EUROPEAN RESPIRATORY *journal*

FLAGSHIP SCIENTIFIC JOURNAL OF ERS

Early View

Original article

Intake of n-3 polyunsaturated fatty acids in childhood, *FADS* genotype, and incident asthma

Mohammad Talaei, Emmanouela Sdona, Philip C. Calder, Louise R. Jones, Pauline M. Emmett, Raquel Granell, Anna Bergström, Erik Melén, Seif O. Shaheen

Please cite this article as: Talaei M, Sdona E, Calder PC, *et al*. Intake of n-3 polyunsaturated fatty acids in childhood, *FADS* genotype, and incident asthma. *Eur Respir J* 2021; in press (https://doi.org/10.1183/13993003.03633-2020).

Intake of n-3 polyunsaturated fatty acids in childhood, *FADS* genotype, and incident asthma

Mohammad Talaei[1], Emmanouela Sdona[2], Philip C. Calder[3,4], Louise R. Jones[5], Pauline M. Emmett[5], Raquel Granell[6], Anna Bergström[2,7], Erik Melén[2,8,9], Seif O. Shaheen[1]

[1] Institute of Population Health Sciences, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London, UK

[2] Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden

[3] Human Development and Health, Faculty of Medicine, University of Southampton, Southampton, UK

[4] NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust and University of Southampton, Southampton, UK

[5] Centre for Academic Child Health, Population Health Sciences, Bristol Medical School, University of Bristol, BS8 1NU Bristol, UK

[6] MRC Integrative Epidemiology Unit (IEU), Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, UK

[7] Centre for Occupational and Environmental Medicine, Region Stockholm, Stockholm, Sweden

[8] Department of Clinical Science and Education, Södersjukhuset, Karolinska Institute, Stockholm, Sweden

[9] Sachs' Children and Youth Hospital, Södersjukhuset, Stockholm, Sweden

Names for PubMed Index: Talaei M, Sdona E, Calder PC, Jones LR, Emmett PM, Granell R, Bergström A, Melén E, Shaheen SO

Correspondence to: Dr Mohammad Talaei, Institute of Population Health Sciences, Barts and The London School of Medicine and Dentistry, 58 Turner Street, London, E1 2AB, UK. E-mail: mohammad.talaei@u.nus.edu; Tel: +44(0)20 7882 2499

Running title: Omega-3 intake from fish and childhood asthma

Word Count (body): 3692, Tables: 4, Figures: 2, Online supplemental materials: Yes.

Take home:

In children with a common fatty acid desaturase (*FADS)* variant, higher intake of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) from fish in mid-childhood was strongly associated with a lower risk of incident asthma up to mid-adolescence.

Abstract

Longitudinal evidence on the relation between dietary intake of n-3 (omega-3) very long-chain polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), in mid-childhood and asthma risk is scarce. We aimed to investigate whether a higher intake of EPA and DHA from fish in childhood is associated with a lower risk of incident asthma.

In the Avon Longitudinal Study of Parents and Children, dietary intakes of EPA and DHA from fish were estimated by food frequency questionnaire at 7 years of age. We used logistic regression, controlling for confounders, to analyze associations between intake of EPA and DHA (quartiles) and incidence of doctor-diagnosed asthma at age 11 or 14 years, and explored potential effect modification by a fatty acid desaturase (*FADS*) polymorphism (rs1535). Replication was sought in the Swedish BAMSE birth cohort.

There was no evidence of association between intake of EPA plus DHA from fish and incident asthma overall (n=4,543). However, when stratified by *FADS* genotype, the odds ratio (95% confidence interval) comparing top versus bottom quartile amongst the 2,025 minor G allele carriers was 0.49 (0.31-0.79) (P-trend 0.006), but no inverse association was observed in the homozygous major A allele group (odds ratio 1.43, 95% confidence interval 0.83-2.46, P-trend 0.19) (P-interaction 0.006). This gene-nutrient interaction on incident asthma was replicated in BAMSE.

In children with a common *FADS* variant, higher intake of EPA and DHA from fish in childhood was strongly associated with a lower risk of incident asthma up to mid-adolescence.

Keywords: eicosapentaenoic acid, docosahexaenoic acid, asthma, childhood, fatty acid desaturases

Introduction

A substantial body of epidemiological evidence has implicated diet early in the life course in the aetiology of asthma and other allergic diseases. However, most evidence in children comes from cross-sectional studies, thus limiting causal inference [1-3]. Fish intake has attracted particular interest, as fish is a rich source of the n-3 (omega-3) very long-chain polyunsaturated fatty acids (VLC-PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have anti-inflammatory effects [4-6]. Other nutrients in fish, such as vitamin D and selenium, may also protect against asthma risk [7].

The few longitudinal studies investigating associations between fish and n-3 VLC-PUFA intake and asthma and allergic diseases have focused on exposures during pregnancy or infancy [8]; one birth cohort reported no association between fish intake in mid-childhood and subsequent asthma, but did not investigate associations with n-3 VLC-PUFA intake [9]. In the Avon Longitudinal Study of Parents and Children (ALSPAC), 25% of children either developed new asthma or had asthma that remitted or persisted between 7 and 14 years of age [10]; dietary intake of fish and n-3 VLC-PUFA in childhood could play an important role in influencing asthma risk at this stage of life. Moreover, any beneficial effects of these exposures on asthma might be most apparent in certain subgroups. Endogenous production of VLC-PUFA depends on the efficiency of conversion of precursor fatty acids by fatty acid desaturase (FADS) [5]. The minor G allele of a *FADS* single-nucleotide polymorphism (SNP), rs1535, predicts lower plasma EPA and DHA concentrations in a meta-analysis of genome-wide association studies [11], and in mothers taking part in a trial of fish oil supplementation in pregnancy [12]. In that trial, a beneficial effect of supplementation on the offspring's risk of asthma was greatest in children of mothers who carried the G allele [12], suggesting that exogenous supply of preformed EPA and

DHA (e.g. from fish or from fish oil supplements) is necessary to achieve both a high status, and the health benefits, of EPA and DHA in individuals with the G allele of this *FADS* SNP.

In this study, we have investigated the associations of intake of fish and n-3 VLC-PUFA from fish at 7 years of age with incident asthma up to mid-adolescence. We have also explored whether these associations were modified by child's *FADS* genotype, in order to strengthen causal inference.

Methods

Study Population

ALSPAC is a population-based birth cohort that recruited predominantly white pregnant women resident in Avon, UK (14,541 pregnancies) with expected dates of delivery from April 1, 1991 to December 31, 1992. The cohort has been followed since birth with annual questionnaires and, since age 7 years, with objective measures in annual research clinics. The study protocol has been described previously [13, 14] and further information can be found at www.alspac.bris.ac.uk, which contains details of all the data that are available (http://www.bristol.ac.uk/alspac/researchers/our-data/). Ethics approval was obtained from the ALSPAC Ethics and Law Committee (IRB 00003312) and the Local National Health Service Research Ethics Committees. Informed consent for the use of data collected via questionnaires and clinics was obtained from participants following the recommendations of the ALSPAC Ethics and Law Committee at the time.

Exposure assessment

We used dietary information collected by food frequency questionnaire (FFQ) at 54 months (~4 years) and 81 months (~7 years) of age, which was completed by the child's mother or the main carer. The FFQ included questions about usual consumption of up to 56 food groups and 12 drinks, with five frequency options ranging from 'never or rarely' to 'more than once a day' [15]. Fish intake was covered by five items: shellfish, white fish in breadcrumbs or batter, white fish without coating, tuna, and oily fish (details in online supplemental materials). Total energy and nutrient intakes were calculated by multiplying estimated food intake (g/day) by their estimated nutrient content from UK food composition tables [16, 17] and summing this across all the foods consumed. Fatty acid composition of fish was based on profiles of typical British species [16]. Accordingly, daily intakes of EPA and DHA from fish, total n-6 fatty acids, and arachidonic acid (an n-6 PUFA which also depends on FADS for endogenous production) were estimated. Maternal intake of EPA and DHA at 32 weeks of gestational age was also estimated similarly by FFQ.

Outcome assessment

Our primary outcome of interest was incident asthma. At 91 months (~7.5 years), 128 months (~11 years), and 166 months (~14 years) of age, we defined current doctor-diagnosed asthma if mothers responded positively to the question "Has a doctor ever actually said that your study child has asthma?", and to at least one of the concurrent following questions which asked if the child had had wheezing, wheezing and whistling in the chest, asthma, or asthma medication in the last 12 months. Among those children who were not identified as having current doctor-diagnosed asthma at 7.5 years, we defined those with current doctor-diagnosed asthma at 11 or 14 years as cases of incident asthma. Further details about secondary outcomes are available in the online supplemental materials.

Genotyping

Among 20 SNPs related to n-3 metabolism in the literature, we selected a SNP in the fatty acid desaturase (*FADS2*) gene, rs1535, as our main candidate variant because of prior strong evidence that it predicts blood levels of EPA and DHA [11] and also modifies the effect of fish oil supplementation [12]. It was imputed with 0.999 imputation quality using the 1000 genomes reference panel (Phase 1, Version 3) (See online supplemental materials for further details). Participants with genetic evidence of non- European ancestry were excluded before imputation.

Statistical analysis

Among 8,140 children with dietary data available at 7 years, data were complete on incident asthma for 4,543 (see **Figure 1** in online supplemental materials). We used logistic regression to examine associations of intakes of fish, and EPA and DHA from fish (in quartiles), with incident asthma using the lowest quartile of intake as the reference category. Linear trend was tested by including median intake of quartiles as a pseudo-continuous variable in the models. We selected known potential confounding factors from the existing literature [18] and then refined our selection by using a directed acyclic graph approach [19] (**Figure 2**). Details of multivariable models and covariates are explained in the online supplemental materials.

We carried out stratified analyses, *a priori*, to explore potential modification of dietary associations by *FADS* genotype [rs1535: major A allele homozygous (AA) vs. heterozygotes plus homozygous for minor G allele (GA/GG); the latter two genotypes were combined for analysis because the number of GG individuals was small: n=393 (10.8% of total) with only 29 cases of incident asthma]. Distribution of allele frequencies for rs1535 was tested for deviation from Hardy-Weinberg equilibrium using a likelihood ratio test (P= 0.26). Potential interactions

were assessed by testing the cross-product term of *FADS* genotype with quartiles (median values) as a continuous variable in regression model.

As *FADS* genes are also involved in the n-6 pathway, leading to the production of arachidonic acid with pro-inflammatory effects, we also explored interactions between intake of total n-6 and arachidonic acid and rs1535 on asthma. We also assessed the relationship of cumulative exposure to EPA plus DHA, defined as being consistently in the top or bottom quartiles of intake at 4 and 7 years of age, with incident asthma. Dietary information at 4 years was not considered as a primary exposure because a lack of asthma diagnosis at this young age meant that subsequent incident asthma could not be defined. We also explored the interaction between maternal intake of EPA plus DHA during pregnancy and maternal *FADS* genotype on incident asthma at 11 or 14 years. Finally, we carried out several sensitivity analyses including further adjustments, exclusions, restricted cubic spline analysis to further examine the dose–response relationship, and inverse probability weighting to correct for potential loss to follow-up bias [20] (see online supplemental materials for further details). All statistical analyses were carried out using Stata version 14.2 (StataCorp, College Station, TX, USA).

Replication cohort

We used the Swedish population-based birth cohort study, BAMSE (Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology), to replicate the main ALSPAC findings. 4,089 infants, born 1994-1996 in Stockholm, were enrolled and have been followed repeatedly [21, 22]. At the 8-year-clinical examination parents were asked to fill in a FFQ containing questions about 98 foods and beverages frequently consumed in Sweden, including six questions on fish intake [23]. Intakes of EPA and DHA from fish were estimated using composition values obtained from the Swedish National Food Administration Database [24]. At

age 8, 12 and 16 years, we defined current doctor-diagnosed asthma, very similarly to ALSPAC, and accordingly cases of incident asthma were determined at 12 or 16 years (n=2,138). We used a similar analytic approach to that used in ALSPAC (Further details in the online supplemental materials).

Results

Median (interquartile range) intake of fish in ALSPAC was estimated as 24.3 (11.1-38.6) g/d, and mainly comprised white fish (on average 74.6% of total fish intake), followed by tuna (18.4%). Intakes of EPA and DHA from fish were 11.2 (5.99-24.0) and 17.6 (9.77-41.4) mg/d, respectively, and were very highly correlated (r>0.95), so we focused our main analyses on combined intakes of EPA plus DHA. Table 1 shows characteristics of children and their mothers across quartiles of child's EPA plus DHA intake. Children with higher intakes of EPA plus DHA from fish at 7 years of age were more likely to be female, had higher total energy intake, a generally more health-conscious dietary pattern, and higher supplement use. These children were also more likely to have been exclusively breast-fed by the third month of life, to have consumed fish before 6 months, and to have a history of food allergy by 7 years of age. Mothers of children who had higher intakes of EPA plus DHA from fish were older, more educated, less likely to live in council rented houses, and had a higher intake of EPA plus DHA from fish during late pregnancy. Among children with data on fish intake, 3,370 (56.3%) carried the minor allele of the *FADS* genotype (rs1535). There was no evidence of a difference in background characteristics between AA and GA/GG genotype groups except for a higher tendency to the health-conscious dietary pattern in the AA group (supplementary **Table E1**).

Fish intake

We did not find any evidence of association between fish intake at 7 years and incident asthma at 11 or 14 years (n=393) in the whole study sample (n=4,543). However, when stratified by *FADS* genotype, a higher intake of fish was associated with a lower risk of incident asthma in the GA/GG group, but not in the AA group (**Table 2**). The inverse association in the minor allele group was substantially attenuated when we further adjusted for intake of EPA plus DHA (adjusted OR, comparing top versus bottom quartile, 0.86, 95% CI 0.48-1.54, P-trend 0.57), but not when adjusted for intake of vitamin D (OR 0.66, 95% CI 0.41-1.05, P-trend 0.08) or selenium (OR 0.61, 95% CI 0.37-0.99, P-trend 0.05).

EPA and DHA intake from fish

Intakes of EPA plus DHA from fish were not significantly associated with risk of incident asthma overall. However, when we stratified by *FADS* genotype, strong inverse associations were observed in the GA/GG group, with evidence of a dose-response, but not in the AA group (P-interaction 0.006) (**Table 3** and supplementary **Figure E2**). In the GA/GG group, the proportion of children developing new asthma was 11.4% in the bottom quartile of EPA plus DHA intake, and 6.6% in the top quartile. Cumulative exposure at 4 and 7 years (correlation r=0.46) showed a stronger association: the OR (95% CI), comparing those who had high intake at both time points with those who had consistently low intake, was 0.33 (0.15-0.70) in the GA/GG group and 1.19 (0.52-2.71) in AA group (P for interaction = 0.02).

Intakes of total n-6 or arachidonic acid were not associated with incident asthma, either overall, or when stratified by *FADS* genotype (supplementary **Table E2**). Rs1535 was not associated with incident asthma (OR per minor G allele 0.91, 95% CI 0.76-1.09). We observed no evidence of associations between intakes of fish or EPA plus DHA from fish at 7 years, and

547 (12.7%) cases of incident eczema or 933 (19.6%) cases of incident hay fever at 11 or 14 years, either overall or when stratified by *FADS* genotype (data not shown).

Sensitivity analyses

Associations with incident asthma, especially those amongst *FADS* minor allele carriers, did not materially change with further adjustment for age at first exposure to fish, health-conscious dietary pattern, any supplement use, BMI at 7 years or 14 years, or genetic markers derived by principal component analysis, nor after exclusion of 59 (1.3%) children of non-white mothers, 749 (16.5%) children with a history of food allergy, 390 (8.6%) with extreme energy intakes, 98 (2.2%) with wheeze at 7 years, and 16 (0.3%) users of fish liver oil or omega-3 supplements (supplementary **Table E3**). The same pattern of associations was observed with EPA and DHA intakes separately (supplementary **Tables E4** and **E5**). Restricted cubic spline analysis showed a non-linear association in carriers of the minor G allele (P for nonlinearity 0.04) but not in the AA group (P for nonlinearity 0.11) or overall (P for nonlinearity 0.50; see **Figure 2**). Furthermore, the main findings did not materially change when we used inverse probability weighting, or energy-adjusted EPA and DHA intakes by the residual method (supplementary **Table E6**).

Maternal intake of EPA plus DHA from fish during pregnancy was weakly associated with a lower risk of incident asthma at 11 or 14 years (adjusted OR comparing top quartile versus bottom quartile 0.69, 95% CI 0.48-0.98); however, there was no evidence of effect modification by *FADS* genotype (supplementary **Table E7**). Importantly, the association between child's intake of EPA plus DHA at 7 years and incident asthma was independent of maternal intake in pregnancy (supplementary **Table E5**).

Finally, we tested all other SNPs as a *post hoc* analysis and found 12 SNPs in strong linkage disequilibrium (10 SNPs with rs1535 and 2 SNPs with rs3734398), thus yielding identical findings (data not shown). We did not find evidence of significant effect modification by the other 7 SNPs, although in line with rs1535, there was weak evidence of an inverse association between intake of EPA plus DHA from fish and incident asthma in carriers of the minor allele for some SNPs (supplementary **Table E8**).

Replication analyses

The characteristics of BAMSE study participants (n=2,138) are summarized in supplementary **Table E9**. Total intake of fish was lower in BAMSE children compared to ALSPAC children, but EPA and DHA intakes from fish were substantially higher in BAMSE as a result of higher oily fish intake (supplementary **Table E10).** In the BAMSE cohort we sought to replicate the n-3 VLC-PUFA-*FADS* interaction on incident asthma, and confirmed that the association between mid-childhood intake of EPA plus DHA and incident asthma was modified by *FADS* genotype (rs1535), with similar effect estimates to those seen in ALSPAC, amongst 1,187 (62.0%) carriers of the minor G allele (P interaction 0.03) (**Table 4**). Similar interactions were also confirmed when we analysed intakes of EPA and DHA separately (supplementary **Table E11**).

Discussion

In more than half of ALSPAC children, who were carrying the minor G allele of a *FADS* polymorphism (rs1535), we found strong inverse associations between intake of fish, and EPA and DHA from fish, in mid-childhood and incident asthma. Replication of these gene-nutrient

interactions on incident asthma in the BAMSE birth cohort confirmed that the main findings are unlikely to have arisen by chance. To our knowledge, these are novel findings which were robust to various sensitivity analyses.

The overall relation between fish intake in mid-childhood and incident asthma has only been investigated in one previous study, namely the BAMSE cohort; in keeping with our findings, no association was observed in that study either [9]. However, we found weak evidence that, in carriers of the minor *FADS* allele, higher fish intake was associated with a lower risk of asthma, which has not been reported before. Whilst fish intake during mid-childhood could potentially reflect similar familial dietary habits earlier in the life course, the findings of our study were unlikely to be confounded by maternal intake of n-3 VLC-PUFA from fish during pregnancy, or by early exposure to fish in infancy.

The inverse association between fish intake and incident asthma in carriers of the minor G allele was largely explained by EPA and DHA. Longitudinal data on the link between dietary intake of EPA and DHA in childhood and incident asthma are lacking. Whilst no previous study has reported effect modification of the association between dietary intake of n-3 VLC-PUFA and asthma by *FADS* genotype in childhood, in a recent randomized trial, the protective effect of fish oil supplementation in pregnancy on risk of early childhood asthma was modified by the same *FADS* gene variant in mothers [12]. Our findings extend those observations and suggest that there may be potential to prevent late childhood onset asthma in some individuals. In contrast to ALSPAC, the inverse associations seen in the BAMSE cohort as a whole may reflect the relatively higher n-3 VLC-PUFA intake and the higher *FADS* minor allele frequency (62% vs 56%).

We showed a stronger association when we compared consistently high versus consistently low intake at 4 and 7 years. This could reflect either the beneficial effect of more prolonged exposure, or reduced exposure misclassification by using dietary data at two time points. Nonetheless, it strengthens the case for a causal association. An important potential concern is reverse causation bias arising from disease-related modification of diet, especially in children with food allergy. However, when we excluded children with any history of food allergy our main findings did not materially change.

Mechanisms

EPA and DHA can modulate inflammatory processes through various pathways, such as increasing mediators that are less pro-inflammatory, anti-inflammatory, or inflammation resolving [4, 25]. Whilst plasma concentrations of n-3 VLC-PUFA at 8 years were inversely associated with incident asthma previously in BAMSE [26], use of biomarkers cannot differentiate between extrinsic (dietary) and intrinsic sources. The aforementioned fish-oil supplement RCT in pregnant women also found effects only for offspring asthma, and not for other allergy-related disorders, which suggests that the anti-inflammatory mechanisms may be confined to the airways [12]. The main endogenous source of n-3 VLC-PUFA is through a pathway mainly regulated by desaturase enzymes encoded by the *FADS* gene, which converts the plant-derived n-3 PUFA precursor, alpha-linolenic acid (ALA), to EPA and then DHA. Carriers of the minor allele of rs1535 (a representative SNP in *FADS2*) have a lower ALA-to-EPA conversion rate. They therefore tend to have lower blood concentrations of EPA and DHA [11] and are thus more dependent on dietary sources. This is likely to explain why a higher dietary intake of EPA and DHA was associated with a lower risk of incident asthma in this genetic sub-group. Of note, whilst *FADS2* influences both n-3 and n-6 PUFA pathways, the effect

modification we observed was specific to intake of EPA and DHA, not n-6 fatty acids or arachidonic acid, further strengthening causal inference.

Strengths and limitations

Strengths of the ALSPAC birth cohort include its population-based prospective design, large size, rich information on diet (at multiple time points) and potential confounders, and availability of the *FADS* genotype data. The detailed, repeated, phenotypic outcome measurements provided an opportunity to study incident rather than prevalent cases. As with any observational study, the possibility of unmeasured or residual confounding cannot be ruled out, although we controlled for numerous potential confounders in the analyses. We could not examine longitudinal associations with atopy and atopic asthma, because skin prick testing was only done at 7 years of age. A proportion of eligible children at 7 years (25.6%) were not included in our analyses due to lack of information on asthma status at any time point later. However, loss to follow-up bias has been shown to only slightly modify associations in longitudinal studies [27], and our inverse probability weighting analysis confirmed that it is unlikely to have biased our results. Although the FFQ that we used had not been formally calibrated against other instruments such as diet diaries, it was based on the one used by Yarnell et al. [28], which has been validated against weighed dietary records, and updated in the light of a local weighed dietary survey [29]. Whilst there is likely to have been some misclassification of the dietary exposures, especially as the FFQ lacked quantitative information on portion sizes, the interaction between n-3 VLC-PUFA intake and *FADS* genotype argues against substantial exposure misclassification. Furthermore, any such misclassification is likely to have been random with respect to asthma, which would tend to bias effect estimates towards the null. In this context, the replication of our findings in an independent cohort study (BAMSE) is strengthened by the fact that preferred fish species and

preparation methods differ between the two countries. However, it would be premature to give clear recommendations regarding the absolute intake of n-3 VLC-PUFA needed to achieve maximum benefit in terms of asthma risk given the semi-quantitative nature of the FFQs, the differences in estimated n-3 intake between the ALSPAC and BAMSE populations, and more importantly the inherent limitations of observational studies in establishing causality.

Current recommendations in the UK are to consume two servings of fish per week (equivalent to 280 g/w for an adult), one of which should be of oily fish [30]. In the last decade, fish consumption in children has slightly increased in the UK [31]; however, only 4.2% of children are achieving the recommended intake [30]. If our findings are causal, this might ultimately lead to a strategy of personalized primary prevention in a large subgroup of the population, according to genotype. In the meantime, public health messages to increase intake of fish should be heeded.

Conclusions

Although the evidence for an association overall was lacking in ALSPAC, we have replicated the finding that in children with a common *FADS* gene variant associated with poorer endogenous synthesis of n-3 VLC-PUFA, a higher intake of EPA and DHA from fish in childhood was associated with a lower risk of incident asthma.

Acknowledgments

We are extremely grateful to all the families who took part in this study, the midwives for their help in recruiting them, and the whole Avon Longitudinal Study of Parents and Children team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists, and nurses. We also thank the children and parents participating in the BAMSE cohort and all staff involved in the study through the years. We would also like to thank Annabelle Bédard for her assistance at the beginning of this project. This paper is the work of the authors, and SOS will serve as guarantor for its contents. SOS had full access to all the data in the study and had final responsibility for the decision to submit for publication. This paper is dedicated to the memory of our late colleague John Henderson.

Conflict of interest: None declared

Author Contributions: MT and SOS conceived the study; MT performed the statistical analyses; MT drafted the manuscript with SOS; PCC, LRJ, and PME advised on dietary and nutritional aspects; RG advised on asthma variables; ES, AB, and EM performed the replication study; all authors assisted in interpreting the data and critically edited the manuscript. All authors have seen and approved the final version of the manuscript.

Funding: This project and Mohammad Talaei were funded by the Rosetrees Trust and The Bloom Foundation (Grant ref: M771). The UK Medical Research Council and the Wellcome Trust (Grant ref: 217065/Z/19/Z) and the University of Bristol provide core support for ALSPAC. This publication is the work of the authors and Raquel Granell and Pauline Emmett will serve as guarantors for the contents of this paper. GWAS data were generated by Sample Logistics and

Genotyping Facilities at Wellcome Sanger Institute and LabCorp (Laboratory Corporation of America) using support from 23andMe. A comprehensive list of grants funding is available on the ALSPAC website (http://www.bristol.ac.uk/alspac/external/documents/grant-acknowledgements.pdf). The BAMSE study was supported by grants from the Swedish Heart-Lung Foundation, the Swedish Research Council, Region Stockholm (ALF project and database management), and the Swedish Research Council for Health, Working Life and Welfare, Formas and the Swedish Asthma and Allergy Research Foundation. EM is supported by an ERC grant (TRIBAL, ref 757919).

References

1. Guilleminault L, Williams EJ, Scott HA, Berthon BS, Jensen M, Wood LG. Diet and Asthma: Is It Time to Adapt Our Message? *Nutrients* 2017: 9(11).
2. Julia V, Macia L, Dombrowicz D. The impact of diet on asthma and allergic diseases. *Nat Rev Immunol* 2015: 15(5): 308-322.
3. Garcia-Larsen V, Ierodiakonou D, Jarrold K, Cunha S, Chivinge J, Robinson Z, Geoghegan N, Ruparelia A, Devani P, Trivella M, Leonardi-Bee J, Boyle RJ. Diet during pregnancy and infancy and risk of allergic or autoimmune disease: A systematic review and meta-analysis. *PLoS Med* 2018: 15(2): e1002507.
4. Miles EA, Calder PC. Can Early Omega-3 Fatty Acid Exposure Reduce Risk of Childhood Allergic Disease? *Nutrients* 2017: 9(7).
5. Minihane AM. Impact of Genotype on EPA and DHA Status and Responsiveness to Increased Intakes. *Nutrients* 2016: 8(3): 123.
6. Willemsen LEM. Dietary n-3 long chain polyunsaturated fatty acids in allergy prevention and asthma treatment. *Eur J Pharmacol* 2016: 785: 174-186.
7. Nurmatov U, Devereux G, Sheikh A. Nutrients and foods for the primary prevention of asthma and allergy: systematic review and meta-analysis. *J Allergy Clin Immunol* 2011: 127(3): 724-733 e721-730.
8. Zhang GQ, Liu B, Li J, Luo CQ, Zhang Q, Chen JL, Sinha A, Li ZY. Fish intake during pregnancy or infancy and allergic outcomes in children: A systematic review and meta-analysis. *Pediatr Allergy Immunol* 2017: 28(2): 152-161.
9. Magnusson J, Kull I, Rosenlund H, Hakansson N, Wolk A, Melen E, Wickman M, Bergstrom A. Fish consumption in infancy and development of allergic disease up to age 12 y. *Am J Clin Nutr* 2013: 97(6): 1324-1330.
10. Granell R, Henderson AJ, Sterne JA. Associations of wheezing phenotypes with late asthma outcomes in the Avon Longitudinal Study of Parents and Children: A population-based birth cohort. *J Allergy Clin Immunol* 2016: 138(4): 1060-1070 e1011.
11. Lemaitre RN, Tanaka T, Tang W, Manichaikul A, Foy M, Kabagambe EK, Nettleton JA, King IB, Weng LC, Bhattacharya S, Bandinelli S, Bis JC, Rich SS, Jacobs DR, Jr., Cherubini A, McKnight B, Liang S, Gu X, Rice K, Laurie CC, Lumley T, Browning BL, Psaty BM, Chen YD,

Friedlander Y, Djousse L, Wu JH, Siscovick DS, Uitterlinden AG, Arnett DK, Ferrucci L, Fornage M, Tsai MY, Mozaffarian D, Steffen LM. Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genet* 2011: 7(7): e1002193.

12. Bisgaard H, Stokholm J, Chawes BL, Vissing NH, Bjarnadottir E, Schoos AM, Wolsk HM, Pedersen TM, Vinding RK, Thorsteinsdottir S, Folsgaard NV, Fink NR, Thorsen J, Pedersen AG, Waage J, Rasmussen MA, Stark KD, Olsen SF, Bonnelykke K. Fish Oil-Derived Fatty Acids in Pregnancy and Wheeze and Asthma in Offspring. *N Engl J Med* 2016: 375(26): 2530-2539.

13. Boyd A, Golding J, Macleod J, Lawlor DA, Fraser A, Henderson J, Molloy L, Ness A, Ring S, Davey Smith G. Cohort Profile: the 'children of the 90s'--the index offspring of the Avon Longitudinal Study of Parents and Children. *Int J Epidemiol* 2013: 42(1): 111-127.

14. Fraser A, Macdonald-Wallis C, Tilling K, Boyd A, Golding J, Davey Smith G, Henderson J, Macleod J, Molloy L, Ness A, Ring S, Nelson SM, Lawlor DA. Cohort Profile: the Avon Longitudinal Study of Parents and Children: ALSPAC mothers cohort. *Int J Epidemiol* 2013: 42(1): 97-110.

15. Emmett P. Dietary assessment in the Avon Longitudinal Study of Parents and Children. *Eur J Clin Nutr* 2009: 63 Suppl 1: S38-44.

16. Holland B, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT. McCance and Widdowson's the composition of foods. 5th ed. Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food, London, UK, 1991.

17. Ministry of Agriculture Fisheries and Food. Fatty Acids supplement to McCance & Widdowson's the Composition of Foods. Royal Society of Chemistry, Cambridge, 1998.

18. Nurmatov U, Nwaru BI, Devereux G, Sheikh A. Confounding and effect modification in studies of diet and childhood asthma and allergies. *Allergy* 2012: 67(8): 1041-1059.

19. Textor J, van der Zander B, Gilthorpe MS, Liskiewicz M, Ellison GT. Robust causal inference using directed acyclic graphs: the R package 'dagitty'. *Int J Epidemiol* 2016: 45(6): 1887-1894.

20. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. *Epidemiology* 2004: 15(5): 615-625.

21. Wickman M, Kull I, Pershagen G, Nordvall SL. The BAMSE project: presentation of a prospective longitudinal birth cohort study. *Pediatr Allergy Immunol* 2002: 13(s15): 11-13.
22. Ekstrom S, Magnusson J, Kull I, Lind T, Almqvist C, Melen E, Bergstrom A. Maternal body mass index in early pregnancy and offspring asthma, rhinitis and eczema up to 16 years of age. *Clin Exp Allergy* 2015: 45(1): 283-291.
23. Magnusson J, Kull I, Westman M, Hakansson N, Wolk A, Melen E, Wickman M, Bergstrom A. Fish and polyunsaturated fat intake and development of allergic and nonallergic rhinitis. *J Allergy Clin Immunol* 2015: 136(5): 1247-1253 e1241-1242.
24. Bergström L, Kylberg E, Hagman U, Eriksson HB, Bruce Å. The food composition data base system KOST: the National Administration's system for nutrive values of food. *Vår Föda* 1991: 43: 439-447.
25. Giudetti AM, Cagnazzo R. Beneficial effects of n-3 PUFA on chronic airway inflammatory diseases. *Prostaglandins Other Lipid Mediat* 2012: 99(3-4): 57-67.
26. Magnusson J, Ekstrom S, Kull I, Hakansson N, Nilsson S, Wickman M, Melen E, Riserus U, Bergstrom A. Polyunsaturated fatty acids in plasma at 8 years and subsequent allergic disease. *J Allergy Clin Immunol* 2018: 142(2): 510-516 e516.
27. Howe LD, Tilling K, Galobardes B, Lawlor DA. Loss to follow-up in cohort studies: bias in estimates of socioeconomic inequalities. *Epidemiology* 2013: 24(1): 1-9.
28. Yarnell JW, Fehily AM, Milbank JE, Sweetnam PM, Walker CL. A short dietary questionnaire for use in an epidemiological survey: comparison with weighed dietary records. *Hum Nutr Appl Nutr* 1983: 37(2): 103-112.
29. Rogers I, Emmett P. Diet during pregnancy in a population of pregnant women in South West England. ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood. *Eur J Clin Nutr* 1998: 52(4): 246-250.
30. Kranz S, Jones NRV, Monsivais P. Intake Levels of Fish in the UK Paediatric Population. *Nutrients* 2017: 9(4).
31. Public Health England. National Diet and Nutrition Survey: time trend and income analyses for Years 1 to 9. 2016 23/01/2019 [cited 2019; Available from: https://www.gov.uk/government/collections/national-diet-and-nutrition-survey#archive-of-ndns-reports

Table 1: Participant characteristics according to quartiles of EPA plus DHA intake from fish at 7† years of age in ALSPAC

	Quartiles of EPA plus DHA intake from fish				
	Q1	**Q2**	**Q3**	**Q4**	**P-value**
n (%)	1050 (23.1)	1028 (22.6)	1325 (29.2)	1140 (25.1)	
EPA plus DHA intake, mg/d	5.46 ± 4.49	21.6 ± 2.46	42.4 ± 11.4	129 ± 77.8	
Male, n (%)	540 (51.4)	543 (52.8)	590 (44.5)	560 (49.1)	<0.001
Total energy intake, kJ/day	7084 ± 1653	7486 ± 1649	7696 ± 1660	7952 ± 1772	<0.001
BMI, kg/m^2	16.1 ± 1.9	16.2 ± 1.9	16.1 ± 1.9	16.1 ± 2.0	0.74
BMI at 13.5 years, kg/m^2	20.2 ± 3.30	20.4 ± 3.42	20.1 ± 3.20	20.0 ± 3.12	0.11
Health conscious dietary pattern score	-0.14 ± 1.08	-0.28 ± 0.80	-0.01 ± 0.84	0.43 ± 0.97	<0.001
Any supplement use, n (%)	360 (34.3)	329 (32.0)	425 (32.1)	431 (37.8)	0.01
Season of dietary information collection, n (%)					0.52
Winter	286 (27.2)	283 (27.5)	326 (24.6)	285 (25.0)	
Spring	323 (30.8)	306 (29.8)	389 (29.4)	338 (29.6)	
Summer	271 (25.8)	275 (26.8)	381 (28.8)	340 (29.8)	
Autumn	160 (15.2)	158 (15.4)	212 (16.0)	166 (14.6)	
Missing	10 (1.0)	6 (0.6)	17 (1.3)	11 (1.0)	
Breastfeeding at 3 months, n (%)					<0.001
Never	177 (16.9)	196 (19.1)	189 (14.3)	114 (10.0)	
Stopped/Non-exclusive	466 (44.4)	486 (47.3)	630 (47.5)	522 (45.8)	
Exclusive	359 (34.2)	313 (30.4)	451 (34.0)	453 (39.7)	
Missing	48 (4.6)	33 (3.2)	55 (4.2)	51 (4.5)	
Age at fish introduction, n (%)					<0.001
≥9 months	316 (30.1)	207 (20.1)	264 (19.9)	168 (14.7)	
6-<9 months	276 (26.3)	288 (28.0)	372 (28.1)	280 (24.6)	
<6 months	450 (42.9)	529 (51.5)	687 (51.8)	686 (60.2)	
Missing	8 (0.8)	-	-	6 (0.5)	
History of food allergy, n (%)	177 (16.9)	148 (14.4)	196 (14.8)	224 (19.6)	0.002
Childcare by day nursery at 15 m, n (%)					0.048
No	950 (90.5)	937 (91.1)	1172 (88.5)	1018 (89.3)	
Yes	65 (6.2)	60 (5.8)	119 (9.0)	92 (8.1)	
Missing	35 (3.3)	31 (3.0)	34 (2.6)	30 (2.6)	
Older siblings, n (%)	585 (55.7)	527 (51.3)	658 (49.7)	583 (51.1)	0.03
Younger siblings, n (%)	487 (46.4)	533 (51.8)	735 (55.5)	581 (51.0)	<0.001
***FADS* genotype (rs1535), n (%)**					0.38
AA	356 (42.9)	351 (43.1)	472 (43.8)	429 (47.1)	
GA	385 (46.4)	383 (47.0)	482 (44.7)	382 (41.9)	

GG	88 (10.6)	81 (9.9)	124 (11.5)	100 (11.0)	
Maternal factors					
Age, year	29.5 ± 4.5	29.3 ± 4.5	29.2 ± 4.3	29.9 ± 4.3	<0.001
Education, n (%)					<0.001
Secondary or vocational	217 (20.7)	217 (21.1)	233 (17.6)	156 (13.7)	
O level	342 (32.6)	392 (38.1)	490 (37.0)	337 (29.6)	
A level or degree	482 (45.9)	404 (39.3)	591 (44.6)	629 (55.2)	
Missing	9 (0.9)	15 (1.5)	11 (0.8)	18 (1.6)	
Housing tenure during pregnancy, n (%)					0.049
Mortgaged/owned	879 (83.7)	863 (83.9)	1138 (85.9)	979 (85.9)	
Council rented	61 (5.8)	73 (7.1)	57 (4.3)	48 (4.2)	
Non-council rented	67 (6.4)	50 (4.9)	68 (5.1)	70 (6.1)	
Missing	43 (4.1)	42 (4.1)	62 (4.7)	43 (3.8)	
Financial difficulty, n (%)					0.07
No	891 (84.9)	898 (87.4)	1113 (84.0)	971 (85.2)	
Yes	153 (14.6)	125 (12.2)	211 (15.9)	163 (14.3)	
Missing	6 (0.6)	5 (0.5)	-	6 (0.5)	
Ethnicity, n (%)					0.004
White	1028 (97.9)	1000 (97.3)	1302 (98.3)	1093 (95.9)	
Non-white	13 (1.2)	10 (1.0)	10 (0.8)	26 (2.3)	
Missing	9 (0.9)	18 (1.8)	13 (1.0)	21 (1.8)	
History of atopy, n (%)					0.45
No	529 (50.4)	551 (53.6)	718 (54.2)	595 (52.2)	
Yes	485 (46.2)	444 (43.2)	563 (42.5)	497 (43.6)	
Missing	36 (3.4)	33 (3.2)	44 (3.3)	48 (4.2)	
Smoking when child 7 years old, n (%)					0.50
No	864 (82.3)	821 (79.9)	1085 (81.9)	929 (81.5)	
Yes	162 (15.4)	168 (16.3)	205 (15.5)	179 (15.7)	
Missing	24 (2.3)	39 (3.8)	35 (2.6)	32 (2.8)	
EPA plus DHA intake from fish at 32 w of gestation, mg/d	91.4 ± 101	107 ± 105	136 ± 116	174 ± 132	<0.001

[†] Child characteristics pertain to 7 years of age unless otherwise stated.

ALSPAC: Avon Longitudinal Study of Parents and Children; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

Table 2: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to intake of fish at 7 years of age, stratified by maternal history of atopy and smoking, and child's *FADS* genotype in ALSPAC

	Quartiles of fish intake				P for trend*	P for interaction
	Q1	**Q2**	**Q3**	**Q4**		
Median (IQR), g/d	6.07 (0.00-8.57)	14.6 (13.7-20.4)	27.2 (24.3-29.3)	46.5 (40.4-58.6)		
Cases/non-cases	104/1034	55/586	138/1518	98/1086		
Model 1	1.00	0.92 (0.65-1.30)	0.86 (0.66-1.13)	0.83 (0.61-1.11)	0.21	
Model 2	1.00	0.92 (0.65-1.29)	0.86 (0.65-1.12)	0.82 (0.61-1.11)	0.20	
Model 3	1.00	0.94 (0.67-1.33)	0.87 (0.66-1.14)	0.83 (0.62-1.13)	0.22	
FADS genotype (rs1535): AA						
Cases/non-cases	28/360	24/198	61/540	32/385		
Model 3	1.00	1.67 (0.92-3.02)	1.41 (0.87-2.30)	1.06 (0.61-1.85)	0.81	
FADS genotype (rs1535): GA/GG						
Cases/non-cases	54/456	23/271	55/671	39/491		
Model 3	1.00	0.66 (0.39-1.12)	0.64 (0.43-0.97)	0.59 (0.37-0.93)	0.03	0.22

ALSPAC: Avon Longitudinal Study of Parents and Children; IQR: interquartile range; FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable.

Multivariable model 1: sex and total energy intake at 7 years;

Multivariable model 2: further adjusted for maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, and maternal ethnicity;

Multivariable model 3: further adjusted for maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 15 months of age, maternal smoking, older sibling, younger sibling, and season when the FFQ was completed.

Table 3: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to intake of EPA plus DHA from fish at 7 years of age, stratified by child's *FADS* genotype in ALSPAC

	Quartiles of EPA plus DHA intake from fish				P for trend*	P for interaction
	Q1	**Q2**	**Q3**	**Q4**		
Median (IQR), mg/d	5.51 (0.00-6.67)	22.1 (22.1-22.1)	41.9 (32.2-48.7)	94.0 (78.9-141)		
Cases/non-cases	100/950	81/947	112/1213	97/1043		
Model 1	1.00	0.80 (0.59-1.09)	0.86 (0.65-1.14)	0.86 (0.64-1.15)	0.56	
Model 2	1.00	0.80 (0.59-1.09)	0.85 (0.64-1.14)	0.86 (0.63-1.16)	0.55	
Model 3	1.00	0.82 (0.60-1.11)	0.87 (0.65-1.16)	0.86 (0.64-1.17)	0.56	
FADS genotype (rs1535): AA						
Cases/non-cases	26/330	28/323	48/424	43/386		
Model 3	1.00	1.10 (0.62-1.95)	1.51 (0.90-2.55)	1.43 (0.83-2.46)	0.19	
FADS genotype (rs1535): GA/GG						
Cases/non-cases	54/419	39/425	43/563	32/450		
Model 3	1.00	0.71 (0.45-1.10)	0.54 (0.35-0.83)	0.49 (0.31-0.79)	0.006	0.006

ALSPAC: Avon Longitudinal Study of Parents and Children; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid, FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable.

Multivariable model 1: sex and total energy intake at 7 years;

Multivariable model 2: further adjusted for maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, and maternal ethnicity;

Multivariable model 3: further adjusted for maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 15 months of age, maternal smoking, older sibling, younger sibling, and season when the FFQ was completed.

Table 4: Odds ratio (95% confidence interval) for incident asthma at 12 or 16 years of age, according to intake of EPA plus DHA from fish at 8 years of age, stratified by *FADS* genotype in BAMSE (replication study)

	Quartiles of EPA plus DHA intake from fish				P for trend*	P for interaction
	Q1	**Q2**	**Q3**	**Q4**		
Median (IQR), mg/d	33.7 (18.5-47.4)	90.5 (70.5-117)	178 (158-197)	291 (248-362)		
Cases/non-cases	45/476	39/486	33/514	33/511		
Model 1	1.00	0.85 (0.54-1.33)	0.67 (0.42-1.07)	0.67 (0.42-1.07)	0.07	
Model 2	1.00	0.85 (0.54-1.33)	0.66 (0.41-1.07)	0.61 (0.38-1.00)	0.04	
Model 3	1.00	0.90 (0.57-1.41)	0.65 (0.40-1.06)	0.58 (0.35-0.96)	0.02	
FADS genotype (rs1535): AA						
Cases/non-cases	16/166	14/174	13/164	15/164		
Model 3	1.00	0.88 (0.41-1.92)	0.75 (0.33-1.70)	0.84 (0.37-1.88)	0.64	
FADS genotype (rs1535): GA/GG						
Cases/non-cases	24/257	22/268	20/298	15/283		
Model 3	1.00	0.96 (0.51-1.79)	0.72 (0.37-1.37)	0.52 (0.25-1.07)	0.05	0.03

BAMSE: Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology; IQR: interquartile range; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable.

Multivariable model 1: sex and total energy intake at 8 years;

Multivariable model 2: further adjusted for maternal education, parental occupation and maternal ethnicity;

Multivariable model 3: further adjusted for maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 2 years of age, maternal smoking, older sibling, and season when the FFQ was completed.

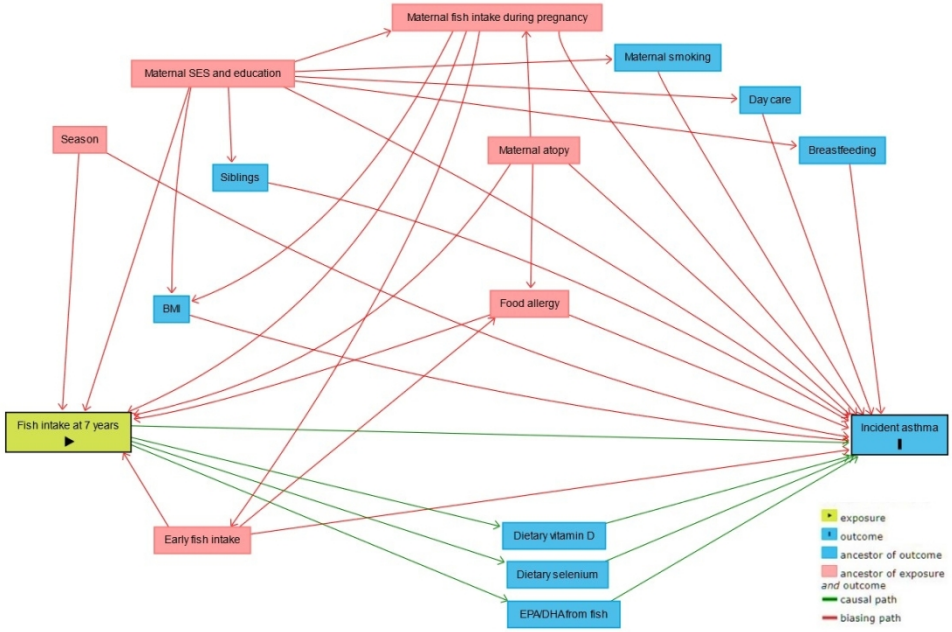
Figure 1. Directed acyclic graph to study covariates and potential structural confounding bias for the association between child's fish intake at 7 years and incident asthma risk.

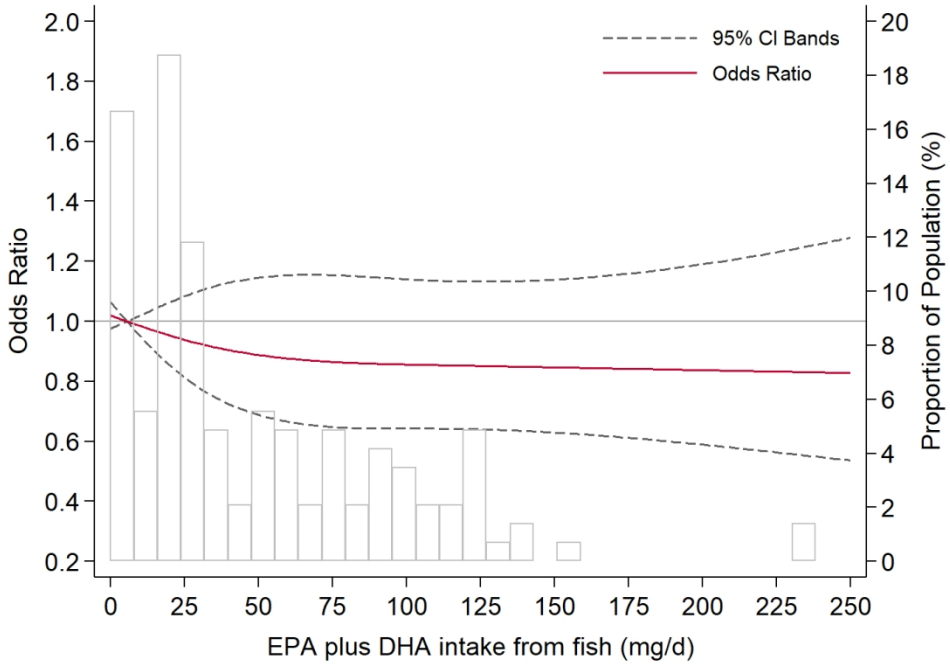
SES: Socioeconomic status; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

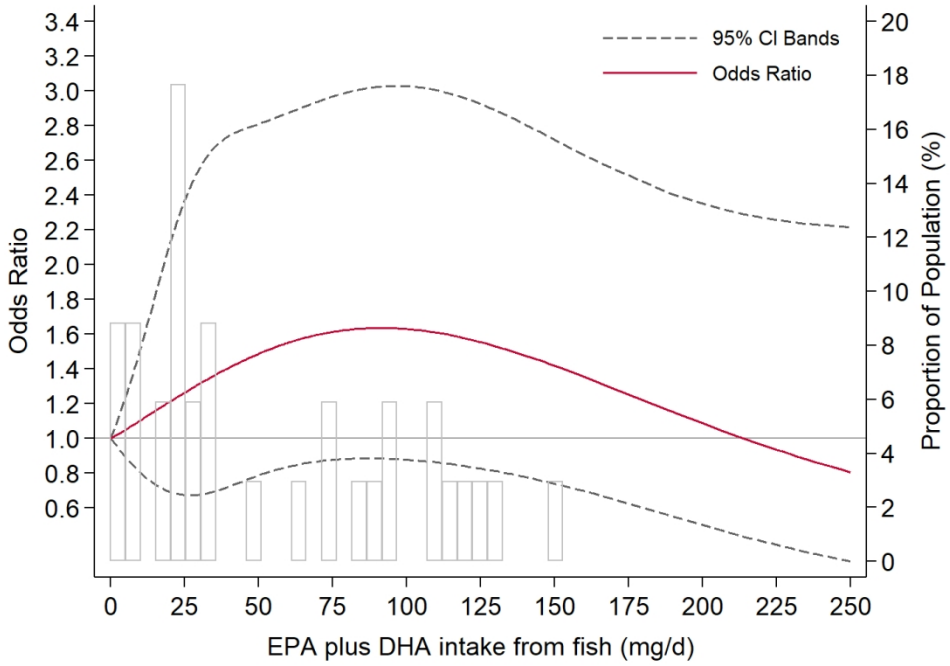
Figure 2. Dose-response relationship between EPA plus DHA from fish and risk of incident asthma overall (A), in those homozygous for the major A allele (B), and in carriers of minor G allele (C) using restricted cubic spline analysis, in ALSPAC.

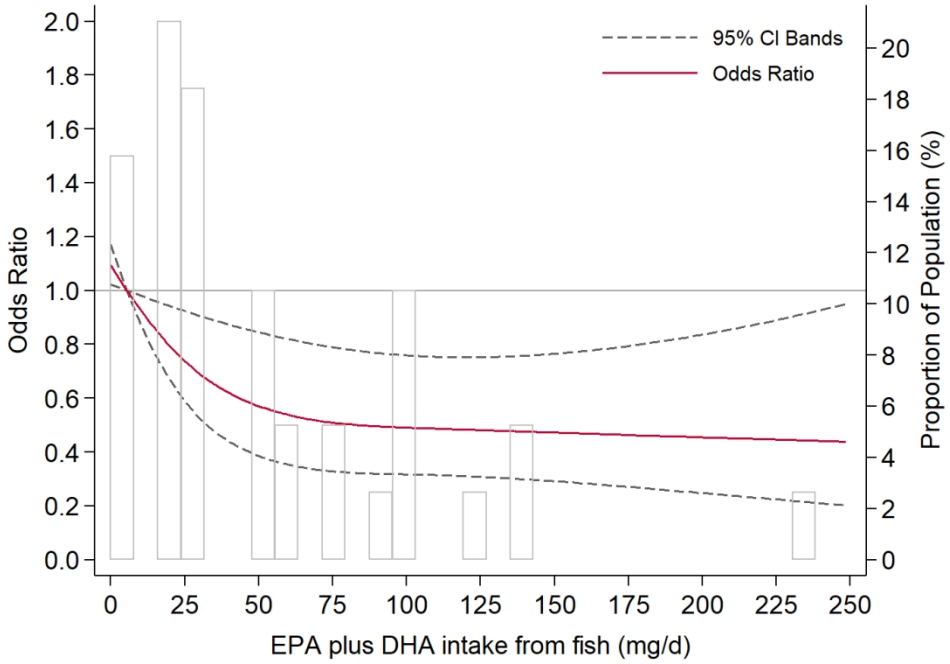
The model was adjusted for sex and total energy intake at 7 years, maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, and maternal ethnicity, maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 15 months of age, maternal smoking, older sibling, younger sibling, and season when the FFQ was completed.

56.3% of children were in the rs1535 GA/GG genotype group. The range of EPA plus DHA intake from fish was 0-675 mg/d with skewness, so this was truncated for presentation purposes.


Maternal fish intake during pregnancy
Maternal SES and education
Maternal smoking
Day care
Season
Maternal atopy
Breastfeeding
Siblings
BMI
Food allergy
Fish intake at 7 years
Incident asthma
Early fish intake
Dietary vitamin D
Dietary selenium
EPA/DHA from fish
exposure
outcome
ancestor of outcome
ancestor of exposure and outcome
causal path
biasing path


95% CI Bands
Odds Ratio
Odds Ratio
Proportion of Population (%)
EPA plus DHA intake from fish (mg/d)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
20
18
16
14
12
10
8
6
4
2
0
0
25
50
75
100
125
150
175
200
225
250


95% CI Bands
Odds Ratio
Odds Ratio
Proportion of Population (%)
EPA plus DHA intake from fish (mg/d)
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
20
18
16
14
12
10
8
6
4
2
0
0
25
50
75
100
125
150
175
200
225
250


95% CI Bands
Odds Ratio
Odds Ratio
Proportion of Population (%)
EPA plus DHA intake from fish (mg/d)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8
2.0
0
2
4
6
8
10
12
14
16
18
20
0
25
50
75
100
125
150
175
200
225
250

Online Data Supplement

Intake of n-3 polyunsaturated fatty acids in childhood, FADS genotype, and incident asthma

Mohammad Talaei, Emmanouela Sdona, Philip C. Calder, Louise R. Jones, Pauline M. Emmett, Raquel Granell, Anna Bergström, Erik Melén, Seif O. Shaheen

Corresponding author:

Prof. Seif Shaheen, Institute of Population Health Sciences, Barts and The London School of Medicine and Dentistry, 58 Turner Street, London E1 2AB. Email: s.shaheen@qmul.ac.uk; Tel: +44 (0)20 7882 2480

Dr Mohammad Talaei, Institute of Population Health Sciences, Barts and The London School of Medicine and Dentistry, 58 Turner Street, London, E1 2AB, UK. E-mail: mohammad.talaei@u.nus.edu; Tel: +44(0)20 7882 2499

Contents

Further details (ALSPAC)

Exposure assessment

Fish intake was covered by five items: shellfish (prawns, crab, cockles, mussels), white fish in breadcrumbs or batter (e.g. fish fingers/shapes, chip shop fish, breaded cod), white fish without coating (e.g. grilled fish, cod in parsley sauce), tuna, and other fish (pilchards, sardines, mackerel, herring, kippers, trout, salmon). Standard portion sizes based on typical consumption patterns in Britain [1] were adapted for the age of children and used to estimate the daily intake of each food group.

Secondary outcomes

Current eczema and hay fever in children at ages 7.5, 11, and 14 years were defined by a positive answer to the question “Has your child had any of the following in the past 12 months" that included eczema and hay fever items. Among those children who were without eczema or hay fever at 7.5 years, we defined incident cases if mothers reported these conditions at 11 or 14 years. Wheeze at 42 months (3.5y) and 91 months (~7.5y) were defined as present if the response to any of the two questions asking about wheezing in the past 12 months was “yes” at each time point. Together with prevalent current doctor-diagnosed asthma at 7 years, presence of wheezing at 3.5 years and 7 years were analysed in relation to maternal n-3 intake during pregnancy. As data on asthma diagnosis were not available for ages below 7 years, we instead considered incident wheeze at 7 years, defined as presence of wheezing at 7 years among those with no report of wheezing at 3.5 years of age. This was analysed in relation to dietary n-3 intake at 4 years of age.

Information on covariates

A maternal history of hay fever, asthma, and eczema was ascertained at 12 weeks of gestation, and any positive response was considered as a maternal history of atopic disease. When the child was 6 months and 15 months old, mothers were asked at what age the child had started eating fish. We defined ‘early fish exposure’ in three categories: before 6 months, 6-9 months, and after 9 months. Mothers were asked

how many cigarettes they smoked per day when the child was 7 years of age. We defined childhood food allergy if there was any such report by mothers at 6 (to milk), 30, 54, or 81 months of age.

Data on maternal ethnicity and indicators of socioeconomic status (maternal education, housing tenure and financial difficulty in pregnancy) were collected at various time points during pregnancy (8, 18, and 32 weeks of gestation) and at 8 weeks postpartum. Maternal age was recorded at delivery. Data on breast-feeding by the 3rd month, and childcare by day nursery, were collected at 6 and 15 months of age, respectively. Number of older and younger siblings was asked at 7 years; if data were missing we used data on parity to calculate the number of older siblings. Child's body mass index was calculated as weight (kg) divided by height squared (m^2), measured at ages 7 and 14 years. A health-conscious dietary pattern was previously defined using principal component analysis and was associated with better nutrient profiles than the processed patterns, which tended to be energy-dense and nutrient-poor [2].

Genotyping

The majority of the children's DNA samples were extracted from cord blood or venous blood collected at age 7 years, with a small number extracted from venous blood collected at 43–61 months. ALSPAC children were genotyped using the Illumina HumanHap550 quad chip genotyping platforms by 23andme subcontracting the Wellcome Trust Sanger Institute, Cambridge, UK and the Laboratory Corporation of America, Burlington, NC, US. The resulting raw genome-wide data were subjected to standard quality control methods. Individuals were excluded on the basis of gender mismatches; minimal or excessive heterozygosity; disproportionate levels of individual missingness (>3%) and insufficient sample replication (IBD < 0.8). Population stratification was assessed by multidimensional scaling analysis and compared with Hapmap II (release 22) European descent (CEU), Han Chinese, Japanese and Yoruba reference populations; all individuals with non-European ancestry were removed. SNPs with a minor allele frequency of < 1%, a call rate of < 95% or evidence for violations of Hardy-Weinberg equilibrium (P < 5E-7) were removed. Cryptic relatedness was measured as proportion of identity by descent (IBD > 0.1). Related subjects that passed all other quality control thresholds were retained during subsequent phasing and imputation. 9,115 subjects and 500,527 SNPs passed these quality control filters. ALSPAC mothers were genotyped using the Illumina human660W-quad array at Centre National de

Génotypage (CNG) and genotypes were called with Illumina GenomeStudio. PLINK (v1.07) was used to carry out quality control measures on an initial set of 10,015 subjects and 557,124 directly genotyped SNPs. SNPs were removed if they displayed more than 5% missingness or a Hardy-Weinberg equilibrium P value of less than 1.0e-06. Additionally SNPs with a minor allele frequency of less than 1% were removed. Samples were excluded if they displayed more than 5% missingness, had indeterminate X chromosome heterozygosity or extreme autosomal heterozygosity. Samples showing evidence of population stratification were identified by multidimensional scaling of genome-wide identity by state pairwise distances using the four HapMap populations as a reference, and then excluded. Cryptic relatedness was assessed using a IBD estimate of more than 0.125 which is expected to correspond to roughly 12.5% alleles shared IBD or a relatedness at the first cousin level. Related subjects that passed all other quality control thresholds were retained during subsequent phasing and imputation. 9,048 subjects and 526,688 SNPs passed these quality control filters.

We combined 477,482 SNP genotypes in common between the sample of mothers and sample of children. We removed SNPs with genotype missingness above 1% due to poor quality (11,396 SNPs removed) and removed a further 321 subjects due to potential ID mismatches. This resulted in a dataset of 17,842 subjects containing 6,305 duos and 465,740 SNPs (112 were removed during liftover and 234 were out of HWE after combination). We estimated haplotypes using ShapeIT (v2.r644) which utilises relatedness during phasing. We obtained a phased version of the 1000 genomes reference panel (Phase 1, Version 3) from the Impute2 reference data repository (phased using ShapeIt v2.r644, haplotype release date Dec 2013). Imputation of the target data was performed using Impute V2.2.2 against the reference panel (all polymorphic SNPs excluding singletons), using all 2186 reference haplotypes (including non-Europeans).

List of n-3 SNPs

Through literature review we found 20 SNPs that were involved in the metabolism of n-3 fatty acids, or were correlated with serum concentration of EPA and/or DHA: rs174547 [3, 4], rs780094 [3], rs3734398 [3], rs12662634 [3], rs3798713 [3], rs174538 [3], rs174535 [3], rs2236212 [3], rs1535 [3, 5, 6], rs174575

[5-8], rs174448 [5], rs11693320 [9], rs174602 [10], rs174556 [7, 8, 11], rs174450 [12], rs174537 [13, 14], rs174576 [13], rs174545 [7, 8, 14], rs174583 [14], rs174561 [7, 8, 14].

Multivariable models

In the multivariable models, we first adjusted for sex and total energy intake (kJ·day−1) at 7 years. The second model additionally included maternal ethnicity (white, non-white) and three indicators of socioeconomic status, namely, maternal education (secondary education, vocational, O level, A level, degree, and missing), housing tenure during pregnancy (mortgaged/owned, council rented, non-council rented, unknown/missing), and financial difficulty during pregnancy (yes/no). In the third model, we further adjusted for maternal history of atopic disease (yes/no), maternal age at delivery (continuous), breastfeeding by the 3rd month (never, stopped/non-exclusive, exclusive), childcare by day nursery at 15 months of age (yes/no), maternal smoking when the child was 7 years of age (none, 1-9, 10-19, and ≥20/day), older sibling (yes/no), younger sibling (yes/no), and season when the FFQ was completed (winter, spring, summer, autumn). Data on potential confounders in multivariable models were missing for 4.2% at most and included in the analyses as separate 'missing' categories.

If evidence for associations with fish intake persisted after adjustment for all potential confounders, we conducted additional adjustment for child's BMI (continuous) and intakes of EPA and DHA from fish, vitamin D, and selenium (quartiles) as potential mediators. When EPA and DHA intakes were the exposures of interest, we further adjusted for dietary intake of vitamin D and selenium as potential confounders.

Sensitivity analyses

We explored the impact of excluding children of non-white mothers, those children with any history of food allergy before 7 years of age, wheeze at 7 years, an extreme total energy intake above the 95th percentile or below the 5th percentile, and those who consumed fish liver oil or omega-3 supplements. We also used the residual method [15] to adjust dietary EPA and DHA for total energy intake and examined the new adjusted variables in the same multivariable models for original ones. The sensitivity of our findings to adjustment for other potential confounders was tested by further adjusting for maternal intake

of n−3 PUFA from fish at 32 weeks of gestation (quartiles), age at first exposure to fish (<6m, 6-<9m, and ≥9m), BMI (continuous), any supplement use (as an indicator of health related behaviours), and health-conscious dietary pattern at 7 years (quartiles, as an indicator of a generally healthy diet) [2]. To address possible residual confounding by population substructure (ancestral differences), we also adjusted for the first 10 principal components (PC) derived by PC analysis from ALSPAC genome-wide data.

Finally, we explored 19 other SNPs involved in n-3 PUFA metabolism and checked for linkage disequilibrium (LD) with our main SNP using LDlink (https://ldlink.nci.nih.gov/). We considered a SNP to be in LD with rs1535 if R^2 >0.90 (9 SNPs) or D´=1 (rs174575). As a *post hoc* analysis, we tested the interaction between EPA plus DHA intake from fish and 7 SNPs not in LD with each other on the risk of incident asthma at 11 or 14 years.

Restricted cubic spline analysis

Restricted cubic spline analysis was used to examine the shape of relationship between EPA plus DHA and incident asthma in multivariable-adjusted models overall and stratified by FADS rs1535. We selected the number of knots based on the values of Akaike information criteria (AIC) to fit the best-approximating model, chose the first knot as reference, and tested for linearity by the Wald-test. Accordingly, the lowest AIC (best fitted model) was obtained by 4 knots in the AA group and by 3 knots in the GA/GG group and overall.

Inverse probability weighting

Inverse probability weighting is a technique to correct for selection bias [16]. In a two-step method, the probability of selection in the study is estimated for everyone based on a given set of covariates and exposure; then the inverse of this probability is included in the analysis as a weight. Inverse probability weighting creates a pseudo-population in which each selected subject accounts for those with similar characteristics who were not selected.

Accordingly, among 7,188 children with data on fish intake who were not diagnosed with current asthma at 7 years, we estimated the probability of selection of 4,543 children for given values of covariates using a logistic regression model. Unselected children were those of unknown asthma status at 7, 11, or 14 years. These covariates included all factors in model 3 (namely, sex, total energy intake, maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, maternal ethnicity, maternal history of atopic disease, maternal age, breastfeeding, childcare, maternal smoking, older sibling, younger sibling, and season of dietary data collection), plus quartiles of fish intake, quartiles of health-conscious dietary pattern score, and history of food allergy. Then, we assigned the inverse of this probability as the weight for each participant, and carried out a multivariable weighted logistic regression analysis to test the associations of fish and n-3 LCPUFA intake with incident asthma in a pseudo-population, which, in contrast to the selected population, is unaffected by selection bias due to these factors. In other words, this approach tests if the observed associations in the main analysis were sensitive to unknown asthma status at baseline or loss to follow-up.

Further details (BAMSE)

Study design

The replication study was conducted within the population-based birth cohort BAMSE (Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology), to which 4,089 children (born 1994-1996, comprising 75% of all eligible children) from predefined areas of Stockholm County, Sweden, have been followed repeatedly from infancy [17, 18]. In brief, baseline information was collected through parental questionnaires when the children were on average two months old, and follow-up questionnaires eliciting information on symptoms of allergic diseases and selected exposures were answered by the parents when the children were 1, 2, 4, 8 and 12 years, and by the adolescents themselves at 16 years. The response rates were 96%, 94%, 91%, 84%, 82% and 78%, respectively. At ages 8 and 16 years, participants were invited to clinical examinations, which included anthropometric measurements, lung function testing and blood sampling using standardized methods. Blood samples

have been analyzed for specific IgE to common inhalant and food allergens, as well as used for DNA extraction. The study was approved by the Ethics Committee of Karolinska Institutet, Stockholm, Sweden, and written informed consent was obtained from parents at 8 and 12 years and study participants at 16 years.

Exposure assessment

At the 8-year-clinical examination, parents (together with their child) were asked to fill in a food frequency questionnaire (FFQ) containing questions about 98 foods and beverages frequently consumed in Sweden, including six questions on fish intake [herring/mackerel and salmon fishes (categorized as oily fish), as well as codfish/pollock/pike, fish fingers, tuna fish and seafood (shrimp, crayfish, crab)]. Most often the FFQ was filled out by a parent (57%) or by a parent together with the child (40%). Children (n = 2,614) were asked how often, on average, they had consumed each type of food or beverage during the past 12 months. There were 10 pre-specified response categories ranging from 'never' to '≥3 times/day' [19].

Intakes of dietary polyunsaturated fatty acids (PUFAs) were computed from the FFQ by multiplying the frequency of consumption of each food item by its nutrient content per serving, using composition values obtained from the Swedish National Food Administration Database and summarized over foods and beverages [20]. Nutrient intakes were adjusted for total energy intake by using the residuals method [15]. Daily intakes of omega-3 (n−3) PUFAs (mg/day) from fish only, including their long chain subtypes (LC-PUFA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), were calculated.

Outcome assessment

At 8, 12 and 16 years, we defined current doctor-diagnosed asthma if mothers responded positively to the question "Has a doctor ever actually said that your study child has asthma?", and to at least one of the concurrent questions which asked if, in the last 12 months, the child had had at least one episode of wheeze, and used asthma medication. Among those children who were not identified as having current doctor-diagnosed asthma at 8 years, we defined those with current doctor-diagnosed asthma at 12 or 16 years as cases of incident asthma.

At 8, 12 and 16 years, we defined eczema if there was a positive reply for eczema symptoms (dry skin in combination with itchy rash and typical localisation) in the last 12 months. Among those children who were not identified as having eczema at 8 years, we defined those with eczema at 12 or 16 years as cases of incident eczema.

Statistical analysis

Among 2,614 children with dietary data available at 8 years, data were complete on incident asthma for 2,159. Participants with baseline questionnaire data, data on total fish and nutrient intake at age 8 years, and complete data on incident asthma, were included in the present study (n=2,138, 52% of the original cohort).

We employed logistic regression to examine associations of n−3 PUFA from fish with incident asthma using the lowest quartile of intake as the reference category. Additionally, we examined associations of fish intake and n−3 PUFA from fish with incident eczema. Linear trend was tested by including median intake of quartiles as a pseudo-continuous variable in the models.

In the multivariable models, we first adjusted for sex and total energy intake (kcal·day−1) at 8 years. The second model additionally included indicators of socioeconomic status, namely, maternal education (9-year compulsory school, 2-year secondary school, 3-4-year secondary school, university or college), parental occupation (blue collar, lower white collar, higher white collar, other), and maternal ethnicity (European, non-European). In the third model, we further adjusted for maternal history of atopic disease (yes/no), maternal age at delivery (continuous), exclusive breastfeeding (less than four months, four months or more), childcare by day nursery at 2 years of age (yes/no), maternal smoking when the child was 8 years of age (none, 1-9, 10-19, and ≥20/day), older sibling (yes/no) and season when the FFQ was completed (winter, spring, summer, autumn).

Fatty acid desaturase (*FADS*) single nucleotide polymorphism (SNP, rs1535) was available from GWAS data in BAMSE for a total of 2,712 subjects (i.e. 66% of the original cohort). The first round of genotyping (Illumina 610k) was done using DNA from 485 asthma cases and controls from the follow-up at eight years of age [21]. Recently, a total of 2,378 16-year-old children were additionaly genotyped with

the Ilumina Infinium Global Screening Array-24.10 BeadChip following the same sample collection procedures, protocols and questionnaires. Quality control (QC) was performed following the Ricopili pipeline [22]. Data were imputed using the Haplotype Reference Consortium 1.1 reference panel [23] with a pre-phasing step using Eagle2. Variants with imputation quality (Rsq) ≥ 0.3 were retained. After QC, a total of 448 (from the eight-year follow-up) and 2,264 subjects (from the 16-year follow-up) with phenotype data available were retained in the genetic analyses for BAMSE.

We carried out stratified analyses, *a priori*, to explore potential modifications of dietary associations by maternal history of atopy, and *FADS* genotype (rs1535 major A allele homozygous vs. heterozygotes plus homozygous for minor G allele, combined). Potential interactions were assessed by testing cross-product terms of these three factors with median values of dietary quartiles as a continuous factor in regression models. Maternal history of atopy was defined as a mother with doctor diagnosed asthma and asthma medication, or doctor diagnosed hay fever, or doctor diagnosed eczema at baseline.

References

1. Ministry of Agriculture Fisheries and Food. Food Portion Sizes, London, HMSO 1991.

2. Emmett PM, Jones LR, Northstone K. Dietary patterns in the Avon Longitudinal Study of Parents and Children. *Nutr Rev* 2015: 73 Suppl 3: 207-230.

3. Lemaitre RN, Tanaka T, Tang W, Manichaikul A, Foy M, Kabagambe EK, Nettleton JA, King IB, Weng LC, Bhattacharya S, Bandinelli S, Bis JC, Rich SS, Jacobs DR, Jr., Cherubini A, McKnight B, Liang S, Gu X, Rice K, Laurie CC, Lumley T, Browning BL, Psaty BM, Chen YD, Friedlander Y, Djousse L, Wu JH, Siscovick DS, Uitterlinden AG, Arnett DK, Ferrucci L, Fornage M, Tsai MY, Mozaffarian D, Steffen LM. Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genet* 2011: 7(7): e1002193.

4. Liu F, Li Z, Lv X, Ma J. Dietary n-3 polyunsaturated fatty acid intakes modify the effect of genetic variation in fatty acid desaturase 1 on coronary artery disease. *PLoS One* 2015: 10(4): e0121255.

5. Harslof LB, Larsen LH, Ritz C, Hellgren LI, Michaelsen KF, Vogel U, Lauritzen L. FADS genotype and diet are important determinants of DHA status: a cross-sectional study in Danish infants. *Am J Clin Nutr* 2013: 97(6): 1403-1410.

6. Steer CD, Hibbeln JR, Golding J, Davey Smith G. Polyunsaturated fatty acid levels in blood during pregnancy, at birth and at 7 years: their associations with two common FADS2 polymorphisms. *Hum Mol Genet* 2012: 21(7): 1504-1512.

7. Standl M, Sausenthaler S, Lattka E, Koletzko S, Bauer CP, Wichmann HE, von Berg A, Berdel D, Kramer U, Schaaf B, Lehmann I, Herbarth O, Klopp N, Koletzko B, Heinrich J, Giniplus, Group LIS. FADS gene cluster modulates the effect of breastfeeding on asthma. Results from the GINIplus and LISAplus studies. *Allergy* 2012: 67(1): 83-90.

8. Standl M, Sausenthaler S, Lattka E, Koletzko S, Bauer CP, Wichmann HE, von Berg A, Berdel D, Kramer U, Schaaf B, Roder S, Herbarth O, Klopp N, Koletzko B, Heinrich J, Giniplus, Group LIS. FADS gene variants modulate the effect of dietary fatty acid intake on allergic diseases in children. *Clin Exp Allergy* 2011: 41(12): 1757-1766.

9. Xu J, Gaddis NC, Bartz TM, Hou R, Manichaikul AW, Pankratz N, Smith AV, Sun F, Terzikhan N, Markunas CA, Patchen BK, Schu M, Beydoun MA, Brusselle GG, Eiriksdottir G, Zhou X, Wood AC, Graff M, Harris TB, Ikram MA, Jacobs DR, Jr., Launer LJ, Lemaitre RN, O'Connor GT, Oelsner EC, Psaty BM, Vasan RS, Rohde RR, Rich SS, Rotter JI, Seshadri S, Smith LJ, Tiemeier H, Tsai MY, Uitterlinden AG, Voruganti VS, Xu H, Zilhao NR, Fornage M, Zillikens MC, London SJ, Barr RG, Dupuis J, Gharib SA, Gudnason V, Lahousse L, North KE, Steffen LM, Cassano PA, Hancock DB. Omega-3 Fatty Acids and Genome-Wide Interaction Analyses Reveal DPP10-Pulmonary Function Association. *Am J Respir Crit Care Med* 2019: 199(5): 631-642.

10. Gonzalez-Casanova I, Rzehak P, Stein AD, Garcia Feregrino R, Rivera Dommarco JA, Barraza-Villarreal A, Demmelmair H, Romieu I, Villalpando S, Martorell R, Koletzko B, Ramakrishnan U. Maternal single nucleotide polymorphisms in the fatty acid desaturase 1 and 2 coding regions modify the impact of prenatal supplementation with DHA on birth weight. *Am J Clin Nutr* 2016: 103(4): 1171-1178.

11. Molto-Puigmarti C, van Dongen MC, Dagnelie PC, Plat J, Mensink RP, Tan FE, Heinrich J, Thijs C. Maternal but not fetal FADS gene variants modify the association between maternal long-chain PUFA intake in pregnancy and birth weight. *J Nutr* 2014: 144(9): 1430-1437.

12. Bernard JY, Pan H, Aris IM, Moreno-Betancur M, Soh SE, Yap F, Tan KH, Shek LP, Chong YS, Gluckman PD, Calder PC, Godfrey KM, Chong MF, Kramer MS, Karnani N, Lee YS. Long-chain polyunsaturated fatty acids, gestation duration, and birth size: a Mendelian randomization study using fatty acid desaturase variants. *Am J Clin Nutr* 2018: 108(1): 92-100.

13. Roke K, Mutch DM. The role of FADS1/2 polymorphisms on cardiometabolic markers and fatty acid profiles in young adults consuming fish oil supplements. *Nutrients* 2014: 6(6): 2290-2304.

14. Gillingham LG, Harding SV, Rideout TC, Yurkova N, Cunnane SC, Eck PK, Jones PJ. Dietary oils and FADS1-FADS2 genetic variants modulate [13C]alpha-linolenic acid metabolism and plasma fatty acid composition. *Am J Clin Nutr* 2013: 97(1): 195-207.

15. Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997: 65(4 Suppl): 1220S-1228S; discussion 1229S-1231S.

16. Hernan MA, Hernandez-Diaz S, Robins JM. A structural approach to selection bias. *Epidemiology* 2004: 15(5): 615-625.

17. Ekstrom S, Magnusson J, Kull I, Lind T, Almqvist C, Melen E, Bergstrom A. Maternal body mass index in early pregnancy and offspring asthma, rhinitis and eczema up to 16 years of age. *Clin Exp Allergy* 2015: 45(1): 283-291.

18. Wickman M, Kull I, Pershagen G, Nordvall SL. The BAMSE project: presentation of a prospective longitudinal birth cohort study. *Pediatr Allergy Immunol* 2002: 13(s15): 11-13.

19. Magnusson J, Kull I, Westman M, Hakansson N, Wolk A, Melen E, Wickman M, Bergstrom A. Fish and polyunsaturated fat intake and development of allergic and nonallergic rhinitis. *J Allergy Clin Immunol* 2015: 136(5): 1247-1253 e1241-1242.

20. Bergström L, Kylberg E, Hagman U, Erikson H, Bruce Å. The food composition database KOST: the National Food Administration's information system for nutritive values of food. *Vår föda* 1991: 43: 439-447.

21. Moffatt M, Gut I, Demenais F, Strachan D, Bouzigon E, Heath S, von Mutius E, Farrall M, Lathrop M, Cookson W, Consortium. G. A large-scale, consortium-based genomewide association study of asthma. *N Engl J Med* 2010: 363(13): 1211-1221.

22. Lam M, Awasthi S, Watson H, Goldstein J, Panagiotaropoulou G, Trubetskoy V, Karlsson R, Frei O, Fan C, De Witte W, Mota N, Mullins N, K. B, Lee H, Wray N, Skarabis N, Huang H, Neale B, Daly M, Mattheissen M, Walters R, Ripke S. RICOPILI: Rapid Imputation for COnsortias PIpeLIne. *Bioinformatics* 2019.

23. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood A, Teumer A, Kang H, Fuchsberger C, Danecek P, Sharp K, Luo Y, Sidore C, Kwong A, Timpson N, Koskinen S, Vrieze S, Scott L, Zhang H, Mahajan A, Veldink J, Peters U, Pato C, van Duijn C, Gillies C, Gandin I, Mezzavilla M, Gilly A, Cocca M, Traglia M, Angius A, Barrett J, Boomsma D, Branham K, Breen G, Brummett C, Busonero F, Campbell H, Chan A, Chen S, Chew E, Collins F, Corbin L, Smith G, Dedoussis G, Dorr M, Farmaki A, Ferrucci L, Forer L, Fraser R, Gabriel S, Levy S, Groop L, Harrison T, Hattersley A, Holmen O, Hveem K, Kretzler M, Lee J, McGue M, Meitinger T, Melzer D, Min J, Mohlke K, Vincent J, Nauck M, Nickerson D, Palotie A, Pato M, Pirastu N, McInnis M, Richards J, Sala C, Salomaa V, Schlessinger D, Schoenherr S, Slagboom P, Small K, Spector T, Stambolian D, Tuke M, Tuomilehto J, Van den Berg L, Van Rheenen W, Volker U, Wijmenga C, Toniolo D, Zeggini E, Gasparini P, Sampson M, Wilson J, Frayling T, de Bakker P, Swertz M, McCarroll S, Kooperberg C, Dekker A, Altshuler D, Willer C, Iacono W, Ripatti S, Soranzo N, Walter K, Swaroop A, Cucca F, Anderson C, Myers R, Boehnke M, McCarthy M, Durbin R, Consortium. HR. A reference panel of 64,976 haplotypes for genotype imputation. *Nat Genet* 2016: 48(10): 1279-1283.

Supplementary tables and figures

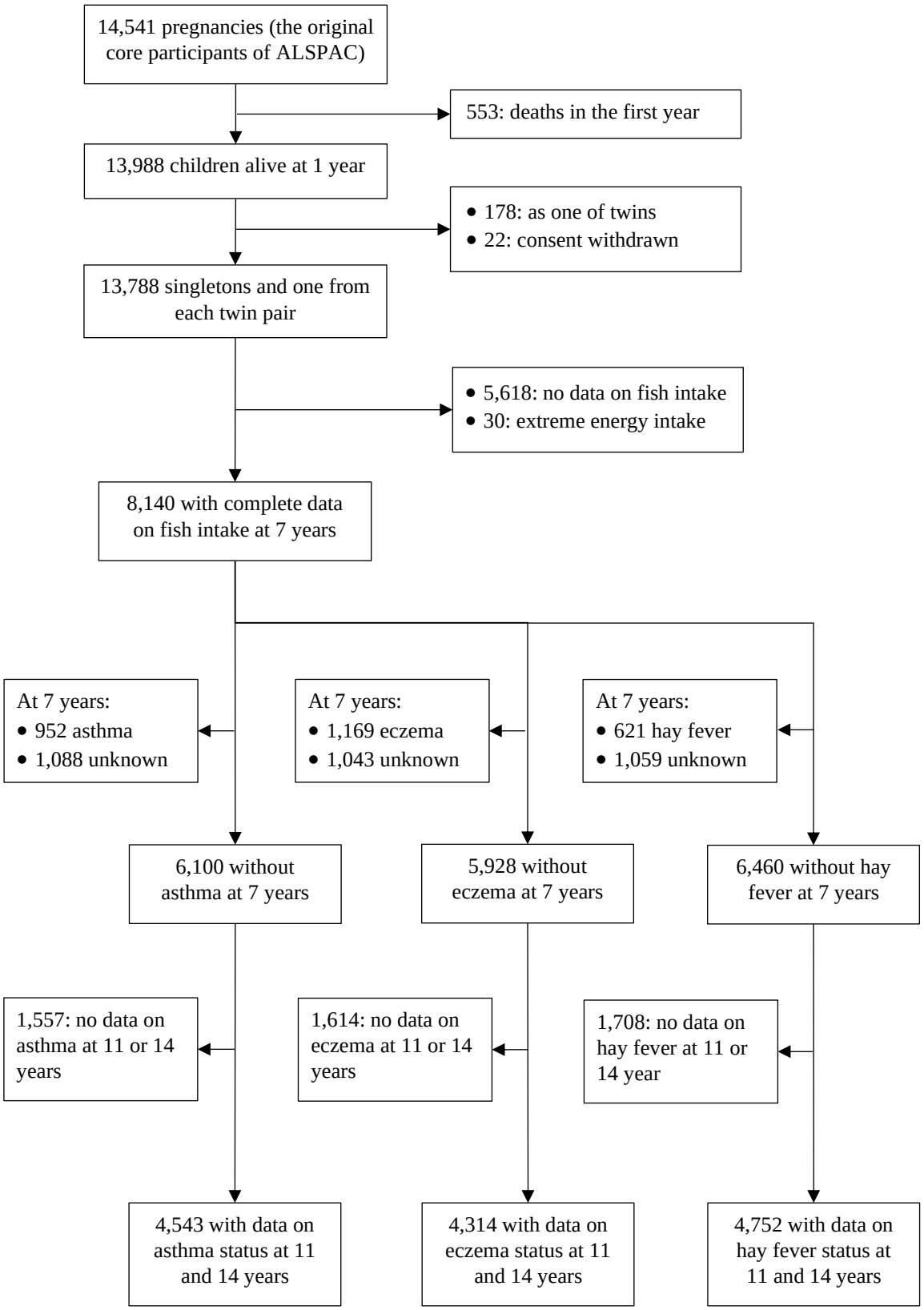


Figure E1. Study profile (ALSPAC).

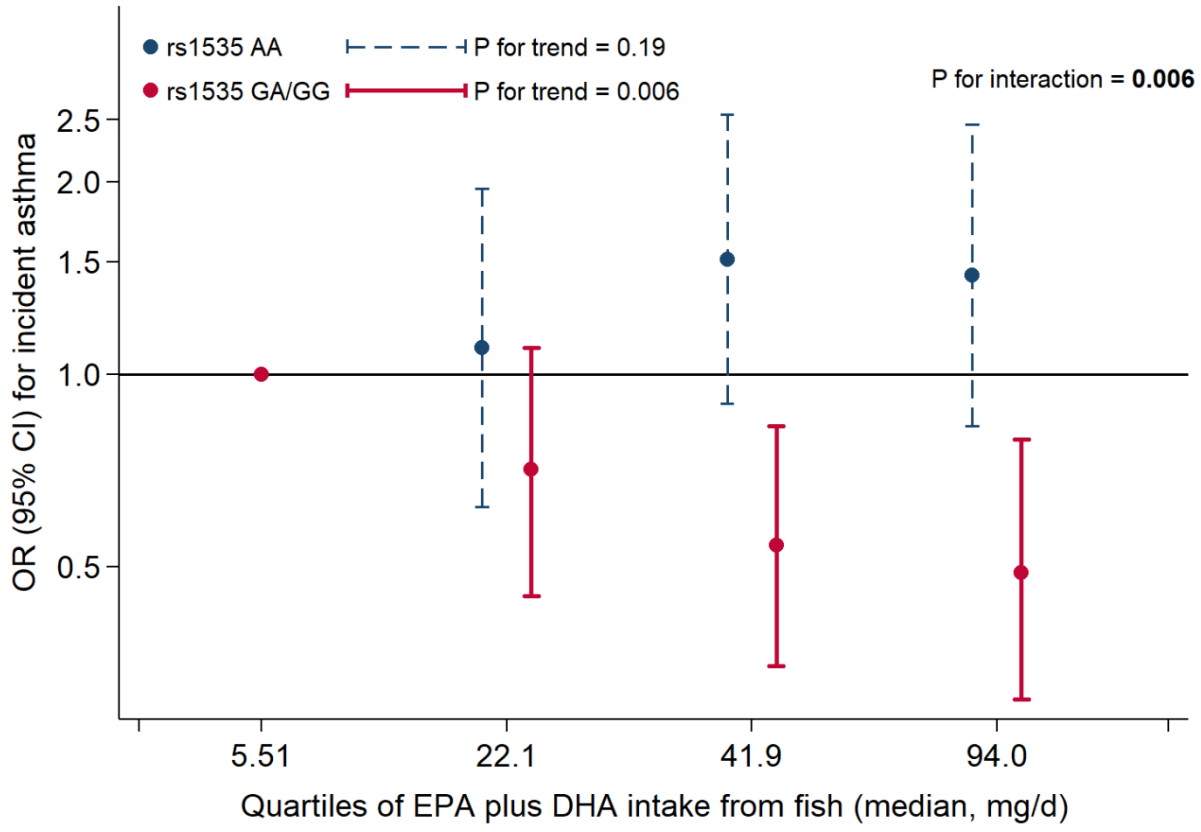


Figure E2: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to intake of EPA plus DHA from fish at 7 years of age, stratified by FADS genotype (rs1535), in ALSPAC

EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; FADS: fatty acid desaturase.
56.3% of children were in the rs1535 GA/GG genotype group.

Table E1: Participant characteristics according to rs1535 genotype in ALSPAC

	rs1535		
	AA	**GA/GG**	**P-value**
n (%)	1607 (44.3)	2024 (55.7)	
EPA plus DHA intake, mg/d	52.15 ± 62.43	49.4 ± 59.35	0.18
Male, n (%)	788 (49.0)	996 (49.2)	0.92
Total energy intake, kJ/day	7578 ± 1701	7578 ± 1672	0.99
BMI, kg/m^2	16.1 ± 1.9	16.1 ± 1.9	0.80
BMI at 13.5 years, kg/m^2	20.1 ± 3.25	20.2 ± 3.28	0.38
Health conscious dietary pattern score	0.05 ± 0.97	-0.02 ± 0.93	0.03
Any supplement use, n (%)	551 (34.3)	670 (33.1)	0.45
Season of dietary information collection, n (%)			0.94
Winter	434 (27.0)	524 (25.9)	
Spring	479 (29.8)	610 (30.1)	
Summer	447 (27.8)	565 (27.9)	
Autumn	233 (14.5)	305 (15.1)	
Missing	14 (0.9)	20 (1.0)	
Breastfeeding at 3 months, n (%)			0.43
Never	208 (12.9)	301 (14.9)	
Stopped/Non-exclusive	750 (46.7)	923 (45.6)	
Exclusive	583 (36.3)	719 (35.5)	
Missing	66 (4.1)	81 (4.0)	
Age at fish introduction, n (%)			0.26
≥9 months	328 (20.4)	421 (20.8)	
6-<9 months	408 (25.4)	559 (27.6)	
<6 months	867 (54.0)	1035 (51.1)	
Missing	4 (0.2)	9 (0.4)	
History of food allergy, n (%)	244 (15.2)	340 (16.8)	0.19
Childcare by day nursery at 15 m			0.40
No, n (%)	1436 (89.4)	1835 (90.7)	
Yes, n (%)	125 (7.8)	141 (7.0)	
Missing	46 (2.9)	48 (2.4)	
Older siblings, n (%)	845 (52.6)	1067 (52.7)	0.94
Younger siblings, n (%)	831 (51.7)	1058 (52.3)	0.74
Outcomes			
Incident doctor-diagnosed asthma at 11 or 14 y, n (%)	145 (9.0)	168 (8.3)	0.44

Incident eczema at 11 or 14 y, n (%)	150 (11.1)	180 (10.6)	0.65
Incident hay fever at 11 or 14 y, n (%)	246 (16.8)	307 (16.6)	0.83
Maternal factors			
Age, year	29.4 ± 4.3	29.6 ± 4.4	0.17
Education, n (%)			0.29
Secondary or vocational	254 (15.8)	359 (17.7)	
O level	567 (35.3)	705 (34.8)	
A level or degree	774 (48.2)	938 (46.3)	
Missing	12 (0.7)	22 (1.1)	
Housing tenure during pregnancy, n (%)			0.99
Mortgaged/owned	1385 (86.2)	1749 (86.4)	
Council rented	79 (4.9)	98 (4.8)	
Non-council rented	85 (5.3)	102 (5.0)	
Missing	58 (3.6)	75 (3.7)	
Financial difficulty, n (%)			0.99
No	1376 (85.6)	1736 (85.8)	
Yes	225 (14.0)	281 (13.9)	
Missing	6 (0.4)	7 (0.3)	
History of atopy, n (%)			0.65
No	855 (53.2)	1047 (51.7)	
Yes	697 (43.4)	909 (44.9)	
Missing	55 (3.4)	68 (3.4)	
Smoking when child 7 years old, n (%)			0.64
No	1335 (83.1)	1663 (82.2)	
Yes	228 (14.2)	309 (15.3)	
Missing	44 (2.7)	52 (2.6)	
EPA plus DHA intake from fish at 32 w of gestation, mg/d	129 ± 119	128 ± 117	0.88

[†] Child characteristics pertain to 7 years of age unless otherwise stated.

ALSPAC: Avon Longitudinal Study of Parents and Children; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

Table E2: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to intake of arachidonic acid and total n-6 fatty acids at 7 years of age, stratified by child's *FADS* genotype in ALSPAC

	Quartiles of arachidonic acid intake				P for	P for
	Q1	**Q2**	**Q3**	**Q4**	**trend***	**interaction**
Arachidonic acid						
Median (IQR), mg/d	26.4 (17.1-31.8)	41.4 (38.6-44.4)	54.3 (50.9-58.6)	76.4 (68.6-89.4)		
Cases/non-cases	93/1023	100/1081	101/1070	99/981		
Model 1	1.00	1.00 (0.74-1.35)	1.00 (0.74-1.36)	1.01 (0.73-1.41)	0.94	
Model 2	1.00	1.00 (0.74-1.35)	1.01 (0.74-1.37)	1.01 (0.73-1.42)	0.93	
Model 3	1.00	1.01 (0.75-1.36)	1.01 (0.74-1.38)	1.03 (0.73-1.43)	0.88	
FADS genotype (rs1535): AA						
Cases/non-cases	28/353	43/397	36/374	38/339		
Model 3	1.00	1.36 (0.81-2.27)	1.25 (0.72-2.17)	1.41 (0.78-2.52)	0.34	
FADS genotype (rs1535): GA/GG						
Cases/non-cases	46/451	42/470	40/498	42/438		
Model 3	1.00	0.85 (0.54-1.32)	0.70 (0.44-1.11)	0.75 (0.45-1.25)	0.23	0.19
Total n-6						
Median (IQR), g/d	7.35 (6.30-8.14)	9.93 (9.34-10.5)	12.2 (11.6-12.9)	15.6 (14.4-17.2)		
Cases/non-cases	95/1074	112/1077	102/1033	84/971		
Model 1	1.00	1.09 (0.81-1.47)	0.97 (0.70-1.34)	0.76 (0.51-1.13)	0.133	
Model 2	1.00	1.10 (0.81-1.48)	0.97 (0.70-1.35)	0.75 (0.50-1.13)	0.129	
Model 3	1.00	1.10 (0.82-1.49)	0.95 (0.68-1.33)	0.75 (0.50-1.13)	0.118	
FADS genotype (rs1535): AA						
Cases/non-cases	35/372	41/391	37/365	32/335		
Model 3	1.00	1.02 (0.62-1.69)	0.90 (0.52-1.58)	0.77 (0.39-1.52)	.414	
FADS genotype (rs1535): GA/GG						
Cases/non-cases	38/484	50/462	45/476	37/435		
Model 3	1.00	1.25 (0.79-1.99)	0.96 (0.57-1.60)	0.78 (0.42-1.45)	.297	0.93

ALSPAC: Avon Longitudinal Study of Parents and Children; FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable

Multivariable model 1: sex and total energy intake at 7 years;

Multivariable model 2: further adjusted for maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, and maternal ethnicity;

Multivariable model 3: further adjusted for maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 15 months of age, maternal smoking, older sibling, younger sibling, and season when the FFQ was completed.

Table E3: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to intake of EPA plus DHA from fish at 7 years of age, stratified by child's *FADS* genotype in ALSPAC (sensitivity analyses)

	Quartiles of EPA plus DHA intake from fish				P for	P for
	Q1	Q2	Q3	Q4	trend*	interaction
***FADS* genotype (rs1535): AA**						
Median (IQR), mg/d	5.51 (0.00-6.67)	22.1 (22.1-22.1)	39.0 (32.2-48.7)	93.4 (78.6-136)		
Cases/non-cases	26/330	28/322	48/424	43/386		
Model 1	1.00	1.13 (0.63-2.02)	1.55 (0.91-2.64)	1.47 (0.84-2.54)	0.18	
Model 2	1.00	1.11 (0.63-1.97)	1.49 (0.88-2.51)	1.37 (0.79-2.37)	0.28	
Model 3	1.00	1.10 (0.62-1.96)	1.51 (0.90-2.54)	1.45 (0.85-2.49)	0.17	
Model 4	1.00	1.03 (0.57-1.88)	1.25 (0.72-2.17)	1.42 (0.82-2.48)	0.16	
Model 5	1.00	0.87 (0.45-1.67)	1.18 (0.67-2.09)	1.30 (0.73-2.33)	0.23	
Model 6	1.00	1.06 (0.58-1.91)	1.64 (0.96-2.81)	1.58 (0.90-2.78)	0.09	
Model 7	1.00	1.17 (0.63-2.16)	1.75 (1.01-3.05)	1.58 (0.89-2.81)	0.13	
Base model						
Exclusion 1	1.00	1.03 (0.58-1.84)	1.47 (0.87-2.46)	1.38 (0.80-2.37)	0.21	
Exclusion 2	1.00	1.12 (0.59-2.12)	1.47 (0.82-2.62)	1.40 (0.76-2.57)	0.28	
Exclusion 3	1.00	0.95 (0.51-1.76)	1.44 (0.84-2.49)	1.40 (0.80-2.47)	0.17	
Exclusion 4	1.00	1.08 (0.59-1.99)	1.42 (0.82-2.45)	1.33 (0.75-2.36)	0.34	
***FADS* genotype (rs1535): GA/GG**						
Median (IQR), mg/d	5.51 (0.00-6.67)	22.1 (22.1-22.1)	42.5 (32.2-51.5)	94.0 (80.3-141)		
Cases/non-cases	54/419	39/425	43/563	32/449		
Model 1	1.00	0.69 (0.44-1.07)	0.52 (0.33-0.80)	0.47 (0.29-0.76)	0.004	0.005
Model 2	1.00	0.70 (0.45-1.09)	0.53 (0.34-0.82)	0.48 (0.30-0.78)	0.005	0.006
Model 3	1.00	0.71 (0.46-1.11)	0.54 (0.35-0.83)	0.49 (0.31-0.79)	0.006	0.006
Model 4	1.00	0.73 (0.46-1.16)	0.57 (0.36-0.91)	0.48 (0.29-0.79)	0.006	0.005
Model 5	1.00	0.69 (0.42-1.14)	0.56 (0.35-0.91)	0.40 (0.23-0.70)	0.002	0.004
Model 6	1.00	0.76 (0.48-1.20)	0.54 (0.34-0.85)	0.52 (0.31-0.86)	0.014	0.004
Model 7	1.00	0.62 (0.39-0.98)	0.49 (0.31-0.77)	0.46 (0.28-0.75)	0.005	0.003
Base model						
Exclusion 1	1.00	0.71 (0.46-1.10)	0.53 (0.34-0.82)	0.48 (0.30-0.77)	0.004	0.007
Exclusion 2	1.00	0.62 (0.37-1.04)	0.52 (0.31-0.86)	0.51 (0.30-0.89)	0.034	0.02
Exclusion 3	1.00	0.69 (0.43-1.10)	0.59 (0.37-0.93)	0.46 (0.28-0.77)	0.005	0.003
Exclusion 4	1.00	0.68 (0.43-1.08)	0.53 (0.34-0.83)	0.51 (0.31-0.82)	0.01	0.02

IQR: interquartile range; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable

Base multivariable model: sex and total energy intake at 7 years, maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, and maternal ethnicity, maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 15 months of age, maternal smoking, older sibling, younger sibling, and season when the FFQ was completed;

Multivariable model 1: further adjusted for age at first exposure to fish;

Multivariable model 2: further adjusted for health-conscious dietary pattern;

Multivariable model 3: further adjusted for any supplement use;

Multivariable model 4: further adjusted for BMI at 7 years;

Multivariable model 5: further adjusted for BMI at 14 years;

Multivariable model 6: further adjusted for maternal intake of EPA plus DHA from fish during pregnancy;

Multivariable model 7: further adjusted for the first 10 principal components (PC) derived by PC analysis from ALSPAC genome-wide data.

Exclusions: 1) children of non-white mothers, 2) children with a history of food allergy, 3) children with extreme energy intakes or users of fish liver oil or omega-3 supplements, 4) children with wheeze at 7 years

Table E4: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to intake of EPA from fish at 7 years of age, stratified by child's *FADS* genotype in ALSPAC

	Quartiles of EPA intake from fish				P for trend*	P for interaction
	Q1	**Q2**	**Q3**	**Q4**		
Median (IQR), mg/d	2.55 (0.00-3.53)	10.2 (6.97-10.2)	14.1 (12.9-16.4)	34.4 (27.9-47.0)		
Cases/non-cases	105/1005	91/1056	99/1038	95/1054		
Model 1	1.00	0.81 (0.61-1.09)	0.89 (0.67-1.19)	0.84 (0.62-1.12)	0.39	
Model 2	1.00	0.81 (0.61-1.10)	0.89 (0.66-1.19)	0.84 (0.62-1.13)	0.39	
Model 3	1.00	0.84 (0.62-1.13)	0.89 (0.67-1.20)	0.84 (0.63-1.14)	0.39	
FADS genotype (rs1535): AA						
Cases/non-cases	28/348	34/362	42/362	41/391		
Model 3	1.00	1.19 (0.70-2.04)	1.49 (0.88-2.51)	1.29 (0.76-2.19)	0.50	
FADS genotype (rs1535): GA/GG						
Cases/non-cases	58/443	39/478	38/483	33/453		
Model 3	1.00	0.59 (0.38-0.91)	0.56 (0.36-0.87)	0.50 (0.32-0.80)	0.01	0.03

ALSPAC: Avon Longitudinal Study of Parents and Children; EPA: eicosapentaenoic acid; FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable

Multivariable model 1: sex and total energy intake at 7 years;

Multivariable model 2: further adjusted for maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, and maternal ethnicity;

Multivariable model 3: further adjusted for maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 15 months of age, maternal smoking, older sibling, younger sibling, and season when the FFQ was completed.

Table E5: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to intake of DHA from fish at 7 years of age, stratified by child's *FADS* genotype in ALSPAC

	Quartiles of DHA intake from fish				P for	P for
	Q1	**Q2**	**Q3**	**Q4**	**trend***	**interaction**
Median (IQR), mg/d	2.96 (0.00-5.69)	11.9 (11.9-11.9)	25.7 (18.7-34.6)	63.8 (48.8-85.4)		
Cases/non-cases	98/938	79/925	117/1211	96/1079		
Model 1	1.00	0.80 (0.59-1.10)	0.91 (0.68-1.20)	0.82 (0.61-1.11)	0.40	
Model 2	1.00	0.81 (0.59-1.10)	0.90 (0.68-1.20)	0.82 (0.61-1.11)	0.38	
Model 3	1.00	0.82 (0.60-1.13)	0.91 (0.68-1.21)	0.83 (0.61-1.13)	0.40	
FADS genotype (rs1535): AA						
Cases/non-cases	25/326	29/315	48/429	43/393		
Model 3	1.00	1.22 (0.69-2.17)	1.55 (0.91-2.62)	1.46 (0.85-2.52)	0.25	
FADS genotype (rs1535): GA/GG						
Cases/non-cases	53/412	36/416	48/560	31/469		
Model 3	1.00	0.67 (0.43-1.06)	0.61 (0.40-0.93)	0.46 (0.29-0.75)	0.004	0.008

ALSPAC: Avon Longitudinal Study of Parents and Children; DHA: docosahexaenoic acid, FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable

Multivariable model 1: sex and total energy intake at 7 years;

Multivariable model 2: further adjusted for maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, and maternal ethnicity;

Multivariable model 3: further adjusted for maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 15 months of age, maternal smoking, older sibling, younger sibling, and season when the FFQ was completed.

Table E6: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to intake of EPA plus DHA from fish at 7 years of age adjusted for total energy intake and applying inverse probability weighting, stratified by child's *FADS* genotype in ALSPAC

	Quartiles of EPA plus DHA intake from fish				P for	P for
	Q1	Q2	Q3	Q4	trend*	interaction
Energy adjusted intakes using residual method						
FADS genotype (rs1535): AA						
Median (IQR), mg/d	7.88 (2.68-12.1)	23.5 (20.0-27.6)	43.2 (36.6-53.3)	93.5 (77.7-134)		
Cases/non-cases	33/354	32/348	39/374	41/386		
Model 3	1.00	1.08 (0.64-1.83)	1.26 (0.76-2.10)	1.23 (0.74-2.03)	0.42	
FADS genotype (rs1535): GA/GG						
Median (IQR), mg/d	7.81 (1.89-12.4)	23.8 (19.7-27.8)	45.3 (37.6-54.6)	95.1 (79.0-137)		
Cases/non-cases	55/437	41/491	42/467	30/461		
Model 3	1.00	0.66 (0.42-1.03)	0.72 (0.46-1.12)	0.50 (0.31-0.80)	0.01	0.02
Inverse probability weighing						
FADS genotype (rs1535): AA						
Model 3	1.00	1.18 (0.60-2.35)	1.51 (0.81-2.79)	1.60 (0.83-3.09)	0.15	
FADS genotype (rs1535): GA/GG						
Model 3	1.00	0.58 (0.34-0.99)	0.61 (0.36-1.03)	0.55 (0.31-0.98)	0.05	0.03

ALSPAC: Avon Longitudinal Study of Parents and Children; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable

Multivariable model 1: sex and total energy intake at 7 years;

Multivariable model 2: further adjusted for maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, and maternal ethnicity;

Multivariable model 3: further adjusted for maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 15 months of age, maternal smoking, older sibling, younger sibling, and season when the FFQ was completed.

Table E7: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to maternal intake of EPA plus DHA from fish at 32 weeks of gestation, stratified by maternal *FADS* genotype in ALSPAC

	Quartiles of EPA plus DHA intake from fish				P for trend*	P for interaction
	Q1	**Q2**	**Q3**	**Q4**		
Median (IQR), g/d	0.0 (0.0-0.0)	37.9 (18.8-69.1)	88.2 (75.1-126)	277 (239-296)		
Cases/non-cases	55/431	126/1354	112/1237	108/1253		
Model 1	1.00	0.74 (0.53-1.04)	0.73 (0.52-1.03)	0.70 (0.49-0.99)	0.26	
Model 2	1.00	0.72 (0.51-1.01)	0.73 (0.51-1.04)	0.69 (0.48-0.98)	0.27	
Maternal *FADS* genotype (rs1535): AA						
Cases/non-cases	18/147	43/449	36/414	36/398		
Model 2	1.00	0.73 (0.40-1.35)	0.76 (0.41-1.43)	0.74 (0.39-1.42)	0.73	
Maternal *FADS* genotype (rs1535): GA/GG						
Cases/non-cases	19/178	57/548	47/500	36/540		
Model 2	1.00	0.96 (0.55-1.69)	0.95 (0.53-1.69)	0.68 (0.37-1.26)	0.10	0.34

IQR: interquartile range; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable

Multivariable model 1: age and total energy intake;

Multivariable model 2: further adjusted for maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, ethnicity, history of atopic disease, age at delivery, smoking during pregnancy, parity, multiple pregnancy, sex of child, season of birth, breastfeeding duration, infections, antibiotics and paracetamol use during pregnancy, and anxiety.

Table E8: Odds ratio (95% confidence interval) for incident asthma at 11 or 14 years according to intake of EPA plus DHA from fish at 7 years of age, stratified by other child polymorphisms related to n-3 fatty acid metabolism (those not in linkage disequilibrium with each other[†]) in ALSPAC

	Quartiles of EPA plus DHA intake from fish				P for	P for
SNP[†] (gene)	**Q1**	**Q2**	**Q3**	**Q4**	**trend[*]**	**interaction**
rs780094 (GCKR)						
TT: Cases/non-cases	8/120	12/112	16/139	13/111		
Model 3	1.00	1.83 (0.67-4.97)	1.35 (0.52-3.53)	1.66 (0.63-4.42)	0.49	
TC/CC: Cases/non-cases	72/629	55/635	75/848	62/724		
Model 3	1.00	0.74 (0.51-1.08)	0.75 (0.53-1.06)	0.71 (0.49-1.02)	0.14	0.13
rs3734398 (ELOVL2)						
TT: Cases/non-cases	11/228	31/307	26/338	22/271		
Model 3	1.00	2.18 (1.05-4.54)	1.45 (0.68-3.10)	1.47 (0.67-3.23)	0.97	
CT/CC: Cases/non-cases	59/443	60/623	55/543	49/567		
Model 3	1.00	0.74 (0.50-1.10)	0.77 (0.52-1.15)	0.62 (0.41-0.94)	0.05	0.15
rs12662634 (ELOVL2-AS1)						
GG: Cases/non-cases	37/451	61/643	57/635	43/564		
Model 3	1.00	1.15 (0.75-1.78)	1.02 (0.66-1.60)	0.85 (0.53-1.37)	0.29	
AG/AA: Cases/non-cases	33/220	30/287	24/246	28/274		
Model 3	1.00	0.68 (0.40-1.18)	0.61 (0.35-1.09)	0.65 (0.37-1.14)	0.21	0.54
rs174448 (between FADS2 and FADS3)						
AA: Cases/non-cases	27/263	37/371	43/379	34/354		
Model 3	1.00	1.06 (0.62-1.82)	1.22 (0.72-2.09)	0.97 (0.55-1.70)	0.87	
GA/GG: Cases/non-cases	37/384	51/528	36/475	35/458		
Model 3	1.00	0.98 (0.62-1.55)	0.73 (0.45-1.20)	0.72 (0.44-1.18)	0.12	0.41
rs11693320 (DPP10)						
AA: Cases/non-cases	47/415	44/578	46/568	44/531		
Model 3	1.00	0.70 (0.45-1.08)	0.69 (0.44-1.07)	0.72 (0.46-1.12)		
GA/GG: Cases/non-cases	20/226	44/323	32/273	26/277		
Model 3	1.00	1.43 (0.80-2.53)	1.23 (0.67-2.26)	0.89 (0.47-1.70)	0.30	0.98
rs174602 (FADS2)						
TT: Cases/non-cases	29/332	44/461	53/458	37/438		
Model 3	1.00	1.07 (0.64-1.77)	1.21 (0.74-1.99)	0.87 (0.52-1.48)	0.51	
CT/CC: Cases/non-cases	16/161	23/211	16/212	16/190		
Model 3	1.00	1.18 (0.58-2.39)	0.82 (0.38-1.76)	0.81 (0.37-1.77)	0.39	0.49
rs174450 (FADS3)						
TT: Cases/non-cases	13/195	30/254	29/251	21/245		
Model 3	1.00	1.96 (0.97-3.96)	1.97 (0.96-4.04)	1.34 (0.63-2.86)	0.99	
GT/GG: Cases/non-cases	47/446	56/628	47/581	47/548		
Model 3	1.00	0.82 (0.55-1.25)	0.72 (0.46-1.11)	0.76 (0.49-1.18)	0.26	0.51

ALSPAC: Avon Longitudinal Study of Parents and Children; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; FADS: fatty acid desaturase

Except for rs780094, carriers of the minor allele (heterozygotes and homozygotes for the minor allele) were combined either because minor alleles were associated with a lower concentration of long-chain

PUFA (5 SNPs), or because a low minor allele frequency resulted in insufficient statistical power (rs11693320).

† The SNPs in linkage disequilibrium with rs1535 were not presented: rs174547, rs174538, rs174535, rs174575, rs174556, rs174537, rs174576, rs174545, rs174561, rs174583. The SNPs in linkage disequilibrium with rs3734398 were also not presented: rs3798713 and rs2236212.

* Linear trend was tested by treating the median values of quartiles as a continuous variable

Multivariable model 1: sex and total energy intake at 7 years;

Multivariable model 2: further adjusted for maternal education, housing tenure during pregnancy, financial difficulty during pregnancy, and maternal ethnicity;

Multivariable model 3: further adjusted for maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 15 months of age, maternal smoking, older sibling, younger sibling, and season when the FFQ was completed.

Table E9. Participant* characteristics in the BAMSE cohort

	N	%
Male sex	1080	50.5
Maternal education		
9-year compulsory school	134	6.3
2-year secondary school	512	24.0
3-4-year secondary school	554	25.9
University or college	928	43.4
Parental occupation		
Blue collar	293	13.7
Lower white collar	959	44.9
Higher white collar	865	40.5
Other	17	0.8
Maternal ethnicity: European	2032	95.0
Maternal history of atopic disease	523	24.5
Exclusive breastfeeding 4 months or more	1739	81.3
Childcare by day nursery at 2 years of age	1574	73.7
Maternal smoking when the child was 8 years of age		
None	1847	88.0
1-9 cigarettes/day	112	5.3
10-19 cigarettes/day	114	5.4
≥20/day	26	1.2
Older siblings	994	46.5
Season of dietary information collection		
Winter	451	21.1
Spring	868	40.6
Summer	178	8.3
Autumn	641	30.0
	Mean	**SD**
Maternal age at delivery, years	31.0	4.4
Total energy intake, kcal/day	1913	463

* Children included in incident asthma analysis (n=2,138).

BAMSE: Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology

Numbers may not add up to the total, due to missing data.

Table E10. Intakes of fish, and EPA and DHA from fish, in BAMSE and ALSPAC.

	BAMSE (n=2,138*)			**ALSPAC** (n=4,543*)		
	Median	**IQR**	**Min-Max**	**Median**	**IQR**	**Min-Max**
Fish intake, times/wk						
Total fish	1.9	1.2	0-15	2.5	1-4	0-42
Oily fish	0.5	0.5	0-7	0	0-0	0-10
n-3 PUFA intake from fish, mg/d						
EPA	41.0	45.4	0-822	11.2	5.99-24.0	0-307
DHA	104	116	0-2266	17.6	9.77-41.4	0-519

BAMSE: Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology; ALSPAC: Avon Longitudinal Study of Parents and Children; IQR: interquartile range; PUFA: polyunsaturated fatty acid; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid

* Children with data on nutrient and fish intake at age 7 (ALSPAC) and 8 (BAMSE) years and data on incident asthma till 14 (ALSPAC) and 16 (BAMSE) years.

Table E11: Odds ratio (95% confidence interval) for incident asthma at 12 or 16 years of age, according to intake of EPA and DHA from fish at 8 years of age, stratified by *FADS* genotype in BAMSE

	Quartiles of EPA and DHA intake from fish				P for	P for
	Q1	**Q2**	**Q3**	**Q4**	**trend***	**interaction**
EPA						
Median (IQR), mg/d	9.9 (8.4)	27.0 (13.4)	50.3 (10.6)	84.7 (33.3)		
Cases/non-cases	43/479	40/481	34/520	33/507		
Model 1	1.00	0.93 (0.59-1.45)	0.72 (0.45-1.15)	0.71 (0.44-1.14)	0.11	
Model 2	1.00	0.93 (0.59-1.46)	0.72 (0.45-1.15)	0.66 (0.40-1.07)	0.06	
Model 3	1.00	0.99 (0.63-1.56)	0.71 (0.43-1.14)	0.63 (0.38-1.05)	0.04	
FADS genotype (rs1535): AA						
Cases/non-cases	14/165	15/173	14/159	15/171		
Model 3	1.00	1.08 (0.49-2.39)	0.94 (0.41-2.16)	0.92 (0.40-2.11)	0.77	
FADS genotype (rs1535): GA/GG						
Cases/non-cases	24/261	22/262	20/311	15/272		
Model 3	1.00	1.01 (0.54-1.90)	0.71 (0.37-1.35)	0.56 (0.27-1.15)	0.07	0.05
DHA						
Median (IQR), mg/d	24.1 (20.4)	62.9 (33.1)	127.4 (28.2)	207.5 (82.7)		
Cases/non-cases	45/477	38/485	35/512	32/513		
Model 1	1.00	0.83 (0.53-1.30)	0.72 (0.45-1.14)	0.64 (0.40-1.04)	0.07	
Model 2	1.00	0.83 (0.53-1.30)	0.71 (0.45-1.13)	0.59 (0.36-0.97)	0.03	
Model 3	1.00	0.87 (0.55-1.37)	0.70 (0.43-1.13)	0.55 (0.33-0.92)	0.02	
FADS genotype (rs1535): AA						
Cases/non-cases	16/162	12/177	15/168	15/161		
Model 3	1.00	0.70 (0.31-1.56)	0.83 (0.38-1.84)	0.81 (0.36-1.83)	0.80	
FADS genotype (rs1535): GA/GG						
Cases/non-cases	24/261	23/262	20/296	14/287		
Model 3	1.00	1.03 (0.55-1.91)	0.74 (0.39-1.41)	0.48 (0.23-0.99)	0.03	0.02

BAMSE: Swedish abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology; IQR: interquartile range; EPA: eicosapentaenoic acid; DHA: docosahexaenoic acid; FADS: fatty acid desaturase

* Linear trend was tested by treating the median values of quartiles as a continuous variable

Multivariable model 1: sex and total energy intake at 8 years;

Multivariable model 2: further adjusted for maternal education, parental occupation and maternal ethnicity;

Multivariable model 3: further adjusted for maternal history of atopic disease, maternal age at delivery, exclusive breastfeeding, childcare by day nursery at 2 years of age, maternal smoking, older sibling, and season when the FFQ was completed.