Letter to the Editor

Genetic Variants in the *ELOVL5* but not *ELOVL2* Gene Associated with Polyunsaturated Fatty Acids in Han Chinese Breast Milk*

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The present study was designed to examine the contributions of the fatty acid elongase (*ELOVL*) gene polymorphisms to the levels of polyunsaturated fatty acids (PUFAs) in breast milk. Two hundred and nine healthy Han Chinese mothers were included in the study. Carriers of minor alleles of SNPs (rs2397142 and rs9357760) in *ELOVL*5 were associated with higher levels of linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), arachidonic acid (AA), docosatetraenoic acid (DTA), docosahexenoic acid (DHA), while in rs209512 of *ELOVL*5 the carriers of minor alleles had lower levels of DTA compared to major homozygote alleles (*P* ranged from 0.004-0.046), and genetically explained variability ranged from 3.2% for eicosapentaenoic acid (EPA) to 6.0% for LA. Our findings demonstrated that common variation in *ELOVL5* gene encoding rate-limiting enzymes in the metabolism of PUFAs contribute to the PUFAs in breast milk.

An adequate supply of long-chain Polyunsaturated fatty acids PUFAs (LC-PUFAs) during pregnancy is important for brain growth as well as visual and cognitive development of the fetus[1]. Breast milk is a major source of food for 0-6 month-old-infants, these PUFAs, which are transferred across the placenta and are present in human milk, are accumulated in the brain and retina during fetal and infant development, and insufficient LC-PUFAs intake may result in visual and cognitive impairment and disturbances in mental functions and could be the main reason for the increasing incidence of different mental disorders in humans[2]. *ELOVL* gene is the elongation of very long chain fatty acids family genes, *ELOVL2* and *ELOVL*5 encode fatty acid elongase-2 and -5 elongases that catalyze the rate-limiting condensation reaction resulting in the synthesis of very long chain fatty acids[3]. The objective of this study was to investigate the association between the levels of PUFAs in breast milk and the common variants of the *ELOVL*2 and *ELOVL*5 genes.

A total of 209 healthy participants from Shirentang House (a Postpartum Care Center, where mothers and babies were taken care after delivery) without any obstetrical complications were enrolled in this study, the age ranged from 22-39 years. They were non-smokers, with a pre-pregnancy BMI ranging from 17.60-26.30 kg/m^2 and they gained weight (18.00 ± 6.90 kg) during pregnancy. Some (55.90%) were breastfed exclusively and the others chose mixed feeding; 68.3% had a caesarean delivery, gestational age was 39.00 ± 1.28 weeks, there are 114 boys and 95 girls, and their average weight was 3.3 kg, and most (56.7%) of the subjects came from middle-income families. There was no significant difference ($P > 0.05$) in the intake of dietary PUFAs between the carriers of minor alleles, compared to the major homozygote alleles. The intake data for each participant were estimated from a 24-h recall questionnaire (*data not shown*).

The distributions of genotypes frequencies in the 209 subjects were in accordance with Hardy-Weinberg equilibrium. The selected SNPs are all in introns (Table 1), the prevalence of the minor alleles was relatively high and ranged from 11%-43% of the population. Hence, one would expect a considerable public health relevance of these genetic variants, which modulate the effects of environmental exposure on human health. To

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determine whether the *ELOVL* genotypes were associated with PUFAs, we evaluated the associations of the genotype of ten SNPs with eight levels of PUFAs by covariate ANOVA and the results are given in Table 2. Significant associations were observed between SNPs in *ELOVL*5 and the levels of PUFAs. Carriers of minor alleles of rs2397142 and rs9357760 in *ELOVL*5 had higher levels of linoleic acid (LA), dihomo-γ-linolenic acid (DGLA), arachidonic acid (AA), docosatetraenoic acid (DTA) and n-3 product docosahexaenoic acid (DHA) compared to major homozygote alleles (*P* changed from 0.004 to 0.046). However, there is an exception that the carrier of a minor allele of SNP rs209512 in *ELOVL*5 was associated significantly ($P = 0.023$) with lower levels of DTA in breast milk compared to major homozygote alleles.

Linear regression analysis was used to investigate the associations of *ELOVL* gene polymorphisms with concentrations of PUFAs. The unadjusted R^2 values, that reflected the variability of PUFA concentrations explained by the genetic variants, ranged from very low for eicosapentaenoic acid (EPA) (3.2%) or GLA (3.4%), to LA (6.0%). The variability of α-linolenic acid (ALA) amounts explained by the analyzed polymorphisms reached 7.4% in adjusted analyses, which included the effect of confounders (maternal age and pre-pregnancy BMI) (Table 3).

The synthesis of LC-PUFAs from LA involves enzyme-mediated desaturation and elongation steps. Δ6-desaturase (D6D, encoded by the *FADS2* gene) catalyzes the conversion of LA into GLA, which is then elongated into DGLA by elongase-5 (encoded by the *ELOVL*5 gene). Moreover, DGLA can be converted into AA by Δ5-desaturase (D5D, encoded by the FADS1 gene)[4]. In the covariate ANOVA analyses, genetic variation in the elongase gene *ELOVL*5 (rs2397142 and rs9357760) was associated with higher levels of LA, DGLA, AA, DTA, and DHA compared to major homozygote alleles. Higher transcription of elongase-5 may increase the conversion of GLA to DGLA, and accumulated AA, which is the substrates for DTA. It may contribute to high efficient synthesis of AA to DTA. This is probably due to *ELOVL*5 gene variants that increased the conversion efficiency or contribute to a high enzyme activity.

The biological effects of LC-PUFAs on brain function are assumed to be mediated by tissue contents of LC-PUFAs with > 20 carbon atoms and more than three double bonds, such as AA, EPA and DHA[5]. AA and DHA have important roles in synaptic transmission and plasticity during early brain development[6]. The intake of AA and DHA during pregnancy and lactation can improve the visual acuity, psychomotor development and mental performance[7]. Although infants are capable of synthesizing AA and DHA, the accumulation of LC-PUFAs in utero is predominantly via placental transfer. Based on the present results, maternal genetic variants influence levels of LC-PUFAs in breast milk and, thus, genetic polymorphisms in the lactating mothers are likely to have important influence on infant brain development.

Table 1. Characteristics of 10 Polymorphisms in the *ELOVL* Gene Cluster

dbSNP	Position (bp) *	Function	Alleles		Genotype		HWE‡	Genotyping Success Rate (%)
			M/m†	MM	Mm	mm		
ELOVL2								
rs2281591	10990260	Intron	A/G	136	62	10	0.40	99.52
rs12332786	10998735	Intron	C/G	115	81	12	0.65	99.52
rs3798713	11008389	Intron	C/G	88	92	23	0.89	97.13
rs3778166	11032931	Intron	G/A	60	108	35	0.25	97.13
rs9468304	11041932	Intron	A/G	103	90	16	0.55	100.00
ELOVL5								
rs2294867	53289156	Intron	C/A	72	100	28	0.47	95.69
rs9357760	53325336	Intron	A/G	88	91	22	0.83	96.17
rs2397142	53335501	Intron	C/G	88	92	20	0.57	95.69
rs209512	53338779	Intron	A/G	59	109	39	0.36	99.04
rs12207094	53339377	Intron	A/T	166	39	4	0.35	100.00

Note. *Position in basepairs was derived from dbSNP Build 126, based on NCBI Human Genome Build 36 of chromosome 6. †M, major allele; m, minor allele. ‡HWE, Hardy-Weinberg equilibrium.

Table 2. Relationship between the 10 SNPs in the *ELOVL2* and *ELOVL5* Gene Region and the Levels of PUFAs in Breast Milk[‡] (g/100 g, $\bar{x}\pm s$)

Gene	SNP	Genotype	LA	GLA[†]	DGLA[†]	AA	DTA[†]	ALA	EPA[†]	DHA[†]
ELOVL2	rs2281591	AA	0.363±0.233	0.194±0.070	0.224±0.077	0.078±0.059	0.128±0.045	0.145±0.083	0.088±0.032	0.211±0.072
		AG+GG	0.371±0.200	0.194±0.061	0.220±0.067	0.081±0.043	0.130±0.040	0.146±0.075	0.084±0.031	0.208±0.063
		P	0.783	0.945	0.722	0.658	0.813	0.978	0.348	0.796
	rs12332786	CC	0.365±0.194	0.192±0.062	0.221±0.069	0.078±0.041	0.128±0.040	0.147±0.079	0.086±0.030	0.207±0.067
		CG+GG	0.370±0.253	0.197±0.072	0.225±0.079	0.080±0.068	0.130±0.046	0.145±0.082	0.089±0.034	0.214±0.072
		P	0.888	0.553	0.717	0.785	0.737	0.888	0.539	0.480
	rs3798713	CC	0.379±0.198	0.191±0.057	0.220±0.067	0.081±0.041	0.130±0.039	0.150±0.079	0.085±0.030	0.209±0.065
		CG+GG	0.358±0.238	0.196±0.073	0.224±0.080	0.078±0.063	0.128±0.046	0.142±0.080	0.089±0.034	0.211±0.073
		P	0.516	0.641	0.753	0.707	0.689	0.476	0.444	0.863
	rs3778166	GG	0.365±0.199	0.192±0.065	0.216±0.071	0.077±0.041	0.126±0.041	0.146±0.077	0.084±0.031	0.208±0.071
		AG+AA	0.371±0.231	0.196±0.067	0.227±0.075	0.081±0.059	0.131±0.043	0.146±0.081	0.089±0.032	0.212±00.069
		P	0.865	0.697	0.351	0.602	0.426	1.000	0.391	0.701
	rs9468304	AA	0.355±0.249	0.190±0.070	0.220±0.080	0.077±0.065	0.128±0.047	0.143±0.088	0.087±0.033	0.208±0.073
		AG+GG	0.378±0.191	0.198±0.063	0.226±0.067	0.081±0.041	0.130±0.038	0.149±0.073	0.087±0.031	0.213±0.066
		P	0.449	0.409	0.614	0.612	0.739	0.639	0.955	0.563
ELOVL5	rs2294867	CC	0.348±0.176	0.194±0.065	0.216±0.064	0.075±0.038	0.124±0.039	0.142±0.073	0.085±0.031	0.204±0.061
		AC+AA	0.378±0.248	0.195±0.068	0.228±0.079	0.083±0.063	0.132±0.046	0.150±0.086	0.089±0.033	0.215±0.075
		P	0.357	0.980	0.278	0.304	0.212	0.501	0.364	0.270
	rs9357760	AA	0.330±0.174	0.187±0.065	0.208±0.066	0.068±0.037	0.120±0.039	0.136±0.075	0.083±0.030	0.198±0.062
		AG+GG	0.405±0.249	0.203±0.067	0.236±0.078	0.089±0.064	0.137±0.044	0.157±0.083	0.091±0.032	0.222±0.073
		P	0.017	0.091	0.008	0.008	0.004	0.057	0.099	0.015
	rs2397142	CC	0.334±0.174	0.187±0.065	0.211±0.067	0.070±0.038	0.121±0.040	0.138±0.076	0.084±0.030	0.200±0.062
		CG+GG	0.397±0.251	0.200±0.067	0.233±0.079	0.087±0.063	0.136±0.045	0.155±0.084	0.089±0.032	0.219±0.074
		P	0.046	0.176	0.034	0.024	0.014	0.131	0.263	0.050
	rs209512	AA	0.394±0.276	0.199±0.062	0.235±0.087	0.090±0.079	0.140±0.049	0.157±0.087	0.090±0.032	0.224±0.072
		AG+GG	0.355±0.196	0.197±0.072	0.217±0.068	0.075±0.040	0.125±0.040	0.141±0.077	0.086±0.032	0.205±0.068
		P	0.253	0.475	0.109	0.067	0.023	0.175	0.365	0.082
	rs12207094	AA	0.367±0.233	0.192±0.066	0.222±0.078	0.079±0.058	0.129±0.046	0.145±0.084	0.087±0.033	0.209±0.072
		AT+TT	0.369±0.173	0.202±0.068	0.228±0.056	0.079±0.036	0.131±0.030	0.150±0.066	0.088±0.029	0.217±0.056
		P	0.954	0.399	0.595	0.996	0.731	0.733	0.885	0.522

Note. SNP, single nucleotide polymorphism; LA, linoleic acid; GLA, γ-linoleic acid; DGLA, dihomo-γ-linolenic acid; AA, arachidonic acid; DTA, docosatetraenoic acid; ALA, a-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexenoic acid; *ELOVL*, *ELOVL* fatty acid elongase. [†]The data were normalized by square root transformation. [‡]Significance of genotype association with concentrations of PUFAs was tested by covariate ANOVA, All *P* values were adjusted by BMI and age.

Table 3. R^2 Across the 10 Genetic Variants for Each Fatty Acid (%)

Fatty Acids		Unadjusted	Adjusted*
n-3			
ALA	C18:3	5.5	7.4
EPA	C20:5	3.2	6.4
DHA	C22:6	4.8	6.4
n-6			
LA	C18:2	6.0	6.5
GLA	C18:3	3.4	3.8
DGLA	C20:3	4.5	5.5
AA	C20:4	5.9	6.0
DTA	C22:4	5.5	6.5

Note. *All R^2 were adjusted by BMI and age.

In addition, not all genetic variants were significantly associated with PUFA synthesis, we find only one n-3 PUFA (DHA) associated with SNP rs9357760 in *ELOVL*5, and the effect of gene variants on n-6 fatty acids was more obvious, compared with n-3 fatty acids, probably due to the low conversion of ALA to DHA (< 4%)[8]; Otherwise, limited sample size also affected the power of the findings.

Associations of the ten SNPs and PUFAs were explained by linear regression. In the initial unadjusted analysis, the genetically explained variability of the amounts of fatty acids ranged from 3.2% for EPA to 6.0% for LA. Lattka et al. reported a genetically explained variability of 28.5% for AA[9] and Tanaka et al reported a variability of 18.6%[10]. In this study, the variability of the AA amounts explained by the ten genetic variants analyzed was 5.9%, which might be because the two earlier studies analyzed fatty acids in serum and plasma, whereas we analyzed fatty acids in breast milk. Our results suggest the amounts of fatty acids in breast milk are less influenced by *ELOVL* genotypes, compared to plasma or serum phospholipid fatty acids, but this is purely speculative. Nevertheless, all the associations remained stable despite adjustments for maternal age and preconception BMI confounders. By including these covariables into the analysis, we were able to explain ≤ 7.4% of the variance in the levels of fatty acids.

Overall, the findings of this study show that the *ELOVL5* gene polymorphisms might affect the levels of PUFAs in the breast milk, accordingly affecting children's growth and development in the future, and it also provides basic data for personalized nutritional intervention, but the underlying mechanism needs further investigation among different ethnic groups and with larger samples.

Authors' contributions to manuscript The authors' responsibilities were as follows. LI Xiang performed the data analyses, and drafted the paper. GAN Zhen Wei, DING Zhen, and LIU Guo Liang aided with the fatty acids measurement. WU Yi Xia, CHEN Xue Yan, TIAN Hui Min, and YANG Ye Tong performed basic information questionnaire and dietary survey. XIE Lin conceived, designed and implemented the study. None of the authors reported a conflict of interest related to the study.

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