Title page

DHA intake interacts with ELOVL2 and ELOVL5 genetic variants to influence polyunsaturated fatty acids in human milk

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Running title: ELOVL Variants and DHA intake for human milk PUFA

Abbreviations: ELOVL, elongation of the very long chain fatty acid; LA, linoleic acid; ALA, \(\alpha\)-linolenic acid; ARA, arachidonic acid; GLA, \(\gamma\)-linolenic acid; DGLA, dihomo \(\gamma\)-linolenic acid; DTA, docosatetraenoic acid; DPA, docosapentaenoic acid.
ABSTRACT

Endogenous synthesis of PUFAs is mediated by genes controlling fatty acid elongases 2 and 5 (ELOVL2 and ELOVL5) and by exogenous DHA intake. Associations between elongases and PUFA levels probably involve genetic variants of ELOVL and changes in DHA intake, but data about their combined effect on PUFA levels are sparse. We hypothesized that each factor would directly affect PUFAs and that interactions between haplotypes and DHA intake would influence PUFAs. We explored four levels of DHA intake in pregnant Chinese Han women and 10 SNPs in the ELOVL genes to determine associations with PUFAs in breast milk. The SNP, rs3798713, and 3-SNP haplotype (rs2281591, rs12332786, and rs3798713) in ELOVL2 were associated with linoleic acid (LA) concentrations. However, carriers of the 3-SNP haplotype with higher DHA intake (second quartile, 14.58 to 43.15 mg/day) had higher concentrations of LA, arachidonic acid, EPA, and DHA compared with the interaction baseline. In ELOVL5, five SNPs (rs2294867, rs9357760, rs2397142, rs209512, and rs12207094) correlated with PUFA changes. Compared with those who had the 5-SNP haplotype C-A-C-G-A and low DHA intake (<14.58 mg/day), carriers with other haplotypes (A-A-C-A-A or C-A-C-A-A) and high DHA intake (≥118.82 mg/day) had increased EPA levels after adjustments for age and body mass index. This study showed that maternal genetic variants in ELOVL2 and ELOVL5 were associated with PUFA levels in breast milk and that the combination of SNP haplotypes and higher DHA intake increased PUFA concentrations.

KEYWORDS

Gene Polymorphism, Diet and Dietary Lipids, Fatty Acid/Synthesis, Nutrition/Lipids,
INTRODUCTION

Polyunsaturated fatty acids (PUFAs), especially long-chain PUFAs (LC-PUFAs), are essential nutrients for human health, and are associated with the development of the human brain and neurotransmitter function (1-3). In particular, the n-3 fatty acid DHA (22:6n-3), present in breast milk, has been identified in animal and human studies as being crucial for the development of the central nervous system during fetal life and early infancy (2, 4). Findings from epidemiological and observational studies substantiate the association between maternal seafood consumption in pregnancy and breastfeeding and improved infant neurodevelopment (5-9). This may be explained partly by the resultant increase in the early supply of LC-PUFAs, especially DHA, which accumulate in the brain during the early growth spurt in children (2, 10). Pregnant women who consumed high amounts of n-3 LC-PUFA in their diet showed positive effects on pregnancy outcomes, such as longer gestational duration and greater birth weight (11). Additionally, LC-PUFAs may increase infant growth and enhance the short- and long-term development of the offspring (11-13). A randomized controlled trial study showed that supplementation with n-3 LC-PUFA in the third trimester of pregnancy reduced the absolute risk of persistent wheeze or asthma and infections of the lower respiratory tract in offspring (14). The fetus is mainly supplied with LC-PUFAs by transfer from the maternal circulation via the placenta (15). Therefore, an adequate supply of LC-PUFA during pregnancy is critical for fetal life onwards.

Elongation of LC-PUFAs in the n-3 family is made possible by enzymes called elongases, which are encoded by the elongation of a very long-chain fatty acid (ELOVL) gene family on
chromosome 6. Elongases catalyze the elongation of aliphatic carbon chains leading to the formation of LC-PUFAs (16, 17). Fatty acid elongase-2 and the fatty acid elongase-5, encoded by the ELOVL2 (6p24.2) and ELOVL5 (6p12.1) gene, respectively, are involved in LC-PUFA synthesis. Plasma percentages of n-3 LC-PUFA have been shown to be associated with single nucleotide polymorphisms (SNPs) in ELOVL2 (18) and ELOVL5 (19, 20), and the enzyme activities are influenced by SNPs within the ELOVL gene family after supplementation of n-3 LC-PUFA (21, 22).

The mechanisms underlying the observed associations between elongases and PUFAs levels are inconsistent, which probably involve genetic variants within ELOVL and changes in levels of DHA intake. Consequently, we hypothesized that ELOVL polymorphisms and DHA intake would each be directly associated with PUFA concentrations. We also hypothesized that the interactions of haplotypes within ELOVL2 and ELOVL5 with DHA consumption during pregnancy may influence PUFA concentration in the breast milk of healthy lactating Chinese Han women.

**MATERIALS AND METHODS**

*Sociodemographic and study design*

The present study recruited four hundred and twenty two healthy Chinese Han pregnant women, 22–40 y of age, who registered for postpartum care at Shirentang house (ChangChun) from March 2012 to December 2014. Only participants with no maternal pregnancy complications were included. Other exclusion criteria included metabolic diseases (including diabetes) and communicable diseases. Sample size varied from a total of 422 participating women to 420 in analyses of ELOVL SNP v. breast milk PUFA as outcome, from 422 to 370.
in analyses of DHA intake v. breast milk PUFA outcomes. All participants gave informed consent according to the procedures approved by the ethics committee of Jilin University, Changchun, China.

*Questionnaire survey and breast milk collection*

We gave each participant a face to face interview and a semi-structured food frequency questionnaire (FFQ). The FFQ was used to assess the dietary intake of enrolled subjects during their pregnancy. It included specific questions about consumption of sources containing DHA, such as freshwater fish, seafood, canned tuna, etc. To better understand the data of DHA intake, in the interview, we also asked participants the DHA supplement’s brand, the supplementing time and daily doses. The investigators checked the content of DHA in supplements and calculated the daily doses of DHA of the enrolled subjects. Five kinds of PUFA dietary intakes were calculated based on the China Food Composition 2009 (23). And, DHA dietary intakes were calculated as mg per day. Twenty milliliter of breast milk was collected between 9:00 a.m. and 11:00 a.m. on one day between the 22nd and 25th day after delivery. The first few drops of milk were discarded, and then the mature breast milk was collected. The samples were stored at-80 °C (24), and detected in one month averagely.

*Fatty acid analysis*

The levels of eight kinds of fatty acids in breast milk were determined by direct methylation (25) with subsequent analysis by gas chromatography-flame ionization detection. Gas Chromatograph (Shimadzu GC-14B, Shimadzu Corp., Honshu, Kyoto, Japan) was equipped with a capillary column (sp-2560, Supelco, Bellefonte, PA, USA; 100 m×0.25 mm×0.20 μm). The internal standard method was used to calculate the levels of fatty acid methyl esters. Fatty
acid methyl esters were prepared from milk by combining 0.2 ml of fat with 2 ml of methanol and benzene (4:1, v/v), 33 µl of internal standard (daturic acid, C17:0) and 200 µl of acetyl chloride in a 10-ml glass tube. Specific experimental steps were described in previous study (26).

**SNPs selection and genotyping**

SNPs in *ELOVL2* and *ELOVL5* were identified using the International HapMap Project SNP database and the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/snp/). Selected SNPs of the *ELOVL* gene cluster (rs2281591, rs12332786, rs3798713, rs3778166, rs9468304, rs2294867, rs9357760, rs2397142, rs209512, rs12207094) have been genotyped using Sequenom Mass Array system (BO MIAO Biological Technological Company, Beijing) with validated primers and the genotyping success rate was above 96%. The genomic location of *ELOVL2* and *ELOVL5* gene region are on the chromosome 6(10.99-11.05 Mb and 53.28-53.34 Mb, respectively), and the selected SNPs are all intron variants. Each SNP has a minor allele frequency (MAF) above 10% in Asian population according to the SNP database of the NCBI. The milk samples were thawed at 4°C and genomic DNA was extracted from 300 µl of the cellular layer using the DNA kit (Beijing, TIANGEN) according to the manufacturer’s instructions.

**Statistical analysis**

Normal distribution of the fatty acids was tested by the Kolmogorov-Smirnov test and distribution plots. Data were expressed as means ± SDs for normal distributed variables, as medians (25th-75th percentiles) for skewed distribution data. The skewed measurements of γ-linolenic acid (GLA, 18:3n-6), EPA (20:5n-3) and DHA concentrations were expressed as
square roots to obtain a normal distribution. Hardy-Weinberg equilibrium was tested by
Chi-square goodness of fit test for each SNP locus. Genotype association with concentration
of PUFAs was tested using the online SNPtrack software (https://www.snpstats.net/start.htm.).
Linear regression analysis was used to investigate the associations of ELOVL gene
polymorphisms with levels of PUFAs. Statistical analysis was performed using SPSS version
16.0 (SPSS Inc., Chicago, IL, 100 USA). Haplotype analyses play an important role in
genetic studies (27, 28). It is impossible to define the combination of haplotypes carried by
any one individual, but all possible combinations can be computed and techniques like the
EM algorithm incorporated in the haplo.stats package of R software
(https://cran.r-project.org/src/contrib/Archive/haplo.stats/) can be used to assign a probability
to each haplotype pair. To explore potential effects of multi-SNPs and DHA intake, the
interactions between ELOVL2/5 haplotype and DHA intake on LC-PUFA levels were
performed by the general linear model using R software (R version 3.5.0) adjusted for
confounding factors. P<0.05 (two-tailed) was considered to indicate a statistical significance.

RESULTS

Characteristics of the study subjects

The 422 healthy lactating mothers included in this study had an average age of 30.29±3.40
years and mainly came from middle income households (54.09%). Gestational age was
39.27±1.00 weeks. The preconception body mass index (BMI) was 20.95±3.33 kg/m² that
was within the normal range. A total of 31.10% of subjects had a vaginal delivery, while the
rest had cesareans. A total of 54.39% of the subject breastfed exclusively while the remaining
45.61% opted for mixed feeding. A total of 50.75% of the mothers had a university education
Effect of DHA intake during pregnancy on the concentration of PUFA in breast milk

We investigated the effect of DHA intake (including dietary and supplement DHA) during pregnancy on the concentration of eight PUFAs in breast milk, and sought to determine whether there was a dose-response relationship. The lactating mothers were classified into four subgroups depending on the quartiles of DHA intake (< 14.58, 14.58–43.15, 43.16–118.82, and ≥ 118.82 mg/day) (Table 2). However, the results showed that there was no significant difference for PUFA composition of breast milk among the four groups.

Frequency of SNPs

The distributions of genotype frequencies in the 422 subjects were in accordance with Hardy-Weinberg equilibrium (P > 0.05). The selected SNPs were all in intron, and the genotyping success rate was > 96%. The characteristics of the detected SNPs, including their positions on chromosome 6 and their genotypes, were summarized in Table 3. Minor allele frequencies (MAF) ranged from 10.7% ~ 46.8% of the population.

The association between ELOVL2 and ELOVL5 genotypes and PUFA concentrations

The association of three genetic models (Codominant model, Dominant model and Recessive model) for each of the 10 SNPs with breast milk PUFA was analyzed using the online SNPstats software adjusted for age, preconception BMI, and DHA intake of subjects. The best fit genetic model of the 10 SNPs was chosen based on the Akaike Information Criterion (AIC) and the Bayesian information criterion (BIC) (Figure 1). Carriers of the minor allele of rs3798713 (P = 0.019) in ELOVL2 gene had lower linoleic acid (LA, 18:2n-6) concentrations of breast milk than homozygous subjects for the major allele. The subjects carrying the minor
allele homozygote of rs2294867 \((P=0.036)\) within \textit{ELOVL5} had higher EPA concentrations than those who carried the major allele. Subjects who carried the minor allele of rs9357760 in \textit{ELOVL5} gene had higher GLA \((P=0.043)\), dihomo \(\gamma\)-linolenic acid (DGLA, 20:3n-6) \((P=0.013)\), arachidonic acid (ARA, 20:4n-6) \((P=0.014)\), and docosatetraenoic acid (DTA, 22:4n-6) \((P=0.009)\) concentrations than homozygotes for the major allele. Carriers of the minor allele of rs2397142 had higher DTA levels compared to homozygotes for the major allele \((P=0.027)\). The subjects homozygous for the minor allele of rs209512 had lower GLA \((P=0.042)\) and DGLA \((P=0.039)\) concentrations than those carrying the major allele. A significant association was also observed between rs12207094 \((P=0.029)\) in \textit{ELOVL5} and the level of GLA; carriers of the minor allele had higher levels compared to homozygotes for the major allele. However, no statistically significant differences were found between the other SNPs and fatty acid levels. The full details of the analytical results are provided in supplemental Table S1.

**Interaction effect between \textit{ELOVL2} and \textit{ELOVL5} haplotypes and DHA intake on breast milk PUFA levels**

The results of the \textit{ELOVL2} and \textit{ELOVL5} haplotype frequencies can be found in supplemental Table S2 and supplemental Table S3. Table 4 showed that a 3-SNP haplotype \((H_2: A-G-G)\) in \textit{ELOVL2} (rs2281591 A/G, rs12332786 C/G, and rs3798713 C/G) associated with a decline in LA \((P=0.046)\), EPA \((P=0.022)\), and DHA \((P=0.008)\) concentrations in breast milk compared with carriers of the baseline haplotype \((H_1: A-C-C)\) adjusted for age and BMI. However, we found that the interaction of haplotype \(H_2\) with the second quartile of DHA intake \((Q_2: 14.58–43.15 \text{ mg/day})\) increased the concentration of LA \((P=0.038)\), ARA
(P=0.025), EPA (P=0.034), and DHA (P=0.004) compared with carriers of haplotype \( H_1 \) with low DHA intake (Q_1: <14.58 mg/day), adjusted for age and BMI.

Compared with those who had a 5-SNP haplotype (H_1: C-A-C-G-A) (rs2294867 C/A, rs9357760 A/G, rs2397142 C/G, rs209512 A/G, and rs12207094A/T) in ELOVL5 with low DHA intake (Q_1), carriers with the haplotype (H_2: A-A-C-A-A) who consumed high DHA (Q_4: ≥118.82 mg/day) had higher levels of DGLA, α-linolenic acid (ALA, 18:3n-3), and EPA (P=0.020, P=0.022, and P=0.042, respectively) in breast milk adjusted for age and BMI. Another interaction between haplotype (H_5: C-A-C-A-A) and DHA intake (Q_2) increased DGLA and EPA concentrations (P=0.036 and P=0.011, respectively). Likewise, haplotype H_5 interacting with DHA intake in Q_4 increased EPA concentrations (P=0.015) in breast milk (Table 4). Table 4 lists the significant results of the haplotype analysis for ELOVL2 and ELOVL5 (P<0.05). Full details of related results can be found in supplemental Table S4 and supplemental Table S5.

**DISCUSSION**

In this study, we analyzed polymorphisms in genes encoding the elongases involved in LC-PUFA synthesis in lactating mothers to disentangle their role in modifying potential nutritional advantages of LC-PUFAs in breastfeeding. Maternal genetic variants in the ELOVL gene family were associated with breast milk levels of LC-PUFA. Furthermore, DHA intake during pregnancy, including dietary intake and supplementation, were not associated with the concentrations of eight LC-PUFAs that were regulated by ELOVL2 and ELOVL5 gene variations. However, we showed an interaction effect between ELOVL2 and ELOVL5 haplotypes and DHA intake on breast milk LC-PUFA levels.
We observed that rs3798713 of ELOVL2 gene influenced the LC-PUFA concentration in present study; carriers of the minor allele had lower LA concentrations than homozygotes for the major allele. Our previous study, however, found no significant association between ELOVL2 variants and LC-PUFA (19), which may be due to a small sample size. Higher EPA levels, in early studies, have been shown in individuals containing minor alleles of rs953413 (29), rs2236212 (18) and rs3798719 (20) with another study showing no effect of the rs3734398 (21) within ELOVL2 gene, while lower DHA levels have been observed in individuals carrying rs953413 (29), rs2236212 (18) and rs3734398 (21) with another study showing no effect of the rs3798719 (20). Inconsistencies between our study and other studies may be because the subjects in our study were all Chinese Han lactating women, and population heterogeneity among studies such as variations in SNP frequencies across the major population groups may also lead to differences in study outcomes.

Regarding the ELOVL5 gene, we found that subjects carrying the minor allele homozygote of rs2294867 had higher EPA concentrations than those who carried the major allele. GLA, DGLA, ARA, and DTA concentrations were significantly affected by rs9357760 in the ELOVL5 gene, and the subjects who carried the minor allele had higher concentrations than those who were homozygous for the major allele. Carriers of the minor allele of rs2397142 had higher DTA levels compared to major allele homozygotes. The subjects homozygous for the minor allele of rs209512 had lower GLA and DGLA concentrations than those carrying the major allele. A significant association was also observed between the minor allele of rs12207094 and a higher level of GLA. In Spanish population-based birth cohorts, a trend was observed for minor allele of rs12207094 and higher EPA levels. Additionally, minor allele
of rs17544159, rs9395855, and rs12207094 were associated with a high EPA/ARA ratio. This study also found that children of mothers carrying the rs17544159-C allele or carrying the rs12207094-T allele had higher cognition scores compared to children of mothers homozygous for the major allele (20). Our results mainly suggest that minor allele of specific SNPs within ELOVL5 could be prior related to n-6 PUFA levels, probably due to increased transcription or to a more enzyme activity of ELOVL5.

In the second half of pregnancy, DHA accumulates rapidly in neural cortex tissues (30) and retinal membrane synapses (31). DHA comes from the diet or adipose stores, and is synthesized from precursor fatty acids circulating in the maternal bloodstream for uptake by the placenta and fetus. It is not clear how much DHA is released from adipose stores or is synthesized, but a remarkable association of maternal DHA intake during pregnancy with maternal circulating DHA shows that diet may be a major source of DHA for the developing fetus (32-34). Thus, we examined the effect of maternal DHA intake during pregnancy on breast milk LC-PUFAs. However, we did not find any difference. It may be the lower intake of DHA (<200mg/d) (35) among most subjects (86.49%) in our study that affected generalizability of the findings to a wider population.

Haplotypes effectively capture joint marker correlations and evolutionary history; a progressive knowledge of haplotype structures holds great promise for the use of haplotype information to understand genetic factors (36). Our results suggested an association of a haplotype (A-G-G) of ELOVL2 with lower LA, EPA, and DHA levels, but not ARA levels. Analysis of the impact of DHA intake on these FA levels, however, showed no differences. Interestingly, there was a significant interaction effect between this haplotype and the second
quartile of DHA intake (Q2) on higher LA, EPA, DHA, and ARA levels.

Zhang et al. reported that minor allele carriers of a 3-SNP haplotype in ELOVL2 were associated with decreased DHA levels (37). However, the association between this haplotype and the plasma percentages of EPA, docosapentaenoic acid [DPA; 22:5(n-3)], and DHA were not significant in the MARINA trial (21).

In the present study, carriers with haplotype (A-A-C-A-A) of 5-SNPs in ELOVL5 who consumed high DHA (Q4) had a higher level of DGLA, ALA, and EPA concentrations compared with those who had the haplotype (C-A-C-G-A) with low DHA intakes (Q1) adjusted for age and BMI. Interaction between the haplotype (C-A-C-A-A) and DHA intake (Q2 and Q4, but not Q3) increased EPA concentrations. The reason may be due to the lower frequency of the haplotype in Q3 group compared to Q2 and Q4 group resulting in lower power. It has been known that DHA supplementation results in an increase in EPA concentrations (38, 39). Metabolically, increases in EPA concentration may be due to increased EPA biosynthesis or decreased EPA degradation. Increased EPA in our study is assumed to result from higher DHA intake via the slowed elongation of EPA. However, there is no significant effect of high DHA intake or haplotype of ELOVL5 on EPA level in our study until high DHA intake interacting with haplotype. This suggests a role for specific ELOVL haplotypes that maybe affect EPA elongation when DHA intake is high.

We speculate that DHA intake for mothers during pregnancy may modulate the ELOVL2 and ELOVL5 gene expression through several mechanisms, such as epigenetic modifications, leading to a change in phenotypes. Consistent with our findings, observational studies have shown gene-diet interaction effects with SNPs within the FADS gene cluster and the ELOVL
gene family. These could potentially modulate the enzyme activities of desaturases and elongases following n-3 FA supplementation (22).

Limitations of our study included the reliance on estimates of n-3 LC-PUFA consumption from food frequency questionnaires and recorded DHA intake, rather than using controlled doses of DHA. In addition, all quartiles of DHA intake in our study were relatively low level, which may be due to the fact that participants came from inland areas and consumed less DHA-rich seafood in their daily diet. It also explained the result that there were no differences in breast milk PUFA levels among the lactating mothers with different quartiles of DHA intake. Therefore, low DHA intake in the study population limits the generalizability of our results, and further studies included DHA supplemented group are needed in the future, which would be more helpful for us to explore the interaction between DHA intake and ELOVL genes. Most genes contributing to quantitative phenotypes confer modest effects, requiring large sample sizes for detection with high power. Our finding of significant gene and diet interactions as a determinant of the breast milk PUFA concentration in 422 subjects must therefore be regarded with caution. Furthermore, we anticipate that the increase in dietary variability would tend to blur the potential effects of ELOVL genes. Studies carry the risk of a false-positive finding, which can arise by chance, for genotyping errors or failure to correct for multiple testing across the number of SNPs or phenotypes tested. However, our genotyping success rate was high and the genotypes met Hardy-Weinberg equilibrium.

In conclusion, the results of our study showed that ELOVL2 and ELOVL5 genetic variants associated with alterations in PUFA levels in Han Chinese lactating mothers. Additionally, we observed interactions between DHA intake during pregnancy and specific haplotypes within
ELOVL2 and ELOVL5 SNPs and an increased level of PUFA levels, especially EPA.

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REFERENCES

1. Lauritzen, L., L. B. Sorensen, L. B. Harslof, C. Ritz, K. D. Stark, and A. Astrup. 2017. Mendelian randomization shows sex-specific associations between long-chain PUFA-related genotypes and cognitive performance in Danish schoolchildren. American Journal of Clinical Nutrition 106: 88-95.

2. Lauritzen, L., P. Brambilla, A. Mazzocchi, L. B. Harslof, V. Ciappolino, and C. Agostoni. 2016. DHA Effects in Brain Development and Function. Nutrients 8: E6.

3. Campoy, C., M. V. Escolano-Margarit, T. Anjos, H. Szajewska, and R. Uauy. 2012. Omega 3 fatty acids on child growth, visual acuity and neurodevelopment. British Journal of Nutrition 107: S85-S106.

4. Anderson, G. J., M. Neuringer, D. S. Lin, and W. E. Connor. 2005. Can prenatal N-3 fatty acid deficiency be completely reversed after birth? Effects on retinal and brain biochemistry and visual function in rhesus monkeys. Pediatric research 58: 865-872.

5. Hibbeln, J. R., J. M. Davis, C. Steer, P. Emmett, I. Rogers, C. Williams, and J. Golding. 2007. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC study): an observational cohort study. Lancet (London, England) 369:
6. Oken, E., M. L. Osterdal, M. W. Gillman, V. K. Knudsen, T. I. Halldorsson, M. Strom, D. C. Bellinger, M. Hadders-Algra, K. F. Michaelsen, and S. F. Olsen. 2008. Associations of maternal fish intake during pregnancy and breastfeeding duration with attainment of developmental milestones in early childhood: a study from the Danish National Birth Cohort. *The American journal of clinical nutrition* **88**: 789-796.

7. Victora, C. G., R. Bahl, A. J. Barros, G. V. Franca, S. Horton, J. Krasevec, S. Murch, M. J. Sankar, N. Walker, and N. C. Rollins. 2016. Breastfeeding in the 21st century: epidemiology, mechanisms, and lifelong effect. *Lancet (London, England)* **387**: 475-490.

8. Cope, M. B., and D. B. Allison. 2008. Critical review of the World Health Organization's (WHO) 2007 report on 'evidence of the long-term effects of breastfeeding: systematic reviews and meta-analysis' with respect to obesity. *Obesity reviews : an official journal of the International Association for the Study of Obesity* **9**: 594-605.

9. Innis, S. M. 2014. Impact of maternal diet on human milk composition and neurological development of infants. *The American journal of clinical nutrition* **99**: 734s-741s.

10. Gould, J. F., L. G. Smithers, and M. Makrides. 2013. The effect of maternal omega-3 (n-3) LCPUFA supplementation during pregnancy on early childhood cognitive and visual development: a systematic review and meta-analysis of randomized controlled trials. *The American journal of clinical nutrition* **97**: 531-544.

11. Carlson, S. E., J. Colombo, B. J. Gajewski, K. M. Gustafson, D. Mundy, J. Yeast, M. K. Georgieff, L. A. Markley, E. H. Kerling, and D. J. Shaddy. 2013. DHA supplementation and pregnancy outcomes. *The American journal of clinical nutrition* **97**: 808-815.
12. Olsen, S. F., and H. D. Joensen. 1985. High liveborn birth weights in the Faroes: a comparison between birth weights in the Faroes and in Denmark. *Journal of epidemiology and community health* **39**: 27-32.

13. Leventakou, V., T. Roumeliotaki, D. Martinez, H. Barros, A. L. Brantsaeter, M. Casas, M. A. Charles, S. Cordier, M. Eggesbo, M. van Eijsden, F. Forastiere, U. Gehring, E. Govarts, T. I. Halldorsson, W. Hanke, M. Haugen, D. H. Høppe, B. Heude, H. M. Inskip, V. W. Jaddoe, M. Jansen, C. Kelleher, H. M. Meltzer, F. Merletti, C. Molto-Puigmarti, M. Mommers, M. Murcia, A. Oliveira, S. F. Olsen, F. Pele, K. Polanska, D. Porta, L. Richiardi, S. M. Robinson, H. Stigum, M. Strom, J. Sunyer, C. Thijs, K. Viljøen, T. G. Vrijkotte, A. H. Wijga, M. Kogevinas, M. Vrijheid, and L. Chatzi. 2014. Fish intake during pregnancy, fetal growth, and gestational length in 19 European birth cohort studies. *The American journal of clinical nutrition* **99**: 506-516.

14. Bisgaard, H., J. Stokholm, B. L. Chawes, N. H. Vissing, E. Bjarnadóttir, A. M. Schoos, H. M. Wolsk, T. M. Pedersen, R. K. Vinding, and S. Thorsteinsdóttir. 2016. Fish Oil-Derived Fatty Acids in Pregnancy and Wheeze and Asthma in Offspring. *N Engl J Med* **375**: 2530-2539.

15. Duttaroy, A. K. 2009. Transport of fatty acids across the human placenta: a review. *Progress in lipid research* **48**: 52-61.

16. Jakobsson, A., R. Westerberg, and A. Jacobsson. 2006. Fatty acid elongases in mammals: their regulation and roles in metabolism. *Progress in lipid research* **45**: 237-249.

17. Barman, M., S. Nilsson, A. Torinsson Naluai, A. Sandin, A. E. Wold, and A. S. Sandberg. 2015. Single Nucleotide Polymorphisms in the FADS Gene Cluster but not the ELOVL2
Gene are Associated with Serum Polyunsaturated Fatty Acid Composition and Development of Allergy (in a Swedish Birth Cohort). *Nutrients* 7: 10100-10115.

18. Lemaitre, R. N., T. Tanaka, W. Tang, A. Manichaikul, M. Foy, E. K. Kabagambe, J. A. Nettleton, I. B. King, L. C. Weng, S. Bhattacharya, S. Bandinelli, J. C. Bis, S. S. Rich, D. R. Jacobs, Jr., A. Cherubini, B. McKnight, S. Liang, X. Gu, K. Rice, C. C. Laurie, T. Lumley, B. L. Browning, B. M. Psaty, Y. D. Chen, Y. Friedlander, L. Djousse, J. H. Wu, D. S. Siscovick, A. G. Uitterlinden, D. K. Arnett, L. Ferrucci, M. Fornage, M. Y. Tsai, D. Mozaffarian, and L. M. Steffen. 2011. Genetic loci associated with plasma phospholipid n-3 fatty acids: a meta-analysis of genome-wide association studies from the CHARGE Consortium. *PLoS genetics* 7: e1002193.

19. Li, X., Z. W. Gan, Z. Ding, Y. X. Wu, X. Y. Chen, H. M. Tian, G. L. Liu, Y. T. Yang, and L. Xie. 2017. Genetic Variants in the ELOVL5 but not ELOVL2 Gene Associated with Polyunsaturated Fatty Acids in Han Chinese Breast Milk. *Biomedical and environmental sciences: BES* 30: 64-67.

20. Morales, E., M. Bustamante, J. R. Gonzalez, M. Guxens, M. Torrent, M. Mendez, R. Garcia-Esteban, J. Julvez, J. Forns, M. Vrijheid, C. Molto-Puigmarti, C. Lopez-Sabater, X. Estivill, and J. Sunyer. 2011. Genetic variants of the FADS gene cluster and ELOVL gene family, colostrums LC-PUFA levels, breastfeeding, and child cognition. *PLoS One* 6: e17181.

21. Alsaleh, A., Z. Maniou, F. J. Lewis, W. L. Hall, T. A. Sanders, and S. D. O’Dell. 2014. ELOVL2 gene polymorphisms are associated with increases in plasma eicosapentaenoic and docosahexaenoic acid proportions after fish oil supplement. *Genes & nutrition* 9: 362.

22. Cormier, H., I. Rudkowska, S. Lemieux, P. Couture, P. Julien, and M. C. Vohl. 2014.
Effects of FADS and ELOVL polymorphisms on indexes of desaturase and elongase activities: results from a pre-post fish oil supplementation. *Genes & nutrition* **9**: 437.

23. Yang YX. 2009. China Food Composition, 2nd ed. *Beijing University Medical Press*, Beijing. (in Chinese)

24. Innis, S. M., J. Gilley, and J. Werker. 2001. Are human milk long-chain polyunsaturated fatty acids related to visual and neural development in breast-fed term infants? *The Journal of Pediatrics* **139**: 532-538.

25. Lepage, G., and C. C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. *Journal of Lipid Research* **27**: 114-120.

26. Ding, Z., G. L. Liu, X. Li, X. Y. Chen, Y. X. Wu, C. C. Cui, X. Zhang, G. Yang, and L. Xie. 2016. Association of polyunsaturated fatty acids in breast milk with fatty acid desaturase gene polymorphisms among Chinese lactating mothers. *Prostaglandins, leukotrienes, and essential fatty acids* **109**: 66-71.

27. Schaid, D. J. 2004. Evaluating associations of haplotypes with traits. *Genetic epidemiology* **27**: 348-364.

28. Tzeng, J. Y., C. H. Wang, J. T. Kao, and C. K. Hsiao. 2006. Regression-based association analysis with clustered haplotypes through use of genotypes. *American journal of human genetics* **78**: 231-242.

29. Tanaka, T., J. Shen, G. R. Abecasis, A. Ksialiou, J. M. Ordovas, J. M. Guralnik, A. Singleton, S. Bandinelli, A. Cherubini, D. Arnett, M. Y. Tsai, and L. Ferrucci. 2009. Genome-wide association study of plasma polyunsaturated fatty acids in the InCHIANTI Study. *PLoS genetics* **5**: e1000338.
30. Martinez, M. 1992. Tissue levels of polyunsaturated fatty acids during early human development. *The Journal of pediatrics* **120**: S129-138.

31. Fleith, M., and M. T. Clandinin. 2005. Dietary PUFA for preterm and term infants: review of clinical studies. *Critical reviews in food science and nutrition* **45**: 205-229.

32. Innis, S. M. 2005. Essential fatty acid transfer and fetal development. *Placenta* **26 Suppl A**: S70-75.

33. Helland, I. B., O. D. Saugstad, K. Saarem, A. C. Van Houwelingen, G. Nylander, and C. A. Drevon. 2006. Supplementation of n-3 fatty acids during pregnancy and lactation reduces maternal plasma lipid levels and provides DHA to the infants. *The journal of maternal-fetal & neonatal medicine : the official journal of the European Association of Perinatal Medicine, the Federation of Asia and Oceania Perinatal Societies, the International Society of Perinatal Obstet* **19**: 397-406.

34. Decsi, T., C. Campoy, and B. Koletzko. 2005. Effect of N-3 polyunsaturated fatty acid supplementation in pregnancy: the Nuheal trial. *Advances in experimental medicine and biology* **569**: 109-113.

35. Chinese Nutrition Society. 2014. Chinese Dietary Reference Intakes (DRIs) Handbook (2013). *Standards Press of China*, Beijing.(in Chinese)

36. Tanaka, T. 2005. International HapMap Project. [http://hapmap.jst.go.jp/index.html.en 63 Suppl 12]: 29-34.

37. Zhang, J. Y., K. S. Kothapalli, and J. T. Brenna. 2016. Desaturase and elongase-limiting endogenous long-chain polyunsaturated fatty acid biosynthesis. *Current opinion in clinical nutrition and metabolic care* **19**: 103-110.
38. Arterburn, L. M., H. Eileen Bailey, and O. Harry. 2006. Distribution, interconversion, and dose response of n-3 fatty acids in humans. *American Journal of Clinical Nutrition* **83**: 1467S-1476S.

39. Allaire, J., W. S. Harris, C. Vors, A. Charest, J. Marin, K. H. Jackson, A. Tchernof, P. Couture, and B. Lamarche. 2017. Supplementation with high-dose docosahexaenoic acid increases the Omega-3 Index more than high-dose eicosapentaenoic acid. *Prostaglandins, leukotrienes, and essential fatty acids* **120**: 8-14.
Table 1: Background characteristics of the lactating mothers

| Variable                                | Value   |
|-----------------------------------------|---------|
| Age (years)                             | 30.29±3.40 |
| Gestational Age (weeks)                 | 39.27±1.00 |
| Preconception BMI (kg / m²)             | 20.95±3.33 |
| Gestational weight gain (kg)            | 18.88±6.30 |
| Feeding patterns                        |         |
| exclusive breast-feeding                | 223(54.39) |
| mixed feeding                           | 187(45.61) |
| Delivery patterns                       |         |
| vaginal delivery                        | 130(31.10) |
| cesarean delivery                       | 288(68.90) |
| Education level (years of schooling)    |         |
| graduate school (≥17 years)             | 37(9.25) |
| university (13-16 years)                | 203(50.75) |
| high school (10-12 years)               | 140(35.00) |
| middle school/ junior high school (7-9 years) | 17(4.25) |
| primary school (≤6 years)               | 3(0.75) |
| Household income (RMB/month)            |         |
| low (≤5,000 RMB)                        | 57(16.67) |
| middle (5,000-10,000 RMB)               | 185(54.09) |
| high (>10,000 RMB)                      | 100(29.24) |

BMI, body mass index.

* Values shown in the table are means ± SDs or n (%).
Table 2: Association between DHA intake and breast milk PUFA by quartile of DHA intake

| PUFA      | DHA intake (mg/day) | P       |
|-----------|---------------------|---------|
|           | Q₁ (< 14.58)       |         |
| n-6       |                      |         |
| LA        | 0.359±0.198         | 0.52    |
| GLA       | 0.039(0.021,0.056)  | 0.67    |
| DGLA      | 0.052(0.030,0.071)  | 0.64    |
| ARA       | 0.076±0.039         | 0.20    |
| DTA       | 0.019(0.011,0.024)  | 0.26    |
| n-3       |                      |         |
| ALA       | 0.143(0.084,0.197)  | 0.87    |
| EPA       | 0.007(0.004,0.011)  | 0.56    |
| DHA       | 0.044(0.028,0.061)  | 0.15    |
| GLA, γ-linolenic acid; DGLA, dihomo γ-linolenic acid; ARA, arachidonic acid; DTA, docosatetraenoic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.
a Q means the quartile level of DHA intake.

b Values are means ± SDs for normal distribution.

c Values are medians (25th-75th percentiles) for skewed distributed variables.
Table 3: Characteristics of 10 polymorphisms in the *ELOVL* gene cluster

| Gene   | SNP      | Position(bp) | Alleles | Genotype | Allele | Genotyping Success Rate (%) |
|--------|----------|--------------|---------|----------|--------|----------------------------|
|        |          |              | M/m     | MM       | M     |                            |
|        |          |              |         | Mm       |        |                            |
|        |          |              |         | mm       |        |                            |
|        |          |              |         |          |        |                            |
|        |          |              |         |          |        |                            |
| ELOVL2 | rs2281591| 10990260     | A/G     | 264      | 663    | 99.53                      |
|        | rs12332786| 10998735     | C/G     | 236      | 632    | 99.53                      |
|        | rs3798713 | 11008389     | C/G     | 180      | 546    | 97.39                      |
|        | rs3778166 | 11032931     | G/A     | 131      | 468    | 98.58                      |
|        | rs9468304 | 11041932     | A/G     | 207      | 585    | 99.29                      |
| ELOVL5 | rs2294867 | 53289156     | C/A     | 156      | 498    | 96.68                      |
|        | rs9357760 | 53325336     | A/G     | 183      | 545    | 97.63                      |
|        | rs2397142 | 53335501     | C/G     | 186      | 549    | 96.45                      |
|        | rs209512  | 53338779     | A/G     | 114      | 445    | 99.05                      |
|        | rs12207094| 53339377     | A/T     | 335      | 750    | 99.76                      |

SNP, single nucleotide polymorphisms; *ELOVL2*, elongase gene 2 of the very long chain fatty acid; *ELOVL5*, elongase gene 5 of the very long chain fatty acid; M, major allele; m, minor allele.
a The number of women carrying M/M, M/m, or m/m genotypes.

b The number of women carrying M or m, the major and minor alleles.
Table 4: Interaction between ELOVL2/5 haplotype and DHA intake on breast milk PUFA level

|          | LA coef | P  | GLA coef | P  | DGLA coef | P  | ARA coef | P  | DTA coef | P  | ALA coef | P  | EPA coef | P  | DHA coef | P  |
|----------|---------|----|----------|----|-----------|----|----------|----|----------|----|----------|----|----------|----|----------|----|----------|----|
| **ELOVL2** |         |    |          |    |           |    |          |    |          |    |          |    |          |    |          |    |          |    |
| Q₁        | Reference |    |          |    |           |    |          |    |          |    |          |    |          |    |          |    |          |    |
| Q₂        | -0.0243  | 0.644 | -0.0161  | 0.344 | -0.0047  | 0.567 | -0.0053  | 0.635 | -0.0012  | 0.654 | -0.0099 | 0.643 | -0.0084 | 0.333 | -0.0031 | 0.876 |
| Q₁-H₁     | Reference |    |          |    |           |    |          |    |          |    |          |    |          |    |          |    |          |    |
| Q₂-H₁     | 0.1107  | 0.038 | 0.0329  | 0.060 | 0.0108  | 0.192 | 0.0255  | 0.025 | 0.0040  | 0.143 | 0.0385 | 0.072 | 0.0190 | 0.034 | 0.0586 | 0.004 |
| **ELOVL5** |         |    |          |    |           |    |          |    |          |    |          |    |          |    |          |    |          |    |
| Q₁        | Reference |    |          |    |           |    |          |    |          |    |          |    |          |    |          |    |          |    |
| Q₂        | -0.0144  | 0.790 | -0.0144  | 0.406 | -0.0023  | 0.780 | 0.0005  | 0.967 | -0.0004  | 0.891 | -0.0327 | 0.128 | -0.0102 | 0.246 | -0.0193 | 0.342 |
| Q₄        | -0.0450  | 0.374 | -0.0126  | 0.437 | -0.0072  | 0.345 | -0.0136  | 0.204 | -0.0024  | 0.351 | -0.0223 | 0.267 | -0.0020 | 0.812 | 0.0036 | 0.851 |
| H₁        | Reference |    |          |    |           |    |          |    |          |    |          |    |          |    |          |    |          |    |
| H₄        | -0.0546  | 0.376 | 0.0280  | 0.157 | -0.0098  | 0.295 | -0.0145  | 0.264 | -0.0040  | 0.205 | -0.0131 | 0.595 | -0.0168 | 0.095 | -0.0329 | 0.157 |
| H₅        | -0.0086  | 0.886 | 0.0081  | 0.672 | 0.0031  | 0.736 | -0.0043  | 0.735 | -0.0011  | 0.721 | 0.0034 | 0.886 | -0.0106 | 0.273 | -0.0172 | 0.473 |
| Q-H | Reference | Q1-H1c | Q2-H2c | Q4-H4c | Q2-H5c | Q4-H5c |
|-----|-----------|--------|--------|--------|--------|--------|
|     |           | 0.0341 | 0.699  | 0.0213 | 0.455  | 0.0030 |
|     |           | 0.0093 | 0.615  | 0.0003 | 0.953  | 0.0679 |
|     |           | 0.0053 | 0.0072 | 0.611  | 0.0434 | 0.185  |
|     |           | 0.1994 | 0.068  | 0.0603 | 0.084  | 0.0385 |
|     |           | 0.020  | 0.0326 | 0.159  | 0.0046 | 0.409  |
|     |           | 0.0998 | 0.022  | 0.0359 | 0.042  | 0.0621 |
|     |           | 0.129  | 0.0424 | 0.011  | 0.0550 | 0.141  |
|     |           | 0.1158 | 0.238  | 0.0250 | 0.452  | 0.0318 |
|     |           | 0.036  | 0.0382 | 0.069  | 0.0083 | 0.099  |
|     |           | 0.0527 | 0.182  | 0.0424 | 0.011  | 0.0550 |
|     |           | 0.141  | 0.0600 | 0.0393 | 0.015  | 0.0600 |
|     |           | 0.0068 | 0.944  | 0.0446 | 0.162  | 0.0059 |
|     |           | 0.0029 | 0.877  | 0.0020 | 0.695  | -0.0055|
|     |           | 0.695  | 0.885  | 0.0393 | 0.015  | 0.0600 |
|     |           | 0.185  | 0.141  | 0.0600 | 0.0393 | 0.015  |

*ELOVL2*, elongase gene 2 of the very long chain fatty acid; *ELOVL5*, elongase gene 5 of the very long chain fatty acid. PUFA, long chain polyunsaturated fatty acids; LA, linoleic acid; GLA, γ-linolenic acid; DGLA, dihomo γ-linolenic acid; ARA, arachidonic acid; DTA, docosatetraenoic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

Q means the quartile level of DHA intake: Q1: <14.58 mg/day (reference); Q2: 14.58–43.15 mg/day; Q4: ≥118.82 mg/day.

H means the type of haplotype. *ELOVL2*, H1: A-C-C (reference); H2: A-G-G. *ELOVL5*: H1: C-A-C-G-A (reference); H3: A-A-C-A-A; H5: C-A-C-A-A; Haplotypes are named according to its frequency.

Q-H means the effect of interaction between DHA intake and haplotype on PUFA levels compared with reference.
Figure 1. Breast milk PUFA levels in optimal genotype model (a) and LD block plots of SNPs in ELOVL2 (b) and ELOVL5 (c). PUFA, polyunsaturated fatty acids; ELOVL2, elongase gene 2 of the very long chain fatty acid; ELOVL5, elongase gene 5 of the very long chain fatty acid; SNPs, single nucleotide polymorphisms; LA, linoleic acid; GLA, γ-linolenic acid; DGLA, dihomo γ-linolenic acid; ARA, arachidonic acid; DTA, docosatetraenoic acid; ALA, α-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; LD, linkage disequilibrium. D in the triangle represents dominant model, ↑ ↓: PUFA changes of Mm+mm compared with MM; R in the circle represents recessive model, ↑ ↓: PUFA changes of mm compared with MM+Mm.