



Patterns of perinatal polyunsaturated fatty acid status and associated dietary or candidate-genetic factors

Aline Abou Assi¹, Barbara Heude^{1,*}, Sabine Plancoulaine^{1,2}, Catherine Sarté³, Muriel Tafflet¹, Wen Lun Yuan^{1,4}, Marie-Aline Charles¹, Martine Armand^{3,*}, and Jonathan Y. Bernard^{1,*}

¹Université Paris Cité and Université Sorbonne Paris Nord, INSERM, INRAE, Centre for Research in Epidemiology and Statistics (CRESS), Paris, France; ²Université Claude Bernard Lyon 1, INSERM, CNRS, Centre de Recherche en Neurosciences de Lyon CRNL U1028 UMR5292, WAKING, Bron, France; ³Aix Marseille Université, CNRS, CRMBM, Marseille, France; and ⁴Singapore Institute for Clinical Sciences, Agency for Science, Technology, and Research (A*STAR), Singapore, Singapore

Abstract Perinatal exposure to omega-3 and omega-6 polyunsaturated fatty acids (PUFAs) can be characterized through biomarkers in maternal or cord blood or breast milk. Objectives were to describe perinatal PUFA status combining multiple biofluids and to investigate how it was influenced by dietary intake during pregnancy and maternal *FADS* and *ELOVL* gene polymorphisms. This study involved 1,901 mother-child pairs from the EDEN cohort, with PUFA levels measured in maternal and cord erythrocytes, and colostrum. Maternal dietary PUFA intake during the last trimester was derived from a food frequency questionnaire. Twelve single-nucleotide polymorphisms in *FADS* and *ELOVL* genes were genotyped from maternal DNA. Principal component analysis incorporating PUFA levels from the three biofluids identified patterns of perinatal PUFA status. Spearman's correlations explored associations between patterns and PUFA dietary intake, and linear regression models examined pattern associations with *FADS* or *ELOVL* haplotypes. Five patterns were retained: "High omega-3 LC-PUFAs, low omega-6 LC-PUFAs"; "Omega-6 LC-PUFAs"; "Colostrum LC-PUFAs"; "Omega-6 precursor (LA) and DGLA"; "Omega-6 precursor and colostrum ALA". Maternal omega-3 LC-PUFA intakes were correlated with "High omega-3 LC-PUFAs, low omega-6 LC-PUFAs" ($r(\text{DHA}) = 0.33$) and "Omega-6 LC-PUFAs" ($r(\text{DHA}) = -0.19$) patterns. Strong associations were found between *FADS* haplotypes and PUFA patterns except for "High omega-3 LC-PUFAs, low omega-6 LC-PUFAs".[■] Lack of genetic association with the "High omega-3 LC-PUFAs, low omega-6 LC-PUFAs" pattern, highly correlated with maternal omega-3 LC-PUFA intake, emphasizes the importance of adequate omega-3 LC-PUFA intake during pregnancy and lactation. This study offers a more comprehensive assessment of perinatal PUFA status and its determinants.

Supplementary key words epidemiology • omega-3 fatty acids • pregnancy • dietary fat • perinatal PUFA status • *FADS* genes • *ELOVL* genes • EDEN mother-child cohort study

The first 1,000 days of life, from conception to two years of age, has been addressed by the World Health Organization as a critical window for the development of the fetus and child and play a pivotal role in the health programming of the future adult (1). Omega-6 and omega-3 polyunsaturated fatty acids (PUFAs) are important nutrients for the growth and development of several key organs, including the nervous and cardiovascular systems of the fetus and the newborn (2). From the 30th to the 38th week of gestation, long-chain PUFA (LC-PUFA) accretion in the fetus increases exponentially, mainly in the fetal brain (3). After birth, breast milk normally provides a substantial amount of crucial LC-PUFAs including arachidonic acid (AA) and docosahexaenoic acid (DHA), which allows to continue the accretion of these two LC-PUFAs in tissues, especially the brain (4, 5).

The two so-called essential fatty acids, linoleic (LA) and alpha-linolenic (ALA) acids, precursors of omega-6 and omega-3 LC-PUFAs, respectively, must be supplied by the diet, as the body is unable to synthesize them. From these precursors, LC-PUFA biosynthesis is catalyzed by desaturase and elongase enzymes. These are encoded by the Fatty Acid Desaturase gene cluster (*FADS1*, *FADS2*, *FADS3*) and Elongation-of-very-long-chain-fatty-acids (*ELOVL2*, *ELOVL4*, and *ELOVL5*) gene family, respectively. Multiple single-nucleotide polymorphisms (SNPs) located on these genes are known to modulate enzyme activity and consequently, maternal and fetal PUFA status, and breast milk composition (6–8).

*These authors contributed equally to this work.

*For correspondence: Barbara Heude, Barbara.heude@inserm.fr.

Perinatal exposure to omega-3 and omega-6 PUFAs over the first 1,000 days has been assessed through PUFA levels mainly from maternal blood, cord blood, or breast milk (9). Maternal blood levels reflect the mother's body PUFA status, which originates from her diet, adipose tissue storage, and metabolism of LA and ALA. On the other hand, cord blood levels reflect the fetus's body PUFA status. Since the fetus has limited desaturase activity to meet its needs, the fetus's body PUFA status depends on the mother's PUFA status, placental transfer, and to a much lesser extent on the fetus' own metabolism (10). Early breast milk (i.e., colostrum) than mature breast milk PUFA levels are the foremost contributors to the PUFA status of newborns when exclusively breastfed.

These three biofluids reflecting distinct but closely related exposure windows have been examined separately in association studies with child health outcomes. Concerning child neurodevelopment, studies on prenatal exposure to PUFAs through maternal or cord blood yielded inconsistent results (11–17), which also diverged from the findings in studies examining postnatal exposure through breast milk PUFAs (18–21). These previous studies analyzing pre- or postnatal biofluids separately may have potentially overlooked confusion that may arise from the high correlation between PUFA across these biofluids, which could explain the lack of consistency in findings. As discussed in a previous study conducted within the EDEN French cohort, which specifically examined the associations between colostrum levels of PUFA and cognitive functions (20), the inverse relationship observed between LA and child IQ could potentially reflect prenatal exposure to LA through maternal status during pregnancy. We aimed to overcome this limitation by adopting an integrative approach that incorporates PUFA levels from maternal and cord blood, and colostrum together to elucidate the specific role of each exposure window while considering their interdependence. This approach intends to enhance the ability to infer causality in forthcoming works. Moreover, the understanding of the mechanisms explaining potential variability in perinatal exposure to PUFAs needs to be further explored, particularly focusing on the role of two major sources influencing perinatal PUFA status: nutritional intake during pregnancy and maternal genetic variants in *FADS* and *ELOVL* genes. To our best knowledge, no such comprehensive study has been previously conducted.

To address this gap, the present study aimed to 1) identify patterns of perinatal PUFA status by combining data from maternal and cord blood erythrocyte membranes, and colostrum and 2) examine the respective role of maternal PUFA intake during pregnancy and maternal candidate SNPs in *FADS* and *ELOVL* genes, on such patterns.

MATERIALS AND METHODS

Design and study population

The 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 women were recruited from February 2003 to January 2006 in two university maternity clinics, in Nancy and Poitiers. A total of 2,002 pregnant women were enrolled before 20 weeks of amenorrhea. Exclusion criteria were multiple births, known diabetes before pregnancy, French illiteracy, and planning to move out of the region in the 3 following years. 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 (CCPPRB) of the Bicêtre university hospital and the National Data Protection Authority (CNIL). More detail about the EDEN cohort study is available elsewhere (22).

Sampling and fatty acids composition

Maternal venous ($n = 1,906$) and cord ($n = 1,437$) blood samples were collected at 24–28th weeks of gestation (mean gestational age \pm SD: 26.3 ± 1.6) and at delivery (mean gestational age: 39.2 ± 1.7), respectively. Additionally, about 5 ml of maternal colostrum ($n=980$) was collected within one week of delivery (3.9 ± 1.1 days after birth). Fatty acid composition of erythrocyte membrane phospholipids in maternal and cord blood, and of colostrum was analyzed using gas chromatography, as described in previous studies (23, 24). In total, 31 and 26 fatty acids (FA), present in proportion $\geq 0.05\%$, including 12 and 11 PUFA species, were detected in erythrocyte membrane and colostrum, respectively, such as LA (C18:2n-6), gamma-linolenic (GLA, 18:3n-6), eicosadienoic (EDA, 20:2n-6), dihomo-gamma-linolenic (DGLA, C20:3n-6), AA (C20:4n-6), docosatetraenoic (DTA, C22:4n-6), docosapentaenoic (DPA, C22:5n-6), ALA (C18:3n-3), stearidonic (STD, 18:4n-3), eicosatetraenoic (ETA, 20:4n-3), eicosapentaenoic (EPA, C20:5n-3), DPA n-3 (C22:5n-3) and DHA (C22:6n-3) acids. FA levels were expressed in the percentage of total fatty acids.

The different time points of sample collection intend to capture the longitudinal status of PUFA in mother-child pairs throughout pregnancy and lactation. Maternal PUFA levels at 24–28 weeks of gestation are expected to mirror both maternal status and fetal exposure during the second trimester of gestation, as erythrocyte PUFA levels are known to reflect long-term PUFA intake (up to the prior 3 months) (25, 26). More specifically, PUFA levels at 24–28 weeks of gestation provide an indication of the mother's baseline status just before the substantial accretion of long-chain PUFA into the fetal tissues, which occurs during the third trimester (3).

Maternal diet during pregnancy

Maternal diet during the third trimester of pregnancy was assessed by a self-administered semi-quantitative Food Frequency Questionnaire (FFQ) completed in the maternity ward in the first few days after delivery. This FFQ has been validated with a series of 24 h recalls (27). The consumption frequency of 137 foods and food groups was estimated with a 7-level scale ranging from “never” to “more than once a day”. In addition, mothers indicated the portion size of 12 food categories (such as meats, French fries, pasta, vegetables, cakes, cheese, and so on) using the food portion pictures booklet

from the « SUPplementation en Vitamines et Minéraux AntioXydants » (SU.VI.MAX) study. For non-available food pictures, standard portions for the French adult population were considered (28). Daily intake of macro- and micro-nutrients (in g/day) including total omega-6, LA, AA, and total omega-3, ALA, EPA, and DHA, were calculated by crossing data frequency, portion size, and nutritional composition of each item derived from the SU.VI.MAX food composition database (29).

Genotyping and selection of genetic variants

Maternal DNA was extracted from maternal blood sample ($n = 1,719$). According to a candidate-gene procedure, 12 SNPs (supplemental Table S1) located in the *FADS* (Chromosome 11) and *ELOVL* (Chromosome 6) gene region encoding for PUFAs metabolic enzymes, were genotyped by the allele-specific polymerase chain reaction (AS-PCR) method on the ABI7900HT platform: rs174546 (*FADS1*), rs174575, rs174589 and rs422249 (*FADS2*), rs174448 and rs174634 (*FADS3*), rs953413, rs10498676 and rs3798719 (*ELOVL2*), rs239532 (*ELOVL4*), rs12207094 and rs17544159 (*ELOVL5*). The selection of the SNPs was based on the knowledge from literature and a Tagging-SNP analysis of *FADS* and *ELOVL* variants from the HapMap database (phase III) using Haploview software v4.2 (Broad Institute of MIT and University of Harvard). The literature review was essentially based on genome-wide association studies performed in plasma or erythrocytes PUFA levels showing significant associations with SNPs from the *FADS* gene cluster or *ELOVL* gene family in linkage disequilibrium with those examined in the present study (30–39).

Study sample

The selection of the study sample is presented in the flow diagram Fig. 1. Among 2,002 women enrolled, 95 left the study during pregnancy or at delivery, leading to 1,907 mother-child dyads followed up after delivery. From this sample, we removed participants with complete missing data for FA in the 3 biofluids ($n = 6$). Among the 1,901 mother-child dyads (whether with breastfeeding or not) having available data for at least one biofluid, missing data on FA levels in one or two of the other biofluids, and dietary intake of PUFAs during pregnancy were imputed. This sample was used for the identification of patterns of perinatal PUFA status. The respective role of dietary intake during pregnancy and maternal genetic variants on the patterns of PUFA status was assessed on 1,677 mother-child dyads after excluding missing data on candidate SNP genotyping (complete missing data $n = 209$; missing data on more than 3 SNPs $n = 15$).

Statistical analyses

Prior to the analyses, the percentage of FAs was log-transformed to improve the normality of the frequency distribution. Missing FA data of biofluids and of dietary intakes were imputed using *MissForest* packages (R software v4.2.1). The *MissForest* is a nonparametric imputation method particularly designed for datasets with mixed types of variables (e.g., continuous and categorical variables). This method employs a random forest algorithm based on an iterative approach to predict missing values. With each successive iteration, the generated predictions are better, refining the imputation process and enhancing the accuracy of the results (40). The *missForest* algorithm has been previously documented and compared to other established imputation methods (40). It has

been demonstrated that *missForest* outperforms other methods by yielding more robust results through a reduction in imputation error. Furthermore, the *missForest* algorithm offers a way to assess imputation quality without the need for setting aside separate test data or conduct cross-validation procedures. This evaluation method is referred to as the out-of-bag (OOB) imputation error estimates and has been calculated in our study (OOB error = 0.065). A lower OOB imputation error, approaching zero, indicates better model performance.

Because erythrocyte FA levels are influenced by gestational age, and colostrum composition shifts during the first few days after delivery, pre-adjustment was carried out to account for these two factors. This consists in applying linear regression models explaining (separately) each PUFA by gestational age (in weeks of gestation) at maternal blood sampling for maternal PUFAs, gestational age at delivery for cord PUFAs, and the time (in days) between birth and colostrum sampling for colostrum PUFAs. The pairwise correlations between PUFA regression residuals from all three biofluids were calculated using Pearson's correlations. Then, a principal component analysis (PCA) including all PUFA regression residuals was performed to identify patterns of perinatal PUFA status. The number of components retained was based on the scree plot curve, and the percentage of variance explained. The conventional loading factor threshold of $[0.30]$ was set to identify high PUFAs contribution (depicting higher or lower biofluid level) to the component. By construct, the component scores of each individual are standardized (mean = 0; SD = 1). Each retained component has been labeled according to the PUFAs contribution as a pattern of perinatal PUFA status, and these labels were further used throughout the paper. In a supplementary analysis, bivariate regressions between each perinatal PUFA pattern and maternal health or sociodemographic characteristics have been performed. As a sensitivity analysis, PCA was also performed with a complete case approach including only mother-child dyads having FA profiles for all three biofluids ($n = 689$).

In order to study the role of maternal diet in the patterns of perinatal PUFA status, a partial Spearman's correlation was estimated between each PUFA pattern selected from the PCA and dietary intake (in g/day) of LA, AA, ALA, EPA, DPA n-3, DHA, during the last trimester of pregnancy, adjusted for study center (Nancy or Poitiers). In a sensitivity analysis, correlations were further adjusted for total energy intake (in calories/day).

Regarding the role of maternal candidate SNPs, due to the high linkage disequilibrium (LD) found among variants from the same chromosome (supplemental Tables S2 and S3), a haplotype-based approach was used. The latter has benefits over individual SNP analysis since it accurately captures the allelic diversity and the pattern of inheritance over the evolution of the presumed causal genes. Therefore, haplotype blocks in chromosome 11 for *FADS* genes and chromosome 6 for *ELOVL* genes were identified using the procedure proposed by Gabriel *et al.* (41) from the *trio* packages—*findLDblocks* function (R software v4.2.1). The settled parameters in this function were the confidence interval of LD coefficient D' for a SNP pair $CI(D') = [0.7; 0.9]$ with $\alpha = 0.1$ and the recombination threshold $cuRecomb = 0.8$ ([rdocumentation.org/packages/trio/versions/3.10.0/topics/findLDblocks](http://rddocumentation.org/packages/trio/versions/3.10.0/topics/findLDblocks)). Then, the associations between patterns of perinatal PUFA status and haplotypes were tested by linear regression model using the *haplo.glm* function (*haplo.stats* packages) and the most frequent haplotype as the reference category. *Haplo.glm* relies on a special expectation-maximization (EM)-based algorithm to estimate haplotype frequencies and assess their association with phenotypic traits

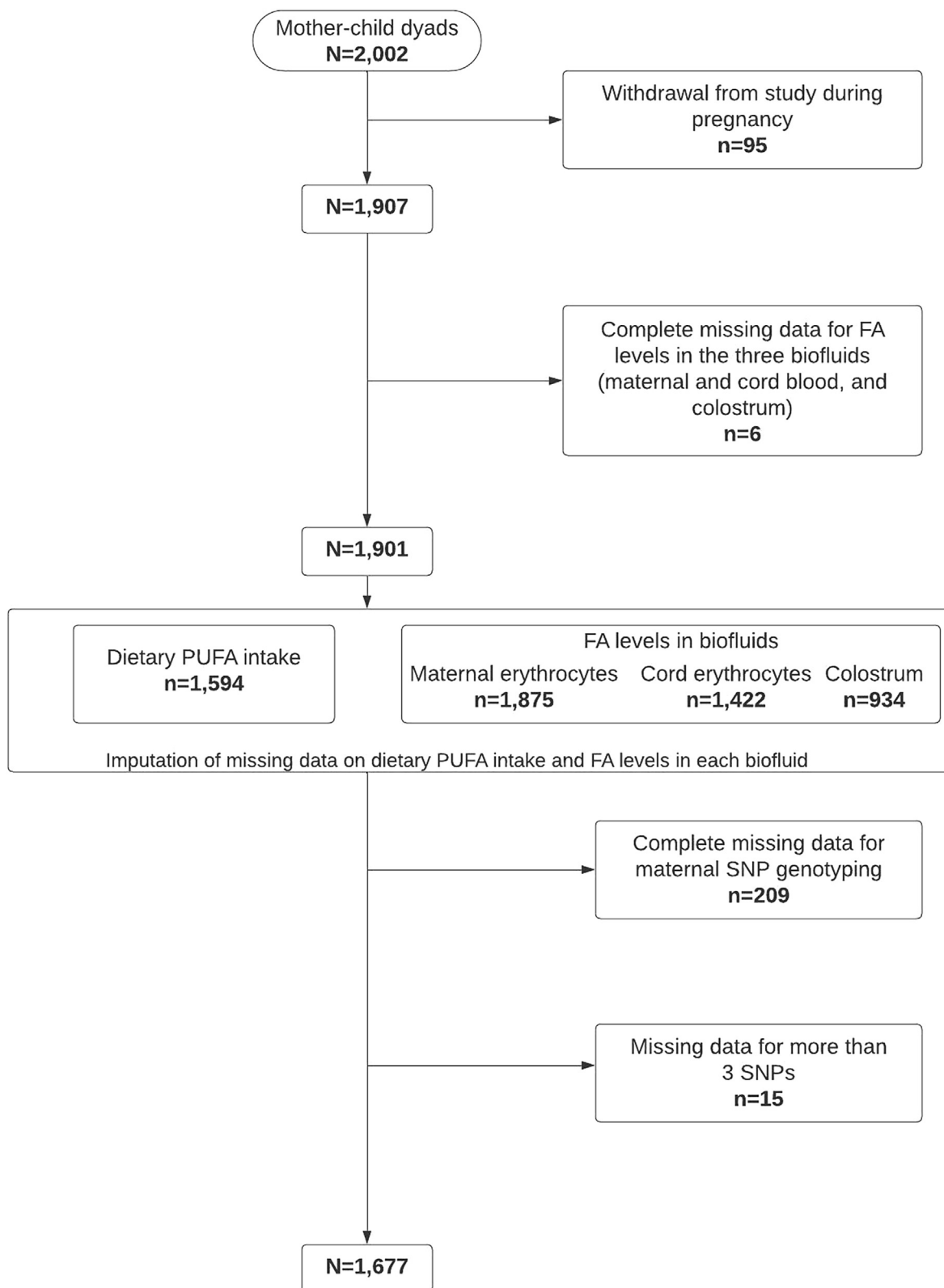


Fig. 1. Flow diagram of study population. FA, fatty acids; PUFA, polyunsaturated fatty acid; SNP, single nucleotide polymorphism.

(42). This method is particularly valuable when the phase of genetic variants is ambiguous, meaning uncertainty about whether alleles are located on the same copy of the chromosome, known as the *cis* configuration, or on different copies, referred to as the *trans* configuration. By integrating statistical

techniques like the EM-based algorithm with generalized linear models (GLMs), *haplo.glm* estimates the probabilities of different haplotypes within the population, allowing for the assessment of their effects on the phenotype. This approach enables the identification of potential genetic associations

while accounting for uncertainty in the haplotype phase. For SNPs being in weak linkage disequilibrium with other SNPs and therefore not included in any haplotype blocks, a simple linear regression model was conducted with each pattern under the codominance assumption.

Imputation and genetic analyses were performed using R software version 4.1.2 (R Foundation for Statistical Computing), and all other analyses were performed using V9.4 SAS software (SAS Institute Inc).

RESULTS

Sample characteristics

Among 1,901 mothers included in the analysis, the mean age at delivery was 29.5 (± 4.9) years. More than 30% of mothers graduated with a five-year university degree and around 27% reported having a mean household income above 3,000 euros per month. The average gestational age at delivery was 39.2 (± 1.8) weeks of gestation.

PUFA correlations across biofluids

The pairwise correlation matrix of PUFAs across the three biofluids is depicted in [Fig. 2](#). Strong correlations, both within and between PUFA families (omega-6 vs. omega-3 and precursors vs. LC), as well as among the different biofluids, were observed. The strongest correlations were observed within the omega-3 LC-PUFAs family in colostrum, particularly between DPA n-3 and DHA (Pearson's correlation coefficient $r = 0.83$), followed by EPA and DHA ($r = 0.67$). Of particular interest were the inter-biofluid correlations observed between omega-3 LC-PUFAs in colostrum and cord blood erythrocytes, ranging from $r = 0.29$ for EPA to $r = 0.52$ for DHA. Similarly, albeit with weaker strengths, correlations were found between omega-3 LC-PUFAs in colostrum and maternal erythrocytes, as well as between cord and maternal erythrocytes.

Identification of patterns of perinatal PUFA status

From the PCA analysis performed on 1,901 mother-child dyads, the first 5 components explaining 50.6% of the total variance in PUFA status were retained ([Fig. 3](#)). These components highlighted notable variations across PUFA species (omega-6 vs. omega-3, precursors vs. LC-PUFAs) and between biofluids and were labeled as follows: pattern 1 "High omega-3, low omega-6 LC-PUFAs"; pattern 2 "Omega-6 PUFAs"; pattern 3 "Colostrum LC-PUFAs"; pattern 4 "Omega-6 precursor (LA) and DGLA"; and pattern 5 "Omega-6 precursor and colostrum ALA".

Pattern 1 corresponded to perinatal exposure to higher levels of omega-3 LC-PUFAs (EPA, DPA n-3, DHA) in the three biofluids altogether, along with lower levels of omega-6 LC-PUFAs (AA, DTA, DPA n-6) in maternal and cord erythrocytes. This pattern captured the most variability in PUFA status with 17.2% of the total variance explained. Pattern 2 (11.7% of total variance) referred to higher levels of omega-6 LC-PUFAs (AA, DTA, DPA n-6) in the three biofluids

combined with lower levels of ALA, EPA, and DHA in maternal erythrocytes. Pattern 3 (9.0% of total variance) was mainly a colostrum-specific pattern with higher levels of omega-6 LC-PUFAs (AA, DTA, DPA n-6) and omega-3 LC-PUFAs, especially DPA n-3 and DHA. However, lower levels were observed in cord erythrocytes for DTA, DPA n-6, and ALA.

Patterns 4 and 5 capturing 7.6% and 5.1% of the total variance, respectively, were defined by higher levels of omega-6 precursor (LA) in all three biofluids. Pattern 4 was also characterized by higher levels of DGLA in all three biofluids, while pattern 5 differed from pattern 4 by presenting a lower level of DGLA in erythrocytes and a higher level of omega-3 precursor (ALA) in the colostrum exclusively. The results based on the complete case sample ($n = 689$) showed similar patterns ([supplemental Fig. S1](#)).

Patterns of perinatal PUFAs status and maternal health or sociodemographic characteristics

Bivariate analyses between identified perinatal PUFA status and maternal health or sociodemographic characteristics are presented in [supplemental Table S4](#). Higher maternal age and education level were associated positively with patterns 1 (High omega-3, low omega-6 LC-PUFAs) and 3 (Colostrum LC-PUFAs) and negatively with patterns 2 (Omega-6 PUFAs) and 4 (Omega-6 precursor (LA) and DGLA). Primiparity was associated positively with pattern 1 and negatively with pattern 3. Prepregnancy underweight, overweight, or obesity status was associated negatively with pattern 1, while overweight and obesity status was positively associated with pattern 2; mothers with an obesity status presented a score of adherence to pattern 1 decreased by -0.26 [-0.42 ; -0.09] SD, and a score of adherence to pattern 2 increased by 0.53 [0.37 ; 0.69] SD, compared to mothers with normal weight. Mothers living in/close to the French town of Nancy were positively associated with patterns 3 and 5, while those living in/close to the town of Poitiers were positively associated with patterns 1 and 2.

None of the maternal health or sociodemographic characteristics was associated with pattern 5 (Omega-6 precursor and colostrum ALA) except for the living place (Nancy/Poitiers).

Maternal diet and patterns of perinatal PUFA status

Mean LA intake during the last trimester of pregnancy was 9.63 ± 4.15 g/day (Min = 1.87 g/day; max = 33.78 g/day) and intake of ALA was on average 0.88 ± 0.33 g/day (Min = 0.24 g/day; max = 2.38 g/day). Regarding LC-PUFAs, mean intake of AA, EPA, DPA n-3 and DHA were 0.16 ± 0.08 , 0.08 ± 0.05 , 0.04 ± 0.02 , and 0.17 ± 0.10 g/day, respectively. Correlations between maternal PUFA intake and patterns of perinatal PUFA status identified from PCA are presented in [Table 1](#). The results of the sensitivity analysis adjusting for total energy intake during pregnancy showed similar results (data not shown). The first two patterns, "High omega-3

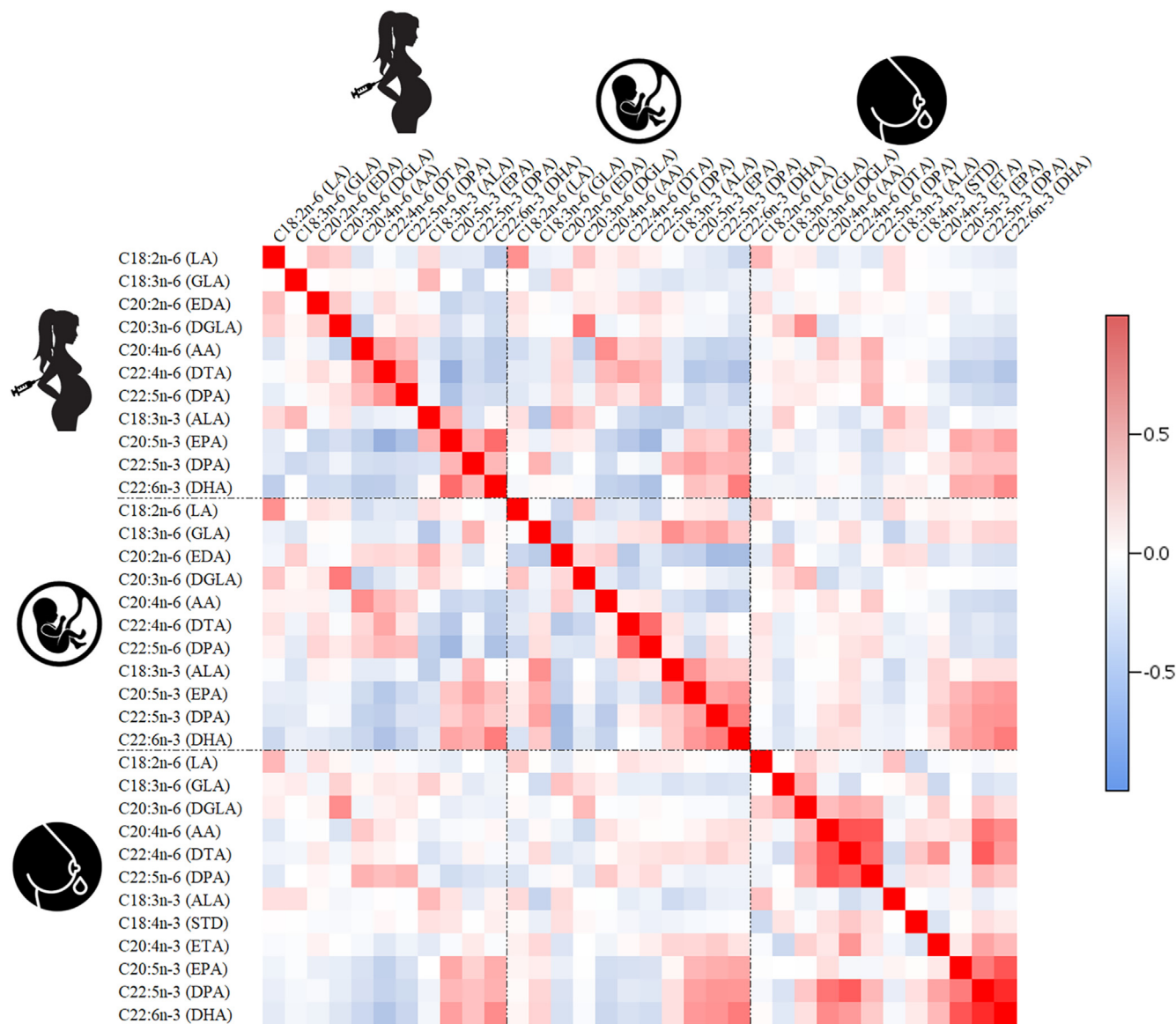


Fig. 2. Pairwise correlation matrix of PUFA levels in maternal and cord blood erythrocytes, and in colostrum - N = 1,901. Pearson's correlations ranging from -1 to 1.

LC-PUFAs, low omega-6 LC-PUFAs" and "Omega-6 LC-PUFAs", were strongly correlated, positively and negatively respectively, with maternal dietary intake of DHA and EPA. Patterns 3 "Colostrum LC-PUFAs" and 4 "Omega-6 precursor (LA) and DGLA" were not correlated with any of the maternal dietary PUFAs, while pattern 5 "Omega-6 precursor and colostrum ALA" showed weak positive correlations with LA intake as well as with the dietary total n-6/n-3 PUFA ratio (intakes of LA+AA/EPA+DPA n-3+DHA).

Maternal candidate single-nucleotide polymorphisms and patterns of perinatal PUFA status

Candidate SNPs located in *FADS* genes were divided into two haplotype blocks (Table 2 - *FADS* gene section). The first block comprised rs174546 (C, T) located in

FADS1, rs174575 (C, G) in *FADS2*, and rs174589 (G, C) in *FADS2*, while the second block included rs422249 (C, T) in *FADS2* and rs174448 (T, C) in *FADS3*. Since rs174634 (G, C) in *FADS3* was in very weak linkage disequilibrium (supplemental Table S2) with the other SNPs, this SNP was left apart from the haplotype blocks.

In the first block (named *FADS1-2*) and the second block (named *FADS2-3*), the most frequent haplotypes were the ones including the major alleles CCG (67.2%) and CT (63.9%), respectively. No association between pattern 1 "High omega-3, low omega-6 LC-PUFAs" and block *FADS1-2* was found. A single, weak association was observed with *FADS2-3* haplotypes, more precisely with the minor alleles TC haplotype. Pattern 2 "Omega-6 LC-PUFAs" and pattern 3 "Colostrum LC-PUFAs" were strongly negatively associated with haplotypes in block *FADS1-2* and *FADS2-3* with the highest association

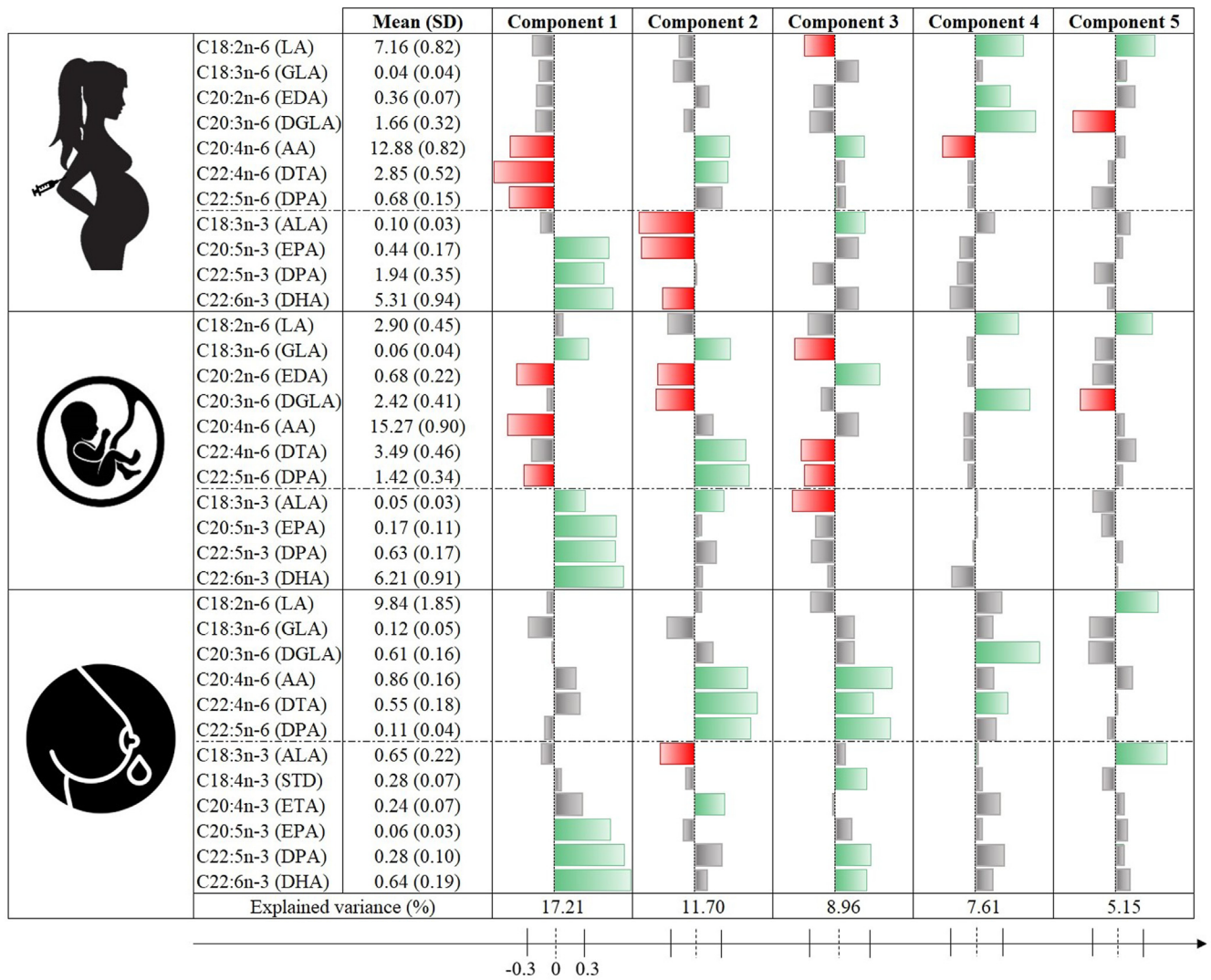


Fig. 3. Principal component analysis including omega-3 and omega-6 PUFAs levels from maternal and cord blood, and colostrum – N = 1,901. Means are expressed as percentage of total fatty acids in each biofluid; a threshold loading factor of |0.30| was set to identify high PUFA contribution to the principal component; According to the contribution of PUFA, components were defined as follows: component 1 “High omega-3 LC-PUFAs, low omega-6 LC-PUFAs”, component 2 “Omega-6 LC-PUFAs”, component 3 “Colostrum LC-PUFAs”, component 4 “Omega-6 precursor (LA) and DGLA”, component 5 “Omega-6 precursor and colostrum ALA”.

observed for TCG haplotype; mother carrying TCG haplotype presented a score of adherence to pattern 2 and pattern 3 decreased by β [95% CI] = -0.65 [-0.78 ; -0.51] and -0.56 [-0.69 ; -0.42] standard deviation, respectively, compared to mothers carrying the reference CCG haplotype. While pattern 4 “Precursor of omega-6 (LA) and DGLA” was strongly positively associated with haplotypes in block *FADS1-2* and *FADS2-3* (0.87 [0.80 ; 0.95] for minor alleles TGC haplotype compared to CCG), pattern 5 “Precursor omega-6 and colostrum ALA” showed consistent negative associations (-0.57 [-0.65 ; -0.48] for the minor alleles TGC haplotype (Table 2 - *FADS* gene section)).

Regarding candidate SNPs located in *ELOVL* genes, the three SNPs in *ELOVL2* (i.e., rs953413 (G, A), rs10498676 (G, A), and rs3798719 (C, T)) were included in a first block haplotype (named block *ELOVL2*), while

two SNPs in *ELOVL5*, rs17544159 (A, C) and rs12207094 (A, T) were comprised in a second one (named block *ELOVL5*) (Table 2 - *ELOVL* gene section). The most frequent haplotypes were the major alleles GGC (56.8%) and AA (86.0%) for the *ELOVL2* and *ELOVL5* blocks, respectively. No association was observed for *ELOVL* haplotypes, except for AGC in block *ELOVL2* which was weakly associated with pattern 3 (-0.26 [-0.45 ; -0.06]) and pattern 5 (0.27 [0.08 ; 0.47]).

DISCUSSION

Owing to the availability of PUFA levels in three biofluids (maternal and cord erythrocytes, and colostrum) in a sample of 1,901 mother-child dyads from the EDEN cohort, five independent patterns of perinatal PUFA status were newly identified. These patterns

TABLE 1. Correlations between maternal intake of PUFAs during the last trimester of pregnancy and patterns of perinatal PUFA status – N = 1,677

Maternal PUFA Intake	Mean (SD)	High Omega-3 LC-PUFAs, low Omega-6 LC-PUFAs	Omega-6 LC-PUFAs	Colostrum LC-PUFAs	Omega-6 Precursor (LA) and DGLA	Omega-6 Precursor and Colostrum ALA
Total n-6/n-3 PUFA ratio	8.80 (2.23)	−0.26 ^c	0.13 ^c	−0.11 ^c	0.04	0.12 ^c
Total PUFA n-6	9.80 (4.21)	0.01	−0.05 ^a	−0.06 ^a	−0.04	0.09 ^b
C18:2n-6 (LA)	9.63 (4.15)	0.01	−0.05 ^a	−0.06 ^a	−0.03	0.09 ^b
C20:4n-6 (AA)	0.16 (0.08)	0.01	−0.02	0.00	−0.05 ^a	0.04
Total PUFA n-3	1.13 (0.42)	0.20 ^c	−0.16 ^c	0.03	−0.06 ^a	0.01
C18:3n-3 (ALA)	0.88 (0.33)	0.09 ^b	−0.11 ^c	−0.01	−0.06 ^a	0.00
C20:5n-3 (EPA)	0.08 (0.05)	0.38 ^c	−0.21 ^c	0.09 ^b	−0.05 ^a	0.03
C22:5n-3 (DPA)	0.04 (0.02)	0.17 ^c	−0.08 ^b	0.03	−0.05 ^a	0.06 ^a
C22:6n-3 (DHA)	0.17 (0.10)	0.33 ^c	−0.19 ^c	0.07 ^a	−0.06 ^a	0.03

Partial Spearman's correlation coefficients adjusted for study Center (Nancy or Poitiers). Correlation coefficient higher than ± 0.05 are significant at α -level 5%.

^a<0.05.

^b<0.001.

^c<0.0001.

differentiate PUFAs not only across the biofluids but also between the two PUFA families (omega-3 vs. omega-6), and within these families (precursors vs. long chains, i.e., FAs with a number of carbon superior to 18). The first pattern “High omega-3 LC-PUFAs, low omega-6 LC-PUFAs” explained the largest proportion of variance in PUFA levels. Maternal intake of EPA and DHA during pregnancy showed positive and negative associations with pattern 1 “High omega-3 LC-PUFAs, low omega-6 LC-PUFAs” and pattern 2 “Omega-6 LC-

PUFAs”, respectively. Haplotypes of the *FADS* gene cluster were strongly associated with all patterns, either negatively or positively, except with the first one.

The methodology applied in the present study contrasts with previous studies in which biofluids were examined separately, considering either individual PUFA or PUFA ratios, when describing PUFA status or analyzing its associations with child health and developmental parameters (9, 11–21). The results of such studies cannot ensure that the identified associations

TABLE 2. Associations between *FADS* and *ELOVL* haplotype blocks and patterns of perinatal PUFA status - N = 1,677

Haplotype	Frequency (%)	High Omega-3 LC-PUFAs, low Omega-6 LC-PUFAs	Omega-6 LC-PUFAs	Colostrum LC-PUFAs	Omega-6 Precursor (LA) and DGLA	Omega-6 Precursor and Colostrum ALA
Block <i>FADS1-FADS2</i>						
CCG	67.2	Ref	Ref	Ref	Ref	Ref
TCG	6.3	−0.02 [−0.16; 0.12]	−0.65 [−0.78; −0.51]	−0.56 [−0.69; −0.42]	0.64 [0.52; 0.77]	−0.11 [−0.24; 0.03]
TGG	4.6	−0.14 [−0.30; 0.02]	−0.45 [−0.60; −0.29]	−0.46 [−0.62; −0.31]	0.63 [0.48; 0.77]	−0.30 [−0.45; −0.14]
TGC	19.3	−0.06 [−0.14; 0.03]	−0.41 [−0.49; −0.33]	−0.41 [−0.49; −0.33]	0.87 [0.80; 0.95]	−0.57 [−0.65; −0.48]
Rare	2.6	0.05 [−0.17; 0.27]	0.11 [−0.10; 0.32]	−0.09 [−0.30; 0.11]	0.21 [0.02; 0.40]	0.02 [−0.19; 0.23]
Block <i>FADS2-FADS3</i>						
CT	63.9	Ref	Ref	Ref	Ref	Ref
CC	3.1	−0.05 [−0.24; 0.15]	−0.35 [−0.55; −0.15]	−0.29 [−0.48; −0.09]	0.77 [0.59; 0.95]	−0.17 [−0.36; 0.03]
TC	32.6	−0.09 [−0.16; −0.02]	−0.27 [−0.35; −0.20]	−0.25 [−0.32; −0.18]	0.52 [0.46; 0.59]	−0.26 [−0.33; −0.19]
Rare (TT)	0.5	0.24 [−0.28; 0.76]	−0.25 [−0.77; 0.27]	0.11 [−0.40; 0.63]	0.38 [−0.13; 0.89]	−0.85 [−1.38; −0.32]
rs174634 (G, C)/ <i>FADS3</i> ^a		−0.01 [−0.09; 0.06]	−0.20 [−0.28; −0.13]	−0.21 [−0.29; −0.14]	0.48 [0.40; 0.55]	−0.17 [−0.24; −0.09]
Block <i>ELOVL2</i>						
GGC	56.8	Ref	Ref	Ref	Ref	Ref
AGC	2.8	0.08 [−0.11; 0.27]	0.10 [−0.10; 0.29]	−0.26 [−0.45; −0.06]	−0.02 [−0.22; 0.17]	0.27 [0.08; 0.47]
AGT	23.7	0.06 [−0.02; 0.14]	0.01 [−0.07; 0.10]	−0.01 [−0.09; 0.07]	−0.03 [−0.11; 0.05]	−0.05 [−0.13; 0.03]
AAC	15.5	−0.01 [−0.11; 0.08]	−0.05 [−0.14; 0.05]	−0.08 [−0.18; 0.02]	−0.003 [−0.10; 0.09]	−0.02 [−0.12; 0.08]
Rare	1.2	−0.10 [−0.42; 0.22]	−0.19 [−0.51; 0.14]	0.07 [−0.25; 0.39]	0.28 [−0.04; 0.60]	−0.02 [−0.35; 0.30]
Block <i>ELOVL5</i>						
AA	86.0	Ref	Ref	Ref	Ref	Ref
AT	6.2	0.09 [−0.04; 0.23]	0.01 [−0.13; 0.14]	0.02 [−0.11; 0.16]	0.09 [−0.05; 0.23]	0.06 [−0.08; 0.20]
CT	7.7	−0.07 [−0.20; 0.05]	−0.06 [−0.18; 0.07]	−0.05 [−0.18; 0.08]	0.11 [−0.01; 0.24]	−0.01 [−0.14; 0.12]
rs239532 (G, C)/ <i>ELOVL4</i> ^a		−0.01 [−0.11; 0.09]	0.06 [−0.04; 0.16]	0.02 [−0.07; 0.12]	−0.05 [−0.16; 0.04]	0.05 [−0.05; 0.15]

Block *FADS1-FADS2* rs174546 (C, T)/*FADS1*, rs174575 (C, G)/*FADS2*, rs174589 (G, C)/*FADS2*; block *FADS2-FADS3* rs422249 (C, T)/(*FADS2*), rs174448 (T, C)/(*FADS3*); block *ELOVL2* rs953413 (G, A)/*ELOVL2*, rs10498676 (G, A)/*ELOVL2*, rs3798719 (C, T)/*ELOVL2*; block *ELOVL5*: rs17544159 (A, C)/(*ELOVL5*), rs12207094 (A, T)/*ELOVL5*. Minor allele is presented in red; rare category includes haplotypes with a frequency of less than 2% each; Associations are represented by β coefficient with corresponding 95% confidence intervals (β [95% CI]) derived from regression models of pattern scores on haplotypes).

^aChange in pattern score in the additive genetic models assuming a trend per copy of the minor allele.

are not confounded by the impact of the same PUFA from another biofluid or of other PUFA strongly correlated. Employing a dimension reduction method such as principal component analysis (PCA) helps avoid correlation bias by generating independent patterns of perinatal PUFA status. The question of using clustering methods instead of PCA has been carefully considered. Clustering methods offer advantages, including a more straightforward interpretation of results. However, as highlighted by Bennette et Vickers, 2012 (43), one of the limitations of categorizing exposure variables is the loss of statistical power. This represents a significant hindrance to the study of the associations with genetic and nutritional factors and to our further research investigation of the clinical implications of perinatal exposure to PUFAs on child neurodevelopment. Therefore, to maintain adequate statistical power, assigning continuous scores to each individual through PCA seemed more appropriate to address our future research objectives.

An additional noteworthy aspect of our study is the measurement of PUFA status in erythrocyte membrane, which has certain advantages over plasma phospholipids used in previous studies (9). The former is known to be a better biochemical index for monitoring long-term PUFA intake (over 2–3 months), whereas plasma phospholipids are more sensitive to short-term changes in PUFA intake (over a few weeks) (25, 26). In addition, it has been suggested that the essential FAs (ALA and LA) and LC-PUFAs found in the erythrocytes of newborns (from cord blood) reliably demonstrate maternal transfer. This has been evidenced by the direct correlations of these fatty acids between term newborns and their mothers, which were stronger than those observed in plasma phospholipids (44).

Examining genetic associations with each pattern of perinatal PUFA status revealed a major role of the *FADS* gene haplotypes in all patterns, except for the “High omega-3 LC-PUFAs, low omega-6 LC-PUFAs” pattern for which weak to no association was observed. These findings are in line with several previous studies that suggested limited associations between SNPs within *FADS* gene cluster and their reconstructed haplotypes, with omega-3 LC-PUFAs especially DHA, while strong associations have been reported for omega-6 LC-PUFAs, and an extremely high genetically explained variance of 28% for AA levels in serum phospholipids has been highlighted (6, 9, 45–48). More precisely, the all-minor haplotype (which is composed of the minor allele at each locus) reconstructed from five loci (rs174544, rs174553, rs174556, rs174561 on *FADS1*, and rs3834458 on *FADS2*) showed significantly decreased proportions of omega-6 fatty acid AA in serum phospholipids (45) as well as in plasma’s and erythrocyte membranes’ phospholipids (47). However, no association was found with omega-3 LC-PUFA such as EPA, DPA, and DHA in both plasma and erythrocytes in this study (47).

The direction of the genetic associations found with pattern 2 “Omega-6 LC-PUFAs”, pattern 3 “Colostrum LC-PUFAs” and pattern 4 “Omega-6 precursor (LA) and DGLA” are consistent with the current understanding of the metabolic pathways of LC-PUFA biosynthesis. The rate-limiting enzymes of the metabolic process, delta-5 (Δ -5) and delta-6 (Δ -6) desaturases, are encoded by *FADS1* and *FADS2*, respectively, and involved in the conversion of the ALA to EPA and DHA and of the LA to AA. Carrying the minor alleles of the *FADS1*/*FADS2* SNPs leads to a reduction of the enzymatic activity and, therefore an accumulation of the precursors and a decrease of de novo LC-PUFA synthesis (9). In pattern 4, the higher levels of DGLA (C20:3n-6) concomitantly with the precursor LA may result from a more pronounced rate-limiting activity of delta-5 desaturase than delta-6 desaturase, resulting in the accumulation of both LA and DGLA. While the function of *FADS3* remains poorly understood, its high sequence homology with *FADS1* (62%) and *FADS2* (70%) suggests that it may encode for an unknown desaturase (49). The elusive nature of its desaturase activity adds to the complexity of unraveling its precise role and which tissue it is expressed in the body. Despite this, in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort, minor alleles of *FADS3* SNPs demonstrated results akin to *FADS1*/*FADS2* SNPs, indicating an elevation in precursor levels and a reduction in LC-PUFA levels in cord blood (50). It is noteworthy that the strength of the associations for *FADS3* SNPs remained comparatively weaker than those observed for *FADS1* or *FADS2* SNPs. Regarding pattern 5 “Omega-6 precursor and colostrum ALA”, the negative associations found with *FADS* gene haplotypes are challenging to explain since contradictory with what is known of the metabolic pathways. Further research is warranted to better understand the intricacies associated with this pattern.

A plausible biological reason that could explain the failure to detect genetic associations with the first pattern “High omega-3 LC-PUFAs, low omega-6 LC-PUFAs” is the competitive inhibition of the desaturase enzymes that are shared by the omega-3 and omega-6 biosynthetic pathways. Since the Western diet is characterized by a 5–15 times higher intake of LA than ALA, the metabolic way of the former largely exceeds that of the latter (51, 52). In our study population, the dietary LA to ALA ratio during the last trimester of pregnancy was approximately 10.9. It is recommended in France as well as in other countries that LA dietary intake should be balanced with ALA at a ratio of around 4:1 (<https://www.anses.fr/fr/system/files/NUT2006sa0359Ra.pdf>) even if it is noteworthy that a ratio of 1:1 to 2:1 has been estimated to be the one used at the beginning of humankind (51). Thus, an imbalanced metabolism favoring omega-6 LC-PUFA biosynthesis could hinder the observation of a potential

genetic variant effect on the pattern “High omega-3 LC-PUFAs, low omega-6 LC-PUFAs”.

Regarding *ELOVL* gene family, studies pertaining *ELOVL* SNPs are comparatively fewer in number than those addressing *FADS* gene cluster. In parallel with our findings showing weak to no association with *ELOVL* gene family, the PREOBE cohort study showed that *ELOVL2* (including rs953413 and rs3798713) and *ELOVL5* SNPs were not associated with any PUFA in plasma phospholipids among normal-weight pregnant women (53). However, genome-wide association studies (GWAS) showed some weak significant associations between plasma PUFA levels in population-based cohorts with *ELOVL2* SNPs especially. Minor alleles of *ELOVL2* SNPs were associated with higher EPA and DPA n-3 levels, and lower DHA levels (30, 31, 33, 35, 36, 38).

The correlation analyses examining the dietary determinants of the PUFA patterns showed that maternal dietary intakes of EPA, DHA, and total omega-3 during the last trimester of pregnancy were associated with both “High omega-3 LC-PUFAs, low omega-6 LC-PUFAs” and “Omega-6 LC-PUFAs” patterns. In contrast, maternal AA and total omega-6 intakes were not associated with these patterns. Some weak associations were found, however, between LA intake and pattern “Omega-6 precursor and colostrum ALA”. These findings are comparable to those from the Project Viva cohort study that included 1,666 mother-child pairs (54). In this study, significant correlations were identified between dietary EPA and DHA intakes during the second trimester of pregnancy and the corresponding biomarkers in maternal (mid-pregnancy) and cord blood. However, no such association was observed for AA. Additionally, in line with our results regarding the pattern “Omega-6 precursor and colostrum ALA”, dietary LA intake appeared to predict LA levels in both maternal and cord blood, whereas dietary ALA intake was not associated with its corresponding biomarker (54). Our findings also align with a prior study in the EDEN cohort, which investigated maternal nutritional factors influencing colostrum PUFAs. The study revealed a positive correlation between dietary intake of EPA and DHA, but not AA, and their respective levels in colostrum (23). A potential explanation for the absence of correlation with AA dietary intake could stem from the dependence of LC-PUFA omega-6 levels in biofluids on sources other than recent dietary intake, including adipose tissue storage (55) and biosynthesis from their precursor. The latter argument has been demonstrated in our study to play a major role in the LC-PUFA omega-6 status in the three biofluids, as evidenced by the strong associations found between the *FADS* gene cluster and the “Omega-6 LC-PUFAs” and “Colostrum LC-PUFAs” (in which AA, DTA, and DPA n-6 contribution were higher than DHA and DPA n-3) patterns. The complementary analyses investigating the relationships between PUFA patterns and dietary or candidate-genetic factors support the

hypothesis that the levels of omega-3 LC-PUFAs are predominantly influenced by dietary sources, while omega-6 LC-PUFAs are more significantly impacted by endogenous synthesis in the EDEN cohort.


To the best of our knowledge, this is the first study investigating perinatal exposure to PUFAs, encompassing data from three relevant biofluids. This has been made feasible due to the availability of measurements for a comprehensive range of omega-3 and omega-6 PUFAs in maternal and cord blood, as well as colostrum, and in a substantial sample size. The use of PUFA levels from erythrocyte membrane, reflecting the cumulative effects of dietary intake and endogenous metabolism, offers a more accurate indicator of the “internal dose” to which tissues are exposed. Data on maternal dietary intake during pregnancy and genotyping for major candidate SNPs involved in PUFA metabolism, enabled us to study the role of two major contributors to PUFA status.

Our study does entail some limitations. The participants in the EDEN cohort exhibit a higher socioeconomic status compared to the general population (22) which may influence dietary habits, PUFA status, and resulting patterns. Since our findings were based on a population with specific intakes of PUFAs and a genetic background primarily derived from European ancestry, it is important to generalize our findings to mother-infant pairs worldwide with caution. Similar studies in populations with different dietary habits or genetic origins could provide additional insights. Moreover, specific candidate SNPs from the *FADS* and *ELOVL* genes were examined in the present study, while other genes associated with PUFA levels (6) might explain potential variability in the identified PUFA patterns. Furthermore, among 1,901 mother-child pairs included in the analyses, colostrum FA levels for potentially non-breastfeeding women were imputed. However, the availability of a large number of 934 colostrum samples renders our study one-of-a-kind. The relatively low value of the OOB imputation error estimates, suggesting that our model's predictions were fairly accurate, coupled with the strong similarity of the patterns observed in the complete case approach and the imputed approach, reinforce our confidence about the reliability of our results.

The chronology of data collection could constitute an additional limitation; the dietary intakes of PUFAs were derived for the last trimester of pregnancy, while maternal blood sampling was collected during the second trimester. We acknowledged that the study design relied on the assumption of a high tracking in dietary intakes between the beginning and the end of pregnancy. Additionally, the protocol for collecting cord blood samples was based on the extraction of blood from either venous or arterial sources during cord cutting. Although, a couple of studies have demonstrated that there was no substantial difference in fatty acid composition between arterial and venous cord blood (56, 57).

In conclusion, this study suggested that perinatal exposure to LC-PUFA omega-3 is predominantly influenced by maternal dietary intakes during pregnancy and is independent of the mother's genetic background. It underscores the importance of maintaining an LC-PUFA ratio more favorable to omega-3 throughout pregnancy and lactation to provide a sufficient supply for the fetus and neonate. SNPs from *FADS* genes seemed to predict more subtle variations in perinatal LC-PUFA status, particularly in omega-6 LC-PUFAs or precursors. This provides a more comprehensive assessment of perinatal exposure to omega-3 and omega-6 PUFAs, paving the way for further association studies between PUFA perinatal exposure and a child's health and development.

Data availability

The data that support the findings of this study are available upon request from the EDEN steering committee. Readers may contact etude.eden@inserm.fr to request the data. 

Supplemental data

This article contains [supplemental data](#).







Acknowledgments

The authors thank the EDEN mother-child cohort study group, whose members are I. Annesi-Maesano, J. Y. Bernard, M.A. Charles, P. Dargent-Molina, B. de Lauzon-Guillain, P. Ducimetière, M. de Agostini, B. Foliguet, A. Forhan, X. Fritel, A. Germa, V. Goua, R. Hankard, B. Heude, M. Kaminski, B. Larroque, N. Lelong, J. Lepeule, G. Magnin, L. Marchand, C. Nabet, F. Pierre, R. Slama, M.J. Saurel-Cubizolles, M. Schweitzer and O. Thiebaugeorges.

Author contributions

J. Y. B., A. A. A., B. H., and M. A. conceptualization; J. Y. B., A. A. A., B. H., and M. A. methodology; J. Y. B., B. H., and M. A. supervision; J. Y. B., A. A. A., B. H., and M. A. validation; J. Y. B., A. A. A., B. H., C. S., S. P., W. L. Y., M. T., M. A., and M.-A. C. writing-review & editing; A. A. A. formal analysis; A. A. A. methodology, A. A. A. writing-original draft; B. H. funding acquisition; C. S., M. T., and M. A. data curation; C. S., M. T., and M. A. resources.

Author ORCIDs

Aline Abou Assi  <https://orcid.org/0009-0004-7317-9168>
Barbara Heude  <https://orcid.org/0000-0002-1565-1629>
Sabine Plancoulaine  <https://orcid.org/0000-0003-0725-8306>
Marie-Aline Charles  <https://orcid.org/0000-0003-4025-4390>
Martine Armand  <https://orcid.org/0000-0002-8712-6620>
Jonathan Y. Bernard  <https://orcid.org/0000-0002-6418-983X>

Funding and additional information

The present study is part of the ODYCE project, funded by AXA mutual insurance company patronage. The EDEN study is supported by Foundation for Medical Research

(FRM), French Ministry of Research: Federative Research Institutes and Cohort Program, INSERM Human Nutrition National Research Program, and Diabetes National Research Program (through a collaboration with the French Association of Diabetic Patients (AFD)), French Ministry of Health, French Agency for Environment Security (AFSSET), French National Institute for Population Health Surveillance (InVS), Paris-Sud University, French National Institute for Health Education (INPES), Nestlé, Mutuelle Générale de l'Éducation Nationale (MGEN), French-speaking Association for the Study of Diabetes and Metabolism (ALFE-DIAM), National Agency for Research (ANR non-thematic program), and National Institute for Research in Public Health (IRESP: TGIR 2008 cohort in health program). Biological analyses of colostrum samples were funded by the PremUp Foundation (French foundation for scientific cooperation in connection with pregnancy and prematurity) and the Groupe Lipides Nutrition (GLN). Biological analyses of erythrocyte membrane phospholipids were funded by the Institut Danone France and Fondation pour la Recherche Médicale (Prix IDF/FRM pour les Sciences de l'Alimentation 2015). The funders had no role in the study design; collection, analysis, interpretation of data, writing of the report, or decision to publish.

Conflict of interest

The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations

AA, arachidonic acid; ALA, alpha-linolenic acid; BMI, body mass index; DGLA, dihomo- γ -linolenic acid; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EDA, eicosadienoic acid; EDEN, étude des déterminants pré-et postnataux du développement de la santé de l'enfant; ELOVL, elongation-of-very-long-chain-fatty-acids; EPA, eicosapentaenoic acid; ETA, eicosatetraenoic; FADS, fatty acid desaturase; FFQ, food frequency questionnaire; GLA, gamma-linolenic acid; HM, human milk; LA, linoleic acid; LC-PUFA, long-chain polyunsaturated fatty acid; LD, linkage disequilibrium; n-3, omega-3; n-6, omega-6; PCA, principal component analysis; SNP, single nucleotide polymorphism; STD, stearidonic acid.

Manuscript received February 20, 2024, and in revised form May 13, 2024. Published, JLR Papers in Press, May 17, 2024, <https://doi.org/10.1016/j.jlrl.2024.100562>

REFERENCES

1. Barker, D. J. P. (1994) The fetal origins of adult disease. *Fetal Matern. Med. Rev.* **6**, 71–80
2. Shrestha, N., Sleep, S. L., Cuffe, J. S. M., Holland, O. J., Perkins, A. V., Yau, S. Y., et al. (2020) Role of omega-6 and omega-3 fatty acids in fetal programming. *Clin. Exp. Pharmacol. Physiol.* **47**, 907–915
3. Al, M. D., van Houwelingen, A. C., and Hornstra, G. (2000) Long-chain polyunsaturated fatty acids, pregnancy, and pregnancy outcome. *Am. J. Clin. Nutr.* **71**, 285S–291S
4. Clandinin, M. T., Chappell, J. E., Leong, S., Heim, T., Swyer, P. R., and Chance, G. W. (1980) Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements. *Early Hum. Dev.* **4**, 131–138
5. Sauerwald, T. U., Demmelmair, H., and Koletzko, B. (2001) Polyunsaturated fatty acid supply with human milk. *Lipids* **36**, 991–996

6. Lattka, E., Illig, T., Koletzko, B., and Heinrich, J. (2010) Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism. *Curr. Opin. Lipidol.* **21**, 64–69
7. Morales, E., Bustamante, M., Gonzalez, J. R., Guxens, M., Torrent, M., Mendez, M., *et al.* (2011) Genetic variants of the FADS gene cluster and ELOVL gene family, colostrums LC-PUFA levels, breastfeeding, and child cognition. *PLoS One* **6**, e17181
8. Koletzko, B., Reischl, E., Tanjung, C., Gonzalez-Casanova, I., Ramakrishnan, U., Meldrum, S., *et al.* (2019) FADS1 and FADS2 polymorphisms modulate fatty acid metabolism and dietary impact on health. *Annu. Rev. Nutr.* **39**, 21–44
9. Conway, M. C., McSorley, E. M., Mulhern, M. S., Strain, J. J., van Wijngaarden, E., and Yeates, A. J. (2020) Influence of fatty acid desaturase (FADS) genotype on maternal and child polyunsaturated fatty acids (PUFA) status and child health outcomes: a systematic review. *Nutr. Rev.* **78**, 627–646
10. Hanebutt, F. L., Demmelmair, H., Schiessl, B., Larqué, E., and Koletzko, B. (2008) Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. *Clin. Nutr.* **27**, 685–693
11. Escolano-Margarit, M. V., Ramos, R., Beyer, J., Csábi, G., Parrilla-Roure, M., Cruz, F., *et al.* (2011) Prenatal DHA status and Neurological outcome in children at age 5.5 Years are positively associated. *J. Nutr.* **141**, 1216–1223
12. van Goor, S. A., Dijck-Brouwer, D. A. J., Erwich, J. J. H. M., Schaafsma, A., and Hadders-Algra, M. (2011) The influence of supplemental docosahexaenoic and arachidonic acids during pregnancy and lactation on neurodevelopment at eighteen months. *Prostaglandins Leukot. Essent. Fatty Acids* **84**, 139–146
13. Bakker, E. C., Ghys, A. J. A., Kester, A. D. M., Vles, J. S. H., Dubas, J. S., Blanco, C. E., *et al.* (2003) Long-chain polyunsaturated fatty acids at birth and cognitive function at 7 y of age. *Eur. J. Clin. Nutr.* **57**, 89–95
14. Brouwer-Brolsma, E. M., van de Rest, O., Godschalk, R., Zeegers, M. P. A., Gielen, M., and de Groot, R. H. M. (2017) Associations between maternal long-chain polyunsaturated fatty acid concentrations and child cognition at 7 years of age: the MEFAB birth cohort. *Prostaglandins Leukot. Essent. Fatty Acids* **126**, 92–97
15. Boucher, O., Burden, M. J., Muckle, G., Saint-Amour, D., Ayotte, P., Dewailly, E., *et al.* (2011) Neurophysiologic and neurobehavioral evidence of beneficial effects of prenatal omega-3 fatty acid intake on memory function at school age. *Am. J. Clin. Nutr.* **93**, 1025–1037
16. Strain, J. J., Davidson, P. W., Thurston, S. W., Harrington, D., Mulhern, M. S., McAfee, A. J., *et al.* (2012) Maternal PUFA status but not prenatal methylmercury exposure is associated with children's language functions at age five years in the Seychelles. *J. Nutr.* **142**, 1943–1949
17. Strain, J. J., Love, T. M., Yeates, A. J., Weller, D., Mulhern, M. S., McSorley, E. M., *et al.* (2021) Associations of prenatal methylmercury exposure and maternal polyunsaturated fatty acid status with neurodevelopmental outcomes at 7 years of age: results from the Seychelles Child Development Study Nutrition Cohort 2. *Am. J. Clin. Nutr.* **113**, 304–313
18. Guxens, M., Mendez, M. A., Moltó-Puigmartí, C., Julvez, J., García-Esteban, R., Forn, J., *et al.* (2011) Breastfeeding, long-chain polyunsaturated fatty acids in colostrum, and infant mental development. *Pediatrics* **128**, e880–e889
19. Bernard, J. Y., Armand, M., Garcia, C., Forhan, A., De Agostini, M., Charles, M. A., *et al.* (2015) The association between linoleic acid levels in colostrum and child cognition at 2 and 3 y in the EDEN cohort. *Pediatr. Res.* **77**, 829–835
20. Bernard, J. Y., Armand, M., Peyre, H., Garcia, C., Forhan, A., De Agostini, M., *et al.* (2017) Breastfeeding, polyunsaturated fatty acid levels in colostrum and child intelligence quotient at age 5–6 years. *J. Pediatr.* **183**, 43–50.e3
21. Zielinska, M. A., Hamulka, J., Grabowicz-Chądrzyńska, I., Bryś, J., and Wesolowska, A. (2019) Association between breastmilk LC PUFA, carotenoids and psychomotor development of exclusively breastfed infants. *Int. J. Environ. Res. Public Health* **16**, 1144
22. Heude, B., Forhan, A., Slama, R., Douhaud, L., Bedel, S., Saurel-Cubizolles, M. J., *et al.* (2016) Cohort profile: the EDEN mother-child cohort on the prenatal and early postnatal determinants of child health and development. *Int. J. Epidemiol.* **45**, 353–363
23. Armand, M., Bernard, J. Y., Forhan, A., Heude, B., Charles, M. A., and EDEN Mother-Child Cohort Study Group (2018) Maternal nutritional determinants of colostrum fatty acids in the EDEN mother-child cohort. *Clin. Nutr.* **37**, 2127–2136
24. Garbi, A., Armand, M., Beltran-Anzola, A. A., Sarté, C., Brévaut-Malaty, V., Tosello, B., *et al.* (2022) Effect of massage with oil balanced in essential fatty acids on development and lipid parameters in very premature neonates: a randomized, controlled study. *Children (Basel)* **9**, 463
25. Cao, J., Schwichtenberg, K. A., Hanson, N. Q., and Tsai, M. Y. (2006) Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clin. Chem.* **52**, 2265–2272
26. Katan, M. B., Deslypere, J. P., van Birgelen, A. P., Penders, M., and Zegwaard, M. (1997) Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J. Lipid Res.* **38**, 2012–2022
27. Deschamps, V., de Lauzon-Guillain, B., Lafay, L., Borys, J. M., Charles, M. A., and Romon, M. (2009) Reproducibility and relative validity of a food-frequency questionnaire among French adults and adolescents. *Eur. J. Clin. Nutr.* **63**, 282–291
28. Hercberg, S., Deheeger, M., and Preziosi, P. (2002) SU.VI.MAX. Portions Alimentaires: Manuel Photos Pour L'estimation des Quantités (Food Portions: A Photo Manual for Estimating Quantities). Polytechnica Edn, Paris
29. Table de composition des aliments: étude NutriNet-Santé. *Economica* **294**, (2013)
30. Tanaka, T., Shen, J., Abecasis, G. R., Kisiailiou, A., Ordovas, J. M., Guralnik, J. M., *et al.* (2009) Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI Study. *PLoS Genet.* **5**, e1000338
31. Lemaitre, R. N., Tanaka, T., Tang, W., Manichaikul, A., Foy, M., Kabagambe, E. K., *et al.* (2011) Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS Genet.* **7**, e1002193
32. Guan, W., Steffen, B. T., Lemaitre, R. N., Wu, J. H. Y., Tanaka, T., Manichaikul, A., *et al.* (2014) Genome-wide association study of plasma N6 polyunsaturated fatty acids within the cohorts for heart and aging research in genomic epidemiology consortium. *Circ. Cardiovasc. Genet.* **7**, 321–331
33. Tintle, N. L., Pottala, J. V., Lacey, S., Ramachandran, V., Westra, J., Rogers, A., *et al.* (2015) A genome-wide association study of saturated, mono- and polyunsaturated red blood cell fatty acids in the Framingham Heart Offspring Study. *Prostaglandins Leukot. Essent. Fatty Acids* **94**, 65–72
34. Dorajoo, R., Sun, Y., Han, Y., Ke, T., Burger, A., Chang, X., *et al.* (2015) A genome-wide association study of n-3 and n-6 plasma fatty acids in a Singaporean Chinese population. *Genes Nutr.* **10**, 53
35. Hu, Y., Li, H., Lu, L., Manichaikul, A., Zhu, J., Chen, Y. D. I., *et al.* (2016) Genome-wide meta-analyses identify novel loci associated with n-3 and n-6 polyunsaturated fatty acid levels in Chinese and European-ancestry populations. *Hum. Mol. Genet.* **25**, 1215–1224
36. Kalsbeek, A., Veenstra, J., Westra, J., Disselkoen, C., Koch, K., McKenzie, K. A., *et al.* (2018) A genome-wide association study of red-blood cell fatty acids and ratios incorporating dietary covariates: framingham Heart Study Offspring Cohort. *PLoS One* **13**, e0194882
37. Marklund, M., Morris, A. P., Mahajan, A., Ingelsson, E., Lindgren, C. M., Lind, L., *et al.* (2018) Genome-wide association studies of estimated fatty acid desaturase activity in serum and adipose tissue in elderly individuals: associations with insulin sensitivity. *Nutrients* **10**, 1791
38. Coltell, O., Sorlí, J. V., Asensio, E. M., Barragán, R., González, J. I., Giménez-Alba, I. M., *et al.* (2020) Genome-wide association study for serum omega-3 and omega-6 polyunsaturated fatty acids: exploratory analysis of the sex-specific effects and dietary modulation in mediterranean subjects with metabolic syndrome. *Nutrients* **12**, 310
39. Yang, C., Veenstra, J., Bartz, T. M., Pahl, M. C., Hallmark, B., Chen, Y. D. I., *et al.* (2023) Genome-wide association studies and fine-mapping identify genomic loci for n-3 and n-6 polyunsaturated fatty acids in Hispanic American and African American Cohorts. *Commun. Biol.* **6**, 852
40. Stekhoven, D. J., and Bühlmann, P. (2012) MissForest-non-parametric missing value imputation for mixed-type data. *Bioinformatics* **28**, 112–118

41. Gabriel, S. B., Schaffner, S. F., Nguyen, H., Moore, J. M., Roy, J., Blumenstiel, B., *et al.* (2002) The structure of haplotype blocks in the human genome. *Science* **296**, 2225–2229
42. Schaid, D. J., Rowland, C. M., Tines, D. E., Jacobson, R. M., and Poland, G. A. (2002) Score tests for association between traits and haplotypes when linkage phase is ambiguous. *Am. J. Hum. Genet.* **70**, 425–434
43. Bennette, C., and Vickers, A. (2012) Against quantiles: categorization of continuous variables in epidemiologic research, and its discontents. *BMC Med. Res. Methodol.* **12**, 21
44. Vlaardingerbroek, H., and Hornstra, G. (2004) Essential fatty acids in erythrocyte phospholipids during pregnancy and at delivery in mothers and their neonates: comparison with plasma phospholipids. *Prostaglandins Leukot. Essent. Fatty Acids* **71**, 363–374
45. Schaeffer, L., Gohlke, H., Müller, M., Heid, I. M., Palmer, L. J., Kompauer, I., *et al.* (2006) Common genetic variants of the FADS1 FADS2 gene cluster and their reconstructed haplotypes are associated with the fatty acid composition in phospholipids. *Hum. Mol. Genet.* **15**, 1745–1756
46. Martinelli, N., Girelli, D., Malerba, G., Guarini, P., Illig, T., Tra-betti, E., *et al.* (2008) FADS genotypes and desaturase activity estimated by the ratio of arachidonic acid to linoleic acid are associated with inflammation and coronary artery disease. *Am. J. Clin. Nutr.* **88**, 941–949
47. Rzehak, P., Heinrich, J., Klopp, N., Schaeffer, L., Hoff, S., Wolfram, G., *et al.* (2008) Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes. *Br. J. Nutr.* **101**, 20–26
48. Schuchardt, J. P., Köbe, T., Witte, V., Willers, J., Gingrich, A., Tesky, V., *et al.* (2016) Genetic variants of the FADS gene cluster are associated with erythrocyte membrane LCPUFA levels in Patients with mild cognitive impairment. *J. Nutr. Health Aging* **20**, 611–620
49. Blanchard, H., Legrand, P., and Pédrone, F. (2011) Fatty acid desaturase 3 (Fads3) is a singular member of the fads cluster. *Biochimie* **93**, 87–90
50. Lattka, E., Koletzko, B., Zeilinger, S., Hibbeln, J. R., Klopp, N., Ring, S. M., *et al.* (2013) Umbilical cord PUFA are determined by maternal and child fatty acid desaturase (FADS) genetic variants in the Avon Longitudinal Study of Parents and Children (ALSPAC). *Br. J. Nutr.* **109**, 1196–1210
51. Simopoulos, A. P. (2009) Omega-6/omega-3 essential fatty acids: biological effects. *World Rev. Nutr. Diet.* **99**, 1–16
52. Baker, E. J., Miles, E. A., Burdge, G. C., Yaqoob, P., and Calder, P. C. (2016) Metabolism and functional effects of plant-derived omega-3 fatty acids in humans. *Prog. Lipid Res.* **64**, 30–56
53. de la Garza Puentes, A., Montes, G. R., Chisaguano Tonato, A. M., Torres-Espínola, F. J., Arias García, M., de Almeida, L., *et al.* (2017) Association of maternal weight with FADS and ELOVL genetic variants and fatty acid levels- the PREOBE follow-up. *PLoS One* **12**, e0179135
54. Donahue, S. M. A., Rifas-Shiman, S. L., Olsen, S. F., Gold, D. R., Gillman, M. W., and Oken, E. (2009) Associations of maternal prenatal dietary intake of n-3 and n-6 fatty acids with maternal and umbilical cord blood levels. *Prostaglandins Leukot. Essent. Fatty Acids* **80**, 289–296
55. Martin, J., Bougnoux, P., Fignon, A., Theret, V., Antoine, J., Lamière, F., *et al.* (1993) Dependence of human milk essential fatty acids on adipose stores during lactation. *Am. J. Clin. Nutr.* **58**, 653–659
56. Ortega-Senovilla, H., Alvino, G., Taricco, E., Cetin, I., and Herrera, E. (2009) Gestational diabetes mellitus upsets the proportion of fatty acids in umbilical arterial but not venous plasma. *Diabetes Care* **32**, 120–122
57. Pankiewicz, E., Cretti, A., Ronin-Walkowska, E., Czeszyńska, M. B., Konefał, H., and Hnatyszyn, G. (2007) Maternal adipose tissue, maternal and cord blood essential fatty acids and their long-chain polyunsaturated derivatives composition after elective caesarean section. *Early Hum. Dev.* **83**, 459–464