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Association of polyunsaturated fatty acids in breast milk with fatty acid desaturase gene polymorphisms among Chinese lactating mothers

Zhen Ding [a], Guo-Liang Liu [b], Xiang Li [a], Xue-Yan Chen [a], Yi-Xia Wu [a], Can-Can Cui [c], Xi Zhang [c], Guang Yang [c], Lin Xie [a,*]

[a] Department of Nutrition and Food Hygiene, School of Public Health, Jilin University, Chang Chun 130021, China
[b] Experimental Center of Prevention, School of Public Health, Jilin University, Chang Chun 130021, China
[c] Medical Law, The grade of 2011, School of Public Health, Jilin University, Chang Chun 130021, China

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ABSTRACT

Background: The fatty acid desaturase (*FADS*) controls polyunsaturated fatty acid (PUFA) synthesis in human tissues and breast milk.
Design: Evaluate the influence of 10 single nucleotide polymorphisms (SNPs) and various haplotypes in the *FADS* gene cluster (*FADS1*, *FADS2*, *FADS3*) on PUFA concentration in the breast milk of 209 healthy Chinese women. PUFA concentrations were measured in breast milk using gas chromatography and genotyping was performed using the Sequenom Mass Array system.
Results: A SNP (rs1535) and 2-locus haplotypes (rs3834458-rs1535, rs1535-rs174575) in the *FADS2* gene were associated with concentrations of γ- linoleic acid (GLA) and arachidonic acid (AA) in breast milk. Likewise, in the *FADS1* gene, a 2-locus constructed haplotype (rs174547-rs174553) also affected GLA and AA concentration ($P < 0.05$ for all). Minor allele carriers of the SNP and haplotypes described above had lower concentrations of GLA and AA. In the *FADS2* gene, the 3-locus haplotype rs3834458-rs1535-rs174575, significantly affected concentrations of GLA but not AA. Pairwise comparison showed that individuals major homozygous for the SNP rs1000778 in the *FADS3* gene had lower concentrations of ALA and linoleic acid (LA) in their breast milk.
Conclusion: Polymorphisms in the *FADS* gene cluster influence PUFA concentrations in the breast milk of Chinese Han lactating women.

1. Introduction

Polyunsaturated fatty acids (PUFAs) constitute the important component of cell membranes and are important for their structure and function. The long-chain polyunsaturated fatty acids (LC-PUFA) arachidonic acid (AA) and docosahexaenoic acid (DHA) are abundant in the membrane phospholipids of cells in the central nervous system [1,2]. LC-PUFAs play a role in early visual [3], cognitive [4,5] and motor development [6], and have also been associated with postpartum depression [7]. Genetic variation and dietary intake largely influence PUFA concentrations in human tissues [8,9]. LC-PUFAs are derived from the elongation and desaturation of the essential fatty acids linoleic acid (LA) and a- linolenic acid (ALA) [10]. Desaturases (D5D and D6D) are the key enzymes in the endogenous synthetic metabolism of LC-PUFAs [11,12]. D5D and D6D are encoded by the fatty acid desaturase genes *FADS1* and *FADS2*, which reside on chromosome 11q12–q13.1, together with the *FADS3* gene in a 91.9-kb cluster [12,13]. *FADS1* and *FADS2* have a head-to-head orientation while *FADS2* and *FADS3* are orientated tail-to-tail. Several single nucleotide polymorphisms (SNPs) in the *FADS* gene cluster are associated with human LC-PUFA concentrations according to genome-wide association studies (GWAS) [14,15]. SNPs and constructed haplotypes have previously been associated with PUFA concentrations in plasma, erythrocyte membranes and breast milk of Caucasian and European [7,11,16–19]. Here, we describe the association of 10 SNPs and various haplotypes in the *FADS* gene cluster with PUFA concentrations in the breast milk of healthy lactating Chinese Han women.

Abbreviations: ANCOVA, analysis of covariance; ALA, a-linolenic acid; AA, arachidonic acid; DGLA, dihomo-γ-linolenic acid; DTA, docosatetraenoic acid; DHA, docosahexenoic acid; df, degrees of freedom; EPA, eicosapentaenoic acid; FADS, fatty acid desaturase; LA, linoleic acid; LD, linkage disequilibrium; NCBI, National Center for Biotechnology; PUFAs, polyunsaturated fatty acids; SNPs, single nucleotide polymorphism; R^2, squared correlation coefficient; GLA, γ- linoleic acid

* Correspondence to: Department of Nutrition and Food Hygiene, School of Public Health, Jilin University, 1163 Xin Min Street, Chang Chun 130021, China.
E-mail address: xielin@jlu.edu.cn (L. Xie).

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2. Materials and methods

2.1. Subjects and design

We screened all pregnant women registered for postpartum care at Shirentang house (ChangChun) from March 2012 to December 2014. 209 healthy Chinese Han lactating mothers aged 22–39 y participated in this study. Only participants with no maternal pregnancy complications were included. Other exclusion criteria included metabolic diseases (including diabetes), communicable diseases and the use of PUFA-containing supplements during pregnancy and lactation. All participants gave informed consent according to the procedures approved by the ethics committee of Jilin University, Changchun, China.

2.2. Questionnaire survey and breast milk collection

Basic information questionnaire and a 24-hour dietary recall questionnaire was implemented preceding milk collection by trained investigators through face to face method. Dietary intakes from the questionnaire were entered into the database built according to Chinese food composition in 2009 [20] and Golden key-maternal nutrition software (Wincome, Shanghai). And, five kinds of PUFA dietary intakes were calculated as g/mg per one day.

20 mL of breast milk was collected by manual expression between 9:00 a.m. and 11:00 a.m. on one day between the 22nd and 25th day after delivery. Abandon the previous few drops of milk and collect the foremilk of the mature breast milk. The samples were stored at $-80\,^{\circ}C$ [21,22], and detected in one month averagely.

2.3. Fatty acid analysis

Internal standard method was used to calculate the levels of fatty acid methyl esters (FAME). The milk samples were thawed at 4 °C and the milk fat (the upper layer) was extracted. FAME were prepared from milk fat by combining 0.2 mL of fat with 2 mL of methanol and benzene (4:1, v/v), 33 μL of margaric acid (C17:0), used as the internal standard (9.844 mg/ml), and 200 μL of acetyl chloride in a 10 mL glass tube. The mixture was incubated in glycerinum at 100 °C for 60 mins. FAME samples were cooled and 5 mL of 6% K_2CO_3 was slowly added to stop the reaction and neutralize the mixture. The solution was centrifuged for 10 min at 3000 rpm and 1 μL of the upper phase was injected into a gas chromatograph with a flame ionization detector.

PUFAs were separated and identified by gas chromatography (Shimadzu 14B, Kyoto, Japan) equipped with a capillary column (SP-2560, Supplco 100 m × 0.25 mm × 0.20 μm). The temperature was kept at 140 °C for the first 5 min, and then increased at a rate of 4 °C/min until 260 °C, then held for 20 min. Nitrogen (99.999%) was utilized as a carrier gas at a flow rate of 1 mL/min. The injection was set at 260 °C with a split ratio of 50:1. The flame ionization detector was maintained at 280 °C and the flame was ignited with hydrogen (flow rate 40 mL/min) and air (flow rate 500 mL/min). The retention times and peak areas were compared to the internal standard and the fatty acid concentration was calculated according to the corresponding conversion coefficient in the national standard [23].

2.4. Genotyping

SNPs were identified using HapMap and the NCBI SNP database (dbSNP Build 126; http://www.ncbi.nlm. nih.gov/). Participants were genotyped for 10 FADS gene cluster variants, including 3 SNPs (rs174553, rs174575, rs3834458) that have previously been reported being associated with PUFA concentrations and 7 tag SNPs with a minor allele frequency above 10%. Among the 7 tag SNPs, 3 tag SNPs with a linkage disequilibrium threshold of $R^2 > 0.8$ (rs174547 tag up to 7 other SNPs in 11kb genomic region of the FADS1, rs1535 tag up to 6 other SNPs in 26 kb genomic region of FADS2 gene and rs1000778 tag up to 5 other SNPs in 11 kb genomic region of FADS3 gene), 4 tag SNPs were selected from different genomic regions (rs174602, rs498793, rs174550, rs7115793).

The milk samples were thawed at 4 °C and the fat layer, skim milk layer and cellular layer were separated. Genomic DNA was extracted from 300 μL of the cellular layer using the DNA kit (Beijing, TIANGEN) according to the manufacturer's instructions. Genotyping was performed using the Sequenom Mass Array system (BO MIAO Biological Technological Company, Beijing).

2.5. Statistics

Normally distributed data were expressed as mean ± SD and skewed distribution data were expressed as median ± IQR. Hardy–Weinberg equilibrium was tested by the Chi-square goodness of fit test for each SNP locus. Lewontin's disequilibrium coefficient D' and the squared correlation coefficient (R^2) were estimated by Haploview software 4.2 [11]. Normal distribution of the fatty acids was tested by the Kolmogorov–Smirnov test and distribution plots. The skewed measurements of n-6 γ-linolenic acid (GLA), Dihomo-γ-linolenic acid (DGLA), docosatetraenoic acid (DTA), n-3 eicosapentaenoic acid (EPA) and docosahexenoic acid (DHA) concentrations were expressed as square roots to obtain a normal distribution. Genotype association with PUFA concentration was tested using covariate ANOVA(ANCOVA, SPSS software version 16.0), with age and BMI included as covariates [19]. Then, the pairwise comparisons were done, yielding P values corrected for multiple comparisons with major allele homozygote. Given the SNP rs174575 and rs498793 with a minor allele frequency below 10%, so the analysis were done in dominant model, which with Mm and mm combined. All statistical tests were two-tailed and the threshold for statistical significance was set at ≤ 0.05. Haplotype analysis of the SNPs in linkage disequilibrium (LD) blocks was performed by the unphased software 3.012 [7]. Rare haplotypes (frequency $< 2\%$) were excluded [24].

3. Results

Informed consent was given by 600 participants at the beginning of the study, but only 514 provided breast milk samples on one day between the 22nd and 25th day after delivery. 86 subjects had withdrawn from the study on account of insufficient lactation. Fatty acids of the 514 breast milk were measured. Then, subjects who were minority or consumed PUFA containing supplements either during pregnancy or lactation were eliminated from the study. DNA was extracted from breast milk samples of 209 women of Han ethnicity who took no PUFA containing supplements during pregnancy or lactation, and then was genotyped. In the end, the data of 209 genotype and fatty acids were analyzed.

3.1. Subject characteristics

The 209 healthy lactating mothers included in this study had an average age of 30 years and mainly came from middle income households (56.70%). Gestational age was 39.00 ± 1.29 weeks. The preconception BMI was 20.40 ± 4.20 kg/m^2 and the weight gain during pregnancy was 18.00 ± 6.90 kg; both were within the normal range. 31.7% of subjects had a vaginal delivery, the rest had cesareans. 55.90% of subjects breastfed exclusively while the remaining 44.10% opted for mixed feeding.