Genetic variants of the fatty acid desaturase gene cluster predict amounts of red blood cell docosahexaenoic and other polyunsaturated fatty acids in pregnant women: findings from the Avon Longitudinal Study of Parents and Children[1–3]

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ABSTRACT

Background: Blood and tissue long-chain polyunsaturated fatty acid (LC-PUFA) amounts, which have been associated with early development and lifelong health, depend on dietary intake and endogenous conversion of precursor fatty acids (FAs) by the enzymes Δ^5-desaturase and Δ^6-desaturase. Polymorphisms in the desaturase encoding genes *FADS1* and *FADS2* have been associated with several n−6 (omega-6) and n−3 (omega-3) FAs and especially with arachidonic acid (AA) amounts. Associations with docosahexaenoic acid (DHA), which is considered particularly important for brain and retina development, are hardly existent.

Objective: We explored the relation between *FADS* gene cluster polymorphisms and red blood cell (RBC) FA amounts in >4000 pregnant women participating in the Avon Longitudinal Study of Parents and Children.

Design: Linear regression analysis of 17 single nucleotide polymorphisms (SNPs) in the *FADS* gene cluster was conducted with RBC phospholipid FAs from 6711 samples from 4457 women obtained throughout pregnancy (mean ± SD gestational age: 26.8 ± 8.2 wk).

Results: Independent of dietary effects, the minor alleles were consistently positively associated with precursor FAs and negatively associated with LC-PUFAs and product:substrate ratios of the n−6 (AA:linoleic acid ratio) and n−3 (eicosapentaenoic acid:α-linolenic acid ratio) pathways. In contrast to previous studies, we also showed significant inverse associations with DHA. Similar but weaker associations were shown for the *FADS3* SNP rs174455.

Conclusions: *FADS* genotypes influence DHA amounts in maternal RBC phospholipids and might affect the child's DHA supply during pregnancy. It is highly likely that a gene product of *FADS3* has a desaturating activity. *Am J Clin Nutr* 2011;93:211–9.

INTRODUCTION

The content of polyunsaturated fatty acids (PUFAs) in blood and tissues is associated with the occurrence of metabolic syndrome and cardiovascular diseases, immunologic and inflammatory responses, and related diseases such as allergies, early visual, cognitive and motor development, and mental health and psychiatric disorders (1, 2). These effects of PUFAs are thought to be primarily mediated by tissue contents of long-chain polyunsaturated fatty acids (LC-PUFAs) with ≥20 carbon atoms and ≥3 double bonds, such as arachidonic acid (AA; 20:4n−6), eicosapentaenoic acid (EPA; 20:5n−3), and docosahexaenoic acid (DHA; 22:6n−3) (3, 4) (**Figure 1**). LC-PUFAs are indispensible components of cell membranes and modulate their integrity and fluidity, they act as second messengers in intracellular signaling pathways or regulate transcription, and they serve as precursors for the synthesis of eicosanoids and docosanoids, which are potent regulators of inflammatory processes (8). LC-PUFA contents in human blood and tissue are determined by their dietary intakes from animal lipids contained in meat, eggs, fish, and human milk and by the intake of the essential fatty acids linoleic acid (LA; 18:2n−6) and α-linolenic acid (ALA; 18:3n−3) primarily from vegetable oils and the subsequent endogenous conversion of these precursors into LC-PUFAs mediated by the enzymes Δ^5-desaturase and Δ^6-desaturase. Genetic association studies of single nucleotide polymorphisms (SNPs) in the desaturase encoding genes *FADS1* and *FADS2* showed significant associations between these SNPs and fatty acid amounts in serum phospholipids with an extraordinarily high

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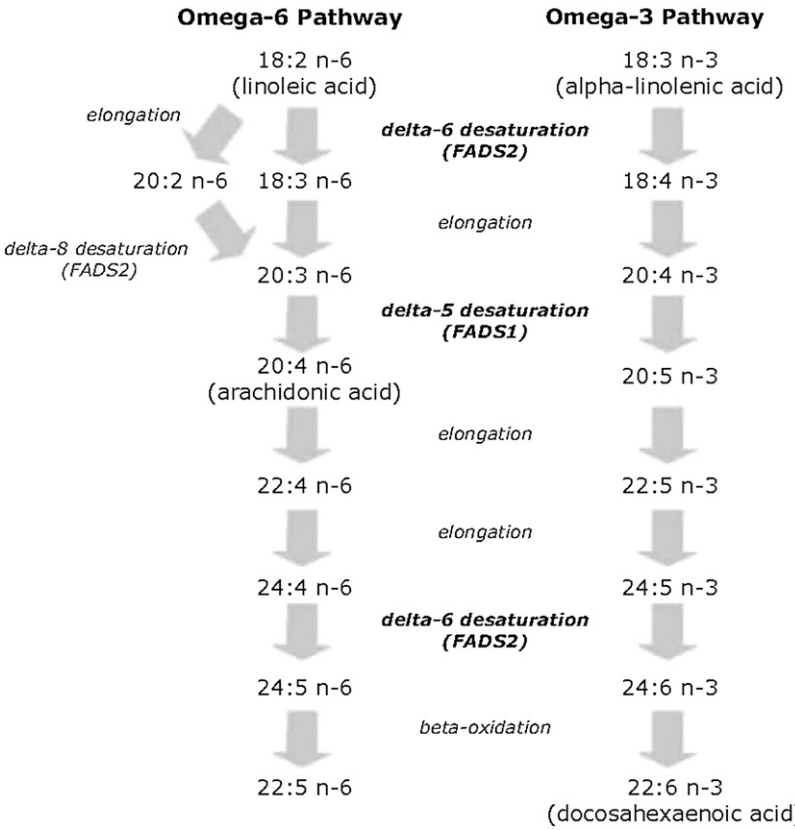

FIGURE 1. The mammalian pathway of n−6 and n−3 long-chain polyunsaturated fatty acid synthesis. Modified and redrawn from references 5–7.

genetically explained variance for AA amounts ≤28.5% (5). Carriers of the minor alleles showed increased amounts of desaturase substrates and reduced amounts of desaturase products, which suggests a decline in desaturase activity because of the polymorphisms. These results were replicated in several independent candidate gene studies that included between 69 and 1820 human subjects (9–13) and in a recent genome-wide association study (14). All studies agreed on the strong association of *FADS1* and *FADS2* genotypes with several n−6 and n−3 fatty acids; however, reports on an association with DHA amounts are hardly existent. DHA is an n−3 LC-PUFA with high incorporation into neural tissues that are considered of particular importance for brain and retina function (15). Only 2 studies reported an association of single *FADS* SNPs with DHA amounts (10, 13); all other studies did not find such associations. There are first indications for an association of *FADS* genotypes with brain-related phenotypes such as intelligence development (16) and attention deficit hyperactivity disorder (ADHD) (17). These effects are thought to be mediated, in part, by DHA availability in the brain, but the question remains how *FADS* genotypes modulate these phenotypes, if not by direct association with DHA amounts. It seems possible that the preliminary studies were too small to detect consistent associations between *FADS* SNPs and DHA amounts. Therefore, we explored the relation between polymorphisms of the *FADS* gene cluster including *FADS3*, the function of which is still unclear, and red blood cell PUFA amounts in a large cohort of >4000 pregnant women participating in the Avon Longitudinal Study of Parents and Children (ALSPAC).

SUBJECTS AND METHODS

Subjects

The ALSPAC (http://www.alspac.bris.ac.uk) is a multipurpose birth cohort study based at Bristol University (Bristol, United Kingdom) and involving over 14,000 pregnancies in the Avon area of England in the early 1990s, the children from which have been followed through childhood (18). Blood samples that had been taken at times of diagnostic venipunctures for clinical care were used with up to 6 samples per woman. Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the local research ethics committees.

Analysis of fatty acids in red blood cell phospholipids

At least one blood sample each was analyzed for fatty acids from 5144 mothers during pregnancy, of whom 4136 mothers had at least one sample taken after 20 wk of pregnancy. Blood samples were collected in heparin-containing tubes and centrifuged at 1500 × *g* for 15 min at 4°C to separate the red cells, which were then stored at −20°C until 1993 and subsequently at −70°C. Frozen red blood cell samples were shipped to the laboratories of Scotia Pharmaceuticals in Canada in 1996 for fatty acid composition analysis as previously described (19). Briefly, lipids from thawed red blood cells were extracted with chloroform and methanol, extracted lipids were redissolved in chloroform (100 μL), and phospholipids were isolated by thin-layer chromatography with a mixture of hexane:diethyl ether: acetic acid (80:20:1; vol:vol:vol). Fatty acid methyl esters were prepared by incubation with 120 g boron trifluoride/L in

methanol at 90°C for 30 min. Fatty acid methyl esters were taken up in hexane, and the relative amounts of 40 fatty acids were measured by gas-liquid-chromatography and expressed as the weight percentage of all measured fatty acids. Fatty acid methyl esters were identified by comparison with authentic standards. Fatty acid amounts below the limit of detection of the assay (0.01%) were recoded to one-half that value.

Genetic analyses

Genomic DNA was extracted from whole blood samples as previously reported (20). SNPs genotyped in this study were chosen on the basis of a minor allele frequency >10% and linkage disequilibrium information provided by HapMap (http://hapmap.ncbi.nlm.nih.gov/). SNPs with $r^2 > 0.8$ were considered to have a high degree of coinheritance in Europeans and, therefore, were selected as tagSNPs. Twelve tagSNPs (rs174576, rs174579, rs174448, rs2727271, rs174634, rs174449, rs968567, rs526126, rs174455, rs174602, rs498793, and rs174570) located in the genomic region spanning *FADS1*, *FADS2*, and *FADS3* were selected for genotyping together with 6 additional SNPs (rs174556, rs174561, rs3834458, rs174548, rs174574, and rs174578) that have already been associated with fatty acid amounts in previous studies. Five nanograms of genomic DNA was subjected to polymerase chain reaction amplification followed by the genotyping procedure by using iPLEX chemistry (Sequenom, San Diego, CA) according to the manufacturer's protocol and a matrix-assisted laser desorption/ionization time-of-flight–based allele-detection method. The procedure has been described in detail elsewhere (12). Genotyping failure rates ranged from 1.1% to 4.1%. Error rates on the basis of 790 duplicate samples ranged from 0% to 0.64%.

Statistics and confounding

All SNPs, except SNP rs174634, passed all quality criteria, and genotypes were used for statistical analyses. Linear regression analysis was used to investigate the associations of *FADS* polymorphisms with fatty acid amounts. In addition, product:substrate ratios for AA:LA and EPA:ALA were also analyzed. Because of the skewness of the data, log transformations were applied. Outcomes were standardized to have a variance of one to produce more comparable effect sizes. Genetic variants were modeled with the assumption of a linear relation with the number of copies of the minor allele. Although genetic effects are unlikely to be confounded with sociodemographic variables (21), the validity was explored in analyses adjusting for 7 confounders as follows: multiple pregnancy (singleton or multiple), parity (primiparous or multiparous), maternal smoking at 32 wk gestation (yes or no), gestation, maternal age, maternal prepregnancy body mass index, and a measure of family adversity, with the latter 4 variables being used as continuous variables. In addition, to exclude possible confounding by differential LC-PUFA intake from the diet, we simultaneously adjusted for 81 dietary variables obtained from a food-frequency questionnaire completed around the 32nd wk of gestation by the mothers. To avoid complications of allele frequencies and effect sizes varying with ethnicity, analyses were restricted to mothers of white ethnic origin. Longitudinal analyses were used to take account of the repeat measurements during pregnancy (xtreg, Stata version 11.0; StataCorp LP, College Station, TX).

RESULTS

Some 6711 samples (91% of total samples) from 4457 women (55% of women genotyped) were obtained at varying times (between the 4th and 44th wk) throughout pregnancy (mean ± SD gestational age at blood sampling: 26.8 ± 8.2 wk). Only 3% of samples were taken in the first trimester, whereas 33% and 64% of samples were taken in the second and third trimesters, respectively.

The composition of red blood cell lipid fatty acids revealed amounts of essential fatty acids similar to a previous report in Dutch pregnant women (22) but with somewhat lower amounts of EPA (0.24% in the ALSPAC cohort compared with 0.39% in midpregnancy in the Netherlands) and DHA (2% compared with 3.9% in the Netherlands) (**Table 1**) perhaps because of a higher habitual fish consumption in the Netherlands.

Genetic associations

Genetic variants were generally in Hardy-Weinberg equilibrium although disequilibrium existed for rs174579 ($P < 0.001$) and rs174570 ($P = 0.001$) (*see* supplemental Table 1 under "Supplemental data" in the online issue.). In general, heterozygotes were underrepresented in this sample, although the reasons for these departures remained unclear. Possible explanations associated with genotyping problems, such as poor cluster separation, were not applicable for these data. Differential fetal survival may provide an alternative explanation (23). But overall, these departures were considered minor, with the

TABLE 1
Polyunsaturated fatty acids (weight % of total fatty acids) in red blood cell lipids of pregnant women of white ethnic origin (6711 blood samples from 4457 women)[1]

Fatty acid	Values
Omega-6	
18:2	11.15 (9.62–12.59)
18:3	0.02 (0.005–0.03)
20:2	0.26 (0.22–0.30)
20:3	1.38 (1.05–1.69)
20:4	6.09 (3.89–8.36)
22:4	0.93 (0.53–1.40)
22:5	0.22 (0.14–0.32)
Omega-3	
18:3	0.14 (0.10–0.18)
20:5	0.24 (0.16–0.36)
22:5	0.66 (0.35–1.05)
22:6	2.01 (1.24–3.05)
Ratios	
AA to LA	0.52 (0.38–0.70)
EPA to ALA	1.83 (1.33–2.53)

[1] All values are medians; interquartile ranges in parentheses. AA, arachidonic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; ALA, α-linolenic acid. Sample reflects observations where genetic data are present for at least one polymorphism. All fatty acids and ratios showed evidence of skewness ($P < 0.001$), although the interquartile ranges suggested that the deviations from symmetry were minor for some outcomes.

significance reflecting the large sample size. It was considered important to report results for these 2 SNPs, but nevertheless, if these departures reflected genotype misclassifications, any associations would be biased toward the null. Hence, reported results would tend to underestimate the true effect sizes.

The minor alleles of variants in the *FADS* genes were positively associated with the precursor PUFAs with 2 or 3 double bonds and negatively associated with the LC-PUFAs with ≥4 double bonds and major product-to-substrate ratios of the n−6 pathway (AA-to-LA ratio) and the n−3 pathway (EPA-to-ALA ratio) (**Table 2**). Associations of *FADS* and intergenic SNPs were strongest in terms of the variability explained and the sizes of the regression coefficients (measured in terms of SDs) for the n−6 metabolite 20:3n−6 followed by the n−6 fatty acid 20:4n−6, 22-carbon metabolites, 20:2n−6, and 18:2n−6. Only 5 of the 17 SNPs showed evidence of an association with 18:3n−6. With respect to n−3 fatty acids, associations were generally weaker than for n−6 fatty acids. Regression coefficients for DHA (22:6n−3) tended to be lower than for 20:4n−6, and regression coefficients for 18:3n−3 tended to be lower than those for 18:2n−6 with the regression coefficients for these n−3 fatty acids being typically one-half for those of the corresponding n−6 fatty acids. Overall, the strongest n−3 associations were observed for DHA. Only 3 of the genetic variants were associated with 22:5n−3. Associations for the *FADS3* SNP rs174455 were weaker than for all *FADS1* and some *FADS2* SNPs but were in the same direction. Perhaps because of the number of SNPs, *FADS2* variants showed greater heterogeneity in results than *FADS1*, with only dihomo-γ-linolenic acid (20:3n−6) showing consistent associations. In contrast, the 3 *FADS1* variants only failed to show consistent associations for γ-linolenic acid (18:3n−6) and docosapentaenoic acid (22:5n−3).

In general, R^2 values that reflected the variability of fatty acid amounts explained by the genetic variants ranged from very low for 22:5n−3 (0.15%) or 18:3n−6 (0.14%) to moderate for 20:3n−6 (5.61%) (**Table 3**). The variability of AA amounts explained by the analyzed polymorphisms reached 1.13% and was 0.51% for DHA amounts.

Adjusted analyses

An adjusted analysis was performed for 81 dietary variables obtained from the food-frequency questionnaire completed around the 32nd wk of gestation by mothers and for 7 potential sociodemographic confounders (*see* supplemental Table 2 under "Supplemental data" in the online issue for the demographics of the sample in the adjusted analysis). Because of missing data, these analyses were restricted to a maximum of 5468 observations. Overall, the associations observed in unadjusted analyses were resilient to controlling for these factors (*see* supplemental Figure S1 under "Supplemental data" in the online issue).

Multiple comparisons

In the main analyses, hypotheses relating to 17 genetic effects for 13 outcomes were being tested. Of these 221 comparisons, 48 comparisons had nominal $P > 0.05$, 173 comparisons had nominal $P < 0.05$, 156 comparisons had nominal $P < 0.01$, 138 comparisons had nominal $P < 0.001$, and 110 comparisons had nominal $P < 0.0001$. With the use of the false discovery rate method of adjusting for multiple comparisons (24), the adjusted 5% critical P value was 0.039. All of the 173 nominally significant P values were also less than this adjusted critical value.

The distribution of the observed P values differed markedly from that expected if all 221 comparisons were null (**Figure 2**). In particular, 78% of observed P values were less than the 5% significance level compared with 5% (by definition) for the null scenario. Even with the use of the overly conservative Bonferroni correction, 53% of tests would have remained significant. A more powerful method, the false discovery rate, suggested a revised critical value of 0.039. The minor adjustment to the nominal value of 0.05 reflected the early divergence in the distributions of observed and expected P values.

The strength of these associations was further illustrated when analyses were repeated for saturated and monounsaturated fatty acids. These fatty acids were not expected to show any associations with genetic variants in the *FADS* genes. In general, no associations after adjustment for multiple comparisons were observed (*see* supplemental Figure S2 under "Supplemental data" in the online issue). Of all saturated and monounsaturated fatty acids, only 16:1n−7 showed consistent and strong associations with most of the analyzed SNPs (*see* supplemental Table 3 under "Supplemental data" in the online issue).

DISCUSSION

The results of this largest available cohort study on the relation between *FADS* polymorphisms and PUFA-status markers showed a consistent association of the minor alleles of the tested SNPs in the *FADS1 FADS2* gene cluster with increased amounts of desaturase substrates, such as 18:2n−6, 20:2n−6, 20:3n−6, and 18:3n−3, decreased amounts of desaturase products such as 20:4n−6 and 22:4n−6, and lower values of the major product-to-substrate ratios of the n−6 pathway (AA-to-LA ratio) and the n−3 pathway (EPA-to-ALA ratio). These data confirmed previous observations in smaller studies (9–13) and suggested a decline in desaturase expression or activity because of the polymorphisms. The prevalence of these minor alleles is relatively high and ranged from 11% to 40% of the population (*see* supplemental Table 1 under "Supplemental data" in the online issue); hence, one would expect a considerable public health relevance of these genetic variants that modulate the effects of environmental exposures on human health, although the genetically explained variability of the fatty acid amounts was relatively low in our study compared with in others and ranged from 0.14% for 18:3n−6 to 5.61% for 20:3n−6. Schaeffer et al (6) reported a genetically explained variability of 28.5% for AA amounts and Tanaka et al (14) showed a variability of 18.6%. In our study, the variability of AA amounts explained by the 17 analyzed genetic variants was 1.13%. The reason for this may lie in the fact that these 2 former studies analyzed fatty acids in serum and plasma, whereas we analyzed red blood cell fatty acids. A recent study by Zietemann et al (25) reported a genetically explained variance for red blood cell AA amounts of 2.6%. These and our results suggest that red blood cell fatty acid amounts are less influenced by *FADS* genotypes than are plasma or serum phospholipid fatty acid amounts, the reason of which remains speculative. Nevertheless, the associations remained stable despite adjustments for sociodemographic

TABLE 2
Regression coefficients from analyses of log n−6 and n−3 fatty acid amounts as a percentage of total fatty acids on *FADS* single nucleotide polymorphisms[1]

	FADS1			Intergenic		*FADS2*	
	rs174548	rs174556	rs174561	rs3834458	rs968567	rs174570	rs174574
Omega-6							
18:2	0.110 (0.020)***	0.112 (0.020)***	0.108 (0.020)***	0.106 (0.019)***	0.018 (0.024)	0.150 (0.027)***	0.113 (0.019)***
18:3	−0.047 (0.020)*	−0.036 (0.020)	−0.035 (0.020)	−0.037 (0.020)	0.012 (0.024)	−0.079 (0.027)**	−0.042 (0.019)*
20:2	0.104 (0.021)***	0.110 (0.021)***	0.104 (0.021)***	0.116 (0.020)***	0.011 (0.025)	0.172 (0.028)***	0.115 (0.020)***
20:3	0.336 (0.020)***	0.347 (0.021)***	0.345 (0.021)***	0.316 (0.020)***	0.403 (0.025)***	0.089 (0.029**	0.305 (0.020)***
20:4	−0.154 (0.021)***	−0.151 (0.021)***	−0.155 (0.021)***	−0.151 (0.020)***	−0.131 (0.025)***	−0.129 (0.029)***	−0.147 (0.020)***
22:4	−0.148 (0.020)***	−0.151 (0.020)***	−0.154 (0.020)***	−0.140 (0.020)***	−0.144 (0.025)***	−0.100 (0.028)***	−0.134 (0.020)***
22:5	−0.141 (0.021)***	−0.134 (0.021)***	−0.137 (0.021)***	−0.140 (0.020)***	−0.085 (0.025)***	−0.166 (0.028)***	−0.146 (0.020)***
Omega-3							
18:3	0.079 (0.021)***	0.084 (0.021)***	0.081 (0.021)***	0.075 (0.020)***	0.013 (0.025)	0.071 (0.028)*	0.073 (0.020)***
20:5	−0.074 (0.021)***	−0.069 (0.021**	−0.074 (0.021)***	−0.075 (0.021)***	−0.091 (0.026)***	−0.037 (0.029)	−0.068 (0.020)***
22:5	−0.039 (0.021)	−0.036 (0.021)	−0.039 (0.021)	−0.036 (0.021)	−0.059 (0.026)*	−0.015 (0.029)	−0.035 (0.020)
22:6	−0.092 (0.021)***	−0.092 (0.021)***	−0.096 (0.021)***	−0.097 (0.020)***	−0.089 (0.025)***	−0.073 (0.028)**	−0.092 (0.020)***
Ratios							
AA to LA	−0.262 (0.021)***	−0.259 (0.021)***	−0.262 (0.021)***	−0.256 (0.020)***	−0.175 (0.025)***	−0.255 (0.028)***	−0.254 (0.020)***
EPA to ALA	−0.165 (0.020)***	−0.165 (0.020)***	−0.166 (0.020)***	−0.161 (0.020)***	−0.111 (0.025)***	−0.117 (0.028)***	−0.151 (0.019)***
n	6565	6524	6560	6558	6666	6583	6554

	FADS2						
	rs2727271	rs174576	rs174578	rs174579	rs174602	rs498793	rs526126
Omega-6							
18:2	0.155 (0.028)***	0.108 (0.019)***	0.111 (0.019)***	0.053 (0.022)*	0.080 (0.023)***	−0.025 (0.018)	−0.011 (0.024)
18:3	−0.056 (0.029)	−0.044 (0.020)*	−0.043 (0.019)*	−0.010 (0.022)	−0.015 (0.023)	−0.001 (0.019)	−0.013 (0.024)
20:2	0.164 (0.030)***	0.115 (0.020)***	0.112 (0.020)***	0.057 (0.023)*	0.080 (0.024)***	−0.019 (0.019)	−0.017 (0.025)
20:3	0.115 (0.031)***	0.304 (0.020)***	0.304 (0.020)***	0.330 (0.023)***	0.118 (0.024)***	−0.056 (0.020)**	0.168 (0.025)***
20:4	−0.114 (0.030)***	−0.144 (0.020)***	−0.146 (0.020)***	−0.120 (0.023)***	−0.037 (0.024)	0.041 (0.020)*	−0.079 (0.025)**
22:4	−0.086 (0.029)**	−0.131 (0.020)***	−0.135 (0.019)***	−0.126 (0.022)***	−0.032 (0.023)	0.033 (0.019)	−0.083 (0.025)***
22:5	−0.126 (0.030)***	−0.147 (0.020)***	−0.145 (0.020)***	−0.111 (0.023)***	−0.035 (0.024)	0.049 (0.019)*	−0.068 (0.025)**
Omega-3							
18:3	0.123 (0.030)***	0.069 (0.020)***	0.070 (0.020)***	0.028 (0.023)	0.063 (0.024)**	0.007 (0.019)	−0.008 (0.025)
20:5	−0.017 (0.030)	−0.064 (0.021)**	−0.064 (0.020)**	−0.066 (0.023)**	−0.012 (0.024)	0.031 (0.020)	−0.067 (0.025)**
22:5	0.006 (0.031)	−0.037 (0.021)	−0.039 (0.020)	−0.054 (0.023)*	0.015 (0.024)	0.021 (0.020)	−0.072 (0.025)**
22:6	−0.052 (0.030)	−0.091 (0.020)***	−0.093 (0.020)***	−0.088 (0.023)***	−0.034 (0.024)	0.045 (0.019)*	−0.092 (0.025)***
Ratios							
AA to LA	−0.241 (0.030)***	−0.248 (0.020)***	−0.252 (0.020)***	−0.183 (0.023)***	−0.096 (0.024)***	0.065 (0.020)***	−0.093 (0.025)***
EPA to ALA	−0.152 (0.029)***	−0.144 (0.020)***	−0.144 (0.019)***	−0.101 (0.022)***	−0.082 (0.023)***	0.026 (0.019)	−0.063 (0.024)**
n	6569	6481	6574	6556	6591	6555	6595

(Continued)

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TABLE 2 (*Continued*)

	Intergenic		*FADS3*				
	rs174448	rs174449	rs174455				
Omega-6							
18:2	0.070 (0.019)***	0.065 (0.019)***	0.059 (0.019)**	—	—	—	—
18:3	−0.025 (0.019)	−0.027 (0.019)	−0.018 (0.020)	—	—	—	—
20:2	0.070 (0.020)***	0.069 (0.020)***	0.062 (0.020)**	—	—	—	—
20:3	0.216 (0.020)***	0.218 (0.020)***	0.196 (0.020)***	—	—	—	—
20:4	−0.095 (0.020)***	−0.103 (0.020)***	−0.099 (0.020)***	—	—	—	—
22:4	−0.096 (0.019)***	−0.103 (0.020)***	−0.096 (0.020)***	—	—	—	—
22:5	−0.097 (0.020)***	−0.103 (0.020)***	−0.102 (0.020)***	—	—	—	—
Omega-3							
18:3	0.043 (0.020)*	0.043 (0.020)*	0.032 (0.020)	—	—	—	—
20:5	−0.043 (0.020)*	−0.050 (0.020)*	−0.051 (0.021)*	—	—	—	—
22:5	−0.033 (0.020)	−0.035 (0.020)	−0.035 (0.020)	—	—	—	—
22:6	−0.076 (0.020)***	−0.082 (0.020)***	−0.062 (0.020)**	—	—	—	—
Ratios							
AA to LA	−0.164 (0.020)***	−0.169 (0.020)***	−0.160 (0.020)***	—	—	—	—
EPA to ALA	−0.093 (0.019)***	−0.101 (0.020)***	−0.088 (0.020)***	—	—	—	—
n	6623	6583	6569	—	—	—	—

[1] SEs are in parentheses. AA, arachidonic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; ALA, α-linolenic acid. Fatty acids and ratios were standardized to have a variance of one. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

confounders and dietary habits. By including these covariables into the analysis, we were able to explain ≤12.1% of the variance in fatty acid amounts (*see* supplemental Table 2 under "Supplemental data" in the online issue).

The strength of the observed associations with n−6 and n−3 fatty acids was further substantiated when analyses were repeated for saturated and monounsaturated fatty acids. These fatty acids were not expected to show any associations with genetic variants in the *FADS* genes because they are synthesized in different pathways. In general, no associations after adjustment for multiple comparisons were observed (*see* supplemental Figure S2 under "Supplemental data" in the online issue). However, 16:1n−7 showed consistent and strong associations with most of the analyzed SNPs (*see* supplemental Table 3 under "Supplemental data" in the online issue), the reason of which was not immediately obvious. The association might have possibly reflected a correlation of 16:1n−7 with 22:4n−6 (r = –0.31).

In contrast to all previous studies cited, we also showed a consistent significant association of the rare SNP alleles with lower amounts of DHA (22:6n−3) in red blood cell phospholipids of pregnant women. Schaeffer et al (5) had reported a similar trend in female and male adults, which, however, failed to reach significance. Two other studies reported isolated weak associations of individual *FADS* SNPs with DHA amounts in serum and breast milk (10, 13). The missing consistent association in previous studies might potentially be due to a lack of statistical power. Moreover, it may reflect a higher rate of DHA synthesis in pregnant women compared with in men or nonpregnant women. The conversion of ALA to EPA and DHA in women was reported to be substantially greater than in a comparable study of men of similar age (26, 27). It has been suggested that estrogen may increase the activity of the desaturation pathway because DHA synthesis was shown to be almost 3 times greater in women who used an oral contraceptive pill that contained 17-ethynylestradiol than in women who did not (26). DHA in plasma cholesteryl esters increased by >40% in male-to-female transsexuals who were given 17-ethynylestradiol, whereas the supplementation of testosterone decreased DHA amounts by >20% in female-to-male transsexuals (28). Thus, exposure to female sex hormones seemed to stimulate DHA synthesis, which appeared to be considerably more active in women and, particularly, in pregnant women exposed to enhanced hormone amounts. This might reflect protective biological mechanisms that contribute to meeting the high fetal demands for DHA. A higher rate of endogenous synthesis in pregnancy could increase the likelihood of detecting effects of SNPs that affect desaturase activity, as shown in this study. Although Xie et al (13) also investigated plasma and red blood cell membrane fatty acids of pregnant women, the reason for the missing association with DHA in their study might have been the very small number of 69 subjects.

Although the genetically explained variability of red blood cell DHA amounts was rather low in this study (0.51%), a modulation of DHA status during pregnancy by frequently occurring *FADS* genotypes may be of major relevance for child outcomes. Several cohort and randomized control studies showed positive (29–34) but also null associations (34–37) between LC-PUFA intake and status in the pre- and postnatal period and developmental outcomes in early childhood. For example, Malcolm et al (29)

TABLE 3
Maximum R^2 across the 17 genetic variants for each fatty acid in unadjusted analyses (reflecting the genetic association) and in adjusted analyses (including the effect of confounders)[1]

	Unadjusted	Adjusted
Omega-6		
18:2	0.64	11.72
18:3	0.14	2.38
20:2	0.72	10.38
20:3	5.61	9.17
20:4	1.13	7.04
22:4	1.21	5.99
22:5	1.30	5.41
Omega-3		
18:3	0.38	6.54
20:5	0.27	12.10
22:5	0.15	8.12
22:6	0.51	6.04
Ratios		
AA to LA	3.28	8.13
EPA to ALA	1.37	9.45
n	6711	5468

[1] AA, arachidonic acid; LA, linoleic acid; EPA, eicosapentaenoic acid; ALA, α-linolenic acid.

provided fish oil during pregnancy and showed that the DHA status of the infants at birth was related to improved visual development at 2.5 and 6 mo of age. Another study reported that eye-hand coordination at the age of 2.5 y was improved in infants whose mothers received high-dose fish oil during pregnancy (30). Trials that investigated the effect of direct infant LC-PUFA supplementation after birth via formulae on potential long-term benefits such as stereoacuity, vision, and intelligence quotient (IQ) did not report significant findings (35–37). Although these contradictory results exist, a sufficient availability of DHA during the perinatal brain growth spurt is widely considered mandatory for normal cognitive, visual, and motor development (32). An average intake ≥200 mg DHA/d for pregnant and lactating women has been recommended (38) because maternal DHA status has a direct effect on DHA availability to the fetus (39, 40), which in turn has been linked to short- and long-term developmental child outcomes (32, 33, 41). Future studies on the association between *FADS* genotypes, DHA amounts, and early childhood development will be needed to evaluate the biological relevance of genotype-dependent DHA amounts. In 2007, Caspi et al (16) reported that a genetic variant in the *FADS2* gene (rs174575) modulated the association between breastfeeding and IQ in 2 large birth cohorts. In both cohorts, previously breastfed and formula-fed children differed in later IQ, but this effect was more pronounced and only significant in children carrying the rs174575 major C allele (IQ point advantage: 6.35 and 7.91, respectively; $P < 0.001$), whereas children with the minor G allele neither gained an advantage nor suffered a disadvantage from being fed breast milk. A recent attempt to replicate these findings in the ALSPAC population showed different results that those of the Caspi study (16), with GG children exhibiting the biggest difference in IQ scores between the formula and breastfed group. These contradictory results require further replication; however, a modification of the breastfeeding effect on IQ scores by *FADS2* genotypes was shown in both studies, even though the direction of effects differed (42). Brookes et al (17) showed an association of SNP rs498793 in the *FADS2* gene with ADHD in 180 ADHD cases compared with control subjects. It is tempting to speculate that genetic heterogeneity in fatty acid metabolism may be one of the reasons besides differing study design and variable quality for the apparent inconsistent results of different studies that investigated effects of a perinatal supply of DHA sources on developmental outcome (43, 44).

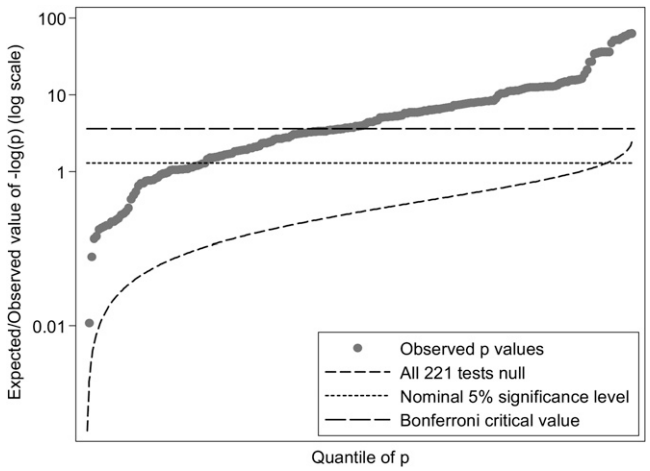

FIGURE 2. Quantile-quantile plot of 221 tests of significance (13 fatty acid outcomes × 17 genetic variants) reported in Table 2. The distribution of the observed *P* values differed markedly from that expected if all 221 comparisons were null. In particular, 78% of observed *P* values were less than the 5% significance level compared with 5% (by definition) for the null scenario. Even with the use of the overly conservative Bonferroni correction, 53% of tests would have remained significant. A more powerful method, the false discovery rate, suggested a revised critical value of 0.039. The minor adjustment to the nominal value of 0.05 reflected the early divergence in the distributions of observed and expected *P* values.

The genes *FADS1* and *FADS2* encoding for the enzymes Δ^5-desaturase and Δ^6-desaturase that are important in the desaturation and elongation pathway of n−6 and n−3 LC-PUFA biosynthesis, respectively, were mapped to chromosome 11q12-13.1 of the human genome in the year 2000 (45). This region shows conserved synteny to the mouse genomic region that contains the murine *fads1* and *fads2* genes on chromosome 19 (46). The 2 human genes are arranged in a head-to-head orientation and build a gene cluster together with a third desaturase gene, *FADS3*, which shows a high degree of sequence homology (47). Protein products of the *FADS3* gene have only recently been identified, but their function is not yet clear (48). Martinelli et al (11) reported the minor allele of rs1000778 mapped in the *FADS3* gene to be weakly associated with both the arachidonic:linoleic ratio and the eicosapentaenoic:α-linolenic ratio at $P <$ 0.05. In this study, we found an association of the minor allele of rs174455 mapped in the *FADS3* gene with higher amounts of the n−6 precursor fatty acids 18:2n−6, 20:2n−6, and 20:3n−6 as well as lower amounts of the LC-PUFA product fatty acids 20:4n−6, 22:4n−6, and 22:5n−6. Moreover, a significant negative association with DHA (22:6n−3) was shown. The strength of the association was comparable with the average of those observed for the *FADS2* SNPs studied. Therefore, it is highly likely that a gene product of *FADS3* has a desaturating activity, which, however, has to be confirmed in functional studies.

In conclusion, this study shows that genetic variants common in the population have effects on pregnant women's blood amounts of PUFAs and LC-PUFAs, including the amounts of DHA considered particularly important for fetal development. Therefore, future studies on the relation between n−3 fatty acid supply and developmental outcomes should aim at inclusion of such genetic analyses to evaluate the biological relevance of genotype-dependent fatty acid amounts.

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