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FADS single-nucleotide polymorphisms are associated with behavioral outcomes in children, and the effect varies between sexes and is dependent on PPAR genotype^{1–3}

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ABSTRACT

Background: Docosahexaenoic acid (DHA), supplied by the diet or endogenous biosynthesis from α -linolenic acid, accretes during the perinatal brain growth spurt. Results regarding a potential programming effect on cognitive function and behavior in humans are inconclusive.

Objective: Here we aimed to investigate whether behavioral outcomes in childhood were associated with *FADS* tag–single-nucleotide polymorphisms (SNPs) previously found to have opposing effects on infant erythrocyte DHA.

Design: At 36 mo, we assessed psychomotor development with the third edition of the Ages & Stages Questionnaire ($n = 256$) and physical activity by accelerometry ($n = 231$) in children from the SKOT [Småbørns Kost Og Trivsel (Diet and Thriving in Young Children)] cohort. Blood samples were taken to determine erythrocyte DHA ($n = 200$), *FADS* tag-SNPs ($n = 255$), and *PPARG*-Pro12Ala ($n = 255$). All outcomes were analyzed in models, including all 3 SNPs, SNP-sex interactions, erythrocyte DHA at 36 mo, and covariates.

Results: As previously shown, the minor allele carriers of the *FADS* SNP rs1535 had increased erythrocyte DHA at 9 mo, whereas DHA decreased in minor allele carriers of rs174448 and rs174575 (effect size around 0.5 percentage points per allele). No overall effects were observed for any of the *FADS* SNPs on the outcomes reported here, but *FADS* SNP-sex interactions were found for a number of DHA-increasing *FADS* alleles on both communication and problem solving ($P = 0.005$ and 0.013). DHA-increasing *FADS* alleles resulted in reduced scores in girls and improved abilities in boys, with an effect size of ~ 1 score-point/allele. No associations were found between current erythrocyte DHA and any of the behavioral outcomes. The P value for the triple interaction between DHA-increasing *FADS* alleles, *PPARG*, and sex for communication was 0.051 , and subsequent analyses showed the *FADS*-sex interaction only in *PPARG* minor allele carriers ($n = 70$). Furthermore, *FADS*-*PPARG* interactions were seen for problem solving in boys and for fine motor skills in girls.

Conclusion: *FADS* SNPs seem to have a sex-specific, possibly peroxisome proliferator-activated receptor-mediated effect on behavior in children, indicating a programming effect of early DHA exposure. *Am J Clin Nutr* 2014;100:826–32.

INTRODUCTION

Several studies have aimed to test the hypothesis that early intake of long-chain PUFAs (LC-PUFAs)⁴, especially DHA

(22:6n–3), improves cognitive development in young children. Breastfeeding has been associated with a higher intelligence quotient (IQ) score later in childhood (1–6), which has been proposed to be attributable to the breast milk content of LC-PUFAs. Early DHA intake, as well as DHA status, has been associated with various cognitive outcomes in longitudinal studies (7–10). However, these associations are likely to be confounded by duration of breastfeeding, healthy diet, and socioeconomic status, for example. Consequently, specific effects of DHA in general as well as the specific effects of DHA intake in early childhood remain to be clarified.

Randomized controlled trials supplementing infants with LC-PUFAs in different stages of the perinatal period have used different types and doses of LC-PUFAs as well as different tests to assess cognitive outcomes. Findings from these studies are therefore difficult to compare and have not shown consistent effects in meta-analyses (11, 12). The largest randomized trial, which allocated 2399 pregnant women to DHA or vegetable oil in the last half of gestation, found no overall effect on Bayley Scale scores at 18 mo (13). However, fewer children in the DHA-supplemented group, especially boys, obtained scores < 85 , and girls in the DHA group scored lower in communication and adaptive behavior. We have previously demonstrated that boys of mothers who were supplemented with fish oil during the first 4 mo of lactation were significantly less physically active at 7 y of age than were boys whose mothers had received olive oil (14). We have also found an

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⁴ Abbreviations used: ASQ-3, third edition of the Ages & Stages Questionnaire; IQ, intelligence quotient; LC-PUFA, long-chain PUFA; MM, homozygous for the major allele; Mm, heterozygous for the minor allele; mm, homozygous for the minor allele; PPAR, peroxisome proliferator-activated receptor; RBC, red blood cell; SNP, single-nucleotide polymorphism.

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inverse association between whole-blood DHA status and physical activity in a cross-sectional study in 10-y-old children (15).

Single-nucleotide polymorphisms (SNPs) in the *FADS* gene have been shown to modify LC-PUFA concentrations in breast milk (16, 17). Mendelian randomization studies have found that *FADS* SNPs modulate the effect of breastfeeding on IQ (18–20), indicating that LC-PUFAs may in part be responsible for the effect on IQ. Studies generally show that minor allele carriers of *FADS* SNPs exhibit a decreased synthesis of LC-PUFAs, as reflected mainly by an increase in dihomo- γ -linolenic acid (20:3n–6) relative to arachidonic acid (20:4n–6) in breast milk and erythrocytes [red blood cells (RBCs)] (16, 17, 21, 22). However, we have demonstrated different effects of 3 *FADS* tag-SNPs on RBC DHA status in 9-mo-old infants, with minor allele carriers of rs1535 having an increased status and those with minor alleles of rs174448 and rs174575 having lower DHA (23). None of the SNPs was associated with RBC DHA status at 36 mo (23). These *FADS* SNPs may therefore be used to investigate a potential programming effect of DHA status in infancy on behavioral outcomes later in childhood. We hypothesize that the effects of rs1535 are opposite to those exhibited by rs174448 and rs174575 and that the effects may be modified by polymorphisms in the *PPARG* gene, because peroxisome proliferator-activated receptor γ (PPAR γ) is a potential mediator of DHA effects. These hypotheses are tested in the children of the SKOT-I [Småbørns Kost Og Trivsel (Diet and Thriving in Young Children)] cohort on behavior assessed at 36 mo of age by the third edition of the Ages & Stages Questionnaire (ASQ-3) and physical activity.

SUBJECTS AND METHODS

The SKOT-I is a prospective cohort study observing 330 children from when they were 9 mo old. The study, conducted at the Department of Nutrition, Exercise and Sports, Faculty of Science, University of Copenhagen, has been approved by the Scientific Ethics Committee of the Capital Region of Denmark (H-KF-2007-0003). Recruitment of the children, from May 2007 through May 2008, was based on random extracts from the National Danish Civil Registry of families with infants in the appropriate age range in the Greater Copenhagen area. The families were invited by letter to participate in the study when the children were 7–8 mo old. Written informed consent was obtained from all parents of the participating children. The inclusion criteria were healthy singletons born after the 37th week of gestation. Infants were excluded if they had or developed disorders that could influence diet, health, or behavior.

The children so far have been examined at 9, 18, and 36 mo of age. Parents completed questionnaires on various background variables (birth data, breastfeeding, health, diet, education, etc) during each examination.

Anthropometric assessments

At the 36-mo visits, which took place from October 2009 through October 2010, height was measured to the nearest 0.1 cm by using a stationary digital height measurer (235 Heightronic Digital Stadiometer; Heightronic), with a total of 3 measurements conducted, and the mean value was used in the subsequent analyses. Weight was assessed to the nearest 0.1 kg on a digital scale (Tanita WB-100MA; Tanita Corporation).

RBC DHA fatty acid analysis

Blood samples were successfully taken from 200 children at 3 y of age. Shortly after blood sampling, RBCs were isolated by centrifugation from a 2-mL lithium-heparin sample (~ 15 min, $2300 \times g$, 4°C). The packed RBCs were washed 3 times in ~ 3 mL 150 mmol/L NaOH and 1 mmol/L EDTA (~ 5 min, $2300 g$, 4°C), followed by a reconstitution at 1:1 in 150 mmol/L NaOH and 1 mmol/L EDTA, with the addition of 2 drops of 0.1% butylated hydroxytoluene in ethanol per milliliter of RBC solution. The entire procedure was carried out on ice, and, last, the samples were treated with N_2 and kept frozen at -80°C until further analysis. Then lipids were extracted according to the Folch procedure (24), and fatty acids were transmethylated with BF_3 in methanolic NaOH, followed by a determination of the fatty acid composition by capillary gas chromatography [on a HP-6800 (Hewlett-Packard) with a SP2380 column] (25). Calculation of the relative content of specific fatty acids in the erythrocytes was based on their contribution to the total chromatogram area and expressed as percentage of all fatty acids from lauric acid (12:0) to DHA (fatty acid percentage).

FADS and PPARG genotyping

FADS and *PPARG* genotypes were determined. The *FADS* SNPs rs1535, rs174448, and rs174575 were chosen because they had been demonstrated to be associated with the content of LC-PUFAs in RBCs (21, 22, 26). The *PPARG*-Pro12Ala SNP (rs1801282) (27) was chosen because it has been shown to reduce transcription of target genes (28). The SNPs were identified from the International HapMap data for European ancestry (Release #28, NCBI build 36). Buffy coat was furthermore isolated from EDTA-treated blood samples and kept at -80°C for later DNA extraction, performed as described by Miller et al (29). Isolated DNA from the blood samples was stored in TrisEDTA buffer at -20°C until real-time polymerase chain reaction genotyping. Genotyping was carried out by using approximately 20 ng DNA in a total volume of 6 μL containing $1 \times$ Mastermix (Life Technologies), 100 nmol/L probes, and 900 nmol/L primers as previously described (23). All *FADS* and *PPARG* samples were genotyped by allelic discrimination on a real-time thermal cycler (ABI model 7500; Life Technologies), and a test run of minimum 24 samples was genotyped by using real-time polymerase chain reaction to identify controls with known genotypes. All samples were genotyped by using allelic discrimination and endpoint readings. Controls were included in each run, and genotyping was repeated in a random 14% subset, which yielded 100% identical genotypes. All *FADS* and *PPARG* SNPs were genotyped with a success rate of 100%.

Assessment of behavior

Behavior was assessed at the age of 3 y by measures of the child's psychomotor development as well as by the average level of physical activity. As previously described (30), the parents were asked to fill out an age-specific edition of the ASQ-3 (31), which was originally designed as a screening tool to detect developmental delays. The questionnaire was translated into Danish in collaboration with a psychologist and consisted of 5 subcategories: communication, gross motor, fine motor, problem solving, and personal/social skills. For each subcategory there

were 6 questions, and for each question, there were 3 possible answers: “yes,” “sometimes,” and “not yet,” each yielding 10, 5, and 0 points, respectively. Therefore, a maximum of 60 points could be obtained in total for each subcategory. The parents were encouraged to fill out the questionnaire when the child was full and rested, preferably making the testing a playful and fun activity for the child. When more than 2 of the 6 questions in a subcategory had not been answered, the entire subcategory was disregarded in the analyses. Data on the children’s psychomotor development from the ASQ-3 questionnaires were obtained from approximately 256 children; however, sample size varied a little for the subcategories (± 3).

Overall physical activity was monitored by accelerometers that captured vertical accelerations (GT3X; ActiGraph) and analyzed by ActiLife software (version 4.4.1; ActiGraph). Activity counts were summarized over 2-s intervals to be able to capture spontaneous activity bursts in 3-y-old children (32). Pre-programmed accelerometers were given to the parents with instructions on how to attach it to the child’s right hip and not to remove it during the following 7 d, except when the child was bathing. The parents were asked to estimate the duration of any interruptions in wearing time and to keep logs of sleeping time, including naps. Sleep time and episodes of >20 min with zero counts were considered as non-wear time and removed from the analyses during manual inspections of the recordings. The accelerometer had to be worn for >8 h daily between 0600 and 0800 and for >3 consecutive days to be included in the analysis. Mean counts per minute were calculated as a proxy of the average level of activity. Accelerometers were returned with good-quality recordings from 231 children; 36 did not meet the physical activity inclusion criteria, and 1 child was excluded because of a severe chronic disorder affecting the physical activity pattern.

Statistics

Data were presented as means \pm SDs for normally distributed continuous variables, as medians (IQRs) for nonnormally distributed continuous variables, and as percentages for categorical variables. Visual inspection of histograms was used to determine whether the variables were approximately normally distributed, and potential outliers were detected by scatter plots and excluded only if the outlying value indicated incorrect measurement. However, no outliers were excluded on this basis. The *t* test was used to compare means between boys and girls for normally distributed continuous variables, and the Wilcoxon rank sum test was used for the ASQ-3 variables. All statistical analyses were carried out with STATA 12.0 (StataCorp LP), and the significance level was set to 0.05.

To assess potential association with the 3 *FADS* SNPs (rs1535, rs174448, and rs174575) and the ASQ-3 scores and to see whether the effects were sex specific, we considered ANCOVA models, including *FADS* SNP-sex interactions, for each of the 6 behavioral outcomes in the ASQ-3 subcategories. Each *FADS* SNP was encoded as a categorical variable with 3 levels: homozygous for the major allele (MM), heterozygous for the minor allele (Mm), and homozygous for the minor allele (mm). Interactions were assessed by *F* tests and quantified through estimated sex differences in the differences between MM compared with Mm and MM compared with mm, respectively. If no

significant interaction was found, we did the analysis combined for boys and girls adjusted for sex.

The analyses were adjusted for birth weight, duration of breastfeeding, older siblings, parental education, and RBC DHA status at 36 mo. The analysis of physical activity was adjusted for duration of breastfeeding, parental education, parental physical activity, single-parent household, and RBC DHA status at 36 mo. The ASQ-3 scores were nonnormally distributed (skewed to the left from the maximum score) and therefore they log-transformed after reversing the scale to ensure acceptable normal distribution of the data.

Sensitivity analyses were carried out by using only 2 categories (*FADS* genotypes: Mm + mm compared with MM), and a shared variable for DHA-increasing alleles for the *FADS* SNPs was constructed as previously described (23). We also explored a potential triple interaction between *PPARG*, *FADS*, and sex. R^2 was used to describe the degree of explained variance in the models. As for the final models of the statistical analyses, residual plots were examined against standardized plots to ensure homogeneity of the variance of the residuals.

RESULTS

Characteristics of the 268 children who participated in the 36-mo examination and had their *FADS* genotypes determined are provided in **Table 1**. Girls obtained significantly higher median scores than did boys in the following ASQ-3 subcategories: fine motor skills (55 and 50, respectively, $P < 0.001$) and personal/social skills (60 and 55, respectively, $P < 0.001$) (30) (*see* Supplemental Table 1 under “Supplemental data” in the online issue). However, on the contrary, boys were significantly more active on average than were girls (*see* Supplemental Table 1 under “Supplemental data” in the online issue).

As previously shown (23), minor allele carriers of the *FADS* SNP rs1535 had increased DHA at 9 mo, whereas DHA was decreased in minor allele carriers of rs174448 and rs174575, and the overall effect size was around 0.5 percentage points per allele. In the model with the 3 SNPs, rs174448 homozygotes had decreased RBC arachidonic acid at 9 mo ($\beta = -1.4 \pm 0.4$, $P = 0.029$), but no associations were seen for rs174575 or rs1535 (data not shown).

FADS SNP-sex interactions

FADS SNP-sex interactions were observed for the association between *FADS* genotypes (Mm + mm compared with MM) and communication and problem-solving skills (**Table 2**). Having a minor allele of rs1535 reduced communication and problem-solving scores in girls relative to boys, whereas a minor allele of rs174448 and, to some extent, rs174575 had the opposite effect. The effect was dose dependent—that is, 2 minor alleles had twice the effect of one, and the effect size was relatively large (~ 6 per allele) (Table 2). No overall effects of the *FADS* SNPs were observed for any of the outcomes, and no *FADS* SNP-sex interactions were found for personal/social skills or fine and gross motor development at 3 y (*see* Supplemental Table 2 under “Supplemental data” in the online issue). There was also no interaction or association between *FADS* SNPs and the mean level of physical activity in either sex or for the sexes combined (data not shown). Furthermore, there was no interaction between *FADS* SNPs and breastfeeding (data not shown).

TABLE 1
Characteristics of the participants¹

	Values	Total <i>n</i>
Sex (% M)	48.1	268
Birth weight (kg)	3.5 ± 0.5 (2.4–4.9) ²	268
Breastfeeding duration (mo)	8.8 ± 4.5 (0–39)	266
Breastfeeding duration (%)		266
<1 mo	6.0	
1–4 mo	11.6	
>4 mo	82.3	
Older siblings (% yes)	41.6	267
Parental education (% of households) ³		267
No formal, vocational, or short education (≤2 y)	13.1	
Academic education (3–4 y)	27.7	
Long academic education (>4 y)	59.2	
Parental physical activity (% of households) ³		258
≥4 h/wk high intensity	34.1	
≥4 h/wk low intensity	58.1	
Primarily sedentary	7.8	
rs1535 (%)		268
With MM	47.8	
With Mm	45.9	
With mm	6.3	
rs174448 (%)		268
With MM	46.3	
With Mm	42.9	
With mm	10.8	
rs174575 (%)		268
With MM	60.4	
With Mm	37.3	
With mm	2.2	
Pro12Ala (%)		268
With MM	72.0	
With Mm	26.9	
With mm	1.1	
RBC DHA status (FA%)		
At 9 mo	6.6 ± 2.0 (2.2–12.6)	234
At 36 mo	7.0 ± 1.3 (3.6–10.5)	200
Fish intake at 3 y (g/d)	9.8 (4.3, 18) [0–64] ⁴	243
Mean physical activity at 3 y (cpm)	554 ± 129 ⁵	231

¹ cpm, counts per minute; FA, fatty acid; MM, homozygous for the major allele; Mm, heterozygous for the minor allele; mm, homozygous for the minor allele; RBC, red blood cell.

² Mean ± SD; range in parentheses (all such values).

³ Households were categorized according to the highest level of education or physical activity of either parent.

⁴ Median; 25th–75th percentiles in parentheses; range in brackets.

⁵ Mean ± SD.

Similar trends in *FADS* SNP-sex interactions were observed in dominant models that combined mm + Mm compared with MM (data not shown) and in models using a combined measure of DHA-increasing *FADS* alleles rather than individual SNPs (Figure 1). Furthermore, the effects of the 3 *FADS* SNPs add up as expected based on their previously observed effect on RBC DHA at 9 mo (Figure 1). No associations were found between current RBC DHA status and any of the behavioral outcomes (numerical effect sizes of 0.1–1.0 and *P* values of 0.58–0.07). Furthermore, there was no interaction between *FADS* SNPs and breastfeeding (data not shown).

The variance explained by *FADS* SNPs was 14% and 12% for communication and 7% and 15% for problem solving in boys

and girls, respectively (see Supplemental Table 3 under “Supplemental data” in the online issue). For personal/social skills, fine and gross motor development, and physical activity, the variance explained by *FADS* SNPs was <9%.

Children generally obtained the highest possible score for most of the individual ASQ-3 subcategory questions (but less so on the overall categories; see Supplemental Table 4 under “Supplemental data” in the online issue), especially within questions on gross motor development, where only ≤12% obtained submaximum scores. The largest variation was found within problem-solving skills, for which 10–29% of the children did not obtain the maximum score on the individual questions. There were no significant *FADS*-sex interactions for the individual problem-solving question scores, although the *P* value for rs1535-sex interaction on the question “Show your child how to make a bridge with blocks, boxes or cans. Does your child copy you by making one like it?” was 0.056, with a direct association in boys and an inverse association in girls. The rs1535-sex interaction for the question “Does your child take turns by waiting while another child or adult takes a turn?” within the personal/social skills subcategory was significant (*P* = 0.009), again with an inverse association in girls and a direct association in boys.

Interactions for DHA-increasing *FADS* alleles, *PPARG*, and sex

The triple interaction between DHA-increasing *FADS* alleles, *PPARG*, and sex had a *P* value of 0.051 for communication. Subsequent separate analyses with *PPARG* major allele homozygotes and all *PPARG* minor allele carriers (Mm + mm), respectively, showed that the DHA-increasing *FADS*-sex interaction was significant only in *PPARG* minor allele carriers (*P* = 0.009, *n* = 70 compared with *P* = 0.158 in *PPARG* MM, *n* = 176). In analyses stratified according to sex, there were significant DHA-increasing *FADS*-*PPARG* interactions in boys for problem solving (*P* = 0.032) as well as for fine motor skills in girls (*P* = 0.050). Furthermore, there were specific *FADS*-*PPARG* interactions for fine motor development for girls for rs1535 and rs174575 (*P* = 0.039 and 0.009, respectively).

DISCUSSION

In this group of mainly breastfed children, *FADS* SNPs alleles were associated with behavior scores in a sex-dependent way. Significant associations were found for communication and problem solving. Among girls, the rs1535 *FADS* SNP, which has been associated with an increased DHA biosynthesis at 9 mo of age (23), was significantly associated with lower communicative and problem-solving skills. Similarly, the rs174448 and rs174575 *FADS* SNPs, which were associated with a decreased DHA synthesis at 9 mo (23), were significantly associated with better scores for communication and problem solving. Opposite associations were found in boys, especially for communicative skills. No associations between *FADS* SNPs and physical activity were demonstrated.

Earlier studies have investigated if several single *FADS* SNPs could modify the association between breastfeeding and IQ in children (18–20). Results from the 2 largest studies are not completely consistent, with one showing lower IQ scores in formula-fed minor allele carriers of *FADS* SNPs (19) and

TABLE 2Estimated associations between 3 *FADS* tag-SNPs and ASQ-3 outcomes at 3 y¹

	rs1535		rs174448		rs174575	
	Mm	mm	Mm	mm	Mm	mm
Communication						
<i>FADS</i> -sex <i>P</i> value	0.010		0.005		0.023	
Estimated difference (girls – boys)	-11.5 ± 4.0 (0.004)	-17.6 ± 7.7 (0.023)	9.3 ± 2.8 (0.001)	7.8 ± 5.5 (0.154)	9.0 ± 3.6 (0.014)	18.6 ± 9.2 (0.046)
Problem solving						
<i>FADS</i> -sex <i>P</i> value	0.016		0.026		0.260	
Estimated difference (girls – boys)	-2.5 ± 3.4 (0.468)	-18.6 ± 6.6 (0.005)	5.8 ± 2.5 (0.021)	10.4 ± 4.7 (0.029)	1.6 ± 3.2 (0.612)	13.2 ± 8.0 (0.102)

¹The associations were analyzed by ANCOVA models, 1 per outcome, including all 3 *FADS* tag-SNPs and *FADS*-sex interactions. Because the *P* values for 1 or more of the *FADS*-sex interactions were significant, data are shown as estimated mean ± SE differences between girls and boys, with the *P* value for this difference in parentheses. Major allele homozygotes were set as the reference for all *FADS* SNPs, and the analyses for ASQ-3 subcategories were adjusted for parental education, older siblings, birth weight, total duration of breastfeeding, and erythrocyte DHA at 3 y. The number of children in the analyses was 179 (91 boys and 88 girls) for communication and 178 (89 boys and 88 girls) for problem solving. ASQ-3, third edition of the Ages & Stages Questionnaire; Mm, heterozygous for the minor allele; mm, homozygous for the minor allele; SNP, single-nucleotide polymorphism.

another demonstrating higher IQ scores in breastfed major allele carriers (18). One study, which carried out multiple analyses with several maternal *FADS* SNPs (33), also found opposing effects between some of the SNPs. Interestingly, maternal minor alleles of rs3834458 [found to be strongly linked with rs1535 (23)] were associated with an increased child IQ at 8 y, whereas other *FADS* SNPs were associated with lower IQ (33). The authors found that ~17% explained variance in models that adjusted for a number of confounders, including parental education, sex, and breastfeeding. In the present study, breastfeeding was not

found to interfere with the effect of the *FADS* SNPs. This could be attributed to the long overall duration of breastfeeding, the relative high fish intake and thus high DHA content of breast milk of Danish mothers (34), and an increase in the n-3 to n-6 PUFA ratio in the formula since the 1990s.

We found no association between *FADS* SNPs and physical activity. Results from an earlier study pointed toward a programming effect of early DHA exposure on physical activity later in childhood (14). However, Asserhøj and colleagues (14) assessed physical activity at 7 y, whereas the children were 36 mo old in the present study, and it is possible that the discrepancy could be attributable to differences in activity pattern at different ages. It is also possible that the association between DHA and physical activity demonstrated by Asserhøj et al was only a chance finding, although the effect is supported by animal studies (35–40) as well as by results from a cross-sectional study of 10-y-old children (15).

An Australian study found that maternal DHA supplementation decreased the number of children, especially boys, who obtained a score <85 on the Bayley Scale at 18 mo compared with the children of mothers who were given the vegetable oil placebo (13). In contrast, more children of supplemented mothers, especially girls, were more likely to obtain lower scores for communication and adaptive behavior (13). This is in agreement with the observed negative association between DHA-increasing *FADS* alleles and communication in girls and the positive association with problem solving in boys in the present study. We have also shown interactions between DHA and sex on the association between whole-blood DHA and blood pressure in Danish schoolchildren aged 8–11 y (15), and we have furthermore demonstrated sex-equalizing effects of fish oil supplementation on appetite in young adults (41). These findings suggest that it is likely that DHA may have sex-specific and, more specifically, sex-opposite effects on various outcomes.

Several of the children obtained maximum ASQ-3 subcategory scores, which obviously lowered the sensitivity to demonstrate differences among the children. However, this sensitivity problem varied among subcategories. As for communication, only 15% and 11% of the boys and girls, respectively, obtained the maximum mean score (60 points), whereas the numbers of

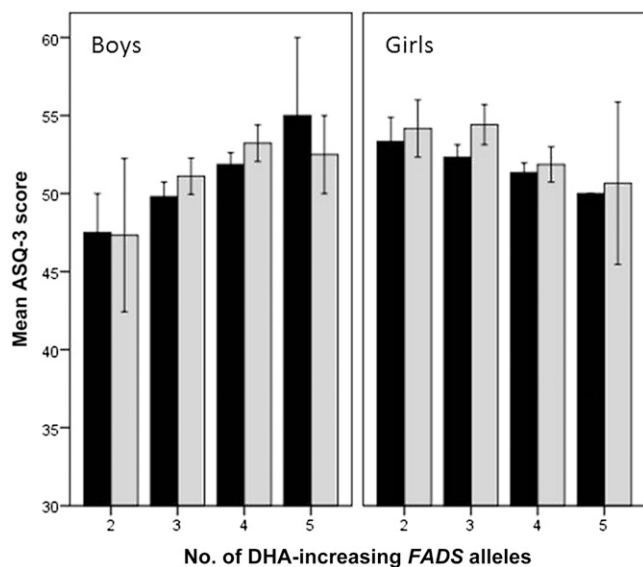


FIGURE 1. Scores from the ASQ-3 in the subcategories of communication (black bars) and problem solving (gray bars) for boys and girls at 36 mo according to the number of DHA-increasing *FADS* alleles. Data are given as mean scores ± SEs with *n* = 121 and *n* = 125 for communication and *n* = 121 and *n* = 126 for problem solving in boys and girls, respectively. The summarizing *FADS* single-nucleotide polymorphism marker is based on the effects of rs1535, rs174448, and rs174575 at infant RBC DHA at 9 mo. The *P* values for the shown interactions between sex and *FADS* DHA-increasing alleles were 0.005 for communication and 0.013 for problem solving in ANCOVAs adjusted for parental education, older siblings, birth weight, total duration of breastfeeding, and RBC DHA at 3 y. ASQ-3, third edition of the Ages & Stages Questionnaire; RBC, red blood cell.

children who obtained 60 points for the other subcategories were >20%. There were no associations between *FADS* SNPs and physical activity, which again suggests that the effect of DHA is mainly on mental and cognitive outcomes.

We previously found an association between RBC DHA status at 9 mo and communication at 3 y, which was significant only in girls (30), which was verified by the present results as the use of *FADS* SNPs which is less prone to be confounded. However, we had only a few minor allele homozygous children, which may have decreased the power to detect significant associations between the SNPs and behavior in the children. This decrease in power is most serious with respect to the *FADS-PPARG*-sex interactions. Furthermore, the ASQ-3 questionnaire was developed in the United States and has been translated into Danish. As a result of cultural differences, some of the questions might be less relevant for Danish children, causing them to obtain a score that did not accurately reflect their developmental stage. The children generally obtained high scores, and it is possible that more variance could have been achieved if the children had been tested at a slightly younger age. It also would have been optimal if we could have validated the results from the present study in another cohort study.

The sex-specific results in the present study suggest that a high DHA status may level out sex differences in the psychomotor development of small children. Girls are traditionally believed to have a faster psychomotor development than boys in several subdomains (eg, fine motor and personal/social skills in this study). However, on the basis of the findings from the present study, it seems likely that some of these developmental advantages in girls may be reversed by *FADS* SNPs that affect DHA status in infancy. An explanation of these sex-opposite effects of early DHA exposure remains to be fully elucidated. In the present study, the associations between the number of DHA-increasing *FADS* alleles and problem-solving and fine motor skills were dependent on *PPARG* genotype for boys and girls, respectively. Our findings indicate that DHA exerted the most pronounced effect in children who exhibited the lowest *PPARG* activity. It is well known that DHA acts as a ligand of nuclear factors belonging to the PPAR superfamily. PPAR γ is involved in the regulation of aromatase, and it has been shown that the *PPARG* polymorphism Pro12Ala is associated with a less efficient transcription (42). This may result in a decrease in estrogen concentrations, and estrogen plays a key role in explaining sex differences in dopaminergic functioning (43), which in turn is well known to be involved in the regulation of cognitive and emotional functions (44, 45).

To our knowledge, this is the first study to demonstrate sex-specific and sex-opposite associations between *FADS* SNPs previously found to determine RBC DHA status in infants and behavioral outcomes later in childhood. The findings from the present study suggest that future studies investigating the association between *FADS* SNPs and IQ in children should base the interpretation of possible associations on their effect on the endogenous synthesis of DHA. Only a few studies have carried out sex-stratified analyses in relation to the effect of DHA on cognitive and behavioral outcome, which may explain the lack of results. The present results indicate that functional outcomes in boys and girls in childhood may be affected oppositely by DHA status in infancy because the *FADS* SNPs do not seem to affect DHA status at the age of 3 y (23). Furthermore, the effect

of the *FADS* SNPs may also indicate a DHA-specific effect, because we did not find the same inverse associations between the SNPs and arachidonic acid. It may be relevant to examine the possible impact of *PPARG* polymorphisms in future studies investigating the association between *FADS* SNPs and cognitive outcomes. Our findings might also be of relevance in research related to the effect of DHA and *FADS* SNPs on attention-deficit hyperactivity disorder (46).

In conclusion, *FADS* SNPs seem to have a sex-specific, possibly PPAR-mediated, effect on behavior in children, potentially indicating a programming effect of early DHA exposure.

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