Maternal single nucleotide polymorphisms in the fatty acid desaturase 1 and 2 coding regions modify the impact of prenatal supplementation with DHA on birth weight[1,2]

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ABSTRACT

Background: Specific single nucleotide polymorphisms (SNPs) in the fatty acid desaturase (*FADS*) gene affect the activity and efficiency of enzymes that are responsible for the conversion of polyunsaturated fatty acids (PUFAs) into their long-chain active form. A high prevalence of SNPs that are associated with slow PUFA conversion has been described in Hispanic populations.

Objective: We assessed the heterogeneity of the effect of prenatal supplementation with docosahexaenoic acid (DHA) on birth weight across selected *FADS* SNPs in a sample of Mexican women and their offspring.

Design: We obtained information on the maternal genotype from stored blood samples of 654 women who received supplementation with 400 mg DHA/d or a placebo from weeks 18 to 22 of gestation through delivery as part of a randomized controlled trial conducted in Cuernavaca, Mexico. We selected 4 tag SNPs (rs174455, rs174556, rs174602, and rs498793) in the *FADS* region for analysis. We used an ANOVA to test for the heterogeneity of the effect on birth weight across each of the 4 SNPs.

Results: The mean ± SD birth weight was 3210 ± 470 g, and the weight-for-age *z* score (WAZ) was −0.24 ± 1.00. There were no intention-to-treat differences in birth weights. We showed significant heterogeneity by SNP rs174602 ($P = 0.02$); offspring of carriers of alleles TT and TC in the intervention group were heavier than those in the placebo group (WAZ: −0.13 ± 0.14 and −0.20 ± 0.08 compared with −0.55 ± 0.15 and −0.39 ± 0.09, respectively); there were no significant differences in offspring of rs174602 CC homozygotes (WAZ: −0.26 ± 0.09 in the intervention group compared with −0.04 ± 0.09 in the placebo group). We showed no significant heterogeneity across the other 3 *FADS* SNPs.

Conclusion: Differential responses to prenatal DHA supplementation on the basis of the genetic makeup of target populations could explain the mixed evidence of the impact of DHA supplementation on birth weight. This trial was registered at clinicaltrials.gov as NCT00646360. *Am J Clin Nutr* 2016;103:1171–8.

Keywords: birth weight, DHA, *FADS*, long-chain PUFAs, prenatal supplementation

INTRODUCTION

The n–3 long-chain PUFA (LC-PUFA)[8] DHA is essential for neurodevelopment and regulates gene expression especially during early development through effects on stem cell proliferation (1). DHA has been suggested to play an important role in fetal growth by increasing the duration of gestation (2, 3) or by increasing blood concentrations of insulin-like growth factor I (4).

Maternal PUFA dietary intake and metabolism determine the availability of essential LC-PUFAs for the offspring during the first months of life (5); hence, an adequate maternal DHA status during pregnancy is an important determinant of offspring development (6). However, results from well-designed randomized controlled trials (RCTs) of prenatal supplementation with DHA have yielded heterogeneous or null effects on birth weight (3). The role of fatty acid desaturase (*FADS*) genes that modulate the conversion of n–3 and n–6 essential fatty acids into their LC-PUFA derivatives (7) could explain this heterogeneity (6, 8–10). The conversion from dietary precursors to LC-PUFAs [including the biologically active DHA and arachidonic acid (AA)] proceeds via a series of desaturations and elongations that are

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[8] Abbreviations used: AA, arachidonic acid; *FADS*, fatty acid desaturase; HWE, Hardy-Weinberg equilibrium; INSP, Mexican National Public Health Institute; LC-PUFA, long-chain PUFA; LD, linkage disequilibrium; MAF, minor allele frequency; RCT, randomized controlled trial; SNP, single nucleotide polymorphism; WAZ, weight-for-age *z* score.

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performed by specific enzymatic complexes in which n–6 and n–3 PUFAs compete for conversion (**Figure 1**). δ-5 Desaturase and δ-6 desaturase are coded in the fatty acid *FADS1*, *FADS2*, and, potentially, *FADS* 3 gene regions (7). Single nucleotide polymorphisms (SNPs) in these genes have been identified as important determinants of an LC-PUFA plasma status through the regulation of this dehydrogenase complex (11–14). Moreover, a recent study identified SNPs in these *FADS* genes as determinants of adult height and weight in Greenlandic Inuit, which suggested that important adaptations to a diet extremely high in n–3 LC-PUFAs are mediated by these *FADS* SNPs (15). Previously, important geographical variations in the prevalence of *FADS* genotypes that are potentially related to genetic adaptation have been described (16, 17). For example, Hispanics (17) and Native American populations have a greater proportion of carriers of the genotype associated with the decreased activity of the δ-desaturase complex (97% compared with 20–50% in Europe and Asia) (16).

On the basis of the important genetic variation in different populations and the lack of evidence from diverse populations of the effect of *FADS* SNPs on LC-PUFA metabolism (16, 17), there has been a call to include genetic assessments in cohort and intervention studies from different settings (10); such assessments might contribute to explain the heterogeneous results across studies. In the current analysis, we address this gap by describing the distribution of 15 *FADS* SNPs and assessing if these SNPs modified the effect of prenatal DHA supplementation on birth weight.

METHODS

Sample description

The Prenatal Omega-3 Supplementation on Child Growth and Development study is a double-blind RCT (clinicaltrials.gov; NCT00646360) that was conducted in Mexico from 2004 to 2006 in which 1094 women were randomly assigned, at 18–22 wk of gestation, to receive 400 mg preformed DHA/d or a placebo through delivery. Eligible women were between 18 and 35 y of age and planned to deliver at the Mexican Institute for Social Security General Hospital in Cuernavaca, to breastfeed for ≥3 mo, and to live in the area for ≥2 y after delivery (18). Blood samples of all participating women were stored and, when technology became available, consent to use these samples for genetic testing was requested from participants; of the 980 women who were still participating in the study at delivery, 654 women provided written informed consent for genetic testing (**Figure 2**) and had valid information for key SNPs.

The study was conducted according to the guidelines of the Declaration of Helsinki. The Emory University Institutional Review Board and the Mexican National Public Health Institute (INSP) ethics committee approved all procedures involving human subjects.

Birth weight-for-age *z* score

Anthropometric measurements were obtained from hospital records ≤24 h after delivery. Birth weight was measured to the nearest 10 g with the use of a pediatric scale. Gestational age at birth in days was determined on the basis of the date of birth, maternal recall of the date of the last menstrual period at recruitment, and a maturity evaluation of Capurro made by the pediatrician. Birth weight was converted into *z* scores relative to WHO Child Growth Standards (19, 20) with the use of an SAS macro (SAS 9.2; SAS Institute Inc.).

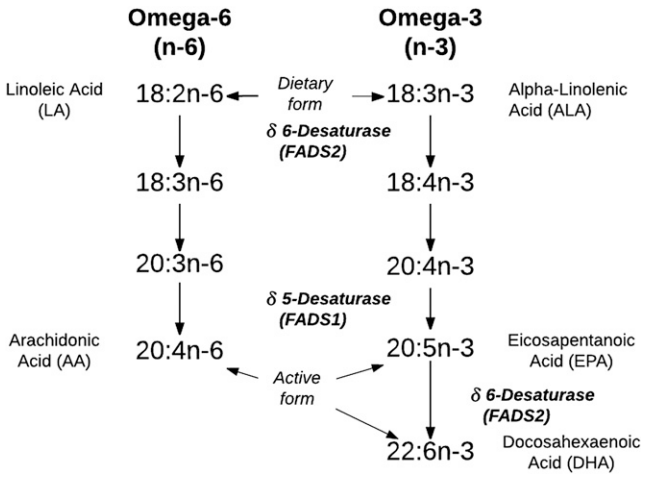

FIGURE 1 Long-chain PUFA metabolism. Conversion from EPA to DHA requires elongation, desaturation (catalyzed by δ-6 desaturase), and β oxidation. Adapted from reference 11 with permission.

Collection and storage of blood samples

Fasting venous blood was obtained at the Mexican Institute of Social Security General Hospital I in Cuernavaca with the use of a evacuated tube system. Samples were centrifuged at 2500 × *g* for 3 min at 4°C. Plasma, buffy coat, and red blood cells were separated and stored at INSP laboratories at −70°C. Plasma was stored in 200-μL aliquots, whereas erythrocytes were stored in cryotubes after being isolated and washed 3 times with saline solution (0.89%). Samples were kept at INSP laboratories before being transported to the University of Munich for a genetic analysis.

Genotyping

A genetic analysis was conducted at the Helmholtz Center, Munich. DNA was extracted from stored buffy coat with the use of a High Pure PCR Template Preparation Kit (Roche). A total of 5 μL DNA was subjected to polymerase chain reaction amplification followed by the genotyping procedure with the use of the MassARRAY system and iPLEX chemistry as suggested by the manufacturer (Sequenom) and previously described in detail (11). Results of the genetic analysis were entered and cleaned at the University of Munich, and the data sets that contained information on 15 *FADS1*, *FADS2*, and *FADS3* SNPs (rs174556, rs174455, rs174561, rs174570, rs174574, rs174575, rs2727271, rs174576, rs174578, rs174579, rs174602, rs498793, rs174448, rs174449, and rs174558) were sent to Emory University in encrypted files. These 15 SNPs were selected on the basis of previous evidence of their effects on LC-PUFA metabolism (6, 11, 14, 21, 22). We included additional SNPs located in the *FADS3* gene because evidence has suggested that they might also play a role in LC-PUFA metabolism (23).

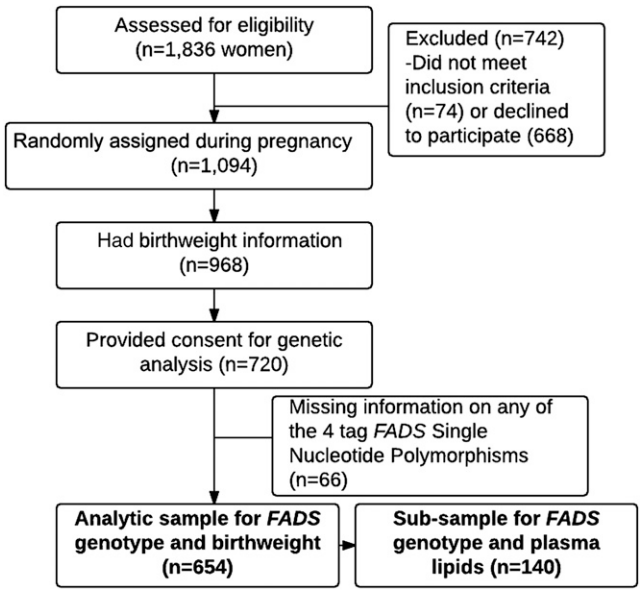

FIGURE 2 Sample selection from the Prenatal Omega-3 Supplementation on Child Growth and Development original trial to the analytic sample for this study. *FADS*, fatty acid desaturase.

Determination of plasma fatty acids

A subsample of 140 women with genetic information also had information on plasma concentrations of LC-PUFA at baseline. Samples from these women were randomly selected for the LC-PUFA analysis and analyzed as part of the original study in 2004–2005. Determinations of total fatty acids in plasma were conducted at the INSP laboratories. Total fat from plasma was extracted with a chloroform:methanol mixture (2:1). The yield was determined with the use of gravimetry and was expressed as g/100 g serum. Fatty acids were derivatized with the use of boron trifluoride and were extracted with pure hexane. The extracts were injected into a gas chromatograph (Hewlett-Packard Model 5890 Series II; Hewlett-Packard) with the use of a 100-m length × 0.25-mm internal diameter Supelco SP 2560 column (Supelco). Chromatographic peaks were identified with the use of reference standards for 37 fatty acids (Supelco).

Dietary intake

Maternal dietary intake was assessed with the use of a 110-item food-frequency questionnaire that was specifically designed to include important PUFA sources (24).

Other demographic characteristics

Maternal age was calculated by subtracting the birth date from the date of the interview; women were asked the number of years they had attended school, and socioeconomic status was calculated with the use of principal components analysis on a list of assets ascertained by interview (18).

Statistical analysis

Baseline characteristics of the included subsample were compared with those of the rest of the birth cohort with the use of *t* tests for continuous variables and chi-square tests for categorical variables. The normality of the concentrations of DHA and AA was assessed with the use of tests for normality by applying PROC UNIVARIATE procedure in SAS 9.2 software (SAS Institute Inc.). Baseline characteristics that differed by intervention groups were included in the models as covariates. $P < 0.05$ was considered significant.

Distribution of 15 FADS SNPs

Allele frequencies, Hardy-Weinberg equilibrium (HWE), and tests on linkage disequilibrium (LD) were calculated with the use of JLIN (Java LINkage disequilibrium plotter, version 1.6.0; University of Western Australia, 2006) (25). Fisher's exact test was used to test for HWE. LD was tested by using the likelihood ratio test of allelic associations and pairwise Lewtontin's D′ squared correlations. JLIN was also used to show LD blocks graphically (**Figure 3**). A single SNP (or tag SNP) was randomly selected from each cluster of SNPs with an LD > 0.8 to represent the clustering of the 15 SNPs that were originally analyzed.

Heterogeneity of impact of prenatal DHA on weight-for-age z score by FADS SNPs

We categorized each allele on the basis of the prevalence in this population as previously described (6, 13, 14) where zero denoted a homozygote minor, one denoted a heterozygote, and 2 denoted a homozygote major. We conducted a multivariate linear regression with birth weight as the outcome and tested for a multiplicative interaction between the intervention (zero denoted the control, and one denoted prenatal DHA) and allele categories. We used an ANOVA to assess differences in weight-for-age *z* scores (WAZs) across intervention groups and *FADS* SNP genotypes. Analyses were first performed separately for each of the 4 candidate SNPs. The models were then adjusted for all other SNPs and relevant covariates (those which varied between groups or by SNP). Significant confounders ($P < 0.05$) were included in the final models. We also conducted a mediation analysis by evaluating models with and without controlling for gestational age.

To account for a potentially increased risk of type 1 error because of multiple testing, we used the Bonferroni correction (26). We tested 4 different SNPs and one outcome (WAZ), and Bonferroni-corrected *P* values <0.013 were considered significant. The sample of $n = 654$ allowed us to look an effect size of the interaction term as little as 0.3 with >85% power [as calculated with the use of Quanto Power and Sample Size software (version 1.2.4; University of South Carolina, 2009) (27)] for minor allele frequencies (MAFs) as small as 0.2.

Association between FADS SNPs and plasma concentrations of AA and DHA

A linear regression analysis of the additive association between each of the 4 candidate SNPs and DHA and AA plasma concentrations was conducted with the heterozygote with the slowest expected rate of conversion as the reference. A multivariate logistic regression was conducted with all 4 SNPs in the model.

RESULTS

Descriptive characteristics of the 654 women and offspring with *FADS* genetic information by supplementation group are shown in **Table 1**. The mean age was 26 y, and the

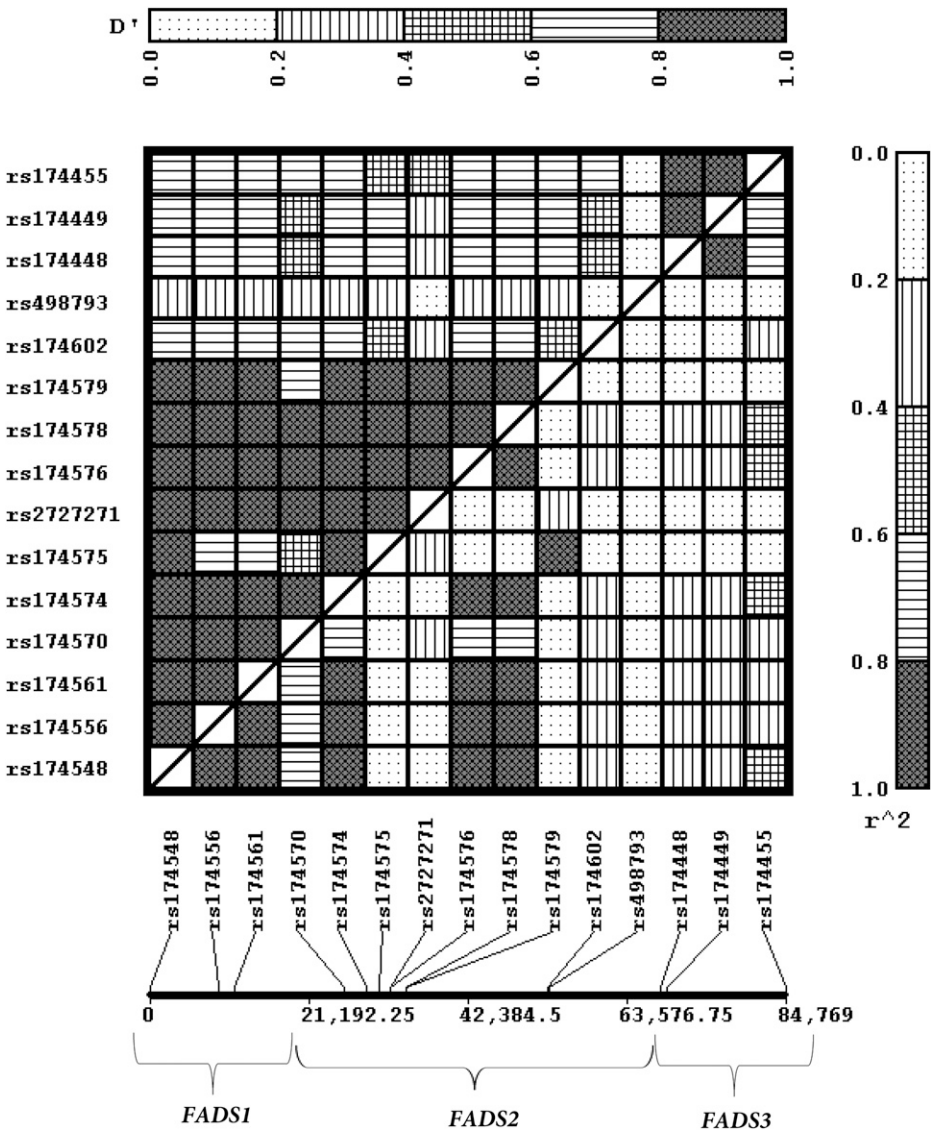

FIGURE 3 LD (D′) plot of 15 *FADS* SNPs. The value 1.0 denotes perfect LD between the SNP pair or 100% power. *FADS*, fatty acid desaturase; LD, linkage disequilibrium; r^2, r^2 power to detect LD; SNP, single nucleotide polymorphism.

mean height was 155 cm. Mean dietary intakes of n–6 linoleic acid and AA were 19.2 and 0.15 g/d, respectively, and intakes of α-linolenic acid and DHA were 1.7 and 0.08 g/d, respectively. A comparison of the analytic sample to the rest of the birth cohort (n = 326) showed small differences in dietary intakes and sociodemographic characteristics whereby women with genetic information were slightly older (26.5 compared with 25.8 y, respectively), a smaller proportion was primagravid (34% compared with 44%, respectively), and a smaller proportion gave birth to a girl (45% compared with 52%, respectively) (all $P < 0.05$) (Table 1)

Distribution of FADS SNPs

The distribution of 15 *FADS* SNPs in the study population is presented in **Table 2**. There were no major violations of the HWE for most of the SNPs (P = 0.005–0.7). The 3 SNPs with a HWE P value <0.05 (rs174449, rs174575, and rs174579) were excluded from additional analyses. Four SNP clusters resulted from the map of LD (Figure 3), and SNPs rs174455, rs174556, rs174602, and rs498793 were selected as tag SNPs representing the *FADS1*, *FADS2*, and *FADS3* gene clusters. These 4 tag SNPs were included for the assessment of the effect modification of prenatal DHA on birth weight and for the subanalysis of plasma concentrations of AA and DHA.

Heterogeneity of the impact of prenatal DHA on birth WAZs across FADS SNPs

In the sample as a whole, the mean ± SD birth weight was 3210 ± 470 g, and the WAZ was −0.24 ± 1.00 with no differences by treatment group (18). We showed a significant interaction by SNP rs174602 ($P < 0.01$). Offspring of carriers of alleles TT and TC in the intervention group were heavier than those in the placebo group (WAZ: −0.13 ± 0.14 and −0.20 ± 0.08 compared with −0.55 ± 0.15 and −0.39 ± 0.09,

TABLE 1
Maternal characteristics at baseline and offspring characteristics at birth for women who were participating in the POSGRAD trial with (n = 654) and without (n = 326) genetic information[1]

	Analytic sample (n = 654)		Sample missing information (n = 326)	
	Control (n = 318)	DHA (n = 336)	Control (n = 172)	DHA (n = 154)
Maternal characteristics				
Age,[2] y	26.4 ± 4.7[3]	26.5 ± 5.0	25.8 ± 4.3	25.8 ± 4.3
Socioeconomic status score	0.06 ± 1.00	0.01 ± 0.99	−0.04 ± 1.04	0.10 ± 0.95
Schooling, y	12.0 ± 3.55	11.8 ± 3.45	11.9 ± 3.63	12.3 ± 3.58
Raven intelligence test score	41.2 ± 9.41	40.6 ± 9.17	41.0 ± 8.95	41.2 ± 8.43
Height, m	155 ± 5.58	155 ± 5.73	156 ± 5.71	155 ± 5.96
BMI, kg/m^2	26.5 ± 4.37	26.0 ± 4.16	25.8 ± 4.04	25.6 ± 4.23
First pregnancy,[2] %	36.2	33.3	43.6	46.8
Dietary intake, g/d				
n–3 Fatty acids	1.81 ± 1.14	1.85 ± 1.04	1.82 ± 0.87	1.71 ± 0.94
ALA	1.69 ± 1.08	1.74 ± 1.00	1.71 ± 0.83	1.59 ± 0.94
DHA	0.08 ± 0.09	0.08 ± 0.07	0.08 ± 0.07	0.08 ± 0.10
n–6 Fatty acids	19.3 ± 10.0	19.7 ± 9.15	18.9 ± 7.79	18.9 ± 8.79
LA	19.2 ± 9.94	19.6 ± 9.11	18.8 ± 7.78	18.8 ± 8.77
AA	0.15 ± 0.11	0.15 ± 0.09	0.14 ± 0.06	0.15 ± 0.07
Offspring characteristics at birth				
Girls,[2] %	45.3	44.9	52.3	51.3
Gestational age, wk	39.0 ± 1.79	39.0 ± 1.83	39.2 ± 1.96	39.0 ± 1.98
Length, cm	50.4 ± 2.45	50.4 ± 2.45	50.3 ± 2.30	50.0 ± 3.18
Weight, g	3190 ± 460	3210 ± 460	3190 ± 520	3172 ± 470
Head circumference, cm	34.2 ± 1.84	34.4 ± 1.50	34.1 ± 2.01	34.2 ± 1.71

[1]Chi-square tests, t tests, and an ANOVA were used to test differences between groups. There were no significant differences between control and intervention (DHA) groups for any of the characteristics included in the table. AA, arachidonic acid; ALA, α-linolenic acid; LA, linoleic acid; POSGRAD, Prenatal Omega-3 Supplementation on Child Growth and Development.
[2]Analytic and missing samples were significantly different, $P < 0.05$.
[3]Mean ± SD (all such values).

respectively); there were no significant differences in the offspring of rs174602 CC homozygotes (WAZ: −0.26 ± 0.09 in the intervention group compared with −0.04 ± 0.09 in the placebo group) (**Figure 4**). These effects remained significant after adjustment for gestational age although there was evidence of some attenuation (WAZ: −0.18 ± 0.14 and −0.22 ± 0.08 in the DHA group compared with −0.48 ± 0.15 and −0.40 ± 0.09 in the placebo group for TT and TC, respectively; P = 0.03). We showed no significant effect modification by the other 3 *FADS* SNPs.

TABLE 2
FADS genotype information of 654 women who were participating in the POSGRAD trial[1]

	Homozygote minor	Heterozygote	Homozygote major	Minor allele frequency	HWE P
FADS1					
rs174548	G (5.1)	GC (36.4)	C (58.6)	0.22	0.05
rs174556	G (5.2)	GA (38.4)	A (56.4)	0.24	0.19
rs174561	A (5.2)	GA (38.2)	G (56.7)	0.24	0.19
FADS2					
rs174570	C (7.0)	CT (40.7)	T (52.3)	0.27	0.47
rs174574	C (4.0)	CA (32.9)	A (62.8)	0.20	0.70*
rs174575	G (14.1)	GC (52.5)	C (33.3)	0.40	0.04
rs174576	C (3.9)	CA (34.6)	A (61.4)	0.21	0.47
rs174578	T (4.1)	TA (35.0)	A (61.0)	0.21	0.40
rs174579	T (11.3)	CT (52.4)	C (36.3)	0.38	<0.01**
rs174602	T (14.7)	TC (44.8)	C (40.4)	0.37	0.49
rs498793	T (13.8)	TC (43.3)	C (42.9)	0.35	0.14
rs2727271	T (15.1)	AT (48.1)	A (36.7)	0.40	0.80
FADS3					
rs174448	T (7.2)	TC (44.5)	C (48.3)	0.29	0.07
rs174449*	T (6.6)	CT (44.3)	C (48.6)	0.28	0.03
rs174455	T (4.9)	TC (35.5)	C (59.6)	0.22	0.49

[1]Values in parentheses are percentages. *P <0.05, **P <0.01. *FADS*, fatty acid desaturase; HWE, Hardy-Weinberg equilibrium; POSGRAD, Prenatal Omega-3 Supplementation on Child Growth and Development.

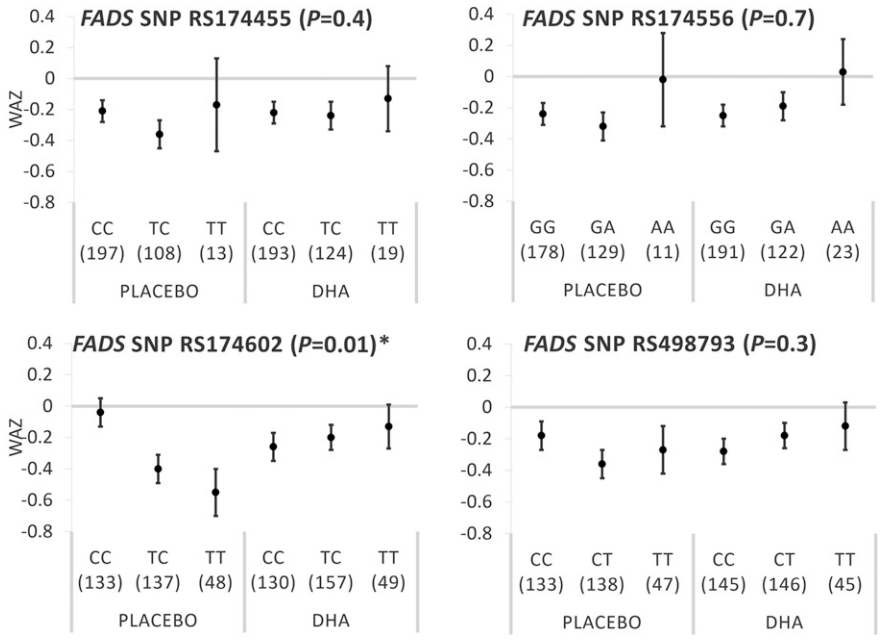

FIGURE 4 Mean ± SEM birth WAZs relative to the WHO Growth Standards of 654 children whose mothers participated in the Prenatal Omega-3 Supplementation on Child Growth and Development trial by 4 tag *FADS* SNPs and prenatal DHA supplementation. *P-interaction < 0.05 after Bonferroni correction for multiple comparisons. *FADS*, fatty acid desaturase; SNP, single nucleotide polymorphism; WAZ, weight-for-age z score.

Association between FADS SNPs and plasma concentration of AA and DHA

Plasma concentrations of AA and DHA were available for 140 women, who were similar to the FADS SNP sample in terms of key demographic characteristics (data not shown). In unadjusted single-SNP models, rs174455 (0.66 ± 0.1 mg/dL), rs174556 (0.79 ± 0.1 mg/dL), and rs174602 (0.28 ± 9.1 mg/dL) were positively associated with AA plasma concentrations ($P <$ 0.05), but only rs174556 was still associated with AA after controlling for the other SNPs. For DHA, the only positive association was with rs174556 both in the unadjusted and adjusted models, and it was inversely associated with rs174602 ($P <$ 0.05) (**Table 3**). The 4 tag SNPs (rs174455, rs174556, rs174602, and rs498793), when included together in the regression analysis, explained 24% and 11% of the variability in plasma concentrations of AA and DHA, respectively.

DISCUSSION

In this sample of Mexican women, the distribution of *FADS* SNPs was consistent with that previously described in other Hispanic populations in whom *FADS* SNPs that were associated with a less-efficient conversion of dietary PUFAs into their active forms were particularly prevalent (16, 17). We showed the heterogeneity of the effect of prenatal supplementation with DHA on birth weight. In carriers of the minor allele of *FADS2* SNP rs174602, women who received prenatal DHA gave birth to significantly heavier offspring than those of women who received the placebo; this difference was not observed in carriers of the major allele. This interaction was only partially explained by an increase in the duration of pregnancy; hence, the specific biological mechanism behind this effect should be further explored.

Results from the fatty acid analysis may contribute to a better understanding of this interaction. The nature and direction of the associations with the plasma LC-PUFAs AA and DHA varied depending on the gene location of each SNP. *FADS1* SNP rs174556 was positively associated with plasma concentrations of AA and DHA, whereas *FADS2* SNP rs174602 was inversely associated with DHA after controlling for the effect of the other tag SNPs. This result contributed to the evidence that carriers of the minor allele of SNP rs174602 in our sample were at greater risk of DHA deficiency. A role for *FADS2* SNPs on DHA metabolism was expected because the enzyme δ-5 desaturase, which is coded in this gene, catalyzes an important step in the conversion from n–3 EPA into DHA that is not in the AA pathway (11, 28) (Table 1). Previous studies of *FADS* SNPs and plasma concentrations of LC-PUFAs, which have mostly been conducted in European cohorts, have reported strong associations between *FADS* SNPs and plasma or erythrocyte concentrations of AA, but the association with DHA has been less clear (9, 10, 11, 21, 22, 28), which is a pattern that is consistent with our results. In a prenatal supplementation trial in the United States (29), women who were homozygous for the minor allele of *FADS1* SNP rs174533 had lower red blood cell concentrations of both AA and DHA at the start of the intervention, and after supplementation with 600 mg preformed DHA/d during the last 2 trimesters of pregnancy, the intervention increased the DHA concentration and decreased the AA:DHA ratio only in carriers of the minor allele. Although we did not analyze SNP rs174533, and information on the LD of this SNP with other FADS SNPS was not provided (26), the results provided additional support for a selective beneficial effect of prenatal DHA supplementation in carriers of some minor *FADS* SNPs alleles.

A potential limitation of this analysis was that a proportion of the women in the original study did not provide consent for the genetic testing; however, there were no major differences

TABLE 3
Associations between 4 selected tag *FADS* SNPs and plasma concentrations of AA and DHA in a randomly selected subsample of 140 women who were participating in the POSGRAD trial[1]

	AA, g/100 g		DHA, g/100 g	
SNPs	Unadjusted[2]	Adjusted[3]	Unadjusted[2]	Adjusted[3]
rs174455	0.67 ± 0.12***	0.28 ± 0.19	0.12 ± 0.04	0.09 ± 0.07
rs174556	0.76 ± 0.12***	0.69 ± 0.20***	0.12 ± 0.04*	0.14 ± 0.07*
rs174602	0.28 ± 0.12*	−0.20 ± 0.13	−0.00 ± 0.04	−0.12 ± 0.05**
rs498793	−0.03 ± 0.12	−0.12 ± 0.11	−0.04 ± 0.04	−0.06 ± 0.04

[1]Values are means ± SDs. Subsample of 140 women was randomly selected and was comparable to the study population and the sample included in the current study. β Coefficients were the results of linear regression models and were interpreted as the changes in plasma fatty acid concentration (mg/dL) per increase in the number of minor alleles (defined as those with the lowest prevalence in this population). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. AA, arachidonic acid; *FADS*, fatty acid desaturase; POSGRAD, Prenatal Omega-3 Supplementation on Child Growth and Development; SNP, single nucleotide polymorphism.

[2]Models represent the linear association of each single SNP and plasma concentrations of AA or DHA independently of the effect of the other 3 tag SNPs.

[3]Models represent the linear association of each SNP and plasma concentrations of AA or DHA adjusted for the other 3 tag SNPs (that were included as covariates).

between these women and the women included in the current study. Similarly, information on plasma concentrations of LC-PUFAs at baseline was only available for a subsample of 140 women. The demographic characteristics of this more-restricted sub-sample were similar to those of the full study population. In addition, we adjusted for variables that were significantly different between groups (maternal age, parity, and offspring sex). Another consideration was that total fatty acids were measured in plasma and not in erythrocytes because this was the method available in Mexico at the time of the intervention. Plasma total LC-PUFA concentrations have been widely used as a proxy for the tissue fatty acid composition and are considered an effective method in adults (30) but have the limitation that they may be affected by fasting status. In our study, all samples were collected in the morning after overnight fasting. Finally, we showed a significant interaction only with SNP rs174602, which was the one with the highest MAF and, hence, the one with the highest power. It is possible that interactions by other SNPs were not detected because of the lack of power. However, the sample size available was enough to detect interactions as small as 0.3 for MAFs as small as 0.2, which is what was observed for SNPs rs174556 and rs174455.

The high prevalence of alleles associated with the slow n–6 LC-PUFA conversion in our sample and other Latin American populations suggested an evolutionary advantage of alleles that have been associated with the slow conversion of n–6 PUFAs into AA within a context where n–6 linoleic acid is highly abundant (16). However, this genetic panorama raised concerns of greater risk of DHA deficiency in this study population because food sources are scarce (31) and a slow conversion of n–3 PUFAs would further exacerbate this deficiency. Our results that showed the heaviest birth weights in the offspring of major allele homozygotes of SNP 174602 together with the selective impact of prenatal DHA in carriers of the minor allele suggest that this SNP located in the *FADS2* gene plays a key role in the adaptation to a diet that is abundant in n–6 PUFAs and poor in n–3. A recent study in Greenlandic Inuit also identified SNP 174602 as showing strong signatures of adaptation to a diet that is high in n–3 PUFAs (15), which provided support of a potentially relevant role of this SNP that induces an intron modification on PUFA metabolism and potentially on long-term growth (15).

Important strengths of this study were as follows: the data collection and laboratory-analysis protocols were standardized, validated, and conducted by trained personnel within a clinical setting; participants of this study were part of a population with high dietary intake of n–6 fatty acids as well as a high prevalence of *FADS* genotypes reportedly associated with the slow conversion of LC-PUFAs; and we were able to test our hypothesis within an RCT with high-quality information on birth outcomes and a wide range of maternal and offspring sociodemographic characteristics. To our knowledge, this is the first study to report a role of the *FADS* genotype in the modification of the impact of prenatal DHA supplementation on birth weight.

In conclusion, this study highlights the importance of incorporating current advances in the field of genetics into the design of future nutrition interventions and in the analysis of existing RCTs. In particular, the maternal *FADS* genotype appears to play a key role in essential LC-PUFA metabolism and availability for the offspring and, consequently, on birth outcomes. Future research should further study the biological mechanisms behind the heterogeneous associations of AA and DHA with different *FADS* SNPs and haplotypes and assess if the effect modification observed in the current study is consistent across populations with different diets and genetic makeups. Similarly, it will be interesting to study if the impact of prenatal DHA supplementation on birth weight in carriers of the minor allele in our study translates into long-term child growth and development outcomes. Differential responses to prenatal DHA supplementation trials on the basis of the genetic makeup of target populations and PUFA dietary intake could explain the mixed evidence of the impact of DHA supplementation on birth weight.

The authors' responsibilities were as follows—IG-C: drafting and editing of the manuscript; IG-C, PR, ADS, RGF, JARD, AB-V, HD, IR, SV, and RM: collection, analysis, and interpretation of the data; IG-C, PR, ADS, JARD, BK, and UR: concept and study design; and all authors: reading and approval of the submission of the final manuscript and responsibility for the reported research and the manuscript. None of the authors reported a conflict of interest related to the study.

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