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

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**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.

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**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.

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**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.

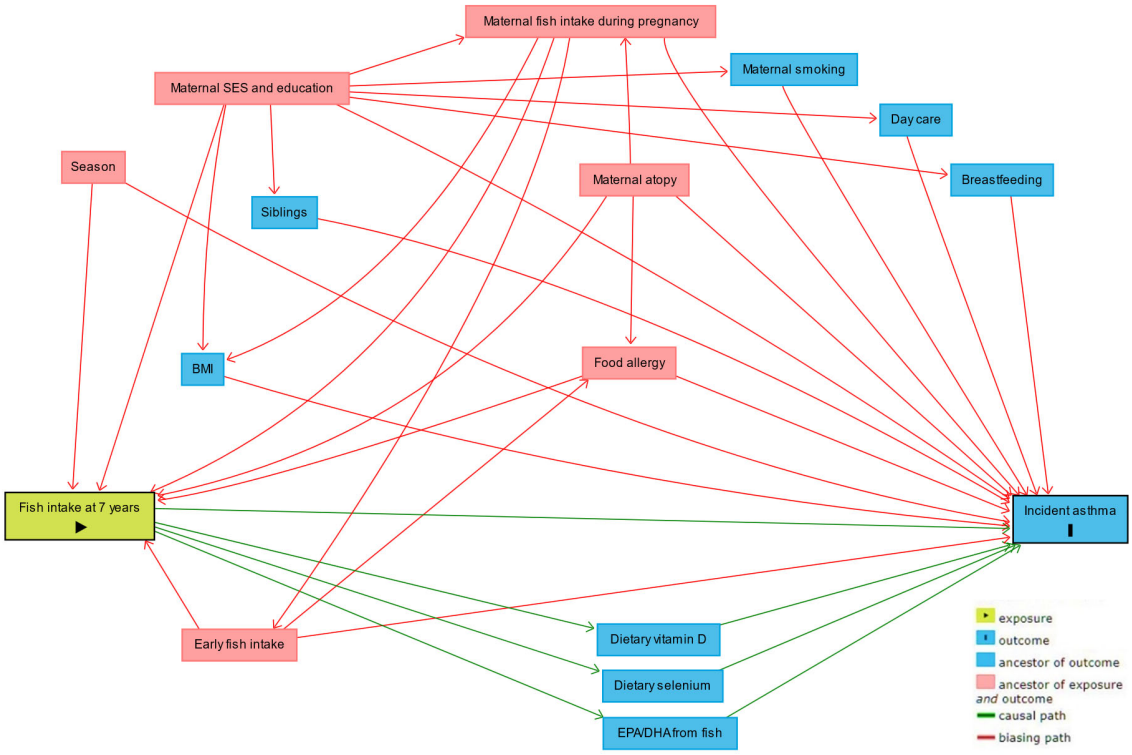
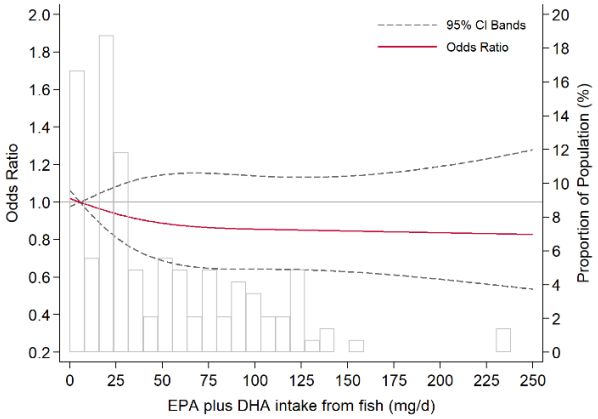


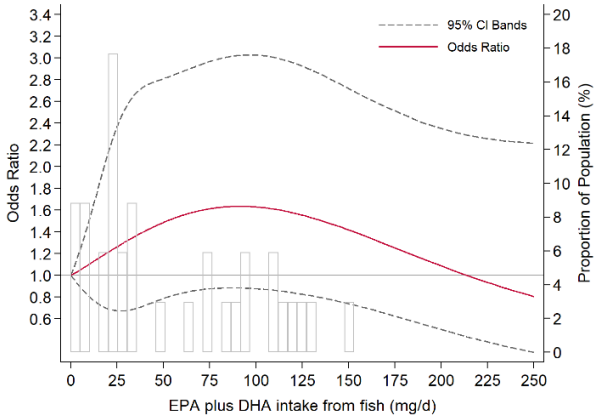

**Figure 1.** Directed acyclic graph to study covariates and potential structural confounding bias for the association between child's fish intake at 7 years and incident asthma risk.

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

**A** (Overall)




**B** (rs1535_AA)




**C** (rs1535_GA/GG)

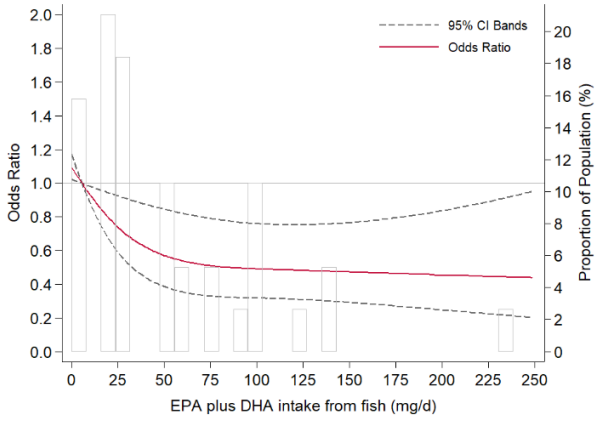



**Figure 2.** Dose-response relationship between EPA plus DHA from fish and risk of incident asthma overall (A), in those homozygous for the major A allele (B), and in carriers of minor G allele (C) using restricted cubic spline analysis, in ALSPAC.

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.