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## Original Research Article

# Patterns of perinatal exposure to PUFAs and child neurodevelopment: evidence from Mendelian randomization using *FADS* cluster variants



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## ABSTRACT

**Background:** The potential causal effects of perinatal exposure to polyunsaturated fatty acids (PUFAs) on child neurodevelopment remains controversial.

**Objective:** To infer causation, we assessed the association of perinatal PUFA patterns and child neurodevelopment by using conventional regression analyses and 1-sample Mendelian randomization (MR).

**Methods:** Among 1096 mother–child pairs from the French *Etude des Déterminants Pré- et Postnatals du Développement de la Santé de L'enfant* cohort, patterns of perinatal exposure to PUFAs were previously identified combining PUFA levels from maternal and cord erythrocytes, and colostrum. Child verbal, performance, and full-scale intelligence quotients (IQs) were assessed at ages 5–6 y. Among maternal fatty acid desaturase (*FADS*) variants genotyped, 2 candidates, rs174546 (*FADS1*) and rs174634 (*FADS3*), were selected, as instrumental variables, for the MR analysis. The association of PUFA patterns with child IQ was examined by conventional multivariable linear regression and 2-stage least-squares MR regression.

**Results:** In the conventional approach, the first pattern “high omega-3 long-chain PUFAs (LC-PUFAs), low omega-6 LC-PUFAs” was positively associated with verbal IQ [ $\beta$  (95% confidence interval) = 1.24 (0.27, 2.21) points per 1 standard deviation (SD) increase in pattern] and full-scale IQ [1.11 (0.18, 2.05)]. This pattern was independent of *FADS* variants, rendering MR analysis inapplicable. The third pattern, “colostrum LC-PUFAs,” was positively associated with verbal [1.11 (0.19, 2.02)], performance [1.01 (0.09, 1.93)], and full-scale IQ [1.13 (0.25, 2.01)]. The MR approach, based on genetic instruments strongly associated with the third pattern, supported the beneficial effect on performance IQ [2.93 (0.05, 5.81) points per 1 SD increase in genetically predicted pattern]. The MR also suggested a deleterious effect of the fourth pattern “linoleic acid (LA) and dihomo-gamma-linolenic acid (DGLA)” on performance IQ [−1.66 (−3.22, −0.09)].

**Conclusions:** These findings supported the potential beneficial effects of perinatal exposure to LC-PUFAs on child neurodevelopment while highlighting possible adverse effects associated with exposure to LA and DGLA.

**Keywords:** polyunsaturated fatty acids, patterns of perinatal exposure to PUFAs, neurodevelopment, *FADS* variants, EDEN mother–child cohort

## Introduction

Long-chain PUFAs (LC-PUFAs), especially DHA (*n*–3) and arachidonic acid (ARA, *n*–6), are crucial for brain development and function, supporting synaptogenesis, neural signaling, neuroinflammation

regulation, and myelin formation [1,2]. During pregnancy, DHA and ARA are transferred from the maternal bloodstream to the fetus via selective placental transfer [3,4], with fetal brain accretion peaking at ~10 mg/d for each during the final weeks of gestation [5]. This process relies on maternal dietary intake, fat stores, and biosynthesis from precursors [i.e.

*Abbreviations:* ALA, *alpha*-linolenic acid; ARA, arachidonic acid; CES-D, Center for Epidemiologic Studies Depression Scale; CI, confidence interval; DGLA, dihomo-gamma-linolenic acid; ELOVL, elongation of very long chain fatty acids; EDEN, Etude des Déterminants Pré- et Postnatals du Développement de la Santé de L'enfant; FA, fatty acid; *FADS*, fatty acid desaturase; FFQ, Food Frequency Questionnaire; HOME, Home Observation for the Measurement of the Environment Inventory-Short Form; IQ, intelligence quotient; LA, linoleic acid; LC-PUFA, long-chain PUFA; MR, Mendelian randomization; *n*–6, omega-6; *n*–3, omega-3; PANDiet, Probability of Adequate Nutrient Intake Diet Quality Index; PCA, principal component analysis; 2SLS, 2-stage least squares; SNP, single nucleotide polymorphism.

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alpha-linolenic acid (ALA,  $n-3$ ) and linoleic acid (LA,  $n-6$ ), respectively]. Postnatal brain accumulation continues until  $\sim 2$  y of age and is primarily driven by DHA and ARA from human milk or formula and dietary intake in early life, as infant synthesis is insufficient to meet demands [6,7].

Despite experimental evidence of the crucial role of PUFAs in neurodevelopment, human studies bring inconsistent findings. Cochrane reviews and meta-analyses of randomized controlled trials have not confirmed significant benefits of omega-3 supplementation during pregnancy [8–11], lactation [12] or through enriched infant formulas [13,14]. Some observational studies have linked maternal seafood consumption—rich in DHA and EPA—to better offspring neurodevelopment [15]. A higher dietary  $n-6:n-3$  PUFA ratio during pregnancy has been associated with poorer neurodevelopment, underscoring the importance of adequate omega-3 PUFA intake [16].

Biomarker-based studies (i.e. PUFA levels in maternal blood [17–23], cord blood [22,24–26], or human milk [27–29]) have produced mixed results, likely due to the variability in the type of biofluids used for biomarker measurement, the specific PUFAs considered in the analyses and residual confounding inherent to observational research. Our previous research identified distinctive patterns of perinatal exposure to PUFAs, integrating maternal and cord erythrocytes and colostrum data, accounting for correlations in levels across these biofluids and within the  $n-3$  or  $n-6$  PUFA families [30].

To mitigate risk of potential bias inherent in conventional observational studies, causal inference designs, such as Mendelian randomization (MR), have emerged. This approach uses genetic variants associated with PUFA exposure as instrumental variables to mitigate confounding and reverse causation, provided key assumptions are met [31,32]. Single nucleotide polymorphisms (SNPs) in the fatty acid desaturase (*FADS*) gene cluster (chromosome 11) and the elongation of very long chain fatty acids (*ELOVL*) gene family (chromosome 6), which encode for PUFA-synthesizing enzymes, modulate PUFA levels in maternal blood, cord blood, and colostrum. This effect is particularly notable in the  $n-6$  family [30,33,34]. Hence, these SNPs are suitable instrumental variables for investigating causal links between perinatal PUFA exposure and child neurodevelopment.

To our knowledge, no study has applied advanced MR analysis to explore this potential causal relationship. Here, we examined the association between previously identified PUFA patterns [30] and child neurodevelopment, using conventional multivariable linear regression analyses and 2-stage least-squares MR regression to strengthen evidence through a triangulation approach.

## Methods

### Study design and population

The *Etude des Déterminants Pré- et Postnataux du Développement de la Santé de L'enfant* (EDEN) mother–child study is an ongoing prospective cohort conducted in France to investigate the early pre- and postnatal determinants of child health and development. Pregnant females were recruited from February 2003 to January 2006 in 2 university maternity clinics, in Nancy and Poitiers. A total of 2002 pregnant females were enrolled before 20 wk of amenorrhea. Exclusion criteria were multiple pregnancies, known diabetes before pregnancy, French illiteracy, and planning to move out of the region in the following 3 y.

At enrolment, the mother signed a written consent for her participation, and at delivery, both parents gave their written consent for their child's participation. The study abides by the Declaration of Helsinki principles and received approval from the ethics committee (*Comité consultatif de*

*protection des personnes dans la recherche biomédicale* - CCPPRB) of Bicêtre University Hospital and from the National Data Protection Authority (*Commission nationale de l'informatique et des libertés* - CNIL). More details about the EDEN cohort study are available elsewhere [35].

### Exposure: patterns of perinatal exposure to PUFAs

Maternal venous blood samples ( $n = 1906$ ) and cord blood samples ( $n = 1437$ ) were collected at 24–28 wk of gestation [mean (SD) gestational age: 26.3 (1.6) wk] and at delivery [mean gestational age: 39.2 (1.7) wk], respectively. In addition,  $\sim 5$  mL of maternal colostrum ( $n = 980$ ) was collected within 1 wk postpartum [mean: 3.9 (1.1) d]. The fatty acid (FA) composition of erythrocyte membrane phospholipids in both maternal and cord blood and of total colostrum lipids ( $\sim 98\%$  triglycerides) was analyzed by gas chromatography with a flame ionization detector (Perkin Elmer Clarus 680, Totalchrom software; hydrogen as gas carrier; column BPX70) following a procedure we previously published elsewhere [28,29,36,37]. An external standard containing different Fatty acid methyl esters (FAMES) at well-known concentrations was used to calibrate peak areas and retention times (GLC 674, Nu-Chek Prep), and an internal standard of a well-known amount (tridecanoic acid, C13:0, Sigma-Aldrich) was added at the step for obtaining sample FAMES (methylation step) as a control of quantification. A total of 31 and 26 FAs, including 12 and 11 PUFA species, present in proportions  $>0.05\%$  of total FAs, were identified in erythrocyte membranes and colostrum, respectively.

Because PUFA levels in the 3 biofluids were strongly correlated [30], analyzing each biofluid separately could introduce confounding bias. To address this limitation, we previously identified 5 statistically independent perinatal PUFA patterns using principal component analysis (PCA), incorporating PUFA levels from the 3 biofluids [30]. Before identifying the patterns, we addressed missing data (1.4% for maternal erythrocytes, 25.2% for cord erythrocytes, and 50.9% for colostrum) by imputing values based on the observed PUFA levels at other time points using the random forest imputation, a nonparametric method available in R (package *missForest*). This approach iteratively imputes missing values by using observed data from other variables—in this case, maternal blood and cord blood FA profiles—as predictors. The method is well-suited for handling mixed data types and accounts for complex interactions and nonlinear relationships, ensuring reliable imputation of the missing colostrum data for our PCA analysis [38].

The 5 patterns explained 50.6% of the total variance in PUFA status [30]. For each mother–child pair, adherence scores to a pattern are defined, by construct, as deviations ( $z$ -scores) from that pattern. These patterns, which serve as the exposure variables in the present study, were labeled as pattern 1 “high omega-3 LC-PUFAs, low omega-6 LC-PUFAs”; pattern 2 “omega-6 PUFAs”; pattern 3 “colostrum LC-PUFAs”; pattern 4 “omega-6 precursor (LA) and dihomo-gamma-linolenic acid (DGLA)”; and pattern 5 “omega-6 precursor (LA) and colostrum ALA.”

### Outcomes: child intelligence quotient assessment

The child neurodevelopment was assessed between ages 5 and 6 y by 2 trained psychologists, 1 in each study center, with the French version of the Wechsler Preschool and Primary Scale of Intelligence-Third Edition [39]. The assessment included the core subtests of the scale, covering areas including information processing, vocabulary, block design, matrix reasoning, picture concepts, and coding. The scores for these subtests were used to derive age-adjusted verbal, performance, and full-scale intelligence quotients (IQs).

## Genetic instruments: FADS genotyping and selection

Maternal and child DNA were extracted from blood samples ( $n = 1719$ , and  $n = 1356$ , respectively). According to a candidate-gene procedure, 12 SNPs located in the *FADS* and *ELOVL* gene regions encoding for desaturase and elongase enzymes involved in the endogenous conversion of the LA and ALA precursors in ARA and DHA, respectively, were genotyped. We selected specific SNPs from the *FADS* gene cluster as instruments for the 1-sample MR analysis according to our previous findings [30], which showed strong associations between identified perinatal PUFA patterns and maternal haplotypes in this gene cluster (except for pattern 1 “high omega-3 LC-PUFAs, low omega-6 LC-PUFAs”).

The selection was based on 2 criteria: 1) the SNP must be strongly associated with the exposure. A linear regression analysis was conducted to evaluate the strength of the genetic instrument in relation to each of the perinatal PUFA patterns. A strong instrument is conventionally indicated by an  $F$ -statistic  $>10$  [40]; 2) the linkage disequilibrium between SNPs should be  $\leq 0.5$ . The 2 SNPs rs174546 (*FADS1*) and rs174634 (*FADS3*) met these criteria and were included individually in the same MR models.

The whole maternal DNA was also genotyped in a subsample of 1050 mothers whose genetic ancestry was derived using PCA [41] and used as a covariate to correct for confounding due to population ethnicity.

## Covariates

Information on covariates other than ethnicity was collected by use of a self-administered questionnaire completed by the mothers at 24–28 wk of gestation. The questionnaire asked about prepregnancy maternal age, primiparity, average household income, maternal lifestyle behaviors (smoking status during the 2 first trimesters of pregnancy, alcohol consumption during the first trimester), and maternal depression symptoms measured with the French version of the Center for Epidemiologic Studies Depression Scale (CES-D) [42]. Maternal education level was determined by the highest diploma reported. Prepregnancy BMI was calculated on the basis of the mother's self-reported weight before pregnancy, and height was measured during the first prenatal clinical visit. The quality of the maternal diet during the third trimester was evaluated with the Probability of Adequate Nutrient Intake Diet Quality Index (PANDiet score), validated in pregnant females [43], which measures adherence to nutritional guidelines based on the adequacy of nutrient intake. This score was derived using a self-administered semi-quantitative Food Frequency Questionnaire (FFQ) completed in the maternity ward in the first few days after delivery, which assessed dietary intake during the third trimester of pregnancy. The FFQ was validated with a series of 24-h recalls [44]. The consumption frequency of 137 foods and food groups was estimated. Daily intake of macro- and micronutrients (in g/day), including LA, ARA, and total omega-6 PUFAs (LA+ARA) and ALA, EPA, DHA, and total omega-3 PUFAs (ALA+EPA+DHA+ docosapentaenoic acid  $n-3$ ), was calculated by crossing data frequency, portion size and nutritional composition of each item from the SU.VI.MAX food composition database [45].

Offspring sex and gestational age were obtained from pediatric and obstetrical records. Children's cognitive stimulation and emotional support and parental involvement were assessed during the follow-up visit at ages 5–6 y by trained research assistants using the French version of the Home Observation for the Measurement of the Environment Inventory-Short Form (HOME). This questionnaire contains 21 items divided into 3 subscales (language stimulation, learning stimulation, and variety in experience) that were summed as a HOME total score [46]. This assessment provides a concise measure of the

home environment's impact on child development by evaluating observable interactions and conditions within the home.

## Study sample

The EDEN study was designed to investigate the relationships between early-life exposures and children's health and developmental outcomes. An a priori power calculation determined that enrolling ~2000 pregnant females would ensure adequate statistical power for postnatal follow-up. This sample size provides 80% power at a 5% significance level to detect associations with quantitative outcomes, as is the case in our study examining child IQ scores.

Of the 2002 women initially recruited, 95 withdrew during pregnancy or at delivery, resulting in a final cohort of 1907 mother–child pairs followed postnatally.

Perinatal PUFA patterns were identified among 1901 of these mother–child pairs, for whom data were available for  $\geq 1$  type of biofluid (erythrocytes membrane from maternal or cord blood, or colostrum), regardless of lactation status. Children with missing data for verbal, performance, or full-scale IQ were then excluded, which led to a sample of 1096 children for assessing the associations between patterns of perinatal exposure to PUFA and child IQ in the conventional regression approach. Causal effects using MR were examined in a subsample of 967 children, after excluding those with missing data for the 2 maternal *FADS* SNPs (rs174546 and rs174634) (Figure 1).

## Statistical analyses

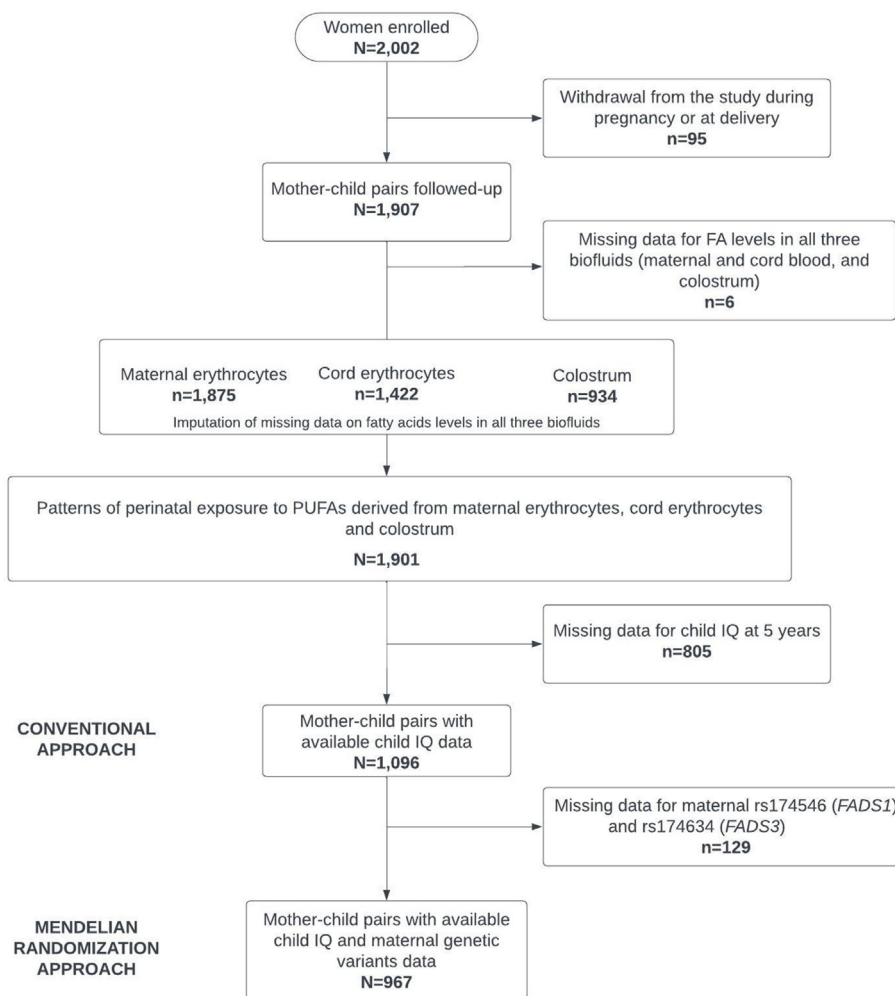
Because the sample sizes for the 2 approaches (conventional regression  $n = 1096$ , and MR analysis,  $n = 967$ ) were similar, the characteristics of the mother–child pairs in the smaller sample (used for the MR analysis) were summarized as percentages and means (SD) and compared with those of the excluded population.

### Conventional regression approach

The associations between adherence to PUFA patterns and child verbal, performance, or full-scale IQ were examined by multivariable linear regression models adjusted for covariates. These covariates were selected based on prior literature and the data available within our cohort. They included study design variables (study center: Nancy/Poitiers), potential confounders [maternal age, maternal education level, household income, child sex, primiparity, prepregnancy BMI, maternal depression score during pregnancy (CES-D), smoking and alcohol use during pregnancy, and PANDiet score during pregnancy], as well as precision variables (associated specifically with neurodevelopmental outcomes), such as the child's HOME score at ages 5–6 y. Before the association analyses, missing data for covariates were imputed by using the *MissForest* package (R v4.2.1) [38]. With sensitivity analysis, we tested for potential interactions between adherence to PUFA patterns and lactation duration, analyzed as a continuous variable, based on the hypothesis that the effects of PUFAs depend on the duration of exposure via lactation because the PUFA composition of colostrum was reported to be significantly correlated with the composition of transitional and mature milk [47]. To ensure the comparability of the results, we conducted an additional sensitivity analysis using this conventional approach with the same subsample as used for the MR analysis ( $n = 967$ ).

### MR approach

The causality of the association between PUFA patterns and child IQ was further investigated by MR analysis. Here, we conducted a 1-sample MR within the EDEN mother–child cohort using the 2-stage least-squares (2SLS) approach [48] with the *ivreg* package (R



**FIGURE 1.** Flow chart of the study population in the EDEN mother-child cohort. EDEN, Etude des Déterminants Pré- et Postnatals du Développement de la Santé de L'enfant; FA, fatty acids; *FADS1* or 3, fatty acid desaturase 1 or 3; IQ, intelligence quotient.

v4.2.1). This method relies on 2 regression stages. We first regressed the 2 *FADS* instruments, rs174546 (*FADS1*) and rs174634 (*FADS3*), which were each classified into 3 groups (0, 1, and 2) according to the number of minor alleles carried, against adherence to each PUFA pattern. Second, we evaluated the associations of the genetically predicted adherence to PUFA patterns from stage 1 with each of our 3 neurodevelopment outcomes (child verbal, performance, and total IQ) using multivariable linear regression adjusted for study center and mother's genetic ancestry [49]. This MR analysis was conducted separately for each PUFA pattern, except for the first one, "high omega-3 LC-PUFAs, low omega-6 LC-PUFAs" which was previously shown to be almost exclusively influenced by maternal PUFA intake and not associated with *FADS* haplotypes [30].

To ensure the reliability of the MR analyses, we addressed potential pleiotropy [31,32] by testing associations between the 2 genetic variants [rs174546 (*FADS1*) and rs174634 (*FADS3*)] and observed confounders. We also performed a sensitivity analysis adjusting the 2SLS for child *FADS* variants to address potential pleiotropy associated with the child genotype [50].

The *ivreg* package also includes 3 diagnostic 2SLS tests [51], which were checked a posteriori: 1) the weak instrument test: a large test statistic and small *P* value indicate a high correlation between instruments and exposure; 2) the Wu-Hausman test [52] for endogeneity: a large test statistic and small *P* value suggest that the ordinary least

squares estimator is inconsistent and the 2SLS estimator is preferred; 3) the Sargan test [53] for overidentification: an overidentified regression equation occurs when there are more instrumental variables than coefficients to estimate. In this case, instruments could provide conflicting information about the values of the coefficients. A large test statistic and small *P* value for the Sargan test indicate a lack of validity of  $\geq 1$  instrument, which could be caused by pleiotropy [54].

Imputation of missing data for covariates and MR analyses involved using R 4.2.1 (R Foundation for Statistical Computing) and all other analyses, SAS 9.4 (SAS Institute Inc.). A 2-sided *P* < 0.05 was considered statistically significant.

## Results

### Characteristics of study population

Characteristics of the 5 patterns of exposure to PUFAs and their corresponding labels are in Table 1. As compared with excluded mothers, included mothers were more frequently from the Poitiers area, had higher socioeconomic status and education level and lower depression scores, were older, smoked less frequently, and drank alcohol more frequently (Table 2). As compared with excluded children, included children had slightly higher HOME scores but comparable average verbal, performance, and full-scale IQ. As compared with

**TABLE 1**

Characteristics of the perinatal patterns of exposure to PUFAs in the EDEN mother–child cohort.

Characteristics	Labels
Pattern 1 Higher levels of omega-3 LC-PUFAs (EPA, DPA <i>n</i> -3, DHA) in the 3 biofluids altogether, along with lower levels of omega-6 LC-PUFAs (ARA, DTA, DPA <i>n</i> -6) in maternal and cord erythrocytes; 17.2% of the total variance.	“High omega-3 LC-PUFAs, low omega-6 LC-PUFAs”
Pattern 2 Higher levels of omega-6 LC-PUFAs (ARA, DTA, DPA <i>n</i> -6) in the 3 biofluids combined to lower levels of ALA, EPA, and DHA in maternal erythrocytes; 11.7% of the total variance.	“Omega-6 LC-PUFAs”
Pattern 3 A colostrum-specific pattern with higher levels of omega-6 LC-PUFAs (ARA, DTA, DPA <i>n</i> -6) and omega-3 LC-PUFAs, especially DPA <i>n</i> -3 and DHA; 9.0% of the total variance.	“Colostrum LC-PUFAs”
Pattern 4 Higher levels of omega-6 precursor (LA) and DGLA in all 3 biofluids; 7.6% total variance.	“LA and DGLA”
Pattern 5 Higher levels of omega-6 precursor (LA) in all 3 biofluids with a lower level of DGLA in erythrocytes and a higher level of omega-3 precursor (ALA) in the colostrum exclusively; 5.1% total variance.	“LA and colostrum ALA”

Abbreviations: ALA, alpha-linolenic acid; ARA, arachidonic acid; DGLA, dihomogamma-linolenic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; LA, linoleic acid; LC-PUFA, long-chain PUFA; *n*-6, omega-6; *n*-3, omega-3.

excluded mother–child pairs, included pairs had higher adherence to pattern 1 “high omega-3 LC-PUFAs, low omega-6 LC-PUFAs” and pattern 2 “omega-6 LC-PUFAs” and lower adherence to pattern 3 “colostrum LC-PUFAs,” and pattern 4 “LA and DGLA”.

**Conventional regression approach**

Linear regression models adjusted for confounders showed positive associations between pattern 1 and verbal IQ [ $\beta$  (95% confidence interval (CI): 1.24 (0.27, 2.21) points per 1 SD increase in pattern adherence score] and full-scale IQ [1.11 (0.18, 2.05)] (Figure 2—conventional approach). Pattern 3 was positively associated with verbal [1.11 (0.19, 2.02)], performance [1.01 (0.09, 1.93)], and full-scale IQ [1.13 (0.25, 2.01)]. A negative trend seemed to appear between pattern 4 and performance IQ.

**MR approach**

The genetic variants rs174546 (*FADS1*) and rs174634 (*FADS3*) were strongly and independently associated with all perinatal PUFA patterns, except pattern 1 (*F*-statistic = 1.5 for pattern 1; 87.4 for pattern 2; 80.5 for pattern 3; 320.4 for pattern 4; and 72.0 for pattern 5). We found no potential pleiotropy between the genetic variants and measured confounders (Supplemental Table 1).

We found a positive causal effect between pattern 3 and child performance IQ [ $\beta$  (95% CI): 2.93 (0.05, 5.81) points per 1 SD increase in genetically predicted pattern adherence score] and a negative causal effect between pattern 4 and performance IQ [−1.66 (−3.22, −0.09)] (Figure 2—MR approach). The results of the weak instrument,

**TABLE 2**

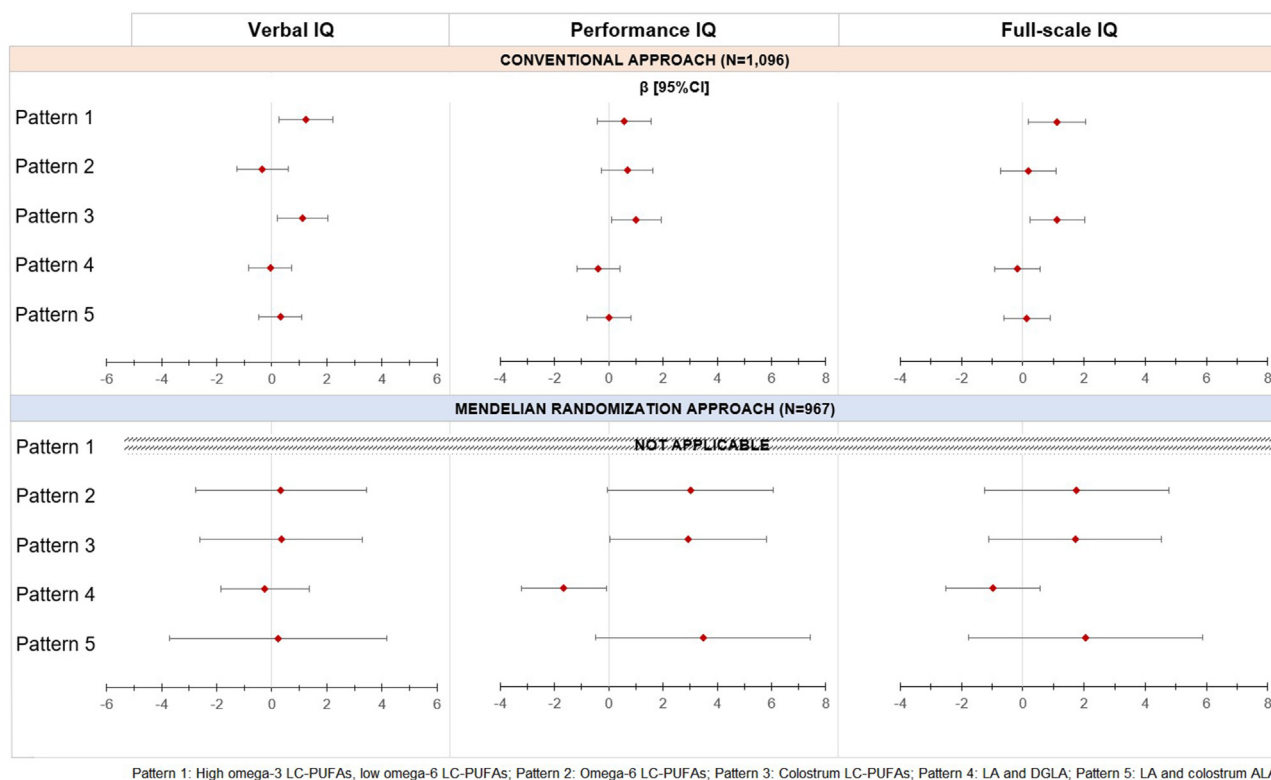
Characteristics of the participants excluded from and included in the Mendelian randomization analysis.

	Excluded ( <i>n</i> = 934)	Included ( <i>n</i> = 967)	<i>P</i>
	% ( <i>n</i> ) or mean (±SD)	% ( <i>n</i> ) or mean (±SD)	
Study center			<0.0001
Poitiers	38.9% (363)	59.7% (577)	
Missing, % ( <i>n</i> )	–	–	
Child sex			0.32
Males	51.3% (477)	53.6% (518)	
Missing, % ( <i>n</i> )	0.4 (4)	–	
Household income (€)			<0.0001
≤1500	21.7% (201)	11.9% (114)	
1501–2300	29.2% (271)	30.2% (291)	
2301–3000	21.7% (201)	30.9% (297)	
>3000	27.4% (254)	27.0% (260)	
Missing, % ( <i>n</i> )	0.7% (7)	0.5% (5)	
Maternal education level			0.0007
<High school diploma	32.9% (303)	24.5% (236)	
High school diploma	17.5% (161)	18.0% (174)	
2 y university degree	20.3% (187)	23.3% (225)	
5 y university degree	29.4% (271)	34.1% (329)	
Missing, % ( <i>n</i> )	1.3 (12)	0.3 (3)	
Maternal age at birth (y)	28.70 (±4.94)	30.23 (±4.73)	<0.0001
Missing, % ( <i>n</i> )	–	–	
Primiparity			0.58
Yes	56.0% (521)	54.7% (528)	
Missing, % ( <i>n</i> )	0.3 (3)	0.2 (2)	
Prepregnancy BMI (kg/m <sup>2</sup> )	23.03 (4.56)	23.43 (4.66)	0.06
Missing, % ( <i>n</i> )	2.7 (25)	1.2 (12)	
Smoking status during pregnancy			<0.0001
Smoker	31.2% (282)	21.3% (200)	
Missing, % ( <i>n</i> )	3.2 (30)	3.0 (29)	
Alcohol drinking status during pregnancy			<0.0001
Consumer	36% (334)	49.3% (476)	
Missing, % ( <i>n</i> )	0.5 (5)	0.2 (2)	
HOME score, range (0–21)	16.79 (2.53)	17.31 (2.23)	0.006
Missing, % ( <i>n</i> )	81.7 (763)	3.8 (37)	
Maternal depression score (CES-D), range (0–60)	12.43 (±8.54)	10.91 (±7.58)	0.00004
Missing, % ( <i>n</i> )	0.5 (5)	0.6 (6)	
Any lactation duration (mo)	3.18 (±3.62)	3.23 (±3.64)	0.77
Missing % ( <i>n</i> )	1.6 (15)	0.1 (1)	
PANDiet score (0–100)	64.19 (6.88)	64.56 (7.02)	0.29
Missing, % ( <i>n</i> )	19.0 (177)	13.4 (130)	
Child IQ			
Verbal, range (40–155)	108.25 (13.37)	106.42 (14.32)	0.16
Performance, range (40–155)	100.97 (12.34)	99.06 (13.98)	0.13
Full-scale, range (40–155)	104.46 (12.15)	102.83 (13.76)	0.20
Missing, % ( <i>n</i> )	85.4 (798)	–	
Mother–child PUFA patterns <i>z</i> -score			
“High omega-3, low omega-6 LC-PUFAs”	−0.18 (±0.99)	0.18 (±0.98)	<0.0001
“Omega-6 LC-PUFAs”	−0.07 (±0.99)	0.07 (±1.00)	0.003
“Colostrum LC-PUFAs”	0.06 (±1.01)	−0.06 (±0.99)	0.006
“LA and DGLA”	0.05 (±0.98)	−0.05 (±1.02)	0.034
“LA and colostrum ALA”	0.00 (±1.00)	0.00 (±1.00)	0.94
Missing, % ( <i>n</i> )	–	–	

Abbreviations: ALA, alpha-linolenic acid; CES-D, Center for Epidemiologic Studies - Depression; DGLA, dihomogamma-linolenic acid; HOME, Home Observation for the Measurement of the Environment Inventory-Short Form; LA, linoleic acid; LC-PUFA, long-chain PUFA; PANDiet, Probability of Adequate Nutrient Intake Diet Quality Index.

The symbol “–” indicates no missing data.

*P* values are based on chi-squared and Student *t* tests for categorical and continuous variables, respectively.



**FIGURE 2.** Associations between perinatal patterns of exposure to PUFAs and child neurodevelopment at 5–6 y using conventional linear regressions and a 1-sample Mendelian randomization in the EDEN mother-child cohort. Conventional regression approach:  $\beta$  (95% CI) IQ points per 1 SD increase in PUFA pattern adherence score; linear regression models adjusted for study center, child sex, household income, maternal age, maternal education level, primiparity, pre-pregnancy BMI, smoking status during pregnancy, alcohol consumption during pregnancy, maternal depression score, HOME score, PANDiet score. Mendelian randomization approach:  $\beta$  (95% CI) IQ points per 1 SD increase in PUFA pattern adherence score predicted genetically by the combined effect of maternal rs174546 (*FADS1*) and rs174634 (*FADS3*) single nucleotide polymorphisms; 2-stage least-squares regression models adjusted for study center and mother's ethnicity. ALA, alpha-linolenic acid; CI, confidence interval; DGLA: dihomo-gamma-linolenic acid; EDEN, Etude des Déterminants Pré- et Postnataux du Développement de la Santé de L'enfant; FADS, fatty acid desaturase; HOME, Home Observation for the Measurement of the Environment Inventory-Short Form; IQ, intelligence quotient; LA, linoleic acid; LC-PUFA, long-chain PUFA; PANDiet, Probability of Adequate Nutrient Intake Diet Quality Index.

Wu-Hausman, and Sargan tests confirmed the reliability of the MR results (Supplemental Table 2).

### Sensitivity analyses

In the conventional regression approach, we found no potential interaction between lactation duration and any of the 5 patterns (Supplemental Table 3). In the subsample of 967 mother-child pairs, pattern 1 remained positively associated with verbal IQ [1.10 (0.05, 2.14)] (Supplemental Table 4). We found a similar, although not significant, association strength for full-scale IQ compared with the main analysis [0.89 (−0.12, 1.9)]. Pattern 3 was positively associated with both verbal IQ [1.02 (0.02, 2.01)] and full-scale IQ [0.99 (0.02, 1.95)]. For performance IQ, pattern 3 also demonstrated a similar association strength as the main analysis [0.82 (−0.19, 1.83)].

Regarding the MR analyses, when adjusting for child rs174546 (*FADS1*) and rs174634 (*FADS3*), we observed a comparable strength of association for pattern 3 [2.56 (−1.43, 6.55)] and pattern 4 [−1.73 (−4.28, 0.81)] with performance IQ (Supplemental Table 5) despite larger CIs due to smaller sample size.

### Discussion

This study examined the relationship between perinatal PUFA exposure patterns [30] and child neurodevelopment at ages 5–6 y,

triangulating conventional regression and 1-sample MR, within the EDEN mother-child cohort. Pattern 1 “high omega-3 LC-PUFAs and low omega-6 LC-PUFAs” was positively associated with verbal and full-scale IQ in conventional analysis but could not be replicated with MR due to a lack of associated candidate SNPs [30]. Pattern 3 “colostrum LC-PUFAs” was positively associated with verbal, performance, and full-scale IQ in conventional analysis, with MR supporting its benefits for performance IQ. MR also indicated a detrimental effect of pattern 4, “LA and DGLA,” on performance IQ, aligning with a trend in conventional analysis.

This study is the first to examine the relationship between perinatal PUFA patterns from 3 biofluids and child neurodevelopment. These patterns address the interdependence among PUFA biofluids (maternal blood, cord blood, and colostrum), complicating the isolation of each biofluid's role. Comparing our results with previous studies on single biofluids is challenging, however. Nonetheless, our findings support existing literature.

Positive associations with pattern 1 align with studies on PUFA levels in maternal or cord blood. Indeed, maternal DHA levels during pregnancy were associated with better full-scale IQ at age 8 y [23], improved psychomotor development in infants [55], greater language skills in 5 y olds [18], and enhanced problem-solving abilities in 12 mo olds [21]. Higher DHA levels in cord blood were associated with improved mental and psychomotor performance at 11 mo [56] and improved memory function

at 11 y [25] in Inuit children. However, some studies found no association with  $n-3$  or  $n-6$  LC-PUFA levels in maternal blood [17,19,20] or cord blood [24], or even a negative association with maternal DHA, potentially due to contaminants in fish consumption [22]. The mechanisms underlying our findings on pattern 1 are not fully understood. Nevertheless, the literature indicates that  $n-3$  and  $n-6$  LC-PUFAs may differentially modulate homeostatic brain functions, particularly by affecting neuroinflammation. ARA-derived eicosanoids promote inflammation, whereas EPA and DHA produce anti-inflammatory and proresolving mediators [57,58]. Chronic ARA-induced inflammation is considered a risk factor for neurodegenerative diseases [59]. Because pattern 1 was mainly associated with maternal dietary intakes of EPA and DHA, with little influence from genetic variants in *FADS* and *ELOVL*, its positive association with full-scale IQ emphasizes the importance of maintaining an LC-PUFA ratio more favorable to  $n-3$  FAs through adequate  $n-3$  LC-PUFA intake during pregnancy and lactation [30]. This can be achieved by consuming fatty fish with high EPA and DHA content while minimizing exposure to heavy metal contaminants by choosing smaller pelagic species (e.g. sardines, mackerel, herring, and anchovies). Currently, French dietary guidelines recommend a daily intake of 250 mg each of EPA and DHA for pregnant and lactating women, based on a 2050/2250 kcal/d diet [60]. However, in the EDEN cohort, maternal EPA and DHA average daily intakes during the last trimester of pregnancy were  $80 \pm 60$  mg (range: 10–390 mg) and  $170 \pm 120$  mg (range: 20–730 mg), respectively, highlighting the fact that recommendations are not always met [36].

The findings for pattern 3 provide additional insights. The positive associations between pattern 3 and all 3 IQs in conventional regression analyses, further supported by MR for performance IQ, indicate the independent beneficial effects of both  $n-3$  and  $n-6$  LC-PUFAs during lactation. These results align with the literature based on human milk PUFA levels. Prior studies in the EDEN cohort showed that children breastfed with colostrum containing the highest levels of ARA or  $n-3$  LC-PUFA had higher full-scale IQs at ages 5–6 y [29], whereas no associations were found with colostrum LC-PUFA levels at earlier stages (2–3 y) [28]. Both studies suggested negative associations between colostrum LA levels with motor and cognitive scores at 2–3 y [28] or verbal IQ at ages 5–6 y, but only when milk DHA levels were low [29]. Similarly, an ecological study linked a higher DHA-to-LA ratio in milk to better academic skills [61]. In a Spanish cohort, higher levels of total  $n-3$  LC-PUFAs and a higher total  $n-3/n-6$  PUFA ratio in colostrum were associated with improved overall neurodevelopment at 14 mo when children received longer milk feeding [27]. In Sweden, higher DHA levels in colostrum were associated with higher full-scale IQ at age 6.5 y whereas higher ARA levels were negatively associated [62].

The conventional regression approach revealed no interaction between lactation duration and pattern 3, failing to support our hypothesis that longer exposure to  $n-3$  and  $n-6$  LC-PUFAs through lactation would increase IQ. This may be due to the short lactation duration (3 mo) in our population, limiting statistical power, or to the child's *FADS* genotype, which may modify the positive association between lactation duration and child IQ [63,64], and influence the association between pattern 3 and IQ in our study.

MR studies on the effects of LC-PUFAs during the perinatal period on child neurodevelopment using maternal *FADS* variants are limited. A systematic review found no conclusive association between maternal *FADS* variants and child cognition, with 3 studies supporting a relationship and 3 findings none [34]. Among the supportive studies, 2 matched our MR findings: 1) the Avon Longitudinal Study of Parents and Children (ALSPAC) showed that infants of mothers with minor alleles of rs174548 (*FADS1*) and rs174455 (*FADS3*) had lower

performance IQ at age 8 y [23], and 2) the minor allele rs174575 (*FADS2*) was associated with poorer memory assessment scores in 16-mo-old toddlers from the southeastern United States [65]. These findings align with the positive associations found with pattern 3 and negative associations with pattern 4 of the MR analyses, where mothers with minor alleles of *FADS1/2/3* had increased levels of precursors ALA and LA and decreased LC-PUFA levels, especially LC-PUFA  $n-6$  [30,34]. Excess LA is suspected to heighten the brain's inflammation risk through its oxidized metabolites [66], and DGLA has been linked to neurodegenerative pathways [67].

When comparing the 2 statistical approaches, MR minimizes confounding bias and measurement errors in exposure but relies on identifying strongly associated genetic variants, which can be challenging, as seen in pattern 1. Nongenetic causal inference methods, such as those described by Frach et al. [68], may help explore causal associations for pattern 1. Both approaches consistently linked patterns 3 and 4 to performance IQ. The lack of association between pattern 3 and verbal IQ in the MR analysis may be due to 1) limited statistical power of the MR sample, as reflected by wider CIs, and 2) potential residual confounding affecting the positive association with verbal IQ in the conventional approach. Indeed, verbal IQ may be more sensitive to environmental factors, such as familial and social interactions, than performance IQ.

Our study strengths include the extensive data from the EDEN cohort on child neurodevelopment, maternal-child genetics, and health and lifestyle factors, enabling robust triangulation of our research question. The availability of child *FADS* variants allowed MR analyses to address potential pleiotropy, with consistent adjusted results enhancing confidence in our findings. However, the 1-sample MR design may overestimate the effect size of genetic variants, introducing bias in causal estimates. The lack of external genome-wide association studies for perinatal PUFA patterns, newly identified in the EDEN cohort, limited alternatives. Nonetheless, the practical impact of this bias appears minimal [69], and risk of false discoveries remains low [70]. Additionally, generalizability is limited, as our cohort comprised individuals of European ancestry with high socioeconomic status, warranting replication in more diverse populations.

In conclusion, the 20th-century shift in Western diets from a balanced intake of  $n-3$  and  $n-6$  PUFAs to an excess of  $n-6$  PUFAs raises concerns about brain development and potential effects on neurodevelopmental disorders [71]. On the basis of our findings, it appears important to: 1) ensure sufficient  $n-3$  LC-PUFA intake during pregnancy and lactation, 2) reduce LA intake to maintain a balance between  $n-6$  LC-PUFAs synthesized from LA and dietary  $n-3$  LC-PUFAs, while also preventing excessive DGLA production.

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## Author contributions

The authors' responsibilities were as follows – AAA, BH, MA, JYB: designed research; BH, M-AC, MA, JYB: conducted research; CS, MT, WLY, HP, MA: provided essential materials; AAA: performed

statistical analysis and had primary responsibility for final content; AAA, BH, MA, JYB: wrote paper; and all authors: contributed to interpretation of data and in editing the manuscript, read and approved the final manuscript.

### Conflict of interest

The authors report no conflicts of interest.

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### Data availability

The data that support the findings of this study are available on request from the EDEN steering committee. Readers may contact [etude.eden@inserm.fr](mailto:etude.eden@inserm.fr) to request the data.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ajcnut.2025.03.014>.

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