

# Do $\omega$ -3 or other fatty acids influence the development of 'growing pains'? A prebirth cohort study

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**To cite:** Golding J, Northstone K, Emmett P, *et al.* Do  $\omega$ -3 or other fatty acids influence the development of 'growing pains'? A prebirth cohort study. *BMJ Open* 2012;**2**: e001370. doi:10.1136/bmjopen-2012-001370

► Prepublication history and additional material for this paper are available online. To view these files please visit the journal online (<http://dx.doi.org/10.1136/bmjopen-2012-001370>).

Received 24 April 2012  
Accepted 24 July 2012

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## ABSTRACT

**Objectives:** To assess whether the prevalence of growing pains varies with indicators of fatty acid exposure. Growing pains (limb pains of no obvious explanation) have been shown to be strongly linked to a family history of arthritis, and are thought to predict an increased risk of the development of arthritis in adulthood. Much has been made of the possibility of fatty acids, particularly the  $\omega$ -3 fatty acids, playing a preventive role in the development of arthritis, but little research has been undertaken to determine whether such fatty acids might reduce the risk of growing pains. We aimed to assess whether the prevalence of growing pains varies with indicators of fatty acid exposures.

**Design:** Case-control study nested within a prospective longitudinal cohort comparing prenatal and postnatal diet, blood measures and variants in fatty acid desaturase (FADS) genes that influence the metabolism of fatty acids. Statistical analysis took account of factors such as gender, smoke exposure, maternal age and education, social class and parity.

**Setting:** Avon Longitudinal Study of Parents & Children.

**Participants:** All children born between 1 April 1991 and 31 December 1992 (approximately 14 000) within the Avon area (only that part of Avon under the South-West Regional Health Authority). This project compared 1676 children who reported 'growing pains' at age 8 with 6155 with no such pain.

**Primary outcome:** Reported limb pains of no apparent origin.

**Results:** There was no indication that the affected children had diets that differed with regard to  $\omega$ -3, plasma levels of fatty acids, or the FADS genetic variants. We also assessed fetal and infant exposure but neither maternal prenatal blood levels nor maternal dietary intake, or duration of breast feeding showed any significant relationships even after adjustment for confounders.

**Conclusions:** Thus, there is no evidence that  $\omega$ -3 fatty acid status protects against the development of growing pains in childhood.

## INTRODUCTION

Growing pains are common in childhood, with about 56% of children reporting them as

## ARTICLE SUMMARY

### Article focus

To assess whether prevalence of growing pains varies.

- With indicators of fatty acid exposure through prenatal and postnatal diet.
- And with variation in fatty acid desaturase genes that influence the metabolism of fatty acids.

### Key message

- There was no evidence that  $\omega$ -3 fatty acid status protects against the development of growing pains in childhood.

### Strengths and limitations of this study

- The strength of the study lies in the fact that the data are collected prospectively; it is population based, with a large sample. It includes biological markers and genetic variants which allow independent assessment of fatty acid metabolism.
- Limitations concern the fact that the information on limb pains is collected from mothers, albeit using structured questions.

occurring frequently between the ages of 5 and 13.<sup>1</sup> Little attention has been paid though as to possible causes. Indeed there has been an assumption that frequent limb pain, like recurrent abdominal pain, has a mainly psychosomatic aetiology.<sup>2</sup> However, an ultrasound study comparing the tibias of 39 children reporting growing pains with a control group showed significant physiological differences in affected boys ( $p=0.004$ ) as well as girls ( $p<0.001$ ).<sup>3</sup> This physical finding suggests that any association with malaise in the child is more likely to be a consequence rather than a precursor of the pain.

Growing pains in childhood are usually self-limiting. However, they may be an indicator of increased risk of arthritis in adulthood, since it has been shown that children with limb pains are more likely to have parents and/or grandparents with a history of arthritis compared to the general population.<sup>1</sup>

Among small studies of selected groups of children, recurrent limb pains have been reported to be particularly prevalent in obese children,<sup>4</sup> and in those with some vitamin D deficiency,<sup>5</sup> though this was not found in a small study in New Mexico.<sup>6</sup> Other possibilities have been summarised by Evans and Scutter.<sup>7</sup>

Adult arthritis has long been considered to be a condition that could be ameliorated or prevented by dietary manipulation, with suggestions of different components of the diet being important—particularly  $\omega$ -3 fatty acids.<sup>8</sup> To our knowledge the only specific dietary factor that has been considered in regard to childhood limb pain concerns a study of 532 Greek children aged 4–12. A quarter of these children had experienced limb pains in the previous year.<sup>9</sup> The non-affected children were substantially more likely to have been breast fed for at least 40 days compared with the affected children ( $p < 0.005$ ). Unfortunately, this study took no account of other factors that may have influenced the relationship, such as socioeconomic background. However, if breast feeding were on the causal pathway, the major differences between breast and artificial milk at the time, such as  $\omega$ -3 fatty acid content, could be prime candidates for prevention.

While the biological aetiology of growing pains is unknown, data on adults indicate that  $\omega$ -3 fatty acids may reduce pain in joints and muscle and may have a role in regulating bone growth: Goldberg and Katz<sup>10</sup> conducted a meta-analysis of 17 trials assessing reduction of pain in rheumatoid arthritis and reported significant reductions in patient-reported joint pain intensity, morning stiffness, number of painful and/or tender joints and analgesic consumption.  $\Omega$ -3 supplementation in adults without arthritis has been shown to reduce perceived pain and increase the range of motion postexercise among untrained men in comparison with placebo.<sup>11</sup> A randomised trial found that in older adults  $\omega$ -3 fatty acids stimulated muscle protein synthesis and were thought to be useful in preventing or treating acropaenia.<sup>12</sup> Fatty acid patterns in serum of healthy 8-year-olds have been associated with bone mineralisation.<sup>13</sup> Arachidonic acid and other  $\omega$ -6 fatty acids influence bone resorption and accretion in a dose-dependent manner while  $\omega$ -3 fats appear to stimulate bone accretion affecting trabecular and cortical bone.<sup>14</sup> Cartilage is also influenced by fatty acid compositions: linoleic acid and arachidonic acid can reduce collagen synthesis while eicosapentaenoic acid has a stimulating effect.<sup>15</sup>

In this study, we examine the possibility of  $\omega$ -3 fatty acids protecting against growing pains in childhood. On the basis of the Greek study and relationships between  $\omega$ -3 and  $\omega$ -6 status and muscle, bone and cartilage health, we hypothesise that beneficial effects may occur from exposure as a fetus, or ingestion in infancy as well as later in childhood. Increases in specific fatty acids may occur from genetic variants and/or dietary intake.

In this study, we are hypothesis free with regard to critical ages at exposure.

## MATERIAL AND METHODS

### Study population

The Avon Longitudinal Study of Parents and Children (ALSPAC) was designed to determine the genetic and environmental factors that influence the health and development of the child.<sup>16</sup> The eligible sample comprised all pregnant mothers resident in a defined area (that part of the County of Avon that was within the South Western Health Authority Area) who had an expected date of delivery between 1 April 1991 and 31 December 1992. The study started in pregnancy and collected information in a variety of ways including questionnaires completed by the parents and, once old enough, by the child. Biological samples were collected from the mother during pregnancy and from the child at various ages.

### Outcome measures

Information on limb pain was collected via self-completion questionnaires sent to the study child's chief carer at 4, 5, 6, 8, 11 and 13 years of age. Questions asked 'does he/she often have aches and pains in his/her arms or legs'; responses were: 'yes arms, yes legs, yes both, no not often'. If yes, parents were asked to describe what they thought was the cause and whether any particular treatment(s) had helped. There was concurrent validity to these questions by virtue of the causes and treatments described being similar to those found in small studies in the literature, as well as by the expected correlations with family history of arthritis ( $p = 0.0001$  for each parent).<sup>1</sup> We also demonstrated test-retest reliability by comparing results over time; of 4491 children for whom responses were obtained on six time points, 43% repeatedly said 'no' to the question and 35% responded positively on more than one occasion.<sup>1</sup> For reasons of space we did not ask supplementary questions that would have allowed some of Peterson's criteria<sup>17</sup> to be used (eg, bilateral nature, occurring exclusively in the late afternoon or evening). However, the major criteria of exclusion of physical causes were included in this project.

For this study, we used the data collected at age 8 and combined all three positive responses and compared them with the group that replied 'no not often'. The children for whom a known physical cause was given were excluded. (The actual descriptions of causes given are described in the supplementary table to ref.<sup>1</sup>) It should be noted that relatively few children were reported as having pain in their arms only (<2% of the population), so pain in the limbs almost entirely comprised pain in the lower limbs.<sup>1</sup>

## Measures of fatty acids

Measures of fatty acids used in this paper are as follows: (1) the maternal prenatal dietary intake of  $\omega$ -3 from seafood in late pregnancy using the data from food frequency questionnaires analysed in Hibbeln *et al.*<sup>18</sup> (2) maternal prenatal red blood cell fatty acids as described in Newson;<sup>19</sup> (3) the duration of breast feeding as assessed from a questionnaire administered at 6 months, categorised as none, <4 months, 4 months or more; (4) the child's  $\omega$ -3 intake from seafood as identified from food frequency questionnaires administered at ages 3 and 7; (5) the relationship with genetic variants in the fatty acid desaturase (FADS) genes that are strongly related to levels of plasma fatty acids (rs numbers 174548, 174556 and 174561 from the FADS 1 gene; rs numbers 3834458 and 968567 from the intergenic region between FADS 1 and FADS 2; rs numbers 174570, 1535, 174574, 174575, 2 727271, 174576, 174578, 174579, 174602, 498793 and 526126 from the FADS 2 gene; rs 174448 and 174449 from the intergenic region between FADS 2 and FADS 3; and rs174455 from FADS 3);<sup>20</sup> and (6) the child's plasma ((blood samples were obtained from the children aged about 7 years at a special ALSPAC clinic). From these samples, plasma was obtained after centrifugal separation and frozen immediately. All samples were stored at  $-70^{\circ}\text{C}$ , thawed once to obtain a 100  $\mu\text{l}$  aliquot, shipped airfreight on dry ice to Rockville MD and thawed a second time for analysis. Transmethylation of lipids with acetyl chloride and methanol was performed using a simplified method based on the Lepage and Roy procedure<sup>21</sup> using a high throughput automated method.<sup>22</sup> Internal calibration was conducted by adding internal standards to each assay. A second standard was used to quantify the exact amount of internal standard in every batch for ongoing assay of experimental variability. Freedom Evo Instrument 200 (TECAN Trading AG, Switzerland) was utilised for the automatic transmethylation and extraction of fatty acids employing the customised control and automation software (EVOware V.2.0, SP1, Patch3). Gas chromatography 6890 Plus LAN system (Agilent Technologies, Inc, Santa Clara, California, USA) coupled with a fused-silica, narrow-bored DB-FFAP capillary column (Agilent 127-32H2, 15 m $\times$ 0.1 mm ID $\times$ 0.1  $\mu\text{m}$  film thickness) which was used for chromatographic separation of the fatty acid methyl esters as reported previously.<sup>22</sup> The assay was linear in the range of 1–600  $\mu\text{g}/\text{ml}$  plasma. The within and between day imprecision was  $3.26 \pm 1.2\%$  and  $2.95 \pm 1.6\%$  for fatty acid concentrations. Assays were undertaken in 2009–2010. In all, 23 FAs were measured including 12 polyunsaturates (Cord plasma was measured 2008–2009).

## Possible confounders

The following factors were taken into account in multi-variable analyses: highest maternal educational achievement, categorised in three groups: low (<O level),

medium (O level) and high (A level or more); socio-economic group as measured by the highest social class categorisation based on the occupation of the parents as recorded in pregnancy; parity of the mother at the time of delivery of the study child, measured as the number of previous pregnancies resulting in a live or stillbirth: 0, 1 and 2+; maternal smoking in mid-pregnancy: none, 1–9 and 10+ cigarettes per day; passive smoke exposure in childhood at 3 years of age, as assessed by the length of time the child was in a room where others were smoking: not at all, low–moderate and high; the child's body mass index (BMI) at age 7 used as a continuous variable.

## Statistical analysis

Differences in the proportions of children reporting growing pains at 8 years by possible confounding factors were examined using  $\chi^2$  tests. Comparisons of mean fatty acid levels were performed using t tests, comparing those with and without growing pains. Logistic regression was performed adjusting for those confounders that showed significant unadjusted associations. All fatty acids were analysed as a percentage of total fatty acids.

## RESULTS

Growing pains of children aged 8 were more likely to be reported by mothers with lower educational achievements, those of lower social class, lower parity and those who smoked in pregnancy. Growing pains were also more likely in children exposed to environmental tobacco smoke in childhood. There was no difference in prevalence of growing pains between the sexes, and the affected children had a similar mean BMI to those without such a history (table 1). These results were similar to the results at other ages.

In table 2, unadjusted indicators of  $\omega$ -3 and  $\omega$ -6 fatty acid status are shown for the fetus and child. There were no unadjusted differences between the children with limb pain and those without with regard to the amount of  $\omega$ -3 consumed as estimated from the mother's dietary intake, the duration of breast feeding or the child's dietary intake at 3 or 6 years; additionally there were no differences in the plasma levels of key  $\omega$ -3 and  $\omega$ -6 fatty acids at 7 years, nor of the maternal prenatal red cell levels. No differences were seen in plasma levels of monosaturated or saturated fatty acids at any age.

Reversing the calculations, table 3 demonstrates the ORs for developing growing pains given the level of exposure to fatty acids, adjusting for the various factors in table 1. Of the 20 factors tested only one showed some evidence of a statistically significant association—there was a slight increase in the risk of growing pains with each unit increase in the child's plasma  $\omega$ -6 linoleic acid levels (OR=1.02; 95% CI 1.00 to 1.05). There was no evidence of any linear relationships between growing

**Table 1** Differences in potential confounding factors and fatty acid measures according to growing pains reported at 8.5 years

	No growing pains (78.6%)	Growing pains (21.4%)	p Value
Gender			
Boy (51%)	3137 (78.6%)	855 (21.4%)	0.972
Girl (49%)	3018 (78.6%)	821 (21.4%)	
Maternal education			
<O level (23%)	1350 (77.3%)	397 (22.7%)	0.022
O level (35.5%)	2115 (78.5%)	579 (21.5%)	
>O level (41.5%)	2539 (80.5%)	616 (19.5%)	
Family social class			
I and II (29.4%)	1710 (80.4%)	417 (19.6%)	0.001
III NM (26.5%)	1545 (80.7%)	370 (19.3%)	
III M (26.9%)	1473 (75.7%)	473 (24.3%)	
IV and V (17.2%)	981 (79.4%)	254 (20.6%)	
Parity			
0 (46.3%)	2700 (77%)	805 (23%)	0.002
1 (35.9%)	2179 (80%)	545 (20%)	
2+ (17.8%)	1091 (80.9%)	258 (19.1%)	
Maternal smoking in pregnancy			
None (85.1%)	5189 (79.4%)	1344 (20.6%)	0.005
1–9 (7.3%)	421 (75.6%)	136 (24.4%)	
10+ (7.6%)	436 (74.8%)	147 (25.2%)	
Child ETS at age 3			
None (62.1%)	3592 (80.4%)	874 (19.6%)	<0.0001
Low–moderate (25%)	1378 (76.8%)	417 (23.2%)	
High (12.9%)	701 (75.8%)	224 (24.2%)	
Mean BMI (SD) at age 7	16.2 (2)	16.3 (2.1)	0.171

BMI, body mass index; ETS, environmental tobacco smoke; M manual; NM, non-manual

pains and the number of alleles of any of the 17 genetic variants examined (data not shown).

We also explored gender differences in the associations. No differences were observed in fatty acid relationships between boys and girls or for the associations with the FADS genetic variants assuming an additive genetic model. However when a recessive model for the minor allele was explored, 47% of the 34 analyses prior to age 7 years showed significant associations for girls compared to 2.9% in boys. A formal interaction test suggested many of these differences could have occurred by chance but nevertheless six of these differences (18%) were nominally significant. After 7 years, differences in the associations by gender persisted to a lesser extent but none exceeded the formal requirements for an interaction.

## DISCUSSION

Growing pains may have deleterious effects on the child's quality of life. For example, a German study of children aged 5–18 showed that 35% of those with limb pain had been absent from school in consequence, 36% had disturbed sleep and 55% were unable to pursue hobbies.<sup>23</sup> Consequently, any way in which the occurrence of these pains can be avoided is likely to have

major benefits on the children's quality of life.  $\Omega$ -3 fatty acids were hypothesised to be candidate substances that might ameliorate or prevent such pains. However, we could find no convincing evidence here that fatty acids have such a part to play in reducing the risk of limb pain at 8 years of age.

In this study we have taken account of factors such as exposure to cigarette smoke, whether in utero or in childhood, socioeconomic status and maternal education level, the child's gender and BMI. Neither the unadjusted markers of fatty acid exposure, nor those demonstrated after adjustment showed more statistically significant results than would be expected by chance. This applied to the duration of breast feeding, the child's dietary exposure as estimated from food frequency questionnaires of the mother during pregnancy and of the child at ages 3 and 6 years. In addition, the levels of 5 different  $\omega$ -3 and  $\omega$ -6 fatty acids measured in the mothers' blood in pregnancy, and in the child's blood at age 7 years, were not associated with limb pain.

It is well documented that any dietary effects shown can be artefacts associated with socioeconomic and personality factors, and the same can be true for lack of associations. We therefore have used the Mendelian randomisation approach to test this further.<sup>24</sup> We considered 17 genetic variants from the FADS 1, 2 and 3 genes

**Table 2** Unadjusted differences in measures of estimated dietary intake of  $\omega$ -3 and mean blood levels according to growing pains at 8.5 years

	No growing pains	Growing pains	p Value
Maternal prenatal FFQ (mean (SD))			
Total $\omega$ -3	0.15 (0.15)	0.16 (0.15)	0.614
DHA	0.07 (0.07)	0.07 (0.07)	0.593
EPA	0.05 (0.05)	0.05 (0.05)	0.654
Breastfeeding duration			
None (20.7%)	1172 (77.7%)	336 (22.3%)	0.193
Up to 3 months (31.2%)	1775 (78.2%)	495 (21.8%)	
>3 months (48.2%)	2797 (79.7%)	712 (20.3%)	
Child FFQ at 3 years (mean (SD))			
Total n3	0.06 (0.06)	0.06 (0.05)	0.443
DHA	0.02 (0.03)	0.03 (0.03)	0.449
EPA	0.02 (0.02)	0.02 (0.02)	0.439
Child FFQ at 6 years (mean (SD))			
Total n3	0.08 (0.09)	0.09 (0.09)	0.173
DHA	0.03 (0.04)	0.03 (0.04)	0.217
EPA	0.02 (0.02)	0.02 (0.02)	0.233
Child plasma levels at 7 years (mean (SD))			
AA (20:4n6)	6.45 (1.32)	6.36 (1.32)	0.087
DHA (22:6n3)	1.89 (0.52)	1.90 (0.53)	0.426
EPA (20:5n3)	0.64 (0.20)	0.64 (0.20)	0.797
LA (18:2n6)	30.6 (3.16)	30.7 (3.25)	0.511
ALA (18:3n3)	0.71 (0.28)	0.72 (0.27)	0.318
Maternal prenatal red cell levels (mean (SD))			
AA (20:4n6)	6.16 (2.98)	6.11 (2.97)	0.727
DHA (22:6n3)	2.32 (1.36)	2.25 (1.31)	0.215
EPA (20:5n3)	0.27 (0.18)	0.27 (0.17)	0.331
LA (18:2n6)	11.10 (2.70)	10.98 (2.82)	0.610
ALA (18:3n3)	0.14 (0.07)	0.14 (0.07)	0.676

AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFQ, calculated from seafood intake using food frequency questionnaire; LA, linoleic acid.

and their intergenic markers. We have already shown that 14 of the minor alleles of these markers are strongly negatively associated with the blood levels of docosahexaenoic acid, and all 17 are associated with the ratios of arachidonic acid to linoleic acid ( $\omega$ -6) and of eicosapentaenoic acid to  $\alpha$ -linolenic acid ( $\omega$ -3).<sup>20</sup> Thus, if there were an association of blood levels between fatty acids and growing pains, one would expect it to be demonstrated by an association with one or more of the genetic variants. No such relationships were found with the number of minor alleles. Some differences were observed in girls using a recessive model. These results might suggest that prepubertal girls homozygous for the minor allele tend to have lower levels of long-chain fatty acids below a requirement to alleviate limb pains. Irrespective of its validity, the increasing (not decreasing) prevalence of limb pain with age in girls and the absence of any genetic associations in boys suggest a different aetiology for the majority of children.

The major strength of this study concerns the large sample size, having objective measures of fatty acid levels from the mother during pregnancy, and of the child at 7 years of age, availability of genetic markers related to the FADS 1, 2 and 3 genes, and the information on the

diet of mother prenatally and of the child at various time points. Here we used the duration of breastfeeding, and the diet of the child at ages 3 and 7, the latter being the closest age at which we had collected dietary information to that of the child's report of growing pains used in this study. The data also benefit from the prospectively collected study design, thus avoiding the biases inherent in retrospective questioning.

A possible weakness concerns the fact that the data were provided by the parents using questions that did not allow the complete categorisation of 'growing pains' using the Peterson's criteria.<sup>17</sup> The child him/herself is the only person who would be able to accurately reveal such a history. However, it is likely to only be parents of 8-year-olds from the most dysfunctional families that would be oblivious to their child's pain. Such families are likely to be poor at responding in general, and are unlikely to have made a detectable difference to the results reported here.

In conclusion, we could find no strong evidence to indicate that variations in fatty acids would prevent the occurrence of growing pains in children. That is not to say, however, that  $\omega$ -3 fatty acids might not be beneficial as a treatment for such pains, just as they seem to be for rheumatoid arthritis.<sup>8</sup>

**Table 3** Unadjusted and adjusted ORs (95% CIs) for growing pains reported at 8.5 years according to various fatty acid measures

	Unadjusted	Adjusted*
Breastfeeding duration		
None	1.00 Reference	1.00 Reference
Up to 3 months	0.97 (0.83 to 1.14)	1.00 (0.82 to 1.22)
>3 months	0.89 (0.77 to 1.03)	0.95 (0.78 to 1.16)
Maternal FFQ in pregnancy		
Total $\omega$ -3	1.10 (0.76 to 1.60)	1.26 (0.81 to 1.97)
DHA	1.24 (0.56 to 2.74)	1.69 (0.66 to 4.34)
EPA	1.30 (0.42 to 4.05)	1.94 (0.50 to 7.52)
Child FFQ at 3 years		
Total $\omega$ -3	1.48 (0.54 to 4.06)	1.85 (0.57 to 6.01)
DHA	2.26 (0.27 to 18.57)	4.35 (0.37 to 50.78)
EPA	3.96 (0.12 to 129.4)	9.73 (0.17 to 568.9)
Child FFQ at 6 years		
Total $\omega$ -3	1.55 (0.83 to 2.91)	1.62 (0.79 to 3.36)
DHA	2.70 (0.56 to 13.04)	3.12 (0.51 to 19.22)
EPA	4.37 (0.39 to 49.32)	5.07 (0.31 to 83.48)
Child plasma levels at 7 years		
AA (20:4n6)	0.95 (0.90 to 1.01)	0.97 (0.91 to 1.03)
DHA (22:6n3)	1.06 (0.92 to 1.22)	1.13 (0.97 to 1.32)
EPA (20:5n3)	1.05 (0.73 to 1.50)	1.15 (0.77 to 1.70)
LA (18:2n6)	1.01 (0.99 to 1.03)	1.02 (1.00 to 1.05)
ALA (18:3n3)	1.14 (0.88 to 1.47)	1.16 (0.87 to 1.55)
Maternal prenatal red cell levels		
AA (20:4n6)	1.00 (0.97 to 1.02)	0.99 (0.95 to 1.02)
DHA (22:6n3)	0.96 (0.90 to 1.02)	0.95 (0.88 to 1.03)
EPA (20:5n3)	0.78 (0.47 to 1.29)	0.63 (0.34 to 1.17)
LA (18:2n6)	0.99 (0.96 to 1.02)	0.99 (0.95 to 1.03)
ALA (18:3n3)	0.76 (0.21 to 2.75)	0.40 (0.09 to 1.83)

\*Adjusted for gender, maternal education, family social class, parity, maternal smoking, childhood environmental tobacco smoke.

AA, arachidonic acid; ALA,  $\alpha$ -linolenic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FFQ, calculated from seafood intake using food frequency questionnaire; LA, linoleic acid.

**Acknowledgements** 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 ALSPAC team, which includes interviewers, computer and laboratory technicians, clerical workers, research scientists, volunteers, managers, receptionists and nurses.

**Contributors** JG, KN and PE had the original idea; KN and CS carried out the data analysis; JG wrote the first draft; all authors contributed to subsequent drafts.

**Funding** The UK Medical Research Council, the Wellcome Trust and the University of Bristol currently provide core support for ALSPAC. The assays of the maternal blood samples were carried out by Scotia at the instigation of the late David Horrobin, to whom we are extremely grateful. Funding for the genotyping and analysis of the genetic variants was partly undertaken with funding from the EU grant no.FP7-212652 and partly by the Waterloo Foundation. The assays of the child's blood were conducted and supported by the intramural research programme of the National Institute on Alcohol Abuse and Alcoholism (NIAAA) with funding from the National Oceanic and Atmospheric Administration (NOAA). Statistical analysis of the information was funded by the Arthritic Association.

**Competing interests** None.

**Ethics approval** Ethical approval for the study was obtained from the ALSPAC Law and Ethics Committee and the Local Research Ethics Committees.

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data sharing statement** ALSPAC is committed to share data with bona fide researchers.

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