons. Significance was taken as P < 0.05 (as confirmed in the multivariate ANOVA).

RESULTS

Characteristics of subjects

Data was available for analysis for 310 of the 367 participants randomized to treatment. The number of participants allocated and dropout rates did not differ significantly between treatment groups (23). Women, mostly postmenopausal, outnumbered men by approximately 1.6:1, and about 20% of the sample was nonwhite, with similar proportions of Asian and black participants. The average BMI was above the desirable range (20–25 kg/m²), and the mean waist circumferences were greater than cut-offs, indicating risk of metabolic syndrome (94 cm in men and 80 cm in women) (23). Table 1 shows the characteristics of subjects, for whom DNA samples were available, after four-week run-in on placebo. There were no significant differences in these measures between the four treatment groups at baseline (P > 0.05).

SNP allele and genotype frequencies

Three SNPs at the FADS1-FADS2 locus, rs174537, rs174561, and rs3834458 were genotyped. The minor allele and genotype frequencies in all subjects who completed the study (n = 310) are shown in supplementary Table I. Genotype distributions did not deviate from Hardy-Weinberg expectations, and minor allele frequencies were in close agreement with those listed for Europeans on the NCBI SNP database (http://www.ncbi.nlm.nih.gov/projects/SNP/, accessed August 2010).

SNP genotype associations with plasma and erythrocyte variables at baseline

Erythrocyte measures previously confirmed establishment of similar levels of n-3 LC-PUFA in all treatment groups during the run-in on placebo and compliance over the intervention period (23). Proportions of fatty acids in plasma and erythrocyte phosphoglycerides were highly correlated (P < 0.01), except for adipic acid (22:4n-6) (supplementary Table II), which is rapidly taken up from plasma by erythrocytes. Table 2 shows total LC-PUFA composition and activities of D5D and D6D estimated in plasma stratified by rs174537, rs174561, and rs3834458 genotypes after a four-week run-in.
on normal diet with placebo supplement. There were significant associations between SNP genotypes and proportions of plasma fatty acids based on a dominant model after adjustment for ethnicity, gender, age, and BMI. In the n-6 series, the minor alleles of all SNPs were associated with a higher proportion of FADS1 substrate dihomo-γ-linolenic acid (DGLA, 20:3n-6) and lower proportions of FADS2 product γ-linolenic acid (GLA, 18:3n-6), FADS1 product AA (20:4n-6), and its derivative adrenic acid. In the n-3 family, minor alleles were significantly associated

### TABLE 1. Characteristics of the sample

| Characteristic          | Placebo | 0.45 g/day | 0.9 g/day | 1.8 g/day |
|-------------------------|---------|------------|-----------|-----------|
| Male n (%)              | 31 (35) | 31 (38)    | 30 (37)   | 31 (38)   |
| Female n (%)            | 42 (48) | 51 (62)    | 51 (63)   | 51 (62)   |
| Age (y)                 | 55.37 ± 6.96 | 55.00 ± 6.79 | 55.16 ± 6.56 | 55.02 ± 6.65 |
| BMI (kg/m²)             | 26.24 ± 3.72 | 25.12 ± 3.86 | 26.13 ± 4 | 25.22 ± 3.49 |

### Plasma fatty acid % total

| Fatty acid (18:2n-6)     | D5D     | D6D   |
|-------------------------|---------|-------|
| Linoleic acid (18:2n-6) | 22.69 ± 4.02 | 27.54 ± 3.62 | 27.69 ± 4.14 |
| γ-Linolenic acid (18:3n-6) | 0.47 ± 0.16 | 0.45 ± 0.18 | 0.44 ± 0.16 |

### Plasma desaturase activity

| Fatty acid % total | D5D | D6D |
|-------------------|-----|-----|
| Docosahexaenoic acid (22:6n-3) | 2.52 ± 0.71 | 2.27 ± 0.70 |

### TABLE 2. Proportions of fatty acids and desaturase activities in plasma stratified by FADS1-FADS2 SNP genotype at baseline

Data show mean (± SD) for each LC-PUFA as a % of total fatty acids and mean surrogate estimates of D5D and D6D activity based on ratio of n-6 product:substrate (± SD).

**Desaturase activity**

- **D5D**: 5.114 ± 1.340, 3.830 ± 1.041, 1.38 × 10⁻¹⁸, 5.075 ± 1.350, 3.769 ± 0.961, 5.84 × 10⁻¹⁹, 5.121 ± 1.353, 3.816 ± 1.026, 4.15 × 10⁻¹⁸
- **D6D**: 0.020 ± 0.008, 0.016 ± 0.008, 3.26 × 10⁻⁷, 0.020 ± 0.008, 0.016 ± 0.008, 6.05 × 10⁻⁸, 0.020 ± 0.008, 0.016 ± 0.008, 4.20 × 10⁻⁷

**Phenotype**

- **GG**: n = 151
- **GT+TT**: n = 125
- **TT**: n = 160
- **TC+CC**: n = 140
- **TT**: n = 160
- **Tdel + deldel**: n = 144

**Fatty acid % total**

| Phenotype | 18:2n-6 | 18:3n-6 | 20:3n-6 | 20:4n-6 | 20:5n-6 | 20:6n-3 | 22:4n-6 | 22:5n-6 | 22:6n-6 |
|-----------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| GG        | 17.27 ± 3.86 | 26.71 ± 4.09 | 0.17    |         |         |         |         |         |         |
| GT+TT     | 0.52 ± 0.17  | 0.42 ± 0.15  | 1.90 × 10⁻⁸ |         |         |         |         |         |         |
| TT        | 1.53 ± 0.28  | 1.70 ± 0.36  | 3.85 × 10⁻⁶ |         |         |         |         |         |         |
| TC+CC     | 2.52 ± 0.72  | 2.26 ± 0.67  | 0.001   |         |         |         |         |         |         |

**Desaturase activity**

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- **D6D**: 0.020 ± 0.008, 0.016 ± 0.008, 3.26 × 10⁻⁷, 0.020 ± 0.008, 0.016 ± 0.008, 6.05 × 10⁻⁸, 0.020 ± 0.008, 0.016 ± 0.008, 4.20 × 10⁻⁷

**Activity estimated by 20:4n-6:20:3n-6 ratio.**

**Activity estimated by 18:3n-6:18:2n-6 ratio.**

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with higher proportion of FADS1 substrate α-linolenic acid (ALA, 18:3n-3) and lower proportions of FADS2 product eicosapentaenoic acid (EPA, 20:5n-3) and its derivatives docosapentaenoic acid (DPA, 22:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Increased substrate and decreased downstream product proportions inferred a reduction in desaturase activities. In line with these observations, carriers of the minor alleles of all three SNPs showed highly significant associations with lower estimated activities of D5D and D6D compared with common homozygotes.

In erythrocytes, DGLA was the only LC-PUFA showing associations with genotypes with significance comparable to those in plasma ($2.97 \times 10^{-19} = P \leq 6.03 \times 10^{-15}$). D5D activity estimated in erythrocytes was much higher than in plasma, a reflection of the higher proportion of AA (16.5% versus 6.9%), but all minor alleles were associated with significantly lower activity ($1.60 \times 10^{-21} = P \leq 2.68 \times 10^{-16}$). D6D activity was not estimated in erythrocytes, as GLA was not detected. Erythrocyte data are presented in supplementary Table III.

In view of observed associations with plasma LC-PUFA proportions, we examined associations with concentrations of plasma total cholesterol, LDL-C, HDL-C, triglyceride, and total cholesterol:HDLC ratio, but none were significant ($P>0.05$).

**Haplotype reconstruction**

Any one of a set of SNPs in LD associated with a phenotype is potentially functional and could be the origin of associations seen with the others. Alternatively, all SNPs could be in LD with an unknown functional site. A third possibility exists, whereby an unknown variant on the same haplotype in LD with a functional site could be responsible for associations seen with analyzed SNPs. Thus, if haplotypes show stronger associations than SNPs independently, an unknown variant(s) could be responsible for the observed association.

Statistical reconstruction of haplotypes in unrelated individuals, for whom phase is unknown, is possible when SNPs are in strong LD. As only rs174537 has been genotyped in NCBI HapMap trios (http://hapmap.ncbi.nlm.nih.gov/), we could not use the NCBI Haploview program to establish pairwise LD between our SNPs. Instead we used the online program CubeX (see Methods) based on diplotypes available for 282 MARINA subjects. Strong LD was confirmed; the pairwise squared correlations $r^2$ ranged from 0.83 to 0.99, and Lewontin’s $D'$ from 0.98 to 1.00 (Fig. 2).

Reconstruction of haplotypes of the three SNPs was based on participants for whom all genotypes were available to avoid errors resulting from missing data. In these, fatty acid composition in plasma and erythrocyte phosphoglycerides was available for, respectively, 250 and 244 and estimated desaturase activities for 254 subjects. Seven of the eight possible haplotypes were represented, with frequencies ranging from 0.2% to 69.3% (supplementary Table IV). The most common (haplotype 1) carried the major alleles G-T-T at all loci (frequency 69.3%). The next most frequent (haplotype 2) carried the minor alleles T-C-del at all loci (frequency 25.8%).

**Haplotype associations with plasma and erythrocyte variables at baseline**

The significance of haplotype associations with plasma and erythrocyte phenotypes and proportions of variance explained by the three most frequent haplotypes, accounting for 98.4% of the total are shown in supplementary Table V. Plasma LC-PUFAs associated with independent SNP genotypes (Table 2) were also associated with haplotype, with similar levels of significance. Variability in fatty acid proportions explained ranged from 26.06% for AA to 2.88% for ALA. The significance of the association between haplotype and estimated D5D desaturase activity ($P = 2.19 \times 10^{-17}$) was similar to that for the

![Fig. 2. Pairwise linkage disequilibrium $D'$ and $r^2$ plots of the three common SNPs in the MARINA study participants ($n = 282$). The positions in bp for rs174537, rs174561, and rs3834458 on chromosome 11 are shown. Derived from NCBI HapMap Data Release 28 Phase II+III August 2010.](image-url)
single SNPs ($5.84 \times 10^{-19} \leq P \leq 4.5 \times 10^{-18}$) (Table 2). However, the significance of haplotype association with estimated D6D desaturase activity ($P=3.59 \times 10^{-28}$) was far greater than those with the single SNPs ($6.05 \times 10^{-17} \leq P \leq 4.20 \times 10^{-7}$).

Among LC-PUFA in erythrocyte phosphoglycerides, DGLA showed by far the strongest association with haplotype, accounting for a much larger proportion of the variance than in plasma (34.6% compared with 10.9%). The significance of association between haplotype and D5D desaturase activity estimated in erythrocytes ($P = 2.01 \times 10^{-19}$) was similar to that for the single SNPs ($1.60 \times 10^{-21} \leq P \leq 2.68 \times 10^{-16}$). No haplotype associations with concentrations of plasma lipids were significant ($P > 0.05$).

Owing to the relatively small number of subjects with complete genotype and phenotype data ($n = 244$–256), only the two haplotypes with an expected frequency of greater than 5% were modeled to determine haplotype effects. Table 3 shows the effect on phenotypes of a single copy of haplotype 2 compared with reference haplotype 1. The most significant effects of haplotype 2 carriage were seen in increased plasma proportions of DGLA and decreased GLA, AA, DPA, and DHA. Carriage of haplotype 2 significantly decreased plasma estimates of D5D and D6D activities compared with haplotype 1. In erythrocytes, only the increase in DGLA and the decrease in D5D activity with respect to haplotype 1 were of comparable significance.

### Changes in proportions of LC-PUFAs and desaturase activities in plasma and erythrocytes after EPA+DHA treatment

In the second part of our study, we investigated effects on plasma and erythrocyte phenotypes of dietary supplementation with EPA and DHA (1.51:1) for six months. We first assessed changes in proportions of LC-PUFAs, estimated desaturase activities, and plasma lipids. We then determined whether genetic associations seen at baseline were modulated by the treatment.

As shown in Table 4, there were significant changes after treatment in the proportions of all plasma LC-PUFAs except adrenic acid, which is rapidly taken up from plasma by erythrocytes. The proportions of all other n-6 LC-PUFAs were significantly decreased, and all n-3 LC-PUFAs were significantly increased. There was a significant increase in D5D and a significant decrease in D6D activity after supplementation. There were no significant changes in the concentrations of plasma lipids after treatment.

There were significant changes in proportions of all LC-PUFAs except ALA in erythrocyte phosphoglycerides after treatment. The proportions of all other n-6 LC-PUFAs significantly increased, and n-3 LC-PUFAs significantly decreased. The change in estimated D5D activity in erythrocytes was barely significant at the $P < 0.05$ level. Erythrocyte data are shown in supplementary Table VI.

### Table 3. Difference in proportion of plasma and erythrocyte fatty acids and desaturase activity per copy of minor allele haplotype compared with reference at baseline

| Haplotype 1 Reference | Haplotype 2 | Haplotype 1 Reference | Haplotype 2 |
|-----------------------|------------|-----------------------|------------|
| 1-1-1                 | 2-2-2      | 1-1-1                 | 2-2-2      |
| Haplotype Frequency % |            | 68.4                  | 27.2       |

| Fatty acids | Intercept | Difference (95% CI) | P |
|-------------|-----------|--------------------|---|
| 18:2n-6     | 20.700    | 0.627 (-0.080 - 1.333) | 0.08 |
| 18:3n-6     | 0.108     | -0.090 (-0.122 - 0.057) | <1.0 x 10^-6 |
| 20:3n-6     | 0.489     | 0.136 (0.076 - 0.196) | 8.0 x 10^-6 |
| 20:4n-6     | 3.250     | -1.138 (-1.425 - 0.851) | <1.0 x 10^-6 |
| 22:4n-6     | 0.415     | -0.036 (-0.064 - 0.009) | 0.01 |
| 18:3n-3     | 0.347     | 0.045 (0.008 - 0.083) | 0.02 |
| 20:3n-3     | 0.144     | -0.178 (-0.307 - 0.050) | 0.097 |
| 22:5n-3     | 0.287     | -0.055 (-0.078 - 0.032) | 2.0 x 10^-6 |
| 22:6n-3     | 1.129     | -0.259 (-0.395 - 0.123) | 1.9 x 10^-4 |

| Desaturase activity | D5D     | D6D     |
|---------------------|---------|---------|
| 18:2n-6             | 2.925   | 0.000   |
| 18:3n-6             | -0.033  | -0.005  |

- Haplotype effects based on subjects with no missing genotype data: plasma fatty acids $n = 250$, erythrocyte fatty acids $n = 244$; D5D and D6D desaturase activity $n = 254$.
- Haplotype alleles in the order rs174537 - rs174561- rs3834458. 1 = major allele; 2 = minor allele.
- The intercept is the proportion of the fatty acid (% total) or desaturase activity (estimated by fatty acid ratio) associated with a single copy of haplotype 1 (major allele 1 at each locus).
- Mean difference (95% CI) relates to the increase or decrease in proportion of a fatty acid or desaturase activity attributed to carriage of a single copy of the haplotype 2 (minor allele 2 at each locus) compared with a single copy of the reference haplotype 1.
- Activity estimated by 20:4n-6:20:3n-6 ratio.
- Activity estimated by 18:3n-6:18:2n-6 ratio. No data available for erythrocytes.

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TABLE 4. Change in proportion of fatty acids and desaturase activities in plasma stratified by treatment group after 6 months randomized treatment

| Fatty acid % total | Placebo | 0.45 g/day | 0.9 g/day | 1.8 g/day |
|--------------------|---------|------------|-----------|-----------|
| 18:2n-6 | 0.23 ± 0.41 | −0.31 ± 0.32 | −0.85 ± 0.36 | −1.30 ± 0.27 | 0.016 |
| 18:3n-6 | 0.00 ± 0.02 | −0.03 ± 0.01 | −0.06 ± 0.02 | −0.14 ± 0.02 | 8.77 × 10⁻⁹ |
| 20:3n-6 | 0.02 ± 0.03 | −0.12 ± 0.02 | −0.19 ± 0.03 | −0.47 ± 0.03 | 3.26 × 10⁻⁶⁶ |
| 20:4n-6 | −0.12 ± 0.12 | −0.32 ± 0.09 | −0.53 ± 0.10 | −0.87 ± 0.12 | 1.86 × 10⁻⁶⁶ |
| 22:4n-6 | 0.00 ± 0.01 | 0.00 ± 0.01 | 0.01 ± 0.01 | 0.03 ± 0.01 | 0.215 |
| 18:3n-3 | −0.05 ± 0.03 | 0.03 ± 0.02 | −0.02 ± 0.02 | 0.04 ± 0.02 | 0.008 |
| 20:5n-3 | −0.23 ± 0.07 | 0.73 ± 0.08 | 1.24 ± 0.08 | 2.75 ± 0.13 | 3.86 × 10⁻⁶⁶ |
| 22:5n-3 | −0.03 ± 0.01 | 0.10 ± 0.01 | 0.14 ± 0.02 | 0.28 ± 0.02 | 1.47 × 10⁻³⁷ |
| 22:6n-3 | −0.14 ± 0.05 | 0.45 ± 0.06 | 1.00 ± 0.07 | 1.60 ± 0.08 | 4.16 × 10⁻²⁵ |

Data show mean change (± SE) for each LC-PUFA as a % of total fatty acids and change in mean surrogate estimates of D5D and D6D activities based on ratio of n-6 product:substrate (± SE) with respect to baseline values. Changes after six months on placebo or EPA+DHA supplements are shown for each randomized treatment group.

Significance of difference in change in proportions of fatty acids with respect to baseline between treatment groups was tested by multivariate ANOVA, yielding $P$ values corrected for multiple comparisons. Significance of difference in change in desaturase activities was tested by univariate ANOVA, yielding $P$ values uncorrected for multiple comparisons. All $P$ values adjusted for BMI, age, gender, and ethnicity.

Activity estimated by 20:tn-6:20:3n-6 ratio.

Activity estimated by 18:3n-6:18:2n-6 ratio.

SNP associations with plasma and erythrocyte variables after dietary intervention

There were no significant differences in SNP genotype frequencies between the four treatment groups: rs174537 $\chi^2 = 6.79, 6$ df, $P = 0.34$; rs174561 $\chi^2 = 9.62, 6$ df, $P = 0.14$; rs3834458 $\chi^2 = 9.00, 6$ df, $P = 0.17$. We found no significant differences in LC-PUFA proportions between common homozygotes and carriers of the minor allele of any of the three SNPs in any treatment group ($P > 0.05$). There were also no effects on plasma lipids dependent on genotype and dose. However, there were significant effects of treatment on estimations of desaturase activity when stratified by genotype. Carriers of the rs174537 minor T-allele had significantly lower D5D activity than GG subjects in the placebo group, but with increasing dosage, activity increased significantly in T-carriers ($P = 3.2 \times 10^{-10}$ in plasma, $P = 4.3 \times 10^{-4}$ in erythrocytes) but not in GG homozygotes ($P = 0.11$ in plasma, $P = 0.76$ in erythrocytes) as shown in Fig. 3. Interaction between rs174537 genotype and intake as a determinant of D5D activity was significant at the $P < 0.05$ level ($P = 0.05$ for plasma and $P = 0.02$ for erythrocyte estimates) after adjustment for age, BMI, ethnicity, gender, and D5D activity at baseline but before correction for multiple comparisons. After correction, interaction remained significant for the plasma estimate ($P = 0.05$) but not for the erythrocyte measure ($P = 0.20$). Interaction between treatment and rs174561 genotype was not significant ($P > 0.05$). Interaction with rs3834458 genotype was significant for the determination in plasma before correction for multiple testing ($P = 0.05$) but not after correction ($P = 0.15$). There was no significant interaction between any of the SNP genotypes and treatment in determining D6D activity.

DISCUSSION

In this sample of healthy subjects, we have shown that genotypes of three SNPs in the FADS1-FADS2 gene cluster were strongly associated with proportions of LC-PUFAs and desaturase activities estimated in plasma and erythrocytes. Minor allele carriage associated with decreased activity of D5D (FADS1) and D6D (FADS2) was reflected in increased proportions of substrates and decreased products in n-6 and n-3 LC-PUFA synthetic pathways. SNP and haplotype associations with LC-PUFA proportions and D5D desaturase activity of similar significance suggested that the analyzed SNPs were in LD with a potential functional site. However, D6D activity was much more strongly associated with haplotype than with single SNPs, suggesting that an unknown variant on the same haplotype might be influential. We have shown that increasing dosage of EPA and DHA in a randomized controlled trial reduced n-6 and increased n-3 LC-PUFA proportions and that D5D desaturase activity increased. SNP genotypes did not interact with treatment in determination of LC-PUFA proportions, but interaction was a significant determinant of D5D activity.

The composition of fatty acids in the tissues reflects the dietary fat composition, but individual differences reflect genetic control of metabolic efficiency. The D5D and D6D genes FADS1 and FADS2 are important regulators of LC-PUFA synthesis, evidenced by the extremely high genetically explained variance of AA (8). As in other investigations (15, 17), we used separate surrogate estimates of D5D and D6D activities based on ratios of LC-PUFA products:substrate in the n-6 pathway to assess the influence of FADS genetic variants. We confirmed highly significant association of FADS SNP minor alleles with a reduction in desaturase.
activities estimated in plasma and erythrocytes. This may indicate a decline in gene transcription and/or enzyme conversion rates in carriers and would result in the increased substrate and decreased product proportions generally observed. At baseline, we found several highly significant SNP minor allele associations with increased proportions of substrates and decreased proportions of products in plasma and a strong association with increased DGLA in erythrocytes. Previous studies have shown association of several SNPs in this region with LC-PUFAs in plasma (4, 9–14) and erythrocyte membranes (4, 8, 10–12, 15), although most did not find associations with proportions of DHA, for which sources are thought to be mainly nutritional (9). The higher D5D activity in erythrocytes than in plasma may have reflected a preferential incorporation of AA into erythrocyte membrane phosphoglycerides. Recent GWAS studies have also identified several genetic loci in the FADS gene cluster that are associated with blood lipid levels (18–21). However, we were unable to replicate previously reported associations with plasma lipids, particularly LDL-cholesterol (13, 22, 30). This most likely relates to the fact that lipids are distal phenotypes that are influenced by many genes and environmental factors in addition to desaturase activity, and our study sample lacked the power to detect significant FADS genotype associations at baseline. As expression is highest in the liver (5, 6), a contribution of FADS1 and FADS2 genetic variation to plasma cholesterol metabolism seems likely.

The FADS1 and FADS2 genes have inverse orientation as a cluster on chromosome 11, with exon 1 of both genes separated by an 11 kb region (3). The proximity of the promoters suggests that their transcription may be coordinate controlled by common regulatory sequences (31). Common genetic variants at the FADS1-FADS2 locus are in strong LD block spanning FADS1 and the intergenic region (4, 8, 9) so that any functional polymorphisms within the block could influence expression of both desaturases. However, our haplotype analysis suggests that control of D5D and D6D activity may have different genetic origins. SNP rs3834458 sited 5′ to FADS2 is a good candidate, but an effect on promoter activity has not been established (32). Proportions of fatty acids and D5D activity in plasma and erythrocytes associated with single SNPs at baseline were also associated with the minor allele haplotype with similar levels of significance. The analyzed SNPs seem therefore to be LD markers of a site influencing D5D activity. However, the significance of haplotype association with D6D activity was substantially greater than that of single SNPs, suggesting that unknown functional SNPs or possibly more than one causal variant on the haplotype influence D6D. Further support comes from a recent GWA study of gene expression, in which rs174546 in LD with our analyzed SNP rs174537 (r² = 0.99) was associated with FADS1 (P = 1.6 × 10⁻⁶) but not FADS2 (P = 0.07) expression in lymphoblastoid cells (33).

As enzymes of the synthetic pathways show a higher affinity for n-3 than n-6 PUFA, the effect of treatment with EPA+DHA was to increase competition from derivatives of the n-3 LC-PUFA supplements and decrease proportions of the n-6 series. The significant increase in D5D activity after treatment reflected a greater reduction in the product AA than in the substrate DGLA. Modulations of the activities of D5D and D6D by intakes of EPA+DHA have been detected in previous controlled intervention studies (34, 35). Interaction between intake of n-3 PUFA or fatty fish and FADS genotypes has been established in larger studies by some (30) but not by others (7, 13, 36). Interaction between genotype and treatment as a determinant of LC-PUFA proportions was not significant in plasma or erythrocytes in our study, most likely owing to insufficient power. For example, to demonstrate a significant difference

Fig. 3. Association between rs174537 and D5D activity stratified by intake of EPA+DHA. D5D activity was estimated by ratio of (AA:DGLA) (20:4n-6:20:3n-6) in (A) plasma (n = 284) and (B) erythrocytes (n = 286). Carriers of the minor T-allele had lower D5D activity estimated from ratio in plasma (P = 0.05) and erythrocytes (P = 6.96 × 10⁻¹⁰) compared with GG in the placebo group (n = 65 plasma, n = 66 erythrocytes). After treatment, D5D activity showed a significant increase with dosage in T-allele carriers (P = 3.2 × 10⁻¹⁰ in plasma and P = 4.5 × 10⁻¹ in erythrocytes), but not in GG homozygotes (P = 0.11 and P = 0.76, respectively). Interaction between genotype and dosage as a determinant of D5D activity determined by univariate ANOVA, yielding P values uncorrected for multiple comparisons, was significant at P < 0.05 (P = 0.05 plasma; P = 0.02 erythrocytes). Interaction determined by multivariate ANOVA, yielding P values corrected for multiple comparisons, remained significant for the plasma estimate (P = 0.05) but not for the erythrocyte measure (P = 0.20).
in proportion of plasma AA with respect to rs174537 genotype across treatments based on a dominant model, a total sample size of 941 would be required for α = 0.05 and a power of 0.95. To demonstrate a significant difference based on an additive model, a total sample size of 1,240 would be required. However, we did find that interaction was a significant determinant of D5D activity estimated in plasma, which increased significantly with dose in variant allele carriers with virtually no change in the common homozygotes. The effect in erythrocytes appeared even more pronounced, but it was not significant after correction for multiple comparisons. D6D activity decreased significantly with treatment, but not after stratification by genotype. LC-PUFAs have previously been shown to downregulate D6D and increase D5D activity in controlled dietary studies (37).

The greatest strength of our study was the strict control of the intakes of EPA and DHA, shown to be correlated with plasma proportions. Long-term compliance was established by measures in erythrocytes. Although we accounted for multiple testing by using multivariate ANOVA, the best insurance that our results are not due to chance lies in replication in an independent sample. However, most associations between LC-PUFA proportions and FADS variants at baseline were highly significant after correction for multiple comparisons and agree with findings in larger studies. The main limitation was the relatively small sample of subjects for genetic analysis (n = 310), which reduced the power to detect some significant genotype associations with phenotypes and interactions with diet. Location of the three SNPs within a strong LD block on the one hand makes identification of the cause of the observed associations difficult, but on the other hand, high correlation between genotypes enabled haplotype reconstruction and analysis. Like other investigators, we used LC-PUFA ratios as surrogate desaturase activities, because direct measures are not possible in population studies.

In summary, we have confirmed that FADS polymorphisms are an important regulator of LC-PUFA synthesis through high genetically explained variance of several fatty acids. Haplotypes carrying three SNP minor alleles were associated with lower D5D and D6D activity, suggesting that any could be in linkage disequilibrium with a functional SNP. However, this study has raised the possibility that another variant on the same haplotype might have more influence on D6D activity than the studied SNPs. In this relatively small sample, we have demonstrated significant interaction between dietary n-3 LC-PUFA intake and rs174537 genotype as a determinant of D5D activity, with potential effects on the composition of LC-PUFA depots and implications for health. Development of individualized strategies to reduce the risk of diseases linked to fatty acid disturbances will require evidence from well-powered replicated studies with accurate dietary data.

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REFERENCES

1. Calder, P. C. 2005. Polyunsaturated fatty acids and inflammation. Biochem. Soc. Trans. 33: 423–427.
2. Nikkari, T., P. Luukkanen, P. Pietinen, and P. Puska. 1995. Fatty acid composition of serum lipid fractions in relation to gender and quality of dietary fat. Ann. Med. 27: 491–498.
3. Latka, E., T. Illig, B. Korte, and J. Heinrich. 2010. Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism. Curr. Opin. Lipidol. 21: 64–69.
4. Martinelli, N., D. Girelli, G. Malerba, P. Guarini, T. Illig, E. Trabetti, M. Sandri, S. Friso, F. Pizzolo, L. Schaeffer, et al. 2008. 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. 88: 941–949.
5. Cho, H. P., M. T. Nakamura, and S. D. Clarke. 1999. Cloning, expression, and nutritional regulation of the mammalian delta-6 desaturase. J. Biol. Chem. 274: 471–477.
6. Cho, H. P., M. Nakamura, and S. D. Clarke. 1999. Cloning, expression, and fatty acid regulation of the human delta-5 desaturase. J. Biol. Chem. 274: 37335–37339.
7. Lemaître, R. N., T. Tanaka, W. Tang, A. Manichaikul, M. Foy, E. K. Kabagambe, J. A. Nettleton, I. B. King, L. C. Weng, S. Bhattacharya, et al. 2011. Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. PLoS Genet. 7: e1002193.
8. Tanaka, T., J. Shen, G. R. Abecasis, A. Kisialou, J. M. Ordovas, J. M. Guralnik, A. Singleton, S. Bandinelli, A. Cherubini, D. Arnett, et al. 2009. Genome-wide association study of plasma unsaturated fatty acids in the InCHIANTI Study. PLoS Genet. 5: e1000358.
9. Schaeffer, L., H. Gohlke, M. Müller, I. M. Heid, L. J. Palmer, I. Kompasser, H. Demmelmaier, T. Illig, B. Korte, and J. Heinrich. 2006. 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. 15: 1745–1756.
10. Rzehak, P., J. Heinrich, N. Klokke, L. Schaeffer, S. Hoff, G. Wolfram, T. Illig, and J. Linseisen. 2009. Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. Br. J. Nutr. 101: 20–26.
11. Malerba, G., L. Schaeffer, L. Xumerle, N. Klokke, E. Trabetti, M. Biscuola, U. Cavallari, R. Galvotti, N. Martinelli, P. Guarini, et al. 2008. SNPs of the FADS gene cluster are associated with polyunsaturated fatty acids in a cohort of patients with cardiovascular disease. Lipids 43: 289–299.
12. Xie, L., and S. M. Innis. 2008. Genetic variants of the FADS1 FADS2 gene cluster are associated with altered (n-6) and (n-3) essential fatty acids in plasma and erythrocyte phospholipids in women during pregnancy and in breast milk during lactation. J. Nutr. 138: 2222–2228.
13. La, T., E. J. Feskens, M. E. Dollé, S. Imholz, W. M. Verschuren, M. Müller, and J. M. Boer. 2010. 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. 92: 258–265.
14. Merino, D. M., H. Johnston, S. Clarke, K. Roke, D. Nielsen, A. Badawi, A. Elsohemy, D. W. Ma, and D. M. Mutch. 2011. Polymorphisms in FADS1 and FADS2 alter desaturase activity in young Caucasian and Asian adults. Mol. Genet. Metab. 103: 171–178.
15. Zietemann, V., J. Kröger, C. Enzenbach, E. Janzen, A. Fritsche, C. Weikert, H. Boeig, and M. B. Schulze. 2010. Genetic variation of the FADS1 FADS2 gene cluster and n-6 PUFA composition in erythrocyte membranes in the European Prospective Investigation into Cancer and Nutrition-Potsdam study. Br. J. Nutr. 104: 1748–1759.
16. Baylin, A., E. Ruiz-Narvaez, P. Kraft, and H. Campos. 2007. alpha-Linolenic acid, Delta-6-desaturase gene polymorphism, and the risk of nonfatal myocardial infarction. Am. J. Clin. Nutr. 85: 554–560.

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17. Bokor, S., J. Dumont, A. Spinneker, M. Gonzalez-Gross, E. Nova, K. Widhalm, G. Moschonis, P. Stehle, P. Amouyal, S. De Henauw, et al., and HELENA Study Group. 2010. 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.* 51:2325–2333.

18. Aulchenko, Y. S., S. Ripatti, I. Lindqvist, D. Boomsma, I. M. Heid, P. P. Pramstaller, B. W. Penninx, A. C. Janssens, J. F. Wilson, T. Spector, et al., and ENGAGE Consortium. 2009. Loci influencing lipid levels and coronary heart disease risk in 16 European population cohorts. *Nat. Genet.* 41:35–46.

19. Sabatti, C., S. K. Service, A. L. Hartikainen, A. Pouta, S. Ripatti, J. Brodsky, C. G. Jones, N. A. Zaitlen, T. Varilo, M. Kaakinen, et al. 2009. Genome-wide association analysis of metabolic traits in a birth cohort from a founder population. *Nat. Genet.* 41:56–65.

20. Chasman, D. I., G. Paré, S. Mora, J. C. Hopewell, G. Peloso, R. Clarke, L. A. Cupples, A. Hamsten, S. Kathiresan, A. Mälarstig, et al. 2009. Forty-three loci associated with plasma lipoprotein size, concentration, and cholesterol content in genome-wide analysis. *PLoS Genet.* 5:e1000730.

21. Kathiresan, S., C. J. Willer, G. M. Peloso, S. Demissie, K. Musunuru, E. E. Schadt, L. A. Cupples, A. Hamsten, S. Kathiresan, A. Mälarstig, et al. 2009. Common variants at 30 loci contribute to polygenic dyslipidemia. *Nat. Genet.* 41:56–65.

22. Nakayama, K., T. Bayasgalan, F. Tazoe, Y. Yanagisawa, T. Gotoh, K. Yamanaka, A. Ogawa, L. Mukhutulga, U. Chimedregze, Y. Kagawa, et al. 2010. A single nucleotide polymorphism in the FADS1/FADS2 gene is associated with plasma lipid profiles in two genetically similar Asian ethnic groups with distinctive differences in lifestyle. *Hum. Genet.* 127:685–690.

23. Sanders, T. A., W. L. Hall, Z. Maniou, F. Lewis, P. T. Seed, and P. J. Chowienczyk. 2011. Effect of low doses of long-chain n-3 PUFAs on endothelial function and arterial stiffness: a randomized controlled trial. *Am. J. Clin. Nutr.* 94:973–980.

24. Sanders, T. A., F. Lewis, S. Slaughter, B. A. Griffin, M. Griffin, I. Davies, D. J. Millward, J. A. Cooper, and G. J. Miller. 2006. Effect of varying the ratio of n-6 to n-3 fatty acids by increasing the dietary intake of alpha-linolenic acid, eicosapentaenoic and docosahexaenoic acid, or both on fibrinogen and clotting factors VII and XII in persons aged 45-70 y: the OPTILIP study. *Am. J. Clin. Nutr.* 84:513–522.

25. Lepage, G., and C. C. Roy. 1988. Specific methylation of plasma nonesterified fatty acids in a one-step reaction. *J. Lipid Res.* 29:227–235.

26. Jebb, S. A., J. A. Lovegrove, B. A. Griffin, G. S. Frost, C. S. Moore, M. D. Chatfield, L. J. Bluck, C. M. Williams, and T. A. Sanders, and RISCK Study Group. 2010. Effect of changing the amount and type of fat and carbohydrate on insulin sensitivity and cardiovascular risk: the RISCK (Reading, Imperial, Surrey, Cambridge, and Kings) trial. *Am. J. Clin. Nutr.* 92:748–758.

27. Tregouet, D. A., and V. Garelle. 2007. A new JAVA interface implementation of THESIAS: testing haplotype effect in association studies. *Bioinformatics*, 23:1038–1039.

28. Tregouet, D. A., S. Escolano, L. Tietj, A. Mallet, and J. L. Golmard. 2004. A new maximum likelihood algorithm for haplotype-based association analysis: the SEM algorithm. *Ann. Hum. Genet.* 68:165–177.

29. Gaunt, T. R., S. Rodríguez, and I. N. Day. 2007. Cubic exact solutions for the estimation of pairwise haplotype frequencies: implications for linkage disequilibrium analyses and a web tool ‘CubeX’. *BMC Bioinformatics*. 8:428.

30. Hellstrand, S., E. Sonestedt, U. Ericson, B. Gullberg, E. Wirfalt, B. Hedblad, and M. Orho-Melander. 2012. Intake levels of dietary long-chain polyunsaturated fatty acids modify the association between genetic variation in FADS and LDL cholesterol. *J. Lipid Res.* 53:1183–1189.

31. Nakamura, M. T., and T. Y. Nara. 2004. Structure, function, and dietary regulation of delta6, delta5, and delta9 desaturases. *Annu. Rev. Nutr.* 24:345–376.

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

33. Dixon, A., L. Liang, M. F. Moffatt, W. Chen, S. Heath, K. C. Wong, J. Taylor, E. Burnett, I. Gut, M. Farrall, et al. 2007. A genome-wide association study of global gene expression. *Nat. Genet.* 39:1202–1207.

34. Zhou, L., and A. Nilsson. 2001. Sources of eicosanoid precursor fatty acid pools in tissues. *J. Lipid Res.* 42:1521–1542.

35. Vessby, B., I. B. Gustafsson, S. Tengblad, M. Boberg, and A. Andersson. 2002. Desaturation and elongation of fatty acids and insulin action. *Ann. N. Y. Acad. Sci.* 967:183–195.

36. Moltó-Puigmartí, C., J. Plat, R. P. Mensink, A. Müller, E. Jansen, M. P. Zeegers, and C. Thijss. 2010. FADS1/FADS2 gene variants modify the association between fish intake and the docosahexaenoic acid proportions in human milk. *Am. J. Clin. Nutr.* 91:1368–1376.

37. Vessby, B., M. Uusitupa, K. Hermansen, G. Riccardi, A. A. Rivellese, L. C. Tapsell, C. Nälsén, L. Berglund, A. Louheranta, B. M. Rasmussen, et al. 2001. Substituting dietary monounsaturated fat impairs insulin sensitivity in healthy men and women: the KANWU study. *Diabetologia*. 44:312–319.

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