Genetic variants in the *FADS* gene cluster are associated with arachidonic acid concentrations of human breast milk at 1.5 and 6 mo postpartum and influence the course of milk dodecanoic, tetracosenoic, and *trans*-9-octadecenoic acid concentrations over the duration of lactation[1–4]

Eva Lattka, Peter Rzehak, Éva Szabó, Viktoria Jakobik, Melanie Weck, Maria Weyermann, Harald Grallert, Dietrich Rothenbacher, Joachim Heinrich, Hermann Brenner, Tamás Decsi, Thomas Illig, and Berthold Koletzko

ABSTRACT

Background: Breastfeeding is considered an optimal nutritional source of n−6 (omega-6) and n−3 (omega-3) fatty acids (FAs) for the proper visual and cognitive development of newborn children. In addition to maternal nutrition as an important regulator of FA concentrations, first results exist on an association of breast-milk FAs with single nucleotide polymorphisms (SNPs) in the *FADS* gene cluster, which encodes the rate-limiting enzymes in the elongation-desaturation pathway of long-chain polyunsaturated fatty acids (LC-PUFAs).

Objective: We analyzed the influence of *FADS* SNPs on breast-milk FA concentrations and their time course during lactation in the Ulm Birth Cohort study, which comprised 772 nursing mothers at 1.5 mo after giving birth, and in a subset of 463 mothers who were still breastfeeding at 6 mo postpartum.

Design: We conducted linear regression analysis of 8 *FADS* SNPs with FA concentrations at both time points separately and assessed the genotype effect over time in a longitudinal analysis by using a generalized estimating equation regression model.

Results: We observed significant associations of *FADS* genotypes with arachidonic acid (AA) concentrations and the 20:4n−6/20:3n−6 ratio at both time points but no association of *FADS* SNPs with the time course of AA concentrations. A longitudinal analysis of FAs other than LC-PUFAs by genotype over time showed associations for dodecanoic acid, *cis*-15-tetracosenoic acid, and *trans*-9-octadecenoic acid.

Conclusions: Maternal *FADS* genotypes are associated with breast-milk AA concentrations and might therefore influence the supply of this FA for children. Furthermore, our data indicate an interrelation between the LC-PUFA pathway and saturated and monounsaturated FAs. *Am J Clin Nutr* 2011;93:382–91.

INTRODUCTION

The supply of the newborn infant with n−6 and n−3 fatty acids by breastfeeding is considered highly beneficial for child health and development. Lipids in human milk are not only an important energy source for the infant but are also considered important for visual and cognitive development (1). Arachidonic acid (AA) and especially docosahexaenoic acid (DHA) are thought to be important long-chain polyunsaturated fatty acids (LC-PUFAs) for developmental processes. AA and DHA are essential membrane constituents, especially in the brain and retina, and AA serves as a precursor to prostaglandins and leukotrienes. In addition to effects on visual and cognitive development, there are also indications that early exposure to dietary LC-PUFAs protects individuals from high blood pressure and cardiovascular risk in later childhood (2), even though controversial data have emerged (3). Moreover, the fatty acid supply with breast milk has been associated with the development of atopic diseases in several studies (4–6).

[1] From the Helmholtz Zentrum München, German Research Center for Environmental Health, Institute of Epidemiology, Neuherberg, Germany (EL, PR, HG, JH, and TI); the Institute of Medical Informatics, Biometrics, and Epidemiology, Ludwig-Maximilians University Munich, Oberschleissheim, Germany (PR); the Department of Paediatrics, University of Pécs, Pécs, Hungary (PR, ÉS, VJ, and TD); the Division of Clinical Epidemiology and Aging Research, German Cancer Research Center, Heidelberg, Germany (M Weck, M Weyermann, and HB); and the Division of Metabolic and Nutritional Medicine, Dr von Hauner Children's Hospital, University of Munich Medical Centre, Munich, Germany.

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[4] Address correspondence to E Lattka, Helmholtz Zentrum München, German Research Center for Environmental Health (GmbH), Institute of Epidemiology, Ingolstaedter Landstrasse 1, 85764 Neuherberg, Germany. E-mail: eva.lattka@helmholtz-muenchen.de.

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The concentrations of long-chain n−6 and n−3 fatty acids in breast milk are highly dependent on the mother's dietary habits (7–9) and are similar to dietary effects on blood fatty acid concentrations (10–12). In addition to their dietary supply, LC-PUFAs can also be endogenously derived from the precursor essential fatty acids linoleic acid (18:2n−6) and α-linolenic acid (18:3n−3) by consecutive desaturation and chain elongation as originally described by Sprecher (13) and Sprecher et al (14). The rate-limiting enzymes in this reaction cascade are the δ-6 desaturase (D6D) and δ-5 desaturase (D5D). A detailed overview of the pathway was shown elsewhere (15, 16). The human desaturase-encoding genes (*FADS1* for D5D and *FADS2* for D6D) are arranged in a head-to-head orientation and build a gene cluster on chromosome 11 together with a third desaturase gene, *FADS3*, the function of which has not yet been revealed. In the past few years, numerous genetic association studies have shown that single nucleotide polymorphisms (SNPs) in the *FADS* gene cluster are associated with n−6 and n−3 fatty acid concentrations in serum, plasma, erythrocyte membranes, and adipose tissue (17–21). Carriers of the minor alleles of the significantly associated SNPs had enhanced concentrations of desaturase substrates and decreased concentrations of desaturase products, which led to the hypothesis that there was a decline in the transcriptional levels or conversion rates of desaturases in minor allele carriers. Associations of *FADS* polymorphisms with fatty acid concentrations in human breast milk have been investigated in 2 previous studies (22, 23). Both of these studies reported significant associations with various fatty acids; however, the study size was rather small in both cases. Also, these studies did not investigate how *FADS* genotypes influence the timely change of fatty acid concentrations over the duration of lactation.

The aim of the current study was to analyze the influence of *FADS* genotypes on breast-milk fatty acid concentrations in a substantially larger German birth cohort that comprised 772 mothers who were breastfeeding their children at 1.5 mo after birth. In addition, breast-milk fatty acid measurements from a subset of 463 nursing mothers at 6 mo postpartum were available. We initially investigated the effect of 8 SNPs in the *FADS* gene cluster on fatty acid concentrations at both time points separately. Because it is known that the concentrations of several fatty acids change during the duration of lactation (7, 24), we also analyzed whether the polymorphisms had an influence on the increase or decrease of fatty acid concentrations during lactation.

SUBJECTS AND METHODS

Study population

Women were recruited during their stay at the Department of Gynecology and Obstetrics at the University of Ulm after delivery of their infants between November 2000 and November 2001. To obtain a birth cohort of healthy and mature infants, exclusion criteria were delivery before 32 gestational weeks, birth weight <2500 g, and transfer to pediatric care immediately after delivery. Also, women with no understanding of the German, Turkish, or Russian language and all women who left the hospital immediately after birth were excluded. In total, 1066 families were included into this study. Participation was voluntary, and written informed consent was obtained in each case. Detailed information on characteristics of study subjects can be obtained elsewhere (25–28). The study was approved by the ethics boards of the University of Ulm and the physicians' boards of the states of Baden-Württemberg and Bavaria.

Data and sample collection

Standardized interviews in German, Turkish, or Russian were conducted by trained interviewers during the hospitalization of mothers after delivery. They included detailed questions about living and housing conditions, lifestyle factors, medical histories, and health status during pregnancy. Furthermore, anthropometric data before and during pregnancy were collected from the pregnancy health charts of mothers ("Mutterpass") by using a standardized form. All participating mothers were contacted 6 wk postpartum and asked if they were breastfeeding at that time. A total of 1024 (96%) mothers were successfully contacted again, and 786 (76.7%) mothers were still breastfeeding their infants. For the collection of milk samples, a trained nurse visited all women who were still breastfeeding and collected 10 mL manually expressed human milk from both breasts before feeding. In some cases, milk was collected with the help of a breast pump. Samples were immediately cooled and frozen at −80°C for ≤24 h. From 786 breastfeeding mothers, 769 (97.8%) milk samples were successfully collected. The women who were breastfeeding after 6 wk were contacted again at 6 mo postpartum and asked if they were still breastfeeding. Milk samples of 98% of mothers who were still breastfeeding were collected successfully by using the same procedure as previously described (n = 463).

Fatty acid analyses

Fatty acids were analyzed by using the procedure previously described (25). In brief, fatty acids were extracted from 100 μl milk, and fatty acid methyl esters were measured by high-resolution capillary gas-liquid chromatography with a 60-m cyanopropyl column and a flame ionization detector. The peak identification was confirmed by comparison with weighted standards. In total, 26 saturated, monounsaturated, n−3, n−6, and *trans* fatty acids with chain lengths between 10 and 24 carbons were measured and used for analyses.

SNP selection and genotyping

Genetic analysis of samples from study participants in an anonymous manner was approved by the ethics committee of the Bayerische Landesärztekammer (the Bavarian Board of Physicians). Ten tagging SNPs in the genomic region spanning *FADS1*, *FADS2*, and *FADS3* were selected by using the HapMap project homepage (http://www.hapmap.org), and 2 additional SNPs were selected based on results of a former association study (17). The genomic DNA of all mothers was extracted from 300 μl breast milk with a High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland) by using the protocol for DNA extraction from whole blood. A total of 5 μL DNA were subjected to polymerase chain reaction amplification followed by a genotyping procedure with the MassARRAY system and iPLEX chemistry as suggested by the manufacturer (Sequenom). The procedure was previously described in detail (18).

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Statistical analyses

Genotype frequencies, allele frequencies, and the Hardy-Weinberg equilibrium were calculated with the statistical software module SAS/Genetics (SAS version 9.1.3; SAS Institute Inc, Cary, NC) by using the proc allele procedure. Deviations from the Hardy-Weinberg equilibrium were tested by using Fisher's exact test. To examine the linkage disequilibrium, Lewontin's D' and pairwise squared correlations r^2 were calculated with the software JLIN (version 1.6.0) (29).

The normal distribution of fatty acids was tested by Kolmogorov-Smirnov tests and evaluated by box plots and quantile-quantile plots (by using the proc univariate procedure of SAS software, version 9.1.3; SAS Institute Inc). Several severely skewed fatty acids (18:3n−6, 22:4n−6, 20:5n−3, 22:6n−3, 22:0, 24:0, 22:1n−9, and 24:1n−9) were log transformed to better approximate the normal distribution for further analysis.

We conducted a linear regression analysis of each of the 8 *FADS* SNPs with each of the measured n−6, n−3, monounsaturated, saturated, and *trans* fatty acids as continuous outcome variables separately at both time points of lactation (1.5 and 6 mo). In addition, D6D and D5D desaturation indexes were calculated as 18:3n−6/18:2n−6 and 20:4n−6/20:3n−6, respectively. We applied an additive model where homozygous minor allele carriers were coded as 2, heterozygous subjects were coded as 1, and homozygous major allele carriers were coded as 0, with the assumption of a linear relation between the fatty acid outcomes and number of minor alleles. For correction for multiple testing, the number of effective loci was calculated with the spectral decomposition method software SNPSpD (Queensland Institute of Medical Research, Herston, Australia; http://genepi.qimr.edu.au/general/daleN/SNPSpd/). For the 8 analyzed SNPs, the number of effective loci was calculated as 6.8269, which resulted in a reduced significance threshold of 0.05/6.8269 = 0.0073. To additionally account for the number of fatty acids in each tested fatty acid group, the significance threshold required to keep the type I error rate at 5% was further reduced to 0.001 (which corresponded to 0.05/6.8269 × 8 analyzed n−6 fatty acids). This threshold was calculated for the group of n−6 fatty acids and was also applied for all other tested fatty acid groups. For longitudinal analysis of fatty acid concentrations by genotype over time between 1.5 and 6 mo of breastfeeding, a generalized estimating equation regression model was applied to account for the correlated data structure (30) in a complete case analysis (which included only those mothers who were breastfeeding at both time points).

RESULTS

General characteristics of the study sample are presented in **Table 1**. Generally, women had a mean age of 31.29 ± 4.76 y, a mean height of 166.59 ± 6.38 cm, and a mean prepregnancy body mass index (in kg/m^2) of 23.03 ±3.86. Most women had

TABLE 1
General characteristics of the sample[1]

	All mothers	Mothers not breastfeeding 6 mo postpartum	Mothers still breastfeeding 6 mo postpartum
No. of subjects	772	309	463
School education before graduation [*n* (%)]			
≥12 y	333 (43.13)	111 (35.92)	222 (47.95)
10 y	287 (37.18)	117 (37.86)	170 (36.72)
≤9 y	143 (18.52)	73 (23.62)	70 (15.12)
No graduation	6 (0.78)	5 (1.62)	1 (0.22)
Missing	3 (0.39)	3 (0.97)	0 (0.00)
Maternal smoking [*n* (%)]			
Ever smoked 100 cigarettes during lifetime			
Yes	343 (44.43)	148 (47.90)	195 (42.12)
No	428 (55.44)	160 (51.78)	268 (57.88)
Missing	1 (0.13)	1 (0.32)	0 (0.00)
During pregnancy			
Yes	65 (8.42)	38 (12.30)	27 (5.83)
No	707 (91.58)	271 (87.70)	436 (94.17)
Missing	0 (0.00)	0 (0.00)	0 (0.00)
1.5 mo postpartum			
Yes	53 (6.87)	34 (11.00)	19 (4.10)
No	716 (92.75)	275 (89.00)	441 (95.25)
Missing	3 (0.39)	0 (0.00)	3 (0.65)
6 mo postpartum			
Yes	67 (8.68)	44 (14.24)	23 (4.97)
No	671 (86.92)	232 (75.08)	439 (94.82)
Missing	34 (4.40)	33 (10.68)	1 (0.22)
Age (y)	31.29 ± 4.76[2]	30.18 ± 4.98	32.20 ± 4.44
Maternal height (cm)	166.59 ± 6.38	166.59 ± 6.41	166.59 ± 6.38
Maternal prepregnancy BMI (kg/m^2)	23.03 ± 3.86	23.41 ± 4.15	22.78 ± 3.65

[1] Number of subjects refers to all mothers for whom demographic data were available. Anthropometric data were available for 770 (age and height) and 746 (weight) mothers.
[2] Mean ± SD (all such values).

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a high education and were nonsmokers. When women were separated into those who were not breastfeeding after 6 mo postpartum and those who were still breastfeeding, women who were still breastfeeding after 6 mo were of higher age, had a higher education, lower body mass index, and less frequently smoked (ever smoked 100 cigarettes during their lifetime as well as during pregnancy and lactation).

Genotyping was successful for all 12 selected SNPs, except for rs174553, for which alleles could not be discriminated. The minor allele frequencies for the successfully genotyped SNPs ranged from 12% to 44% and matched those reported in the Single Nucleotide Polymorphism database (http://www.ncbi.nlm.nih.gov/projects/SNP/). The overall mean genotyping success rate was 92.1%. The distribution of genotypes of 8 SNPs was consistent with the Hardy-Weinberg equilibrium, whereas 3 SNPs showed a deviation from the Hardy-Weinberg equilibrium (rs174561, $P = 0.0042$; rs2072114, $P = 0.0039$; and rs174627, $P = 0.0179$) and, therefore, were excluded from further analysis. Eight SNPs were included in the final analysis, and the characteristics of these SNPs are listed in **Table 2**, including their position on chromosome 11, their location, and their genotype and allele frequencies. Genotype and allele frequencies did not differ between the group of women who were not breastfeeding at 6 mo postpartum and those women who were still breastfeeding at 6 mo postpartum.

Fatty acid concentrations and CVs at time points of 1.5 and 6 mo with fatty acids expressed as the percentage of weight divided by the weight of total fatty acids are shown in **Table 3**. Some fatty acids had higher CVs compared with others at the 1.5-mo time point (ie, 18:3n−6, 18:3n−6/18:2n−6, 20:5n−3, 22:5n−3, 22:6n−3, and 24:1n−9).

Association of *FADS* SNPs with n−6 and n−3 fatty acids in breast milk

Significant associations were observed for milk AA (20:4n−6) concentrations with SNPs rs174547 and rs174556 at 6 mo after birth ($P < 0.001$; **Table 4**). Before correction for multiple testing, both SNPs also showed significant associations with AA at the 1.5-mo time point ($P = 0.0031$ and 0.0025). Three additional SNPs (rs174626, rs1000778, and rs174455) were associated with AA concentrations at both investigated time points; however, this occurred without taking multiple testing into account ($P = 0.0022$–0.0090). For all associated SNPs, carriers of the minor alleles had lower concentrations of AA in breast milk compared with those of carriers of the major alleles. SNPs rs174602, rs498793, and rs526126 did not show significant associations, even without correction for multiple testing. When we looked at the 20:4n−6/20:3n−6 ratio, which was a measure of the D5D activity, associations remained essentially the same. Sensitivity analysis that excluded all potential outliers defined as the mean ± (1.5 × the interquartile range) confirmed the significant results obtained in the original analysis. Significant associations with other fatty acids were not observed, except for 22:4n−6, which showed an association with rs1000778 ($P = 0.0007$) 6 mo after birth; however, this association was not stable in the outlier sensitivity analysis. The ratio 18:3n−6/18:2n−6, which approximated the D6D activity, was not significant for any SNPs at either of the 2 time points. For n−3 fatty acids, no significant associations were observed after correction for multiple testing (*see* supplemental Table S1 under "Supplemental data" in the online issue).

Associations of *FADS* genotypes with saturated, monounsaturated, and *trans* fatty acids

Because Xie and Innis (22) reported an association of *FADS* polymorphisms with the saturated fatty acid 14:0 and the monounsaturated fatty acid 18:1n−7, we tested associations of the genotyped SNPs with all measured saturated fatty acids (10:0, 12:0, 14:0, 16:0, 18:0, 20:0, 22:0, and 24:0), monounsaturated fatty acids (16:1n−7, 18:1n−9, 22:1n−9, and 24:1n−9), and *trans* fatty acids (*t*-16:1, *t*-18:1n−9, and *tt*-18:2n−6) in our study.

After correction for multiple testing, no significant associations were observed for saturated, monounsaturated, and *trans* fatty acids (*see* supplemental Tables S2–S4 under "Supplemental data" in the online issue for summary of results of saturated, monounsaturated, and *trans* fatty acids).

TABLE 2
Characteristics of 8 analyzed polymorphisms in the *FADS* gene cluster region[1]

					No. of subjects at 1.5/6 mo with				
					Genotype[2]			Allele[2]	
dbSNP	Position (bp)	Gene	Alleles (major/minor) 1/2	No. at 1.5/6 mo	11	12	22	1	2
rs174547	61327359	*FADS1*	T/C	716/423	353/208 (49)	294/178 (41)	69/37 (10)	1000/594 (70)	432/252 (30)
rs174556	61337211	*FADS1*	C/T	714/424	393/236 (55)	270/161 (38)	51/27 (7)	1056/633 (74)	372/215 (26)
rs174602	61380990	*FADS2*	A/G	714/423	463/276 (65)	214/127 (30)	37/20 (5)	1140/679 (80)	288/167 (20)
rs498793	61381281	*FADS2*	G/A	701/415	253/146 (36)	334/203 (48)	114/66 (16)	840/495 (60)	562/335 (40)
rs526126	61381461	*FADS2*	C/G	718/421	482/279 (67)	213/129 (30)	23/13 (3)	1177/687 (82)	259/155 (18)
rs174626	61393633	Intergenic *FADS2/3*	T/C	710/419	234/137 (33)	326/195 (46)	150/87 (21)	794/469 (56)	626/369 (44)
rs1000778	61411881	*FADS3*	G/A	714/425	406/244 (57)	261/157 (37)	47/24(7)	1073/645 (75)	355/205 (25)
rs174455	61412693	*FADS3*	A/G	711/417	311/182 (44)	302/184 (42)	98/51 (14)	924/548 (65)	498/235 (35)

[1] dbSNP, Single Nucleotide Polymorphism database (http://www.ncbi.nlm.nih.gov/projects/SNP/); bp, base pairs.
[2] Numbers in parentheses indicate genotype or allele frequencies at 1.5 mo (%).

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TABLE 3
Raw fatty acid (FA) concentrations in breast milk of mothers at 1.5 and 6 mo postpartum[1]

	1.5 mo (n = 769)		6 mo (n = 463)	
	Mean ± SD	CV	Mean ± SD	CV
n−6 FA				
18:2n−6	11.02 ± 4.06	36.85	11.65 ± 4.14	35.59
18:3n−6	0.17 ± 0.29	172.02	0.18 ± 0.10	55.83
20:2n−6	0.23 ± 0.11	45.54	0.25 ± 0.09	33.86
20:3n−6	0.33 ± 0.21	63.01	0.33 ± 0.17	52.55
20:4n−6	0.44 ± 0.24	52.92	0.54 ± 0.29	54.63
22:4n−6	0.08 ± 0.08	96.62	0.13 ± 0.10	75.65
18:3n−6/18:2n−6	0.02 ± 0.03	203.63	0.02 ± 0.01	58.49
20:4n−6/20:3n−6	1.46 ± 0.80	54.69	1.76 ± 1.02	58.01
n−3 FA				
18:3n−3	0.79 ± 0.46	58.21	0.89 ± 0.45	50.49
20:3n−3	0.05 ± 0.04	81.90	0.06 ± 0.04	67.05
20:5n−3	0.06 ± 0.07	114.81	0.08 ± 0.06	74.88
22:5n−3	0.15 ± 0.19	127.85	0.20 ± 0.12	62.88
22:6n−3	0.22 ± 0.23	107.76	0.25 ± 0.16	64.91
Saturated FA				
10:0	2.23 ± 0.86	38.50	2.16 ± 1.21	56.02
12:0	6.61 ± 2.35	35.56	6.89 ± 2.18	31.64
14:0	7.19 ± 2.02	28.10	7.49 ± 2.06	27.50
16:0	22.38 ± 2.94	13.13	23.26 ± 2.98	12.80
18:0	8.15 ± 2.25	27.58	8.37 ± 2.14	25.56
20:0	0.28 ± 0.13	45.29	0.26 ± 0.09	35.12
22:0	0.16 ± 0.11	68.19	0.16 ± 0.09	54.35
24:0	0.16 ± 0.16	97.47	0.12 ± 0.08	70.73
Monounsaturated FA				
16:1n-7	2.72 ± 0.90	33.14	2.58 ± 0.68	26.56
18:1n−9	30.89 ± 3.84	12.42	29.74 ± 3.83	12.88
22:1n−9	0.07 ± 0.05	76.95	0.10 ± 0.11	108.57
24:1n−9	0.10 ± 0.11	113.44	0.09 ± 0.06	60.22
trans FA				
t-16:1	0.43 ± 0.23	53.17	0.42 ± 0.23	54.26
t-18:1n−9	1.16 ± 0.94	81.26	0.95 ± 0.90	94.49
tt-18:2n−6	0.40 ± 0.35	88.59	0.29 ± 0.14	48.26

[1] Values are presented as the percentage of weight divided by the weight of total FAs.

Association of SNPs with the timely change in fatty acid concentrations during lactation

Milk contents of fat and of most fatty acids change during lactation (7, 24). Therefore, we asked whether *FADS* genotypes influence the change in fatty acid concentrations from 1.5 to 6 mo of lactation and investigated the interaction between SNPs and the time effect on fatty acid concentrations. We used a complete case design that included only those mothers who were breastfeeding at both investigated time points to avoid problems of a generalized estimating equation regression models with missing data.

FADS SNPs were not associated with the timely change in AA concentrations during lactation

With the use of a longitudinal model, we examined whether *FADS* genotypes modulated the change of AA concentrations over time in women who were breastfeeding at both investigated time points. There was no significant association of any of the tested SNPs with AA concentrations or the ratio (20:4n−6/20:3n−6) that estimated the D5D activity over time accounting for multiple testing.

Longitudinal analysis indicated a role of *FADS* polymorphisms in the regulation of monounsaturated, saturated, and *trans* fatty acid concentrations

In addition to the longitudinal analysis for AA, we also analyzed the time course of all other measured fatty acids dependent on the *FADS* genotypes in women who were breastfeeding at both investigated time points. Although we did not observe any significant associations below the significance threshold of 0.001, we observed some significant associations before correction for multiple testing. We observed time-genotype interactions for 12:0 (dodecanoic acid) and SNP rs174626 (P for interaction = 0.0186) with a difference in dodecanoic acid concentrations between the 3 genotype groups only at the 6-mo time point (**Figure 1**). Homozygous carriers of the minor allele exhibited a remarkable increase in dodecanoic acid concentrations over the duration of breastfeeding. This effect was also visible in heterozygous subjects, although it was less pronounced. The dodecanoic acid concentrations in homozygous carriers of the major allele remained rather stable during the lactation period. A similar effect was observed for 14:0 (tetradecanoic acid) and the same SNP (P for interaction = 0.0287) as well as for the association between SNP rs526126 and the timely

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TABLE 4
Results of linear regression analysis of 8 *FADS* single nucleotide polymorphisms with n−6 fatty acid concentrations and ratios that estimated desaturase activity in human breast milk after 1.5 and 6 mo of lactation[1]

	18:2 n−6		log 18:3 n−6		20:2 n−6		20:3 n−6		20:4 n−6		log 22:4 n−6		log 18:3 n−6/18:2 n−6		20:4 n−6/20:3 n−6	
	1.5 mo	6 mo	1.5 mo	6 mo	1.5 mo	6 mo	1.5 mo	6 mo	1.5 mo	6 mo	1.5 mo	6 mo	1.5 mo	6 mo	1.5 mo	6 mo
rs174547																
Intercept	11.0726	11.6758	−2.2459	−1.8670	0.2293	0.2566	0.3308	0.3369	0.4709	0.5966	−3.0741	−2.2208	−4.5820	−4.2717	1.5756	1.9080
$\beta \pm$ SE	−0.0559 ± 0.2342	0.1145 ± 0.3193	−0.0274 ± 0.05314	−0.0520 ± 0.0460	0.0053 ± 0.00605	−0.0035 ± 0.0065	0.0029 ± 0.0111	−0.0050 ± 0.0136	−0.0391 ± 0.0132	−0.0838 ± 0.0226	0.0767 ± 0.0653	−0.1149 ± 0.0539	−0.0266 ± 0.0538	−0.0595 ± 0.0484	−0.1682 ± 0.0459	−0.2168 ± 0.0791
P	0.8116	0.7201	0.6060	0.2589	0.3836	0.5903	0.7914	0.7150	0.0031	0.0002[2]	0.2405	0.0334	0.6206	0.2196	0.0003[2]	0.0064
n	713	423	713	423	713	423	713	423	713	423	713	423	713	423	713	423
rs174556																
Intercept	11.1261	11.7266	−2.2365	−1.9180	0.2301	0.2554	0.3361	0.3310	0.4711	0.5893	−3.0494	−2.2477	−4.5800	−4.3276	1.5459	1.9382
$\beta \pm$ SE	−0.1029 ± 0.2454	−0.1479 ± 0.3185	−0.0408 ± 0.0565	0.0238 ± 0.0489	0.0020 ± 0.0061	−0.0039 ± 0.0067	0.0020 ± 0.0127	0.0062 ± 0.0141	−0.0424 ± 0.0140	−0.0808 ± 0.0237	0.0470 ± 0.0689	−0.1214 ± 0.0562	−0.0333 ± 0.0571	0.0335 ± 0.0510	−0.1430 ± 0.0485	−0.3127 ± 0.0821
P	0.6752	0.6425	0.4699	0.6266	0.7445	0.5608	0.8751	0.6603	0.0025	0.0007[2]	0.4949	0.0312	0.5595	0.5109	0.0033	0.0002[2]
n	711	424	711	424	711	424	711	424	711	424	711	424	711	424	711	424
rs174602																
Intercept	10.7271	11.5937	−2.2228	−1.8648	0.2242	0.2547	0.3350	0.3320	0.4508	0.5661	−3.0672	−2.2634	−4.5293	−4.2655	1.4893	1.8202
$\beta \pm$ SE	0.6490 ± 0.2548	0.1749 ± 0.3399	−0.0447 ± 0.0598	−0.1022 ± 0.0502	0.0125 ± 0.0064	−0.0034 ± 0.0073	0.0033 ± 0.0137	0.0030 ± 0.0151	−0.0130 ± 0.0151	−0.0496 ± 0.0255	0.0713 ± 0.0737	−0.1295 ± 0.0600	−0.1100 ± 0.0601	−0.1144 ± 0.0531	−0.0628 ± 0.0522	−0.0999 ± 0.0889
P	0.0111	0.6072	0.4549	0.0423	0.0495	0.6361	0.8065	0.8439	0.3900	0.0528	0.3336	0.0314	0.0676	0.0317	0.2293	0.2614
n	711	423	711	423	711	423	711	423	711	423	711	423	711	423	711	423
rs498793																
Intercept	11.1477	11.6647	−2.3002	−1.8739	0.2319	0.2517	0.3304	0.3346	0.4325	0.5176	−3.0070	−2.3044	−4.6439	−4.2840	1.4182	1.6705
$\beta \pm$ SE	−0.1205 ± 0.2224	0.0399 ± 0.2883	0.0440 ± 0.0509	−0.0248 ± 0.0439	−0.0022 ± 0.0055	0.0039 ± 0.0062	0.0005 ± 0.0107	−0.0002 ± 0.0129	0.0152 ± 0.0127	0.0359 ± 0.0217	−0.0549 ± 0.0628	0.0123 ± 0.0512	0.0509 ± 0.0515	−0.0232 ± 0.0459	0.0650 ± 0.0447	0.1298 ± 0.0757
P	0.5881	0.8900	0.3874	0.5721	0.6941	0.5271	0.9631	0.9855	0.2330	0.0990	0.3830	0.8101	0.3238	0.6124	0.1463	0.0868
n	699	415	699	415	699	415	699	415	699	415	699	415	699	415	699	415
rs526126																
Intercept	11.1388	11.9742	−2.2721	−1.9055	0.2330	0.2562	0.3366	0.3327	0.4501	0.5629	−3.0409	−2.3125	−4.6146	−4.3352	1.4994	1.7916
$\beta \pm$ SE	−0.3531 ± 0.2768	−0.7557 ± 0.3604	0.0529 ± 0.0647	0.0011 ± 0.0538	−0.0091 ± 0.0069	−0.0047 ± 0.0077	0.0026 ± 0.0148	0.0069 ± 0.0161	−0.0091 ± 0.0162	−0.0382 ± 0.0273	0.0260 ± 0.0796	−0.0083 ± 0.0644	0.0747 ± 0.0655	0.0580 ± 0.0570	−0.0990 ± 0.0556	−0.0353 ± 0.0952
P	0.2026	0.0366	0.4135	0.9841	0.1865	0.5467	0.8584	0.6672	0.5737	0.1621	0.7438	0.8978	0.2547	0.3097	0.0751	0.7106
n	715	421	715	421	715	421	715	421	715	421	715	421	715	421	715	421
rs174626																
Intercept	10.9464	11.7751	−2.2119	−1.8793	0.2308	0.2597	0.3433	0.3349	0.4753	0.6000	−3.0177	−2.2295	−4.5429	−4.2991	1.5830	1.9351
$\beta \pm$ SE	0.0350 ± 0.2067	−0.1573 ± 0.2707	−0.0493 ± 0.0488	−0.0265 ± 0.0410	−0.0018 ± 0.0052	−0.0060 ± 0.0058	−0.0084 ± 0.0111	−0.0009 ± 0.0122	−0.0345 ± 0.0122	−0.0583 ± 0.0205	−0.0312 ± 0.0597	−0.0890 ± 0.0478	−0.0507 ± 0.0491	−0.0085 ± 0.0432	−0.1343 ± 0.0421	−0.1712 ± 0.0714
P	0.8656	0.5614	0.3132	0.5182	0.7345	0.3057	0.4489	0.9436	0.0047	0.0047	0.6015	0.0634	0.3019	0.8446	0.0015	0.0168
n	707	419	707	419	707	419	707	419	707	419	707	419	707	419	707	419
rs1000778																
Intercept	11.1477	11.8562	−2.2227	−1.8621	0.2346	0.2551	0.3376	0.3313	0.4674	0.5832	−3.0174	−2.2189	−4.5695	−4.2789	1.5276	1.9089
$\beta \pm$ SE	−0.0984 ± 0.2512	−0.2635 ± 0.3385	−0.0615 ± 0.0572	−0.0735 ± 0.0484	−0.0058 ± 0.0064	−0.0045 ± 0.0068	−0.0018 ± 0.0130	0.0030 ± 0.0143	−0.0378 ± 0.0143	−0.0745 ± 0.0242	−0.0240 ± 0.0697	−0.1951 ± 0.0569	−0.0496 ± 0.0580	−0.0568 ± 0.0514	−0.1159 ± 0.0494	−0.2649 ± 0.0839
P	0.6954	0.4367	0.2827	0.1294	0.3649	0.5093	0.8920	0.8347	0.0086	0.0022	0.7313	0.0007	0.3933	0.2698	0.0193	0.0017
n	711	425	711	425	711	425	711	425	711	425	711	425	711	425	711	425
rs174455																
Intercept	10.9748	11.7296	−2.2338	−1.8838	0.2282	0.2513	0.3399	0.3321	0.4700	0.5924	−3.0700	−2.2658	−4.5676	−4.2947	1.5548	1.9349
$\beta \pm$ SE	0.1078 ± 0.2202	−0.0590 ± 0.2917	−0.0297 ± 0.0506	−0.0197 ± 0.0430	0.0039 ± 0.0055	0.0049 ± 0.0062	−0.0031 ± 0.0115	0.0043 ± 0.0130	−0.0330 ± 0.0126	−0.0635 ± 0.0218	0.0473 ± 0.0620	−0.0602 ± 0.0523	−0.0353 ± 0.0511	−0.0155 ± 0.0457	−0.1328 ± 0.0434	−0.2243 ± 0.0760
P	0.6245	0.8397	0.5580	0.6471	0.4802	0.4361	0.7845	0.7388	0.0090	0.0038	0.4453	0.2504	0.4902	0.7352	0.0023	0.0033
n	708	417	708	417	708	417	708	417	708	417	708	417	708	417	708	417

[1] Intercept, mean fatty acid concentration in homozygous carriers of the major allele; β, change in the fatty acid concentration with each minor allele copy; *n*, number of subjects used in analysis. Uncorrected *P* values are shown.

[2] $P < 0.001$ was significant with correction for multiple testing.

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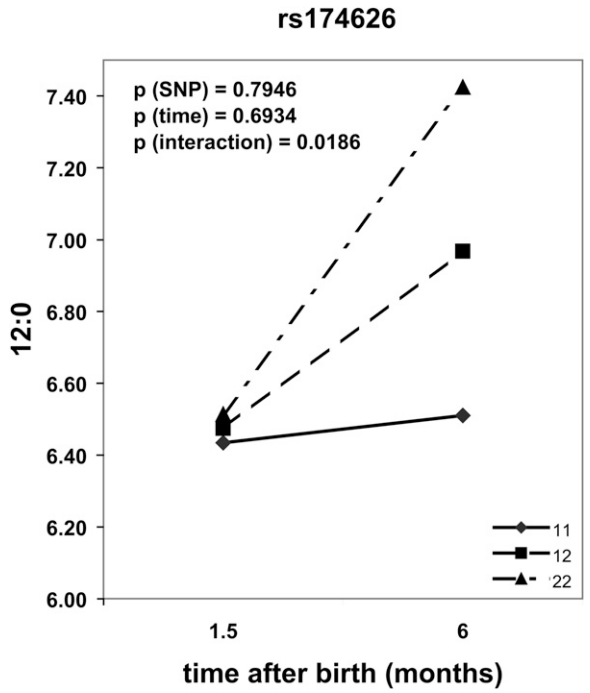

FIGURE 1. Longitudinal association analysis of single nucleotide polymorphism (SNP) rs174626 with dodecanoic acid (12:0) concentration by using a generalized estimating equation regression model. Results were based on 418 subjects. β-Coefficients (±SEs) were as follows: 0.0399 ± 0.1532 for SNP, 0.0762 ± 0.1931 for time, and 0.4172 ± 0.1751 for interaction. 11, homozygous major allele; 12, heterozygous; 22, homozygous minor allele.

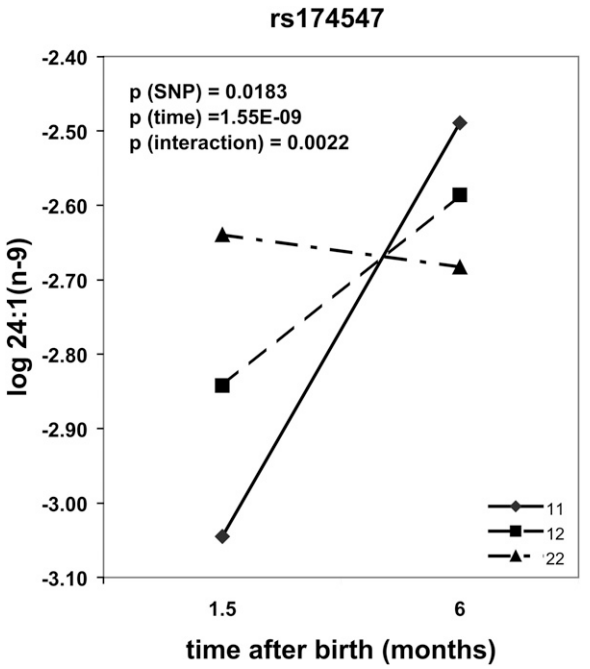

FIGURE 2. Longitudinal association analysis of single nucleotide polymorphism (SNP) rs174547 with log-transformed *cis*-15-tetracosenoic acid (log 24:1n−9) concentration by using a generalized estimating equation regression model. Results are based on 422 subjects. β-Coefficients (±SEs) were as follows: 0.2026 ± 0.0837 for SNP, 0.5555 ± 0.0850 for time, and −0.2994 ± 0.0946 for interaction. 11, homozygous major allele; 12, heterozygous; 22, homozygous minor allele.

change of 16:0 (hexadecanoic acid) concentrations (P for interaction = 0.0194).

Another interaction between time and genotype was observed for *cis*-15-tetracosenoic acid (24:1n−9) and SNP rs174547 (P for interaction = 0.0022). Carriers of the major allele showed an increase of this fatty acid over the lactation duration, whereas the concentrations in homozygous carriers of the minor allele were stable (**Figure 2**). Similar effects were observed for SNP rs174556 (P = 0.0059), which was in a high linkage disequilibrium with rs174547, and for SNPs rs174626 (P = 0.0455) and rs174455 (P = 0.0059).

The third fatty acid that showed a genotype-dependent change over time was *trans*-9-octadecenoic acid (*t*-18:1n−9) (P for interaction = 0.0032). Only carriers of the major allele of SNP rs174455 showed a remarkable decrease of this fatty acid over the breastfeeding period (**Figure 3**). A similar trend was observed for SNP rs174626; however, the P value for the interaction was not significant (P for interaction = 0.0835).

All reported associations remained significant before correction in a sensitivity analysis that excluded all potential outliers [defined as the mean ± (1.5 × the interquartile range)].

DISCUSSION

In the current study, we analyzed the effect of 8 SNPs in the *FADS* gene cluster on breast-milk fatty acids concentrations of breastfeeding women after 1.5 and 6 mo of lactation in a birth cohort that was larger than in previous studies. *FADS* genotypes were consistently associated with breast-milk AA concentrations, but not with other n−6 or n−3 fatty acids. The time course of AA concentrations during lactation was independent of the *FADS* genotype. Furthermore, our results suggested a relation between the n−6/n−3 fatty acid pathway and concentrations and time course of saturated, monounsaturated, and *trans* fatty acids.

In contrast to previous reports (22, 23), we showed no associations with n−6 or n−3 fatty acids except for an association with AA, which was significant at both investigated time points. There are several potential reasons for this discrepancy between the results of our study and the 2 previous studies. Breast-milk samples in the previous studies were collected 1 mo postpartum, whereas our first time point of collection was 1.5 mo after birth. The biggest changes in milk fatty acid concentrations occur during the first month of breastfeeding (31, 32), and possibly the genetic effect on breast-milk composition is more pronounced during this early stage of lactation. Also, the maternal dietary fatty acid intake is known to affect the fatty acid composition of breast milk (33, 34), which might modulate the strength of the genetic effect on milk fatty acid concentrations. In a recent study, associations between *FADS* genotypes and cholesterol concentrations were only observed in subjects with high intakes of n−3 LC-PUFAs, whereas this effect was not present in the low-intake group (35). In the study of Moltó-Puigmartí et al (23), the difference in breast-milk DHA concentrations between genotype groups was more pronounced in people with a higher number of fatty fish portions per week. In our study, we investigated a German study population from the area of Ulm in south Germany where, typically, a relatively low amount of sea fish is consumed, whereas in the 2 previous studies, Canadian (22) and Dutch (23) populations were investigated, which were

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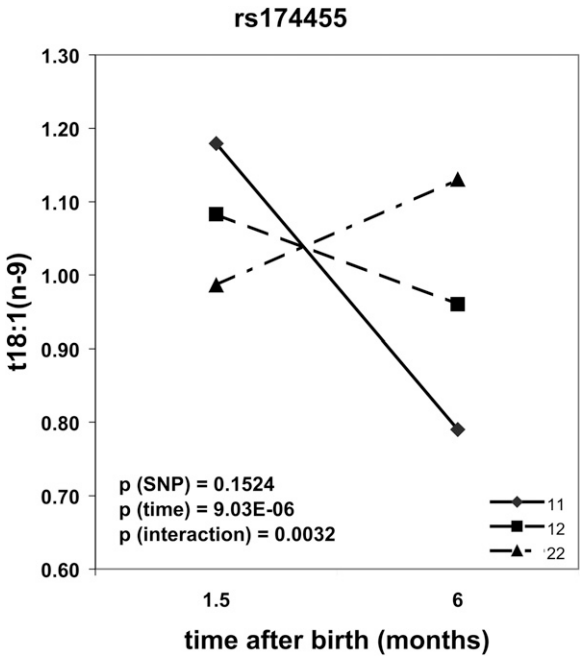

FIGURE 3. Longitudinal association analysis of single nucleotide polymorphism (SNP) rs174455 with *trans*-9-octadecenoic acid (t18:1n−9) concentration by using a generalized estimating equation regression model. Results are based on 416 subjects. β-Coefficients (±SEs) were as follows: −0.0965 ± 0.0668 for SNP, −0.3892 ± 0.0844 for time, and 0.2666 ± 0.0873 for interaction. 11, homozygous major allele; 12, heterozygous; 22, homozygous minor allele.

likely to differ in their dietary habits, especially regarding fish consumption. Furthermore, the lack of an association with n−3 fatty acids might have been caused by more imprecise measurements leading to higher CVs (Table 3) of the quite low abundant longer-chain n−3 fatty acids such as 22:6n−3 compared with the more abundant n−6 fatty acids. Furthermore, we used a more conservative statistical approach by correcting our *P* values for multiple testing to reduce the number of significant results obtained by chance. Such a correction was not reported by the 2 previous studies.

To our knowledge, one of the strengths and novelties of our study is the availability of fatty acid data at 2 time points of lactation (1.5 and 6 mo). Therefore, we conducted a longitudinal analysis to detect differences in time courses of fatty acid concentrations dependent on the *FADS* genotype. The time course of AA concentrations was independent of the *FADS* genotype in our complete case approach that comprised 463 mothers who were breastfeeding at both investigated time points. However, AA concentrations were markedly higher in carriers of the major alleles at both investigated time points compared with in carriers of the minor alleles, which suggested lower D5D expression rates or enzyme activity in minor allele carriers. It is not clear whether this was attributed to an altered synthesis rate in the mammary gland itself or whether it was due to a lower D5D activity in other tissues such as the liver and, consequently, diminished the import into the mammary gland. In several tracer studies in humans and animals, it was suggested that the mammary gland plays an important role in the synthesis of LC-PUFA itself (33, 36). In addition, it is known that the mammary gland expresses D6D and D5D (37, 38). Further studies are needed to understand the role of the mammary gland in fatty acid synthesis and the influence of the *FADS* genotype. Moreover, whether the different AA concentrations of the 3 genotype groups had any influence on the breast-fed infant could not be inferred from this study and needs further investigation.

To our knowledge, a function of the D6D and D5D in the biosynthesis of saturated and monounsaturated fatty acids has not been reported. Therefore, the reason for the association of *FADS* genotypes with the longitudinal change of dodecanoic, *cis*-15-tetracosenoic, and *trans*-9-octadecenoic acid concentrations, was not immediately apparent. It has been shown that polyunsaturated fatty acids are able to regulate pathways involved in lipid, energy, and carbohydrate metabolism by modifying gene expression in different tissues through binding to nuclear receptors such as peroxisome proliferator–activated receptor α (39, 40). In addition, in tracer studies in nonhuman primates, it was previously shown that n−6 and n−3 fatty acids could be oxidized, and their carbons could be recycled to saturated and monounsaturated fatty acids, which were detected in milk and other tissues of the animals (41). The authors argued that pregnant and lactating nonhuman primates use excess LC-PUFA from the diet for energy production and storage of saturated and monounsaturated fatty acids for later use. However, the mechanism that caused the apparent relation between n−6 or n−3 LC-PUFAs and saturated and monounsaturated fatty acids remained unclear, and our findings need to be replicated. The association of SNP rs174455 with *trans*-9-octadecenoic acid over time with major allele carriers that showed a decrease of this fatty acid in contrast to homozygous minor allele carriers was not less surprising because the source of *t*-18:1n−9 was exclusively nutritional. It was recently shown that concentrations of *trans* fatty acids decrease during the duration of lactation, possibly because of a decreased maternal dietary intake of *trans* fatty acids during the lactation period, and the concentration of *trans* fatty acids was inversely related to AA and other LC-PUFA concentrations (24, 25). We saw a decrease of *t*-18:n−9 concentrations only in carriers of the major allele of rs174455, which might suggest a differential eating behavior dependent on the genotype. Further replication including dietary data are needed.

To our knowledge, this is the largest study on *FADS* genotypes and breast-milk fatty acid concentrations [n = 772 at 1.5 mo and n = 463 at 6 mo compared with n = 54 in the study by Xie and Innis (22) and n = 309 in the study by Moltó-Puigmartí et al (23)] and the only one that additionally genotyped SNPs in *FADS3*. The biological function of the protein product of *FADS3* has not been completely clarified, but because of high a homology between all 3 *FADS* genes, a function in the desaturation pathway has been suggested. In the current study, SNPs in *FADS3* showed significant associations with AA before correction, which corroborated a functional role of *FADS3* in the fatty acid desaturation. In contrast to the 2 previous studies that analyzed the breast-milk fatty acid composition at one single time point only, we measured fatty acid concentrations at 1.5 and 6 mo of lactation and performed a longitudinal analysis of fatty acid concentrations dependent on the genotype.

Although, compared with previous studies, the availability of fatty acid data at 2 different time points was a clear strength of this study, it would be desirable to include even more time points to study the exact time course of fatty acid concentrations during

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lactation, which might not be linear as assumed in our study. The longitudinal analysis included only those women who were breastfeeding at both investigated time points, and the observed associations might have been specific for this special group of women. Whether the associations can also be observed in women breastfeeding, eg, until the fourth month postpartum requires additional studies. Moreover, more subjects might be required for a longitudinal analysis to not lose too much power because of the problem of missing cases. Another limitation of our study was the lack of nutritional data to test the interaction between genes and diet on the course of fatty acid concentrations in human breast milk, which might be a task in a future study. Because fatty acid data were expressed as a percentage of total fatty acids, one might assume that the percentage change of low abundant fatty acids was highly influenced by changes in high-abundant fatty acids such as 18:2n−6. However, if the contribution of major fatty acids markedly increased by 50%, from 10% of total fatty acids to 15% of total fatty acids, the relative contribution of all other fatty acids would be expected to be equally lowered by less than a relative 5% (eg, from 0.1% to 0.095% of total fatty acids). This means that a minor change of a low-abundant fatty acid would require a very high change of a high-abundant fatty acid, which makes the assumption that low-abundant fatty acids are very much influenced by major fatty acids unlikely.

In conclusion, we showed the clear influence of *FADS* polymorphisms on breast-milk AA concentrations at 1.5 and 6 mo postpartum. The time course of AA concentrations during lactation was not influenced by the *FADS* genotype. The effect of *FADS* polymorphisms on saturated, monounsaturated, and *trans* fatty acid concentrations in human breast milk awaits further investigation.

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The authors' responsibilities were as follows—EL: wrote the first version of the manuscript and was responsible for the design, writing, and final content; M Weck, M Weyermann, DR, and HB: initialized and conducted the Ulm Birth Cohort Study; ÉS, VJ, and TD: performed DNA extraction and fatty acid analyses; EL, HG, and TI: performed genetic analyses; EL, PR, and HG: analyzed data; PR, JH, TI, and BK: contributed to the interpretation of results; and all authors: contributed to the writing of the manuscript and read and approved the final manuscript. None of the authors had a conflict of interest.

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