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Maternal FADS2 single nucleotide polymorphism modified the impact of prenatal docosahexaenoic acid (DHA) supplementation on child neurodevelopment at 5 years: Follow-up of a randomized clinical trial

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Abstract:	Background Variability in the FADS 2 gene, which codifies the Delta-6 Desaturases and modulates the conversion of essential n-3 and n-6 polyunsaturated fatty acids into their active metabolites, might modify the impact of prenatal supplementation with n-3 docosahexaenoic acid (DHA) on neurodevelopment. Objective To assess if maternal FADS2 single nucleotide polymorphisms (SNPs) modified the effect of prenatal DHA on offspring development at 5 years. Design We conducted a post-hoc interaction analysis of the POSGRAD randomized controlled trial (NCT00646360) of prenatal supplementation with algal-DHA where 1,094 pregnant women originally randomized to 400 mg/day of preformed algal DHA or a placebo from gestation week 18-22 through delivery. In this analysis, we included offspring with information on maternal genotype and neurodevelopment at 5 years (DHA=316; Control=306) and used generalized linear models to assess interactions between FADS2 SNPs rs174602 or rs174575 and prenatal DHA on neurodevelopment at 5 years measured with McCarthy Scales of Children's Abilities (MSCA). Results Maternal and offspring characteristics were similar between groups. At baseline, mean (± standard deviation) maternal age was 26 ± 5 years and schooling was 12 ± 4 years. Forty-six percent (46%) of the children were female. Maternal minor allele frequencies were 0.37 and 0.33 for SNPs rs174602 and rs174575, respectively. There were significant interactions by SNP rs174602 where only among offspring of TT (minor allele homozygotes), those in the intervention group had higher quantitative (DHA: mean=22.6 ± SEM=0.9 vs. Control= 19.1 ± 0.9, mean difference (Δ)= 3.45; p=0.01) and memory (DHA= 27.9 ±1.1 vs. Control= 23.7 ± 1.1, Δ=4.26; p=0.02) scores. Conclusions Maternal FADS2 SNP rs174602 modified the effect of prenatal DHA on cognitive development at 5 years. Variations in the genetic make-up of target populations could be an important factor to consider for prenatal DHA supplementation interventions.
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APPLIED HEALTH SCIENCE
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Editorial Board AJCN:

On behalf of my co-authors, I am submitting this manuscript titled "**Maternal *FADS2* single nucleotide polymorphism modified the impact of prenatal docosahexaenoic acid (DHA) supplementation on child neurodevelopment at 5 years: Follow-up of a randomized clinical trial"**. We conducted a post-hoc interaction analysis of a randomized controlled trial and found that maternal genotype modified the impact of prenatal DHA on neurodevelopment at 5 years. These findings have important implications for clinical nutrition that will be of interest to the readers of AJCN.

The authors declare no conflict of interest and the manuscript has not been published elsewhere.

Please let me know if you require any further information.

Sincerely,

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Maternal *FADS2* single nucleotide polymorphism modified the impact of prenatal docosahexaenoic acid (DHA) supplementation on child neurodevelopment at 5 years: Follow-up of a randomized clinical trial

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Data described in the manuscript, code book, and analytic code will be made available upon request to the corresponding author pending application and IRB approval.

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Trial Registration: https://clinicaltrials.gov/ct2/show/NCT00646360

Running head: Maternal genotype on the impact of DHA on neurodevelopment

Abbreviations: ARA-Arachidonic Acid, DHA- Docosahexaenoic Acid, MSCA- McCarthy Scales of Child Abilities, Single Nucleotide Polymorphism (SNP),

Abstract

Background: Variability in the *FADS*2 gene, which codifies the Delta-6 Desaturases and modulates the conversion of essential n-3 and n-6 polyunsaturated fatty acids into their active metabolites, might modify the impact of prenatal supplementation with n-3 docosahexaenoic acid (DHA) on neurodevelopment.

Objective: To assess if maternal *FADS2* single nucleotide polymorphisms (SNPs) modified the effect of prenatal DHA on offspring development at 5 years.

Design: We conducted a post-hoc interaction analysis of the POSGRAD randomized controlled trial (NCT00646360) of prenatal supplementation with algal-DHA where 1,094 pregnant women originally randomized to 400 mg/day of preformed algal DHA or a placebo from gestation week 18-22 through delivery. In this analysis, we included offspring with information on maternal genotype and neurodevelopment at 5 years (DHA=316; Control=306) and used generalized linear models to assess interactions between *FADS2* SNPs rs174602 or rs174575 and prenatal DHA on neurodevelopment at 5 years measured with McCarthy Scales of Children's Abilities (MSCA).

Results: Maternal and offspring characteristics were similar between groups. At baseline, mean (± standard deviation) maternal age was 26 ± 5 years and schooling was 12 ± 4 years. Forty-six percent (46%) of the children were female. Maternal minor allele frequencies were 0.37 and 0.33 for SNPs rs174602 and rs174575, respectively. There were significant interactions by SNP rs174602 where only among offspring of TT (minor allele homozygotes), those in the intervention group had higher quantitative (DHA: mean=22.6 ± SEM=0.9 vs. Control= 19.1 ± 0.9, mean difference (Δ)= 3.45; p=0.01) and memory (DHA= 27.9 ±1.1 vs. Control= 23.7 ± 1.1, Δ=4.26; p=0.02) scores.

Conclusions: Maternal *FADS2* SNP rs174602 modified the effect of prenatal DHA on cognitive development at 5 years. Variations in the genetic make-up of target populations could be an important factor to consider for prenatal DHA supplementation interventions.

Introduction

Long-chain polyunsaturated fatty acids are conditionally-essential nutrients and during the prenatal period are obtained from the mother through placental transfer. Both docosahexaenoic acid (DHA, 22:6n-3) and arachidonic acid (ARA, 20:4n-6) accrete in the fetal brain, where they have important membrane structural, cell signaling, and gene expression regulatory functions. [1,2] In particular, DHA is important for the process of myelination, for visual functioning, and brain development in general.[3]

In observational studies, self-reported maternal DHA intake and plasma concentrations of DHA during pregnancy have been associated with heavier birth weights, extended gestational age, lower odds of preterm birth, and higher scores in mental development tests during infancy and early childhood.[4] However, except for preterm or high-risk populations, human experimental studies have failed to show consistent benefits of prenatal supplementation with DHA on a range of birth outcomes or on global cognitive development during childhood.[5,6]

Recent evidence suggests that variants in the *FADS*2 gene, which encodes for the fatty acid delta-6-desaturase enzyme (D6D) responsible for converting n-3 and n-6 PUFAs into their active metabolites, may modify dietary requirements.[7] Two distinct haplotypes have been identified to date: one, more prevalent in European populations, that has been associated with more efficient conversion of n-6 dietary precursor linoleic acid (LA, 18:2n-6) into ARA; and the other, more prevalent in Native Americans and Mexican American populations, that has been associated with less efficient conversion.[8,9] This variation in the geographic distribution of single nucleotide polymorphisms (SNPs) in the *FADS* genes has been attributed to high selective pressure based on climate and fatty acid composition of the diet.[10]

Two *FADS2* SNPs (rs174575 and rs174602) have been identified as potential modulators of the impact of dietary intake on child growth and development. [11-13] We have previously reported that in a randomized controlled trial of prenatal supplementation with DHA in Mexico, the intervention improved birth weight only among offspring of carriers of the *FADS2* SNP rs174602 minor allele for Mexican populations (T).[11]

In observational studies from high-income countries (New Zealand and Britain), rs174575 modified the association between early feeding practices and cognitive development at 8 years.[12,13] The potential role of *FADS2* genotype modifying the impact of prenatal supplementation with DHA on child development has not been studied in randomized controlled trials. Hence, the objective of this study was to assess if maternal *FADS2* SNPs rs174575 and rs174602 modified the impact of prenatal DHA supplementation on global cognitive development at 5 years among Mexican children whose mothers participated in a DHA-supplementation trial during their pregnancy.

Methods

Parent study and sample selection

Data from this study came from POSGRAD (Prenatal Omega-3 Supplementation on Child Growth and Development), a double-blind randomized controlled trial (NCT00646360) conducted in Mexico. The original trial methodology has been described elsewhere; [11] briefly, between 2002 and 2006, 1,094 pregnant women in their 18-22 week of gestation were randomized to receive 400 mg/day of algal DHA or a placebo mixture of corn and soybean oil through delivery; on average women consumed 88% of the capsules provided. [11] Among the 968 women who completed the study, there were 973 live births (including 5 pairs of twins). For the purpose of this analysis, we included 622 mother-child pairs in which the women consented to genetic testing and singleton children had valid measures of global cognitive development at the 5-year data collection time point (Figure 1).

The Emory University Institutional Review Board and the Ethics Board of the Mexican National Institute of Public Health reviewed and approved this study. Written informed consent was obtained from the mothers at enrollment and again on behalf of the child at the 5-year follow-up.

Cognitive Development Assessment

We used the Spanish version of the McCarthy Scales of Children's Abilities (MSCA) to assess cognitive development at 5 years of age. The MSCA is designed to assess development in children 2.5 to 8.5 years and includes six different scales: Verbal, Perceptual-Performance, Quantitative, Memory, Motor, and General Cognitive (which is derived from the Verbal, Perceptual-Performance, and Quantitative Scales). These scales are assessed through 18 subtests.[14] The MSCA was applied by three trained psychologists in a quiet setting within a hospital; application of the entire battery took on average 1 hour. Administration of the test was supervised by the study lead psychologist through random observations and a full review of all tests was performed on site before data were entered.[15] Raw scores were computed by adding the results of the individual tests and were used for this analysis. The MSCA has been validated in Spain[16] and used by others to assess the impact of dietary and environmental exposures in Mexican children.[17,18]

Genotyping and Tag SNP selection

Stored blood samples that had been collected from the mothers at baseline were shipped from the Mexican National Institute of Public Health to the Hemholtz Center (Munich, Germany) for genetic analysis. Polymerase chain reaction amplification and genotyping procedures were carried out on extracted DNA (total of 5μL) using the MassARRAY system and iPLEX chemistry (6). Fifteen candidate SNPs were assessed based on evidence suggesting that they might play a role in LCPUFA metabolism (6-11) and to represent the *FADS1 (*rs174556, rs174561, rs174558), *FADS2 (*rs174570, rs174575, rs2727271, rs174576, rs174578, rs174579, rs498793, rs174602), and *FADS3* (rs174455, rs174448) genes. Genotype data were sent to Emory University in encrypted files for statistical analysis. The two SNPs for this analysis (rs174575, rs174602) were selected based on evidence of clinical significance, location in the *FADS2* cluster, Hardy-Weinberg Equilibrium in the sample[11] and frequency of minor allele.

Statistical analysis

The analysis included all children with maternal genetic information and MSCA measurement at 5 years. Maternal baseline characteristics, child characteristics, and cognitive development measurements were

compared between the analytic sample and those with missing information using chi-square, t-tests, or ANOVA as needed.

Generalized linear models (MANOVA) were used to assess differences in MSCA cognitive development scores by *FADS SNPs* (Categorized into major allele homozygotes, heterozygotes, and major allele homozygotes) and to test for interactions between each of the two *FADS* SNPs and DHA supplementation groups with each of the MSCA as an outcome. Additionally, we conducted an exploratory analysis testing for heterogeneity by sex. For SNP*intervention interactions significant at $p<0.05$, we conducted stratified analysis and tested pairwise comparisons between the DHA and placebo groups by allele combinations. We adjusted all estimates by age at measurement and sex and by potential maternal and child confounders (Supplemental table 1) selected using PROC GLMSELECT with backwards stepwise elimination. Multiple imputation was used to account for missing covariates. PROC MI was used to generate twenty imputed datasets using fully conditional specification. Generalized linear models were then conducted for each of the twenty imputed datasets and the estimates were pooled by PROC MIANALYZE.

The sample had 80% power to detect minimum SNP*diet interaction beta coefficients of 0.45 with a conservative minor allele frequency of 0.3 at an alpha-level of 0.05. For the three-way interaction with sex, we had 70% power to detect a minimum interaction beta coefficient of 1.2.

Power calculations were conducted using Quanto 1.2.4 (Los Angeles, CA)[19] and statistical analyses were conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Significance was set at $p<0.05$. Results are presented as marginal means with standard error of the mean.

Results

The final sample for this study included 622 children (306 in the control group and 316 in the intervention group) with maternal genetic information and measures of cognitive development at 5 years (Figure 1).

At randomization, mothers were on average 27 years (26.5 ± 4.8), had approximately 12 years of schooling, and approximately a third were primigravid (34%). Mean offspring birth weight (SD) was 3.2 (0.5) kg and gestational age was 39 weeks (1.7). Exclusive breastfeeding at 3 months was 24%, with most children receiving mixed breastfeeding (58% combining breastmilk and formula). The intervention and control groups were well-balanced on maternal and child characteristics (Supplemental Table 1).

There were no significant differences in offspring MSCA scores at 5 years by intervention group (Table 1), and maternal *FADS*2 SNPs rs174602 or rs174575 were not associated with children's MSCA scores at 5 years (Table 2).

We found evidence of an interaction between the intervention and maternal SNP rs174602 in the Quantitative (p=0.02) and Memory scales (p=0.01) of the MSCA test (Table 3). After adjustment for baseline socioeconomic status score, maternal intelligence and schooling, and child sex and age at measurement, offspring of women who were homozygous for the minor allele TT for SNP rs174602 and received prenatal DHA had higher Quantitative and Memory scores when compared to those born to homozygous women in the control group. For Quantitative scores, among children whose mothers were homozygotes for the minor allele T, those in the intervention group had on average 3.5 higher scores than those in the control, which is equivalent to a difference of 0.6 SD. Similarly, for Memory scores, among offspring of T homozygotes, those who received the intervention had scored on average 4.3 points higher, which also represents a difference of 0.6 SD (Figure 2).

There was no interaction between the intervention and SNP rs174575 for any outcome measured (Table 3). There was no observed heterogeneity by sex (data not shown).

Discussion

In this analysis, we assessed if two maternal *FADS2* SNPs modified the impact of prenatal supplementation with n-3 DHA on measures of global cognition at age 5 years, and found that the intervention selectively improved quantitative and memory scores among offspring of homozygotes for

FADS2 SNP rs174602 minor allele TT. This is consistent with previous results from this trial where we observed an impact of the intervention on birthweight only among carriers of the minor allele for this same SNP.[11]

The importance of DHA for neurodevelopment is well-established,[1,20] however the positive impact of supplementation on child development during the preschool years and beyond remains controversial.[4,21] Clarification of this important question is further complicated by different doses and composition of the supplements that have been tested in clinical trials, as well as by the diversity of tools to assess child cognition and the different brain regions and functions that they target. Previous trials that have reported effects of DHA supplementation on visual acuity have had mixed results for different domains of childhood cognitive functioning.[21] For example, there is evidence of a potential negative impact of DHA and other n-3 fatty acid supplementation on verbal development, especially in girls.[22,23]

In this trial, we had previously showed an impact of prenatal supplementation on attention at 5 years measured by the Conners Kiddie Continuous Performance Test, where offspring of women who received the intervention committed fewer omissions, which is consistent with an impact on visual acuity and attention.[15] There was however no overall impact on measures of mental or motor functioning at 18 months[24] or 5 years.[15] In this analysis, we found a post-hoc gene-supplement interaction for the Memory and Quantitative scales, which are processes related to the parietal, pre-frontal, and frontal cortices[25,26] where there is evidence of DHA accretion in early life and of attention-related activation.[27,28]

Our results highlight the role of genetic variations as another factor modifying the impact of prenatal supplementation with DHA on development. We found an interaction of prenatal DHA supplementation with *FADS2* SNP rs174602, a functional intron variant that substitutes an amino acid (N/A) in the region encoding for D6D, which is responsible for a step in the synthesis of n-6 AA and n-3 EPA, and two steps in the synthesis of n-3 DHA. While other SNPs in the *FADS2* gene have been associated with D6D activity using plasma or erythrocyte concentrations of n-6 LA, AA or their ratio as proxies, *FADS2* SNP rs174602 does not seem to be associated with n-6 concentrations or with LA to AA ratios.[29] In contrast, it

has been identified as showing strong signatures of adaptation to a diet high in n-3 in Greenlandic Inuit populations.[10] We have previously shown that the presence of the rs174602 minor allele T predicts lower plasma concentrations of DHA in our study sample after adjusting for other *FADS* SNPs, [11] supporting that the subgroup carrying this minor allele has higher requirements for pre-formed DHA, which can explain the selective positive impact of this prenatal intervention on child development observed only among homozygotes for the minor allele TT. In this context where n-6 fatty acids have traditionally been abundant in the diet and n-3 is scarce, only a minority of the study population saw improvements on cognitive development after an intervention providing pre-formed n-3 long- chain polyunsaturated fatty acid DHA.

In contrast, we found no effect modification by *FADS2* SNP rs174575, another intron variant coding an amino acid (N/A) that had been identified as a potential effect modifier of the impact of infant feeding practices (breastfeeding or formula) on childhood IQ. The results on gene-diet interactions for this SNP have not been consistent: Caspi et al. found that the impact of breastfeeding on IQ was only present among carriers of the mayor allele (C),[13] while Steer et al found that breastfeeding was particularly important for minor allele homozygotes (GG)[12]. A smaller study from the United States found associations between maternal rs174575 genotype and declarative memory ability at 16 months, where toddlers whose mothers carried the allele C performed better than those whose mothers were GG homozygotes, and these associations were mediated by methylation in the child DNA supporting the role of programming.[30] We did not find any effect modification of prenatal supplementation with DHA by maternal *FADS2* SNP rs174575; it is possible that the child genotype and diet, including breastfeeding or the intake of other essential fatty acids, are more relevant to study the role of this SNP on child development.

The impact of prenatal DHA supplementation on childhood cognitive function can be explained by a metabolic impact on prenatal brain development because the intervention addressed the requirements for this fatty acid involved in myelination, gene expression and signaling during a critical period of brain

development.[20] However, a potential continued effect during the continuing rapid brain development after birth cannot be excluded because we expect maternal prenatal DHA status to modulate neonatal body DHA stores, given that maternal DHA serum concentrations in pregnancy predict neonatal cord blood DHA levels. [31]

Even though the minor allele frequencies of 0.38 for SNP rs174602 and 0.33 for SNP rs174575 allowed us sufficient power to detect gene-diet interactions,[32] we had limited power to examine these relationships stratified by sex. In this sense, missing data is another limitation: attrition in the cohort was only 15% through five years but not every woman consented to the genetic analysis. Similarly, lack of information on offspring genotypes is another potential limitation of this study, although we do not expect it to differ by intervention group, and previous studies have found the role of the maternal genotype more important for young children's cognitive functioning. In this sense, a potential continued effect of the prenatal supplementation during the continuing rapid brain development after birth cannot be excluded because we expect maternal prenatal DHA status to modulate neonatal body DHA stores, given that maternal DHA serum concentrations in pregnancy predict neonatal cord blood DHA levels.[31] We were also able to determine that breastfeeding practices and offspring fatty acid intake at 4 y did not differ by maternal *FADS2* SNPs and intervention subgroups. Further studies with larger sample sizes and including the offspring genotype will be important to fully elucidate the role of *FADS2* genotype moderating the impact of essential fatty acids on child development before this can be translated into precision nutrition applications.

An important strength of this study is that were able to assess effect modification by maternal genotype within the design of a randomized controlled trial. Also, we had a sufficient sample size to assess tag SNP interactions with the intervention, a follow up of the birth cohort through the preschool years, standardized protocols for data collection and information on several maternal and child characteristics. To our knowledge, this is the first study to report a role of the *FADS2* genotype moderating the impact of

prenatal DHA on childhood cognitive development as part of the follow-up of a randomized controlled trial.

In summary, we found prenatal DHA supplementation benefits only for children whose mothers were homozygotes for the FADS2 SNP rs174602 minor allele T. These results need to be confirmed through additional studies with larger sample sizes, in diverse populations, and with different dosage of DHA and other essential fatty acids. If results are consistent, that would mean that the beneficial impact of prenatal DHA supplementation at a population level depends on the prevalence of *FADS2* SNP rs174602 T homozygotes. This could have important implications for the design of n-3 and n-6 prenatal supplementation or dietary interventions, where genetic screening could help target groups at risk of deficiency. Further, there is evidence that excessive amounts of dietary DHA can have negative effects on early development especially in the absence of sufficient n-6 fatty acids,[33] which would make genetic targeting of essential fatty acid supplementation interventions even more relevant.

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Figure 1: Flowchart for the maternal *FADS2* single nucleotide polymorphism effect modification analysis of the POSGRAD supplementation trial

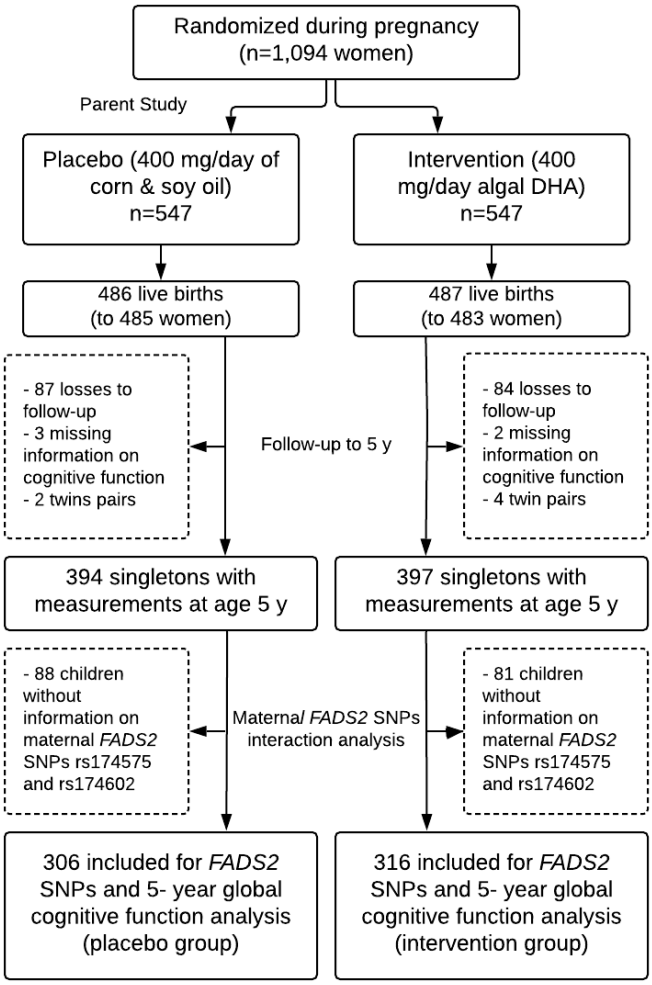


Table 1: McCarthy Scales of Child Abilities scores at 5 years group among Mexican children whose mothers participated in the POSGRAD randomized controlled trial of prenatal Docosahexaenoic n-3 fatty acid (DHA) supplementation, by intervention group.

	Placebo (n= 306)	**Intervention (n= 316)** [b]	**p-value** [a]
McCarthy Scales of Child Ability, 5 years			
Composite Score [c]	121.0 (1.3)	121.4 (1.3)	0.86
Quantitative Score	20.0 (0.4)	20.0 (0.4)	0.95
Verbal Score	54.3 (0.7)	53.6 (0.7)	0.49
Perceptual Score	46.5 (0.5)	47.3 (0.5)	0.28
Memory Score	25.2 (0.4)	25.3 (0.4)	0.95

[a] Values are raw score means (standard error of the mean) and are result of generalized linear models testing mean differences by supplementation group adjusted for age at measurement and child sex.
[b] The intervention was 400mg/day of algal n-3 docosahexaenoic acid and the placebo was 400 mg/day of soy and corn oil from week 18-22 of pregnancy through delivery.
[c] The composite score is the sum of verbal, perceptual and quantitative scores.

Table 2: Child cognitive function at 5 years by *FADS2* SNPs among Mexican children whose mothers participated in the POSGRAD randomized controlled trial of prenatal n-3 Docosahexaenoic acid (DHA) supplementation.

	SNP rs174602				**SNP rs174575**			
	CC (n=259)	**CT (n=273)**	**TT (n=90)**	**p-value**	**CC (n=213)**	**GC (n=322)**	**GG (n=87)**	**p-value**
McCarthy Scales of Child Ability, raw scores[a]								
Composite Score[b]	120.9 (1.3)	121.1 (1.3)	120.6 (2.2)	0.81	119.9 (1.5)	121.2 (1.2)	123.3 (2.3)	0.43
Quantitative Score	20.0 (0.4)	19.9 (0.4)	20.8 (0.6)	0.40	19.9 (0.4)	20.0 (0.3)	20.6 (0.6)	0.62
Verbal Score	53.9 (0.7)	53.9 (0.7)	53.9 (1.2)	0.58	53.2 (0.8)	54.2 (0.6)	55.3 (1.2)	0.32
Perceptual Score	47.1 (0.6)	47.3 (0.5)	45.8 (0.9)	0.36	46.8 (0.6)	47.1 (0.5)	47.4 (1.0)	0.54
Memory Score	25.3 (0.4)	25.1 (0.4)	25.8 (0.8)	0.75	25.0 (0.5)	25.4 (0.4)	25.9 (0.8)	0.54

[a] Values are raw score means (standard error of the mean) and are result of generalized linear models testing mean differences by supplementation group adjusted for child sex and age at measurement and maternal SES, Raven Progressive Matrices score and years of schooling.

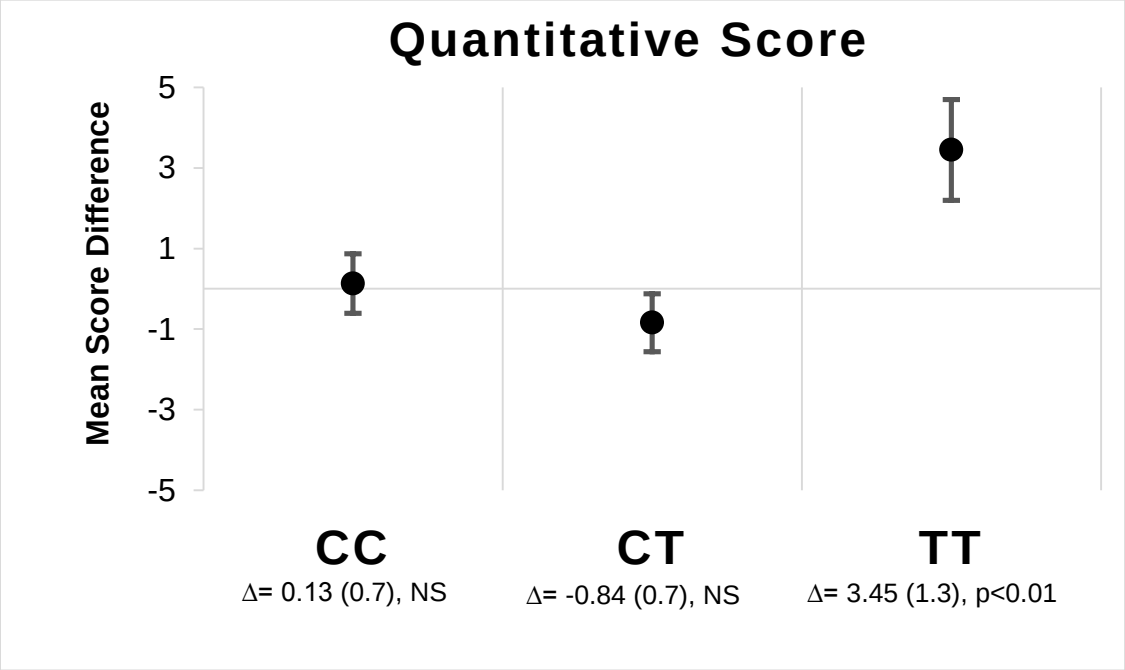
[b] The composite score is the sum of verbal, perceptual and quantitative scores.

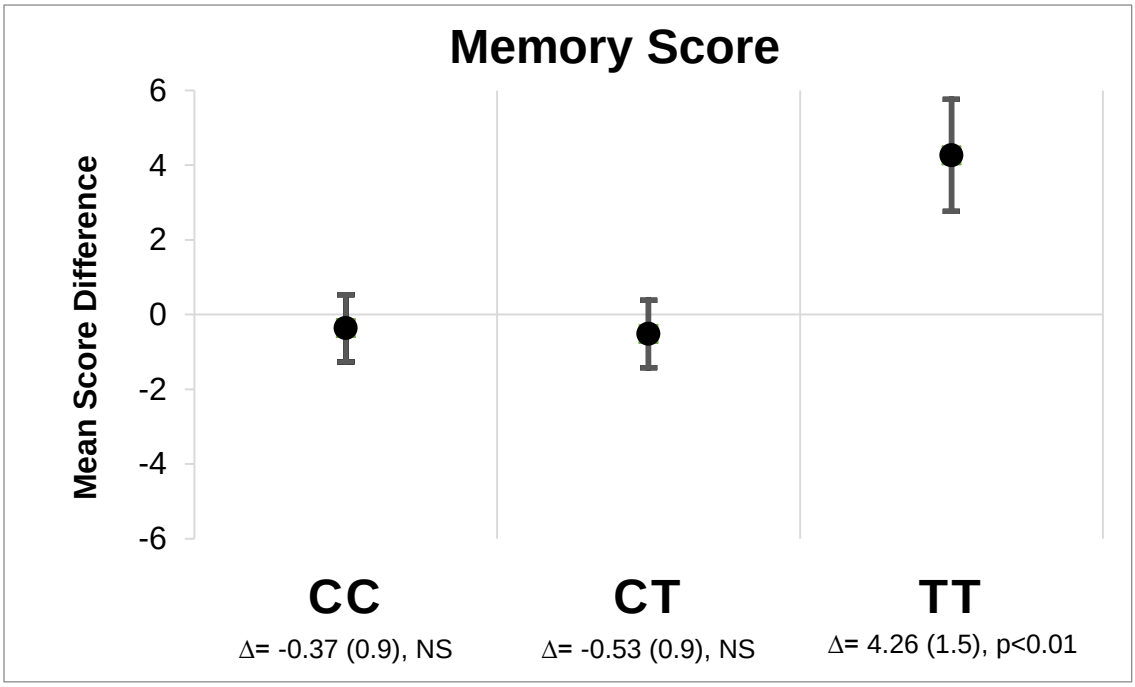
Table 3: McCarthy Scales of Child Abilities scores at 5 years by maternal *FADS2* SNPs and supplementation group among Mexican children whose mothers participated in the POSGRAD randomized controlled trial of prenatal n-3 Docosahexaenoic acid (DHA) supplementation.

	Placebo[b] (n= 306)			**Intervention[b] (n= 316)**			
FADS SNP rs174602 [a]	**CC (n=132)**	**CT (n=129)**	**TT (n=45)**	**CC (n=127)**	**CT (n=144)**	**TT (n=45)**	**p-value[d]**
Composite Score [c]	121.0 (1.8)	121.7 (1.9)	115.4 (3.1)	120.9 (1.9)	120.6 (1.8)	125.8 (3.1)	0.11
Quantitative Score	19.9 (0.5)	20.3 (0.5)	19.1 (0.9)	20.0 (0.5)	19.4 (0.5)	22.6 (0.9)	0.01
Verbal Score	54.2 (1.0)	54.7 (1.0)	52.0 (1.7)	53.7 (1.0)	53.2 (1.0)	55.7 (1.7)	0.16
Perceptual Score	46.9 (0.8)	46.7 (0.8)	44.3 (1.3)	47.2 (0.8)	48.0 (0.8)	47.3 (1.3)	0.41
Memory Score	25.5 (0.6)	25.4 (0.6)	23.7 (1.1)	25.1 (0.6)	24.9 (0.6)	27.9 (1.1)	0.02
FADS SNP rs174575 [a]	**CC (n=119)**	**CG (n=149)**	**GG (n=38)**	**CC (n=94)**	**CG (n=173)**	**GG (n=49)**	**p-value[d]**
Composite Score [c]	118.2 (1.9)	121.6 (1.7)	122.8 (3.4)	121.5 (2.2)	120.8 (1.6)	123.8 (3.0)	0.55
Quantitative Score	19.6 (0.5)	20.0 (0.5)	20.8 (1.0)	20.3 (0.6)	20.0 (0.5)	20.4 (0.9)	0.69
Verbal Score	52.7 (1.0)	55.0 (0.9)	54.9 (1.8)	53.7 (1.2)	53.3 (0.9)	55.6 (1.6)	0.34
Perceptual Score	46.1 (0.8)	46.6 (0.7)	47.1 (1.5)	47.5 (0.9)	47.6 (0.7)	47.8 (1.3)	0.39
Memory Score	24.5 (0.7)	25.5 (0.6)	26.2 (1.2)	25.5 (0.7)	25.3 (0.5)	25.6 (1.0)	0.60

[a] Values are raw score means (standard error of the mean) and are result of generalized linear models testing the interaction between FADS2 single nucleotide polymorphism and supplementation group on cognitive development scores measured using the McCarthy Scales of Infant Abilities at 5 years adjusted for child sex and age at measurement (months), and maternal SES, Raven Score and years of schooling.
[b] The intervention was 400mg/day of algal n-3 docosahexaenoic acid and the placebo was 400 mg/day of soy and corn oil from week 18-22 of pregnancy through delivery.
[c] The composite score is the sum of verbal, perceptual and quantitative scores.
[d] P-values refer to the interaction term (intervention*FADS2 SNP, error=615 df)

Figure 2- Quantitative and memory scores contrast-specific mean differences (Δ) between intervention and placebo by *FADS2* SNP rs174602





Models were adjusted for child sex and age at measurement, and maternal SES, Ravens Progressive Matrices score, and years of schooling.

Supplemental Table 1: Additional maternal and child characteristics considered for the models

	Placebo[b] (n= 306)	**Intervention[b] (n= 316)**
Maternal Characteristics		
Age, years	26.5 ± 4.7	26.5 ± 5.0
Socioeconomic status (SES), score	0.1 ± 1.0	0.0 ± 1.0
Schooling, years	11.9 ± 3.6	11.8 ± 3.4
Raven Progressive Matrices, score	41.0 ± 9.4	40.5 ± 9.2
Height, cm[a]	155.3 ± 5.6	154.7 ± 5.5
Body Mass Index, kg/m^2	26.4 ± 4.4	26.0 ± 4.2
First pregnancy, %	36.1	33.2
Dietary Intake, g/day		
n-3 Fatty Acids	1.8 ± 1.1	1.8 ± 1.1
ALA	1.7 ± 1.1	1.7 ± 1.0
DHA	0.1 ± 0.1	0.1 ± 0.1
n-6 Fatty Acids	19.5 ± 10.1	19.8 ± 9.3
LA	19.4 ± 10.0	19.6 ± 9.2
AA	0.2 ± 0.1	0.2 ± 0.1
Maternal *FADS* Genotype		
rs174575 (*FADS2*), %		
CC	38.9	29.7
CG	48.7	54.7
GG	12.4	15.5
rs174602 (*FADS2), %*		
CC	43.1	40.2
CT	42.2	45.6
TT	14.7	14.2
Child Characteristics at Birth		
Female, %	45.3	45.0
Gestational Age, weeks	39.0 ± 1.7	39.1 ± 1.8
Preterm, <37 weeks, %	8.6	9.6
Length, cm	50.4 ± 2.3	50.4 ± 2.3
Weight, kg	3.2 ± 0.5	3.2 ± 0.4
Low birth weight, <2.5 kg	3.4	3.4
Head Circumference, cm	34.4 ± 1.8	34.5 ± 1.5
Child Characteristics, postnatal		
Breastfeeding, 3 mo		
Exclusive or predominantly (with water), %	24.9	23.3
Mixed (breastmilk and formula), %	57.6	59.5
Not breastfed, %	17.5	17.2
HOME Score, 12 mo	37.0 ± 4.4	36.7 ± 4.3
Dietary Intake, g/day, 4 years		
n-3 Fatty Acids	0.2 ± 0.1	0.2 ± 0.2
ALA	0.1 ± 0.1	0.1 ± 0.1
DHA	0.1 ± 0.1	0.1 ± 0.1
n-6 Fatty Acids	2.8 ± 1.4	2.8 ± 1.7

[a] Intervention and placebo samples are significantly different ($p<0.05$);

[b]Intervention group received 400 mg/day of algal DHA from pregnancy week 18-22 through delivery, placebo group received 400 mg/day of a mix of corn and soy oil; alpha-linolenic acid (ALA),

docosahexaenoic acid (DHA), linoleic acid (LA), Arachidonic Acid (AA). Chi-squared tests, t-tests, and ANOVA were used to test the differences between groups.

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