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Original article

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Maternal seafood consumption during pregnancy and child attention outcomes: a cohort study with gene effect modification by PUFA-related genes

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Abstract

Background: There is a need to test the fetal programming theoretical framework in nutritional epidemiology. We evaluated whether maternal seafood intake during pregnancy was associated with 8-year-old attention outcomes after adjusting for previous child seafood intake and cognitive function. We also explored effect modification by several single nucleotide polymorphisms (SNPs) related with polyunsaturated fatty acid (PUFA) metabolism.

Methods: Our final analyses included 1644 mother-child pairs from the prospective INMA (INfancia y Medio Ambiente) cohort study (Spain, recruitment between 2003 and

2008). We used food frequency questionnaires to assess prenatal and postnatal seafood consumption of the mother-child pairs. We evaluated attention function of the children through the computer-based Attention Network Test (ANT) and we used the number of omission errors and the hit reaction time standard error (HRT-SE). Parents reported child attention deficit hyperactivity disorder (ADHD) symptoms using the Revised Conners' Parent Rating Scale Short Form (CPRS-R: S). We measured seven candidate SNPs in a subsample of 845 children. We estimated associations using regression models, adjusting for family characteristics, child seafood intake and cognitive functions at early ages, and to explore SNP effect modifications.

Results: Higher total seafood intake during early pregnancy was associated with a reduction of child ANT omission errors, 5th quintile (median = 854 g/week) vs 1st quintile (median = 195 g/week), incidence risk ratio (IRR) 0.76; 95% CI = 0.61, 0.94. Similar results were observed after adjusting the models for child seafood intake and previous cognitive status. Lean, large and small fatty fish showed similar results, and generally similar but less robust associations were observed with the other attention outcomes. Shellfish and canned tuna showed weaker associations. The association patterns were weaker in late pregnancy and null in child seafood consumption. Child rs1260326 (glucokinase regulator, *GCKR*) and child/maternal rs2281591 (fatty acid elongase 2, *ELOVL2*) polymorphisms showed nominal *P*-value for interactions <0.10 between total seafood intake and ANT outcomes.

Conclusions: After adjusting for previous child cognitive functions and child seafood intake, high pregnancy consumption (total, lean, small and large fatty fish) was independently associated with improvements of some 8-year-old attention outcomes. Genetic effect modification analyses suggest PUFA intake from seafood as a potential biological mechanism of such association.

Key words: Seafood intake, fish intake, pregnancy, cohort study, essential fatty acids, PUFAs, attention function, ADHD symptoms, genetic polymorphisms, SNPs

Key Messages

- There is a need to empirically test whether the association between maternal seafood intake in pregnancy and child attention outcome is independent.
- There is a need to explore whether polyunsaturated fatty acid (PUFA)-related genes play a role in this association.
- Maternal seafood intake during pregnancy was independently associated with long-term child attention outcomes.
- Some PUFA-related single nucleotide polymorphisms modified the association.
- This original contribution empirically tested and supported the fetal programming theoretical framework between maternal seafood intake and child attention outcomes.
- Furthermore, the gene-environmental interaction analyses suggested the potential PUFA involvement in this beneficial association.

Introduction

There is increasing interest in the link between maternal diet during pregnancy and child neurodevelopment.[1] Main brain development occurs during crucial time windows during pregnancy with highly active biological processes, such as neuron formation, migration, axon and dendritic growth, synaptogenesis and myelination.[2] Essential nutrients, such as polyunsaturated fatty acids (PUFAs), are required for the development of a healthy newborn brain, and the neuro-functional consequences of low PUFA levels during pregnancy could affect child development several years after birth.[1,2] Docosahexaenoic (DHA) and

eicosapentaenoic (EPA) acids are the main omega-3 PUFAs involved in the human neurodevelopment.[3,4] Seafood is the major human source for DHA and EPA intake. Recent reviews concluded that maternal seafood intake was positively associated with child neurodevelopment, even though seafood-related methylmercury exposure could increase the developmental neurotoxic risk.[1,5,6]

We previously reported protective associations between high seafood consumption during pregnancy, including large fatty fish, and child neurodevelopment outcomes at ages 1 and 5 years.[7] In this study, we investigate whether these longitudinal associations are still observed at older ages. We focused on attention function because attention deficit and hyperactivity disorder symptoms (ADHD symptoms) are common neuropsychiatric problems during school age. However, whereas this may provide the possibility of greater statistical power via larger effect sizes, it is also possible that the data at this older age will also exhibit greater noise due to the multicausal routes affecting ADHD symptoms. Attention is a complex cognitive function involving processes like selectively attending a specific stimulus, focusing for prolonged periods and monitoring of actions. Attention precedes and may alter the performance of other complex functions like memory and executive function. Indeed, attention development in children has implications on learning, academic achievement and social functioning.[8]

Further empirical evidence from nutritional epidemiology is needed to test the concept of prenatal fetal programming and long-term health consequences, commented on in the developmental origins of health and disease hypothesis (DOHaD).[9] In this analysis, we gained sufficient longitudinal data to formally test whether there is a direct association between pregnancy seafood consumption and 8-year-old attention, independent of child seafood consumption (5 years) and child cognitive status (1 and 5 years) collected in previous follow-ups of the cohort.[7]

Seafood intake-neurodevelopment associations are potentially linked to the DHA and EPA availability in the fish. Gene-environment analyses of candidate single nucleotide polymorphisms (SNPs) involving PUFA metabolism could provide additional support from observational studies for this hypothesis. Some studies reported breastfeeding duration interactions with fatty acid desaturase (*FADS*) gene cluster and fatty acid elongase (*ELOVL*) gene family, and child cognition.[10] However, no studies reported similar interactions with pregnancy seafood intake.

In this study, we investigated the association between maternal seafood intake during pregnancy and 8-year-old attention outcomes in a longitudinal cohort in Spain (INMA Project, INfancia y Medio Ambiente).[11] We; (i) analysed associations with different seafood subtypes, since PUFA levels are different between seafood species;[7] (ii) adjusted for child seafood consumption and cognitive functions at early ages; and (iii) explored potential interactions with several child and maternal candidate SNPs related to PUFA metabolism.

Methods

Participants

Participants were from the Spanish Environment and Childhood Project (Proyecto Infancia y Medio Ambiente, INMA [http://www.proyectoinma.org/en_index.html]), a population-based birth cohort study established between 2003 and 2008 in four regions of Spain: Asturias, Gipuzkoa (the Basque Country), Sabadell (Catalonia) and Valencia.[11] A total of 2644 eligible women were recruited during prenatal visits in the first trimester of pregnancy. Women were included if they agreed to participate, met the inclusion criteria (≥16 years of age, singleton pregnancy, intention to deliver at the reference hospital), but not the exclusion criteria (communication handicap, fetuses with malformations, assisted conception). Women were followed up during pregnancy and their children were enrolled at birth and followed up until age 8 years. After excluding women who withdrew, were lost to follow-up or underwent abortions or fetal deaths, a total of 2506 pregnant women were monitored through delivery and 2282 presented data on maternal seafood intake during pregnancy. Main final analyses included 1641 children at 8 years with at least one neuropsychological measurement. From delivery, 740 (28%) children were lost to follow up at age of 8 years. The remaining missing cases ($n = 125$) are attributable to missing data on some study variables and covariables. All participants provided written informed consent, and the study was approved by hospital and institutional ethics committees in each region. Further information is published elsewhere.[7]

Seafood consumption

We used a semi-quantitative food frequency questionnaire (FFQ) of 101 food items to assess the usual daily intake of foods and nutrients at 10–13 weeks of pregnancy.[12] Women reported their usual intake of foods from the last menstruation to the first prenatal visit (12th week), using reference portions and nine frequency categories ranging from never/less than once a month to more than six times per day. The questionnaire included 10 seafood items. The response to each seafood item was converted to average weekly (w) intakes in grams (g); then summed to compute the total and seafood subtypes (in g/w). Seafood was classified a priori[7] as follows: (i) large fatty fish (one item, item descriptions are in Supplementary Appendix I, available as

Supplementary data at *IJE* online); (ii) smaller fatty fish species (two items); (iii) lean fish (two items); (iv) canned tuna (one item); (v) shellfish (three items); and (vi) overall (total) seafood intakes, calculated as the sum of consumption of all previous items plus smoked fish consumption (this type of fish was not included as a separate group due to a low consumption level reported here). Extended description about the 10 seafood items and subtype group creation is in Supplementary Appendix I, available as Supplementary data at *IJE* online and previously published.[7] Intakes were adjusted for energy intake using the residual method;[13,14] and analysed primarily in quintile categories of weekly grams for total seafood, and quartile categories for seafood subtypes, due to constrained distributions by lower intake frequency. In the analyses, we kept non-consumers as the reference group (0 g per week), except for total and lean seafood variables due to their higher intake frequencies. The variable categories were created prior to analysis. The same FFQ and similar methods were applied to estimate seafood consumption of mothers at 28–32 weeks of pregnancy. The FFQ used for the mothers was validated using the data from the two different time assessments during pregnancy. In the study validation,[12] dietary changes from the first to third trimester were reported. We used a similar FFQ validated for children of age 5 years to estimate seafood consumption.[13]

Child and family characteristics

Trained interviewers administered in-person questionnaires twice during pregnancy and at child ages of 1, 5 and 8 years. An extended description of child and family's sociodemographic data collected is in Supplementary Appendix II, available as Supplementary data at *IJE* online. At child's age 1 and 5 years, neuropsychological assessments using Bayley and McCarthy Scales were undertaken by psychologists.[7] Cord blood mercury ($n = 1276$) and total fatty acid levels ($n = 870$) were measured following standard methods previously reported.[7,15] In relation to total fatty acids, these were analysed in a subsample of cord plasma by fast-gas chromatography; 100 µl of sample were saponified by adding 1 ml of sodium methylate (0.5% w/v) and heating to 100 °C for 15 min. Cord plasma levels of DHA and EPA were measured for this study. Maternal consumption of large fatty fish showed the strongest Spearman coefficients for correlation with cord blood mercury levels ($r = 0.27$) and DHA levels ($r = 0.17$). Total seafood consumption showed moderate Spearman correlation coefficients with cord blood mercury levels ($r = 0.22$) and DHA levels ($r = 0.11$). A similar pattern was observed with EPA levels. All the Spearman correlation coefficients between DHA and EPA and maternal seafood subtypes are shown in Supplementary Table 1, available as Supplementary data at *IJE* online.

Attention function assessment

In-person examinations were conducted at the 8-year visit. The Attention Network Test (ANT) is a computer-based test designed to assess attention function; an extensive description of ANT is published elsewhere.[16] The main ANT outcomes selected were omissions, which is the count number of omission errors on target stimuli, an indicator of selective and sustained attention; and hit reaction time standard error (HRT-SE), which is the time (ms) variability. Lower HRT-SE indicates good attention performance with consistent reaction times.[8,17] During the 8-year visit, parents reported child ADHD symptoms using the Revised Conners' Parent Rating Scale Short Form (CPRS-R: S).[18] For all three outcomes, higher scores indicate worse attention performance and behaviour. Further information is in Supplementary Appendix III, available as Supplementary data at *IJE* online.

Genotyping

Seven independent SNPs known to be quantitative trait loci (QTLs) for otPUFA [PUFA levels other than linoleic acid (18: 2)] and/or DHA were retrieved from [Mrbase.org] (released May 2017), which is based on the genome-wide association study by Kettunen *et al.*[19,20] (Supplementary Table 2, available as Supplementary data at *IJE* online).

Child genotypes were obtained from genome-wide genotypic data assessed with the Illumina HumanOmni1-Quad v1.0 Beadchip (INMA Sabadell and Valencia) or the Infinium Global Screening Array (GSA) (INMA Gipuzkoa) (total $n = 853$). The standard quality control thresholds were applied.[21] All the SNPs were in Hardy-Weinberg equilibrium (Supplementary Table 2, available as Supplementary data at *IJE* online). Maternal genotypes were only available for two out of the seven loci in INMA Sabadell ($n = 410$). More details of this section are described in Supplementary Appendix IV, available as Supplementary data at *IJE* online.

Statistical analysis

Associations between seafood consumption (including subtypes) and child attention scores were evaluated using multivariable regression analyses. Linear regression models were applied for ANT HRT-SE. ANT omission errors and CPRS-R: S scores were evaluated using negative binomial regression models to account for over-dispersion of the

data, and results are shown as incidence rate ratios (IRR).[22] Seafood consumption was evaluated as ordinal (quintiles and quartiles) variables. Tests for linear trend were performed by including median values of consumption within each variable category in the regression models. Based on our previous study,[7] minimally adjusted regression models included age and gender of the child, cohort and maternal total energy intake (kcal). Fully adjusted models further included maternal age, education level, occupation, parity, prepregnancy body mass index (BMI), country of origin/birth and a modified relative Mediterranean diet score. This score was constructed based on the consumption of vegetables, fruits and nuts, cereals, legumes, olive oil, meat and dairy products obtained from the FFQ.[23] We modified the original score by excluding fish and alcohol consumption. The covariables were retained in the final models because of previous knowledge that they were considered to be the main outcome-related covariables (child gender and age) and the main confounders (all the other variables listed above). Nevertheless, important covariables were excluded from main analyses, due to pre-analysis confounder prioritization (gestational age, birthweight, breastfeeding duration and family urban vulnerability index), or further selected to test the prenatal programming hypothesis (child early cognition scores and seafood intake), or excluded due to an important reduction of the observation numbers, i.e. maternal verbal IQ proxy, mental health, cord blood mercury levels and paternal social class (occupation level). However, all of them were further considered in sensitivity analyses.

Several sensitivity analyses on the association of first trimester total seafood intake were conducted additionally adjusting for: (i) 5-year-old total seafood intake; (ii) child 1- and 5-year-old general cognition (Bayley and McCarthy Scales); (iii) concentrations of cord blood mercury; (iv) gestational age, birthweight, breastfeeding duration, 4-year visit maternal verbal IQ proxy (WAIS-IV Similarities subtest) and psychopathological symptoms (SCL-90-R), family urban vulnerability index and paternal occupation at child's birth. In secondary analyses, the main models were: (i) rebuilt using inverse probability weighting (IPW) to control the potential selection bias induced by restricting the analysis to complete cases (i.e. individuals with no missing values);[24] (ii) stratified by geographical location, Cantabric sea (Asturias and Gipuzkoa) vs Mediterranean Sea (Sabadell and Valencia); and (iii) replicated using maternal seafood consumption during the third trimester of pregnancy and child seafood consumption (at 5 years old) as independent variables.

The seven SNPs in PUFA-related genes were analysed in an interaction model. A dominant genetic effect was tested, where the minor homozygous and heterozygous genotypes were grouped. When testing interactions between SNPs and total seafood intake, multiple comparisons were addressed by correcting nominal *P*-values using Bonferroni criteria (0.05/7 SNPs = 0.007). Furthermore, for those SNPs showing effect modifications between total seafood intake and the outcomes, we explored their pattern again with DHA and EPA as exposure variables. All analyses were conducted with the STATA 12.0 statistical software package (College Station, TX).

Results

Average maternal total seafood consumption during the first trimester of pregnancy was 498 g/week. Average child age was 7.6 [standard deviation (SD) = 0.6] years, and females were 49% of the total sample; extended characteristics of the study population are presented in Supplementary Table 3, available as Supplementary data at *IJE* online. The participation rate was of 62% in this follow-up.

Table 1 describes the medians of maternal seafood consumption by 8-year-old ANT and CPRS-R: S ADHD index outcomes. Higher maternal seafood intakes were observed in the groups with better scorings of the three attention outcomes; they were particularly relevant with total seafood and large fatty fish subtype.

Maternal seafood subtypes were moderately mutually correlated, particularly lean fish, large fatty fish and total seafood. Similar correlation patterns, but at lower degree, were observed between child total seafood intake and maternal seafood subtypes (Supplementary Table 4, available as Supplementary data at *IJE* online).

In Tables 2 and 3 and in Supplementary Table 5 (available as Supplementary data at *IJE* online), the minimally and fully adjusted regression models were fit for consumption of total seafood and seafood subtype (independent variables) and the three attention outcomes. Table 2 refers to ANT omission errors, Table 3 refers to CPRS-R: S ADHD index and Supplementary Table 5 (available as Supplementary data at *IJE* online) refers to ANT HRT-SE. In Table 2, higher total seafood intake was associated with a reduction of ANT omission errors; 5th quintile (median = 854 g/w) vs 1st quintile (median = 195 g/w) incidence rate ratio (IRR) 0.76; 95% CI = 0.61, 0.94. The association patterns were similar for total seafood, large fatty fish, small fatty fish and lean fish. Weaker associations were observed for canned tuna and shellfish (Table 2). CPRS-R: S ADHD index was also associated with total maternal seafood intake, large fatty fish and lean fish (Table 3). However, ANT HRT-SE was weakly associated with the large and small fatty fish subtypes (Supplementary Table 5, available as Supplementary data at *IJE* online). Generally in the main models, the inclusion of all the selected covariables in the regressions increased the

Table 1. Maternal seafood consumption during first trimester by child attention outcomes at age of 8 years (INMA Study, 2003–08)

Child outcomes[b]	*N*	Median seafood intake, g/week					
		Total	Large fatty fish	Smaller fatty fish	Lean fish	Canned tuna	Shellfish
ANT HRT-SE (ms)							
(median, range: 313, 85–597)							
>PC80	310	435[a]	0[a]	26	195	44[a]	45
<=PC80	1332	461[a]	48[a]	26	201	54[a]	52
ANT omissions							
(2, 0–95)							
>PC80	301	411[a]	31[a]	20	152[a]	41[a]	46
<=PC80	1341	468[a]	47[a]	28	206[a]	54[a]	51
Conner's ADHD index							
(6, 0–36)							
>PC80	329	419[a]	31[a]	21	163[a]	48	44[a]
<=PC80	1345	467[a]	47[a]	28	205[a]	53	52[a]

ANT, Attention Network Test; HRT-SE, hit reaction time standard error; CPRS-R: S, Revised Conners' Parent Rating Scale Short Form; ms, milliseconds.
[a]Wilcoxon rank-sum test *P*-value <0.05. PC80 = 80th percentile.
[b]Higher scores indicate worse attention performance or behavior

adjusted R^2 from 0.01 to 0.12. This indicates a moderate degree of variability added after these covariable adjustments.

Table 4 presents results from sensitivity analyses on the association between first trimester total seafood consumption and ANT omission errors. We observed that total seafood consumption during early pregnancy was more strongly associated with the outcome than the associations observed with seafood consumption during the third pregnancy trimester and with the child's intake at 5 years. Further adjustment for 5-year-old seafood intake or child cognitive functions at early ages (Bayley Scales at 1 year and McCarthy Scales at 5 years) did not change the early pregnancy intake association (Table 4). The results indicate that child characteristics did not mediate the prenatal association. However, child Bayley and McCarthy Scales were strongly associated with ANT omission errors (Supplementary Table 6, available as Supplementary data at *IJE* online).

Similarly, additional adjustments for cord mercury concentration, child gestation age, birthweight and breastfeeding duration, 5-year-visit maternal mental health and verbal IQ proxy, family urban vulnerability index and paternal occupation, as well as re-analyses using IPW correction (Supplementary Table 7, available as Supplementary data at *IJE* online), did not change the conclusions. Furthermore, we observed slightly stronger associations in Mediterranean regions (Sabadell and Valencia) than Atlantic regions (Asturias and Gipuzkoa), but no statistically proved interaction was observed (*P* for interaction = 0.651) (Supplementary Table 7, available as Supplementary data at *IJE* online). Finally, the regression coefficient associations between total seafood intake and the standardized mental Bayley Scale, cognitive McCarthy Scale and ANT HRT-SE were essentially similar in the three settings, with a moderate increase in the last setting (Supplementary Table 8, available as Supplementary data at *IJE* online).

Figure 1 presents the results of analyses testing for modification of the association between maternal total seafood consumption in pregnancy and offspring ANT scores by selected genotypes in PUFA-related SNPs. The interaction *P*-value for the effect modification of the child rs1260326 (glucokinase regulator, *GCKR*) on omission errors was 0.006, significant after Bonferroni correction (adjusted interaction *P*-value = 0.042) (Figure 1). At low levels of maternal seafood consumption, children with the CC genotype at rs1260326 (*GCKR*) had higher omission rate. On the other hand, both maternal and child SNPs at *ELOVL2* showed marginal interaction *P*-values for HRT-SE and/or omissions (Figure 1), this did not survive Bonferroni correction (adjusted interaction *P*-values ranged from 0.266 to 1) (Figure 1). At high levels of maternal seafood consumption, carriers of the G allele at rs2281591 (*ELOVL2*) or born from mothers with the T allele at rs3798719 (in linkage disequilibrium with rs2281591) had worse performance on ANT outcomes. Results of the other five SNPs, which did not show any effect modification, can be found in Supplementary Table 9, available as Supplementary data at *IJE* online, which includes the seven SNP interactions. There were no effect modifications with CPRS-R: S ADHD index as an outcome (data not shown). The SNP interactions with maternal total seafood intake, shown in Figure 1, were replicated with DHA and EPA as exposure variables, but the results were not replicated (Supplementary Table 10, available as Supplementary data at *IJE* online).

Table 2. Associations between maternal seafood consumption during first trimester and child ANT omission errors at 8 years (INMA Study, 2003–08)

Maternal first trimester seafood intake, quantiles (median[e])		ANT omission errors as a count variable[f]			
		Minimally adjusted[a]		Fully adjusted[b]	
	N	IRR	95% CI	IRR	95% CI
Total seafood					
Quintiles: 1 (median = 195 g/w)	321	1.00	Referent	1.00	Referent
2 (338 g/w)	328	1.02	0.83, 1.25	1.04	0.85, 1.27
3 (461 g/w)	314	0.79[d]	0.64, 0.97	0.82[c]	0.66, 1.01
4 (600 g/w)	334	0.84	0.68, 1.04	0.84	0.69, 1.04
5 (854 g/w)	315	0.76[d]	0.61, 0.94	0.76[d]	0.61, 0.94
P for trend	1612	0.003		0.002	
Large fatty fish					
Quartiles: 1 (none)	699	1.00	Referent	1.00	Referent
2 (48 g/w)	300	0.98	0.82, 1.18	1.01	0.84, 1.20
3 (92 g/w)	303	0.92	0.77, 1.11	0.92	0.77, 1.11
4 (238 g/w)	310	0.75[d]	0.62, 0.91	0.76[d]	0.63, 0.92
P for trend	1612	0.003		0.004	
Small fatty fish					
Quartiles: 1 (none)	745	1.00	Referent	1.00	Referent
2 (37 g/w)	284	0.80[d]	0.66, 0.97	0.83[c]	0.69, 1.01
3 (69 g/w)	301	0.82[d]	0.68, 0.98	0.81[d]	0.68, 0.97
4 (147 g/w)	282	0.77[d]	0.63, 0.92	0.79[d]	0.65, 0.95
P for trend	1612	0.003		0.005	
Lean fish					
Quartiles: 1 (52 g/w)	406	1.00	Referent	1.00	Referent
2 (148 g/w)	398	0.78[d]	0.65, 0.94	0.81[d]	0.67, 0.98
3 (261 g/w)	402	0.71[d]	0.59, 0.86	0.73[d]	0.60, 0.88
4 (413 g/w)	406	0.74[d]	0.61, 0.89	0.77[d]	0.64, 0.93
P for trend	1612	0.002		0.007	
Canned tuna					
Quartiles: 1 (0 g/w)	386	1.00	Referent	1.00	Referent
2 (37 g/w)	418	1.07	0.89, 1.29	1.09	0.91, 1.30
3 (72 g/w)	410	0.81[d]	0.67, 0.98	0.79[d]	0.66, 0.96
4 (167 g/w)	398	1.12	0.92, 1.35	1.09	0.90, 1.32
P for trend	1612	0.312		0.503	
Shellfish					
Quartiles: 1 (none)	383	1.00	Referent	1.00	Referent
2 (35 g/w)	393	1.09	0.90, 1.31	1.10	0.91, 1.33
3 (63 g/w)	414	0.91	0.75, 1.11	0.95	0.79, 1.15
4 (128 g/w)	422	0.97	0.79, 1.18	0.94	0.77, 1.15
P for trend	1612	0.503		0.338	

N, number of subjects with ANT test, total seafood intake and adjustment confounders available.

[a]Regression models adjusted for: gender of the child, age during testing, cohort and maternal energy (Kcal) intake during pregnancy.

[b]Regression models additionally adjusted for: maternal age, education level and occupation, prepregnancy body mass index, parity, country of origin/birth and Mediterranean diet score without seafood items during pregnancy.

[c]*P*-value <0.10.

[d]*P*-value <0.05.

[e]Median of seafood intake within quantile category.

[f]Higher scores indicate worse attention performance.

Discussion

We observed a longitudinal association between high seafood consumption during early pregnancy and improvements in most of the 8-year-old attention outcomes in the Spanish INMA cohort study. Total seafood, large fatty fish, lean fish and to a lesser degree small fatty fish, were similarly associated with the outcomes; however canned tuna and shellfish showed weaker associations.

Table 3. Associations between maternal seafood consumption during first trimester and child CPRS-R: S ADHD index at 8 years (INMA Study, 2003–08)

Maternal first trimester seafood intake quantiles (median[e])		CPRS-R: S ADHD index as a count variable[f]			
		Minimally adjusted[a]		Fully adjusted[b]	
	N	IRR	95% CI	IRR	95% CI
Total seafood					
Quintiles: 1 (median=195 g/w g)	323	1.00	Referent	1.00	Referent
2 (338 g/w)	336	1.01	0.88, 1.15	1.04	0.91, 1.19
3 (461 g/w)	322	0.86[d]	0.75, 0.99	0.91	0.79, 1.04
4 (600 g/w)	334	0.91	0.79, 1.04	0.94	0.82, 1.08
5 (854 g/w)	326	0.80[d]	0.70, 0.92	0.84[d]	0.73, 0.97
P for trend	1641	0.001		0.004	
Large fatty fish					
Quartiles: 1 (none)	708	1.00	Referent	1.00	Referent
2 (48 g/w g)	308	0.93	0.82, 1.05	0.97	0.86, 1.09
3 (92 g/w)	310	0.98	0.87, 1.11	1.00	0.88, 1.13
4 (238 g/w)	315	0.84[d]	0.74, 0.95	0.86[d]	0.76, 0.97
P for trend	1641	0.008		0.02	
Small fatty fish					
Quartiles: 1 (none)	757	1.00	Referent	1.00	Referent
2 (37 g/w)	288	1.08	0.95, 1.23	1.13[c]	0.99, 1.28
3 (69 g/w)	303	0.98	0.87, 1.11	1.00	0.89, 1.12
4 (147 g/w)	293	0.92	0.81, 1.04	0.94	0.83, 1.07
P for trend	1641	0.188		0.326	
Lean fish					
Quartiles: 1 (52 g/w)	414	1.00	Referent	1.00	Referent
2 (148 g/w)	409	0.91	0.80, 1.03	0.95	0.84, 1.07
3 (261 g/w)	405	0.80[d]	0.71, 0.91	0.86[d]	0.75, 0.97
4 (413 g/w)	413	0.84[d]	0.74, 0.95	0.89[c]	0.78, 1.01
P for trend	1641	0.002		0.034	
Canned tuna					
Quartiles: 1 (0 g/w)	391	1.00	Referent	1.00	Referent
2 (37 g/w)	423	0.97	0.85, 1.09	0.99	0.88, 1.12
3 (72 g/w)	417	0.99	0.88, 1.28	1.01	0.89, 1.14
4 (167 g/w)	410	0.93	0.82, 1.06	0.92	0.81, 1.05
P for trend	1641	0.319		0.180	
Shellfish					
Quartiles: 1 (none)	390	1.00	Referent	1.00	Referent
2 (35 g/w)	401	0.93	0.82, 1.05	0.94	0.83, 1.06
3 (63 g/w)	424	1.02	0.91, 1.16	1.04	0.92, 1.17
4 (128 g/w)	426	0.88[c]	0.78, 1.01	0.88[c]	0.78, 1.01
P for trend	1641	0.114		0.104	

N, number of subjects with CPRS-R: S test, total seafood intake and adjustment confounders available.

[a]Regression models adjusted for: gender of the child, age during testing, cohort and maternal energy (Kcal) intake during pregnancy.

[b]Regression models additionally adjusted for: maternal age, education level and occupation, prepregnancy body mass index, parity, country of origin/birth and Mediterranean diet score without seafood items during pregnancy.

[c]*P*-value <0.10.

[d]*P*-value <0.05.

[e]Median of seafood intake within quantile category.

[f]Higher scores indicate worse attention behaviour.

Adjustment for child total seafood consumption and child cognitive functions at young ages did not change the prenatal associations; indicating an independent association between maternal seafood consumption during early pregnancy and 8-year-old attention outcomes. Furthermore, PUFA-related genotypes in rs1260326 (*GCKR*) (*P*-for-interaction = 0.006) and rs2281591/ rs3798719 (*ELOVL2*) (*P*-for-interaction = 0.061/0.050)

Table 4. Adjusted[a] associations between seafood consumption and child ANT omission errors at 8 years (INMA Study, 2003–08): a sensitivity analysis based on maternal seafood intake during third trimester of pregnancy and child intake and additional adjustment for child 5-years-old intake, and 1- and 5-year-old general cognition

	ANT omission errors as a count variable[f]											
	N	IRR	95% CI	*N*	IRR	95% CI	*N*	IRR	95% CI	*N*	IRR	95% CI
Total seafood intake	**Maternal third-trimester total seafood intake**			**Child 5-year-old total sea food intake**			**Maternal first-trimester total seafood intake adjusted for child 5-year-old total seafood intake**			**Maternal first-trimester total seafood intake adjusted for 1- and 5-year-old general cognition[e]**		
Quintiles[b] 1	317	1	Referent	281	1	Referent	302	1	Referent	290	1	Referent
2	313	1.03	0.84, 1.27	289	0.92	0.74, 1.14	305	1.04	0.84, 1.29	308	0.98	0.80, 1.20
3	311	0.84	0.68, 1.04	307	0.88	0.70, 1.10	292	0.76[d]	0.61, 0.95	279	0.77[d]	0.62, 0.96
4	315	0.83[c]	0.67, 1.03	312	0.92	0.74, 1.16	301	0.85	0.68, 1.06	301	0.85	0.68, 1.04
5	317	0.88	0.71, 1.09	301	1.03	0.82, 1.30	290	0.75[d]	0.60, 0.94	278	0.73[d]	0.58, 0.91
P for trend	1573	0.102		1490	0.646		1490	0.002		1456	0.002	

N, number of subjects with ANT test, total seafood intake and adjustment confounders available. All the association patterns were unchanged when seafood subtypes were lean, small and large fatty fish (data not shown).

[a]Regression models adjusted for: gender of the child, age during testing, cohort, energy (Kcal) intake, maternal age, education level and occupation, prepregnancy BMI, parity, country of origin/birth and maternal and child Mediterranean diet score without seafood items at first or third pregnancy trimester or at child age 5 years.

[b]Median of total seafood intake within quintile (Q), g per week: maternal first trimester, Q1 = 195, Q2 = 338, Q3 = 461, Q4 = 600, Q5 = 854; maternal third trimester, Q1 = 196, Q2 = 337, Q3 = 455, Q4 = 585, Q5 = 829; child 5 years old, Q1 = 84, Q2 = 162, Q3 = 213, Q4 = 271, Q5 = 377.

[c]*P*-value <0.10.

[d]*P*-value <0.05.

[e]Bayley and McCarthy general cognitive scales. Similarly unchanged findings were observed when the model was additionally adjusted for 5-year-old ADHD-DSM-IV symptom scores (data not shown).

[f]Higher scores indicate worse attention performance.

polymorphisms modified the seafood-outcome associations. Finally, the overall findings were not changed after applying IPW corrections, in order to control for both selection bias and bias due to missing data.

The results here confirmed our previous findings.[7] High maternal seafood consumption, including large fatty fish, was associated with 8-year-old attention outcomes after adjusting for cord mercury concentration, probably due to a compensatory effect of higher DHA-EPA concentrations in fatty fish. The mercury levels in cord blood of this sample were 8.4 μg/L; 64% percent of the children exceeded the reference level established by the EPA.[6,15] However, the adjustment for mercury concentrations did not change the maternal seafood intake coefficients, as we would have expected due to potential negative confounding effect. Nevertheless, since the mercury exposure is positively correlated with seafood intake, there may be an attenuating effect on seafood coefficients by the amplifying effect of mercury exposure.[6,7]

These results are reported in a population sample from Spain, a country with one of the highest seafood consumption levels globally. This is relevant for public health, because it enables us to better investigate dose-response patterns and check for differential effects by seafood subtypes.[7] In relation to lean fish and large fatty fish, we observed similar association patterns with the attention outcomes. We would have expected less beneficial associations with large fatty fish, since large predatory fatty fish (i.e. tuna or swordfish) tend to accumulate more mercury levels.[15] One explanation for this finding may be the higher DHA levels normally found in fatty fish,[6,7] as we reported here. However, the main finding here is related to the theoretical framework of prenatal fetal programming and long-term neurodevelopment consequences. This concept could only be considered if a continuum of postnatal environment and behaviour is taken into account, as we did when the models were additionally adjusted for child seafood consumption and cognitive functions at previous ages.[9] In our previous study,[7] we did not fully develop this approach since the main measurements of child seafood consumption and child cognitive outcomes were cross-sectional.

The nutrient intake during pregnancy, as fish-related DHA, programmes the structure and the function of the highly developing brain, which has an impact on later neuropsychological development.[25] Alterations in this process could lead to attention dysfunction since mono-aminergic systems, which are highly related to attention functioning, have been found to be affected by DHA status.[4] Furthermore, in rodent experiments, authors described alterations of neurobiological pathways during a developmental time window equivalent to the human uterine period.[26] As in our previous study,[7] it seems that seafood

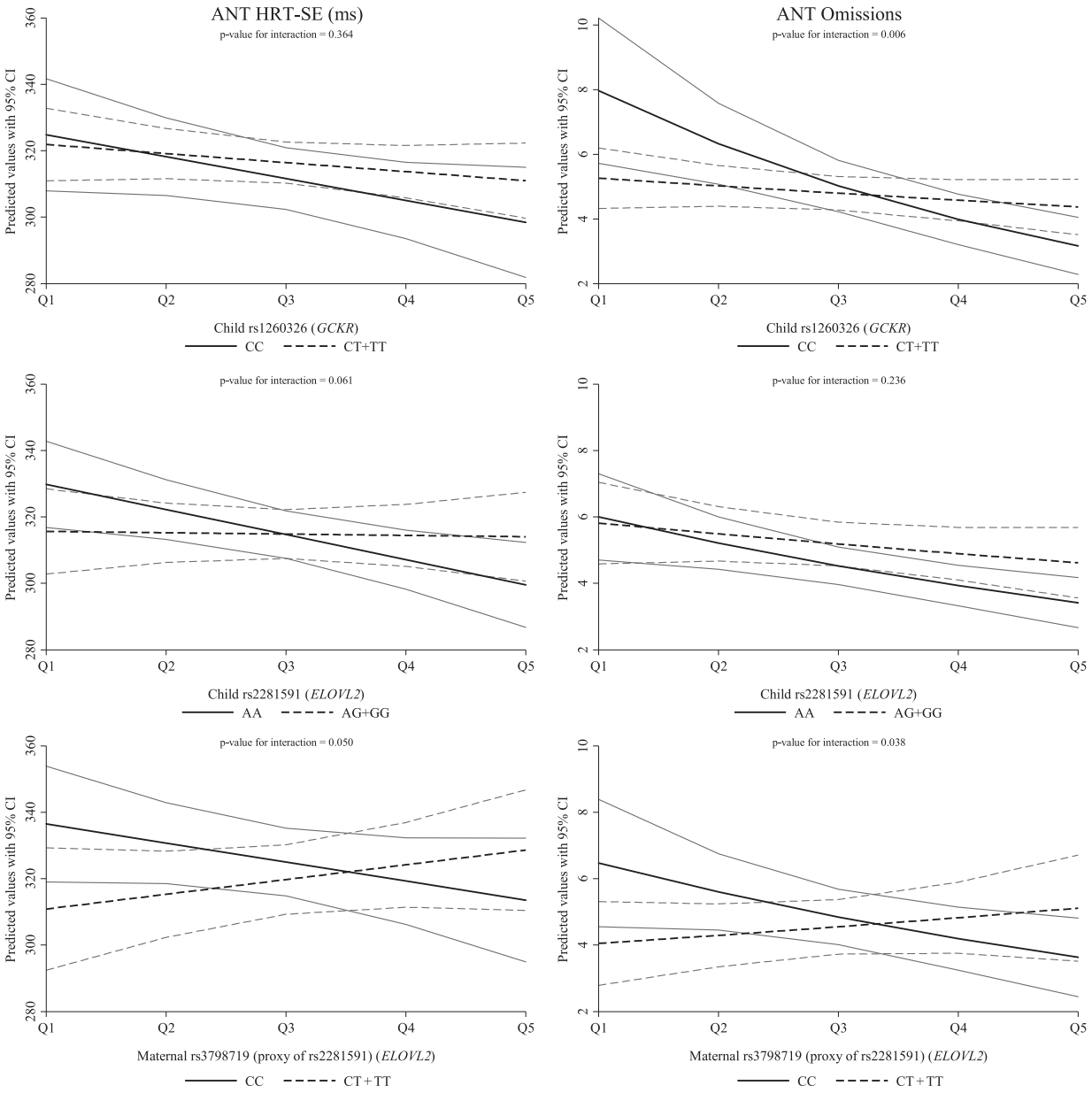

Figure 1. Adjusted[a] associations between maternal pregnancy first trimester seafood consumption and child Attention Network Test outcomes[b] by genotypes in polyunsaturated fatty acid-related single nucleotide polymorphisms (INMA Study, 2003–08). A total of seven SNPs were tested. Figure 1 only shows SNPs with *P*-values for interactions <0.10. The results for all the seven SNPs can be found in Supplementary Table 9, available as Supplementary data at *IJE* online. [a]All regression models adjusted for: gender of the child, age during testing, cohort and maternal energy (Kcal) intake during pregnancy, maternal age, education level and occupation, prepregnancy BMI, parity, country of origin/birth and Mediterranean diet score without seafood items during pregnancy. [b]Higher scores indicate worse attention performance.

consumption during early pregnancy is more important than intakes during late pregnancy, when some of the neurodevelopment processes would be less crucial at this time point.[2]

There is growing evidence that seafood consumption during pregnancy is beneficial to infant and child cognitive development.[7,27] However, few studies explored specifically attention phenotype,[1,6,28,29] which is a complex behaviour a child needs for learning and further development cognitively and socially.[17] Two previous studies reported a protective association between maternal seafood consumption during pregnancy and child ADHD symptoms at school age, assessed by Conner's Rating Scale–Teacher's Version and Strength and Difficulties Questionnaire (SDQ).[28,29] Indeed, a recent randomized clinical trial with DHA supplementation during pregnancy found children of the intervention group committed fewer omission errors than the placebo group in the Continuous Performance Test (CPT), a similar test to ANT.[30] This evidence is consistent with animal studies on early neurodevelopment,

linking DHA to functions of prefrontal cortex such as selective and sustained attention.[30] Our results are in the line with these previous findings, where we combined cognitive and behavioural-symptom attention outcomes in the same setting at school age. This period is considered to be the appropriate time window for attention assessments, as children rapidly develop in behaviour complexity, and it is commonly the start of ADHD symptom visibility.[8]

Seafood may enhance neurodevelopment by being the main source of DHA and EPA, which are essential nutrients for brain development.[3] Based on this hypothesis, we tested gene-environment interactions of 7 PUFA QTLs.[19] A recent cohort study with high seafood consumption reported *FADS* genes were associated with some PUFA levels in maternal serum during pregnancy, but they did not find any gene association with child cognitive scores.[31] However, they did not aim to analyse gene effect modifications with maternal seafood intake.[31] To our knowledge, no previous studies have explored gene-environment interactions with seafood consumption and child neurodevelopment. We found that child rs1260326 (*GCKR*) and child/maternal rs2281591/rs3798719 (*ELOVL2*) modified the seafood-outcome associations.

ELOVL2 enzyme controls the elongation of EPA (20: 5) to docosapentaenoic acid (DPA, 22: 5), and of DPA to tetracosapentaenoic (TPA, 24: 5), which after other modifications results in DHA. The individuals with the rs2281591 AA genotype in *ELOVL2* are better metabolizers, exhibiting higher levels of DHA[19,32,33] and lower levels of EPA[32] and DPA.[32,33] In our study, this genotype was associated with better attention scores only when there was a high supply of EPA and DPA (high fish consumption during pregnancy). Of note, SNPs in the *FADS1* and *FADS2* desaturases, which are the rate limiting step upstream EPA, did not modify the effect of fish intake.

GCKR, glucokinase regulator that inhibits glucokinase, plays an important role controlling hepatic triglyceride and glucose metabolism.[33] In our study, children with the rs1260326 CC genotype, which has been associated with lower levels of PUFA[19] and of DPA,[34] had worse attention scores at low fish maternal consumption. However, when their mothers had high fish consumption, these poor metabolizers recovered and improved their attention scores. A previous study among healthy Chinese adolescents found that a *GCKR* gene polymorphism in linkage disequilibrium with rs1260326 (rs780094) modified the effect of fish intake on plasma triglycerides levels.[35] Another study found an interaction between rs1260326 polymorphism and plasma n-3 PUFA levels modulating insulin resistance and inflammatory markers in metabolic syndrome subjects.[36]

The combination of previously published and present findings, including the gene-exposure interaction, is suggestive that pregnancy seafood consumption may improve long-term child attention functioning through increasing DHA supply to the fetus. To disentangle whether the effect on DHA synthesis is driven by maternal or by fetal genotypes would require a bigger sample size. However, some studies in humans and rats suggest that the fetus is able to synthesize PUFA.[37] Moreover, when we explored whether the associations between cord plasma levels of DHA and EPA and attention outcomes were modified by PUFA metabolizing SNPs, we detected null results, probably due to the smaller sample size of the biomarker measurements. Besides PUFA, other nutrients present in the fish and not studied here, such as selenium, iodine and vitamin D, could also be beneficial for neurodevelopment.[6,7,38–41]

This longitudinal study has several strengths, such as the focus on several aspects of a behavioural construct such as attention functions and ADHD symptoms. Furthermore, the use of seafood subtypes, as in our previous study, was still relatively unexplored due to a lower seafood intake reported in other studies.[1,6,7] Our results were robust across multiple sensitivity analyses recommended by recent reviews in the field,[1,6] such as controlling for cord mercury levels, child seafood intake, breastfeeding duration, maternal mental health and IQ proxies, and further corrections by IPW. The adjustments for child cognitive functions at early ages provide support for the hypothesis related to fetal prenatal programming. Finally, the use of gene-environment interaction methods gives further insights of potential biological pathways in nutritional epidemiology, a specific angle of research not yet deeply explored.[42]

The use of an FFQ, despite being a validated tool, is subjected to measurement errors that may lead to the attenuation of the effect estimate. During the pregnancy study period, there were no specific Spanish guidelines for seafood intake during pregnancy.[43] We did not expect changes in the consumption levels of the pregnant women in this study, nor in the implications for the observed associations. Beside the study availability of many confounders, residual confounding is a structural problem in epidemiological studies, particularly with complex neuropsychological outcomes, which are environmentally dependent. The fact of observing a moderate increase in the proportion of variability, after adding the study confounders to the main models, may be indicative of important factors have been controlled. Since most of the gene-environment *P*-values did not pass the strict Bonferroni corrections, more studies with larger sample sizes are needed to further confirm PUFA involvement in the seafood-neurodevelopment association.

Future research should look at pregnancy seafood subtype consumptions and adolescent neurodevelopment; and investigate whether these associations are statistically confirmed by direct pathways in other world regions, since seafood methylmercury levels are regionally different.[44]

In conclusion, high maternal consumption of seafood during early pregnancy was associated with improvements in 8-year-old offspring's attention function and ADHD symptoms. Lean and fatty seafoods, large and small, are associated with this important outcome related to a successful cognitive and social development. Our results support the fetal programming framework, since we could take into consideration the role of the previous child nutritional and cognitive status. The effect modification of rs1260326 (*GCKR*) and rs2281591/rs3798719 (*ELOVL2*) polymorphisms, both SNPs related with PUFA levels, suggest that PUFA may mediate part of the association between maternal seafood consumption in pregnancy and offspring neurodevelopment.

Supplementary Data

Supplementary data are available at *IJE* online.

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