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GENETIC VARIANTS OF THE FADS GENE CLUSTER ARE ASSOCIATED WITH ERYTHROCYTE MEMBRANE LC PUFA LEVELS IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT

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Abstract: *Background:* Long-chain (> 20 C-atoms) polyunsaturated fatty acids (LC PUFAs) of both the omega-6 (n-6) and omega-3 (n-3) series are important for the functional integrity of brain and thereby cognition, memory and mood. Clinical studies observed associations between altered LC PUFA levels and neurodegenerative diseases such as Alzheimer´s disease and its prodromal stage, mild cognitive impairment (MCI). *Methods:* The present study examined the LC PUFA status of MCI patients with specific view on the relative LC n-3 PUFA levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in erythrocyte membranes (omega-3 index). 12 single nucleotide polymorphisms (SNPs) of the FADS1, FADS2, and FADS3 gene clusters were genotyped in 111 MCI patients and evaluated associations with PUFA levels in erythrocyte membranes (primary outcome). In addition, the associations between FADS SNPs and LC PUFA levels with serum lipid levels as well as depressive symptoms were examined (secondary outcomes). *Results:* Minor allele carrier of rs174546, rs174548 (FADS1), rs3834458, rs1535, rs174574, rs174575, rs174576, and rs174578 (FADS2) showed significant higher n-6 and n-3 precursor PUFA levels (linoleic acid, and alpha-linolenic acid, respectively) and lower arachidonic acid (AA) levels in erythrocyte membranes compared to the major allele carriers. Differences in EPA and DHA levels were not significant. Minor allele carriers of rs174574, rs174576 and rs174578 (FADS2) and rs174455 (FADS3) exhibited significant higher triglyceride levels, whereas minor allele carriers for rs174449 and rs174455 (FADS3) exhibited significant higher total- and LDL-cholesterol levels compared to the more common variant. The mean omega-3 index of the study cohort was 6.19 ± 1.55 %. In more than 85 % of the patients, the omega-3 index was below 8 % and in 23 % below 5 %. Moreover, it was shown that a low DHA status and omega-3 index was associated with depressive symptoms (Beck's depression-inventory). *Discussion and conclusion:* These findings indicate an association between several FADS genotypes for higher n-6 and n-3 precursor PUFA and lower AA levels in erythrocyte membranes in minor compared to major allele carriers. To what extent FADS genotypes and a lower conversion of LA and ALA to biologically important LC PUFAs such as AA, EPA and DHA contributes to cognitive decline should be investigated in further trials. Nevertheless, the omega-3 index in this cohort of MCI patients can be classified as insufficient.

Key words: FADS genotype, long-chain polyunsaturated fatty acids, polymorphism, mild cognitive impairment.

Introduction

The prevalence and incidence of Alzheimer´s disease (AD) and its prodromal stage, mild cognitive impairment (MCI) is rapidly rising in parallel to the extended life expectancy worldwide (1). Risk factors for AD are older age, genetic susceptibility (i.a. APOE ε4), cardiovascular disease (CVD) and vascular risk factors (cigarette smoking, high blood pressure, obesity, diabetes, and cerebrovascular lesions) (2). Long-chain (> 20 C-atoms) polyunsaturated fatty acids (LC PUFAs) of both the omega-6 (n-6) and omega-3 (n-3) series play a pivotal role in neuronal membrane integrity and function, and together with their metabolites regulate several processes within the brain (i.e. neurotransmission, cell survival and neuroinflammation), and thereby mood and cognition (3). The LC n-3 PUFAs eicosapentaenoic acid (C20:5n-3 [EPA]) and docosahexaenoic acid (C22:6n-3 [DHA]) are widely acknowledged to have neuroprotective and cognitive-enhancing effects due to their modulating effects on synaptic plasticity and neuroinflammation (4). Likewise, EPA, DHA, and the LC n-6 PUFA arachidonic acid (C20:4 [AA]) also affect cardiovascular health and inflammation (5). Epidemiology studies observed that neurological disorders such as AD and MCI are associated with altered LC PUFA levels (5, 6). For example, it has been shown that cognitive impairment with aging is associated with decreased DHA plasma levels (7, 8) and that MCI patients exhibit lower EPA levels in erythrocyte membranes as well as higher LC n-6 PUFA (i.a. AA; dihomo-gamma-linolenic acid, C20:3 [DGLA]; docosapentaenoic acid, C22:5n-6 [DPA]) levels compared to healthy controls (9). To improve the LC n-3 PUFA status and memory function in older adults, several RCTs with LC n-3 PUFA supplementation have been taken out (10,

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11) or are on-going (12).

The LC PUFA status in the body is dependent on the direct intake of these FAs via the diet. While the intake of AA via meat and meat products in western countries such as Germany is generally high, the intake of EPA and DHA is low due to low intake of fish, which is the main source for LC n-3 PUFAs (13). Beside the diet, the endogenous status of LC PUFAs is greatly influenced by genetic heritability (14). The precursor FA alpha linolenic acid (C18:3n-3 [ALA]) can be enzymatically converted to the physiologically more active LC n-3 PUFAs EPA and DHA via elongation and desaturation (14). The same applies for the n-6 precursor FA linoleic acid (C18:2n-6 [LA]) which can be converted to AA. In a typical western diet, the dietary supply of LA via several vegetable oils (i.e. soy oil, rapeseed oil) is high, while intake of ALA is low since it is only contained in a few foodstuffs (i.e. linseed oil, chia oil, walnuts) (13). Moreover, both precursor FA compete for the same enzymes in the conversion process. Consequently, the ratio between n-3/n-6 is very high in favour of the n-6 PUFAs. The rate-limiting enzymes of the conversion process are delta-5 (Δ-5) and delta-6 (Δ-6) desaturases encoded by respectively FADS1 and FADS2 located on the desaturase gene cluster on chromosome 11 (11q12–13.1) (15–17). This cluster also includes the FADS3 gene revealing 62 and 70 % nucleotide sequence identity with FADS1 and FADS2, respectively, as well as a cytochrome b5-like domain and a multiple membrane-spanning desaturase region. It is assumed that FADS3 encodes for another desaturase, which is yet unidentified (15).

Several studies reported an association between single nucleotide polymorphisms (SNPs) in the FADS gene clusters and the PUFA status (reviewed in (14)). Furthermore, SNPs in FADS are associated with blood lipid levels (18), glucose levels (19) as well as allergic diseases (20) and CVD (21). Whereas genotypes in FADS gene clusters have been associated with intelligence (22) or attention deficit hyperactivity disorder (23), studies on their impact on brain integrity and related cognitive functions in older people with cognitive decline are lacking.

The present study aimed to close knowledge gaps in the relationship between FADS genotypes and the endogenous LC PUFA status in older adults with MCI. Thus, we evaluated the association of SNPs in the FADS1, FADS2 and FADS3 genes with the relative erythrocyte membrane LC PUFA levels (primary outcome) in a cohort of MCI patients. It is hypothesized that minor allele carriers of FADS SNPs show higher n-6 and n-3 precursor PUFA levels (LA and ALA, respectively) and lower LC PUFA levels (EPA, DHA and AA, respectively) in erythrocyte membranes compared to the major allele carriers. To decipher potential pathophysiological links, we examined associations between a) FADS genotypes and b) single LC PUFAs with serum lipid levels and depressive symptoms (secondary outcomes). Since LC PUFAs affect lipid levels, it is hypothesized that likewise FADS genotypes influence lipid levels. Also, it is hypothesized that a low LC n-3 PUFA supply status is linked to depressive symptoms. Finally, we assessed the LC n-3 PUFA status of the MCI collective by using the omega-3 index (secondary outcome), defined as the combined percentage of EPA and DHA on total FAs in erythrocyte membranes. This enables a comparison of the LC n-3 PUFA supply status on population basis.

Material & Methods

Study design

Cross-sectional data derive from a randomised double-blind placebo-controlled intervention study in which the effect of dietary interventions in combination with exercise and cognitive training on neuropsychological functions in MCI patients was examined. The study was conducted in accordance with good clinical practice guidelines and ethical principles of the Declaration of Helsinki. The Ethics Committee of the Charité – University of Medicine, Berlin, Germany, approved the study. Written informed consent was obtained from all participants. The study and selected SNPs in FADS1, FADS2, and FADS3 gene clusters was planned in 2009.

Participants

The study cohort consisted of 111 patients (54 men, 57 women) with MCI who were recruited between 2011 and 2014 in Berlin (memory clinic of the Department of Neurology of the Charité University Hospital and Neurology specialist practice) and Frankfurt am Main (Institute of General Practice), Germany. Beside diagnosed MCI, major criteria for inclusion were an age between 50 and 80 years and a body mass index (BMI) between 19 – 35 kg/m^2. MCI diagnosis (amnestic; single and multiple domain) was made according to Mayo criteria based on subjective cognitive complaints and objective memory impairment in standardized tests (performing at least 1.5 SD below age- and education-specific norm in relevant subtests (Total Word List, Delayed Recall Word/Figures) of the CERAD- (Consortium to Establish a Registry for Alzheimer's Disease)-Plus test battery (24)), relatively preserved general cognition, no impairment in activities of daily living, and no dementia (25). The exclusion criteria were serious diseases (cancer, coronary heart disease, bleeding disorders), BMI > 35 kg/m^2, gastrointestinal disorders, ingestion of LC n-3 PUFA supplements or daily fish consumption. At the first visit, anthropometric measures and blood samples were taken. Venous blood was sampled after overnight fasting of at least 10 hours for analysis of FA profile in erythrocytes, total cholesterol (TC), high- and low-density lipoprotein-cholesterol (HDL-C and LDL-C), and triglycerides (TG) in serum as well as SNP genotyping in whole blood. Patients additionally completed a dietary questionnaire to obtain information on usual fish intake among other things. In addition, patients were tested on memory and cognitive performance using the Alzheimer's Disease Assessment Scale (ADAS) – memory (maximum 22 scores) and cognitive (maximum 70 scores; including and memory scores) subscale. The Beck's depression-inventory

Table 1
Characterisation of study collective

	Total population (n = 111) mean ± SD	Female (n = 57) mean ± SD	Male (n = 54) mean ± SD
Age (years)	69 ± 7.6	70 ± 6.0	68 ± 8.9
Anthropometric data			
Body weight (kg) *	73 ± 12	67 ± 10	80 ± 11
BMI (kg/m²)	25.6 ± 3.4	25.1 ± 3.4	26.2 ± 3.3
Cognitive and depression status			
ADAS memory score *	7.1 ± 2.5 a	6.6 ± 2.5	7.6 ± 2.4
ADAS cognitive score	8.0 ± 2.9 a	7.5 ± 2.8	8.5 ± 2.9
BDI	9.5 ± 5.9 b	10.0 ± 5.9	9.0 ± 6.0
Serum lipids			
TG (mg/dl)	111 ± 51 c	105 ± 50	117 ± 51
TC (mg/dl)	218 ± 37 c	226 ± 34	210 ± 39
LDL-C (mg/dl)	137 ± 31 c	140 ± 29	134 ± 33
HDL-C (mg/dl) *	64 ± 16 c	71 ± 17	57 ± 11
LDL/HDL ratio *	2.3 ± 0.8 c	2.1 ± 0.8	2.4 ± 0.8

* Values differ significantly between female and male population ($p \leq 0.05$; calculated by t-test for independent samples); a n = 109 (♀ n = 55, ♂ n = 51); b n = 101 (♀ = 50, ♂ = 51); c n = 109 (♀ = 57, ♂ = 52); Abbreviations: ADAS, Alzheimer's Disease Assessment Scale; BDI, Beck's depression-inventory; BMI, body mass index; TC, total cholesterol; TG, triglycerides.

(BDI) was measured to assess depressive symptoms. All testings were conducted by trained staff members according to standard procedure.

Analysis of fatty acids and omega-3 index in erythrocyte membranes

FA composition in erythrocyte membranes was analyzed according to the omega-3 index methodology as described previously (26). FA methyl-esters were generated from erythrocyte membranes by acid transesterification and analyzed by gas chromatography using a GC2010 Gas Chromatograph (Shimadzu, Duisburg, Germany) equipped with a SP2560, 100-m column (Supelco, Bellefonte, PA), using hydrogen as the carrier gas. FAs were identified by comparison with a FA standard mixture characteristic for erythrocyte membranes. Results for the omega-3 index are given as EPA + DHA expressed as a percentage of the total identified FAs after response factor correction. The coefficient of variation for EPA + DHA was 5 %. Quality was assured according to DIN ISO 15189. FA analyses were performed by Omegametrix Laboratory, Martinsried, Germany.

SNP genotyping

DNA was extracted from whole blood using a blood mini-kit (Qiagen, Hilden, Germany) and stored at -80°C until analysis. We selected 12 SNP of the FADS1, FADS2, and FADS3 gene cluster in which the promoter region was included for SNP analysis. Genotyping of the SNPs rs174546, rs174548 (FADS1), rs3834458, rs1535, rs174574, rs174575, rs174576, rs174578, rs174579, rs526126 (FADS2), rs174449 and rs174455 (FADS3) were performed on a Sequenom® MassARRAY iPLEX system at the laboratory of Prof. Dr. Dan Rujescu (University of Halle, Germany) following procedures described previously (27).

Statistical analysis

Statistical analyses were processed with SPSS software version 21.0 (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± SD unless otherwise specified. Differences between men and women were calculated by the t-test for independent samples. Patients were divided into three groups dependent on their FADS genotype (genotypic model) and relative erythrocyte membrane LC PUFA levels were compared among genotypes (primary outcome). In addition, serum lipid levels were compared among genotypes (secondary outcome). Furthermore, patients were divided into tertiles dependent on their relative PUFA levels (LA, AA, C22:4n-6, ALA, EPA, C22:5n-3, DHA, omega-3 index) in erythrocyte membranes and BDI measures were compared among the PUFA tertiles. All group differences were tested using the univariate ANOVA and Scheffé post hoc test. Pearson's correlation coefficients were used to assess the degree of linkage between the 12 SNPs in the study cohort. P-values ≤ 0.05 were considered significant. All comparisons other than primary outcome were

considered as exploratory analysis. Hence, a correction for multiple comparisons was not conducted.

Results

Characterisation of the study cohort is presented in Table 1. One hundred eleven amnestic MCI patients with a mean age of 69 ± 7.6 years were included. All subjects were of white origin. The study cohort was balanced in terms of gender. Subjects were slightly overweight (BMI > 25 kg/m²) and hypercholesterolemic (TC > 200 mg/dl).

The ADAS cognitive and memory subscale scores of both female and male patients represent typical values for patients with mild cognitive impairment (2). Note that higher scores indicate a worse performance in memory performance and cognition. It should be kept in mind that the ADAS is designed to assess cognitive functioning in people with dementia and not necessarily very sensitive to cognitive functioning in people with MCI.

Mean BDI values of the study cohort were among the cut-off value of 10 between minimal depression (< 10) and mild depression (> 10). While male MCI patients observed a mean BDI of 9.0 ± 6.0 indicating a minimal depression, female MCI patients had a mean BDI of 10.0 ± 5.9 indicating a mild depression. However, differences in BDI values between male and female were not significant. The evaluation of the dietary questionnaire showed that two patients were ovo-lacto-vegetarians, 31 patients eat fish once a week and 15 patients eat fish several times a week.

Characterisation of LC PUFA status

The characterisation of the FA status (table 2) showed that there were no systematic differences between male and female, except C20:1n-9 and C24:1n-9. For all physiologically important PUFAs (including LA, AA, C22:4n-6, ALA, EPA, C22:5n-3, DHA) and the omega-3 index, no differences were found between male and female. Levels of LA and AA in erythrocyte membranes were negatively correlated (r: -0.546, p<0.001). The average omega-3 index was 6.19 ± 1.55 %. More than 85 % of the patients had an omega-3 index below 8 %, while 23 % had an omega-3 index < 5 %.

SNPs in the FADS1, FADS2 and FADS3 gene clusters

The genomic location of SNPs in the FADS1, FADS2 and FADS3 gene cluster is shown in table 3. The genotypes of rs174546, rs174548, rs3834458, rs1535, rs174574, rs174575, rs174576, rs174578, rs526126, rs174449, and rs174455 were determined in 100 % (n = 111) of the patients, while rs174579 was determined in 99 % (n = 110) of the patients. All genotyped SNPs were found to be polymorphic. The genotype distribution for each SNP was consistent with Hardy-Weinberg equilibrium. The minor allele frequency (MAF) of most SNPs ranged between 20 and 25 %, with only one SNP (rs174449) showing a MAF < 10 % (9.0 %). Pairwise correlations (r) between the 12 SNPs in the study cohort are shown in table 4. The major and minor alleles of rs174574, rs174576 and rs174578 were present in the same patients without exception, meaning that the results for erythrocyte FA levels of the patients grouped by rs174574, rs174576 and rs174578 alleles were the same. This result confirms the accuracy of the genotyping.

Table 2
Erythrocyte membrane fatty acid composition in MCI patients

	Total population (n = 111) mean ± SD	Female (n = 57) mean ± SD	Male (n = 54) mean ± SD
C14:0	0.28 ± 0.19	0.28 ± 0.16	0.27 ± 0.22
C16:0	22.64 ± 1.12	22.62 ± 1.05	22.67 ± 1.20
C16:1n-7t	0.15 ± 0.05	0.16 ± 0.05	0.14 ± 0.05
C16:1n-7	0.57 ± 0.27	0.60 ± 0.25	0.55 ± 0.29
C18:0	15.97 ± 1.15	16.05 ± 1.08	15.87 ± 1.23
C18:1t	0.42 ± 0.20	0.41 ± 0.19	0.43 ± 0.22
C18:1n-9	15.76 ± 1.14	15.67 ± 1.08	15.86 ± 1.19
C18:2n-6tt	0.08 ± 0.06	0.08 ± 0.06	0.08 ± 0.06
C18:2n-6ct	0.03 ± 0.03	0.03 ± 0.04	0.03 ± 0.02
C18:2n-6tc	0.11 ± 0.05	0.11 ± 0.05	0.11 ± 0.05
C18:2n-6 (LA)	12.32 ± 2.12 a	12.27 ± 1.94	12.41 ± 2.31
C20:0	0.15 ± 1.32 a	0.16 ± 0.05	0.15 ± 0.05
C18:3n-6 (GLA)	0.10 ± 0.04	0.10 ± 0.04	0.09 ± 0.04
C20:1n-9 *	0.26 ± 0.06	0.24 ± 0.05	0.27 ± 0.06
C18:3n-3 (ALA)	0.25 ± 0.16	0.25 ± 0.17	0.25 ± 0.14
C20:2n-6	0.22 ± 0.04 a	0.21 ± 0.04	0.23 ± 0.04
C22:0	0.40 ± 0.17	0.39 ± 0.17	0.40 ± 0.17
C20:3n-6	1.60 ± 0.34	1.60 ± 0.36	1.60 ± 0.33
C20:4n-6 (AA)	15.20 ± 1.52 a	15.41 ± 1.43	14.99 ± 1.60
C24:0	0.85 ± 0.33 a	0.80 ± 0.30	0.89 ± 0.35
C20:5n-3 (EPA)	1.06 ± 0.47	1.11 ± 0.51	1.01 ± 0.44
C24:1n-9 *	0.92 ± 0.28	0.87 ± 0.26	0.98 ± 0.28
C22:4n-6	2.49 ± 0.57	2.45 ± 0.59	2.53 ± 0.56
C22:5n-6	0.50 ± 0.17	0.50 ± 0.17	0.50 ± 0.17
C22:5n-3	2.56 ± 0.34	2.55 ± 0.38	2.57 ± 0.30
C22:6n-3 (DHA)	5.13 ± 1.24	5.14 ± 1.31	5.12 ± 1.17
Omega-3 index	6.19 ± 1.55	6.24 ± 1.63	6.14 ± 1.47

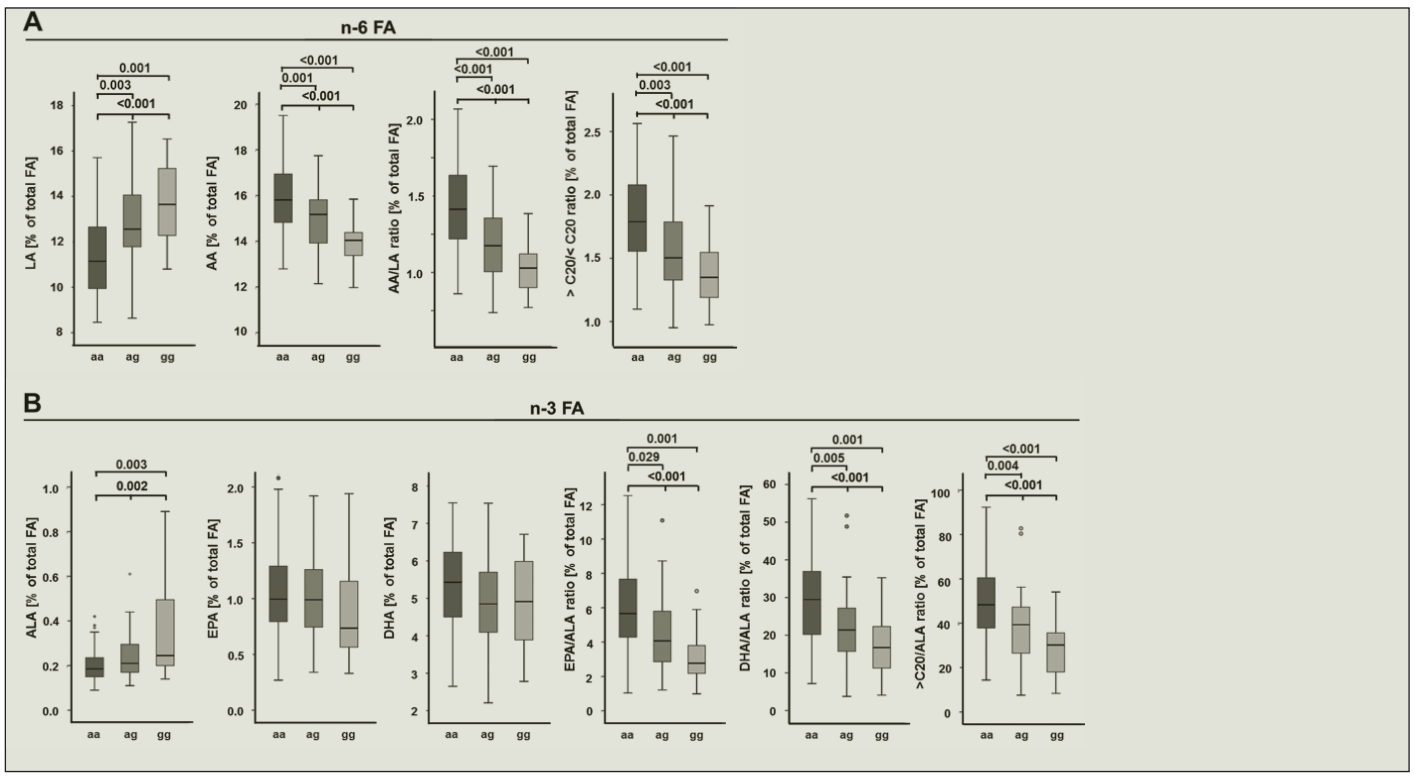
* Values differ significantly between female and male population ($p \leq 0.05$; calculated by t-test for independent samples); a n = 110 (♀ n = 56)

Table 3
Occurrence of SNPs in the FADS1, FADS2 and FADS3 gene cluster in MCI patients (n=111)

dbSNP	Position (bp)	Gen	Allel major/ minor	Genotype													
				major/major				major/minor				minor/minor				MAF	H-W *
				n	%	♂ %	♀ %	n	%	♂ %	♀ %	n	%	♂ %	♀ %	%	Χ *
rs174546	61569830	FADS1 intron	C/T	50	45.0	40.0	60.0	44	39.6	59.1	40.9	17	15.3	47.1	52.9	25.2	0.170
rs174548	61571348	FADS1 intron	C/G	58	52.3	41.4	58.6	39	35.1	53.8	46.2	14	12.6	64.3	35.7	21.4	0.080
rs3834458	61594921	FADS2 intergenic	T/D	54	48.6	44.4	55.6	41	36.9	53.7	46.3	16	14.4	50.0	50.0	23.7	0.086
rs1535	61597972	FADS2 intragenic	A/G	52	46.8	42.3	57.7	43	38.7	55.8	44.2	16	14.4	50.0	50.0	24.4	0.158
rs174574	61600342	FADS2 intron	C/A	50	45.0	40.0	60.0	44	39.6	56.8	43.2	17	15.3	52.9	47.1	13.8	0.170
rs174575	61602003	FADS2 intron	C/G	69	62.2	44.9	55.1	35	31.5	54.3	45.7	7	6.3	57.1	42.9	14.2	0.380
rs174576	61603510	FADS2 intragenic	C/A	50	45.0	40.0	60.0	44	39.6	56.8	43.2	17	15.3	52.9	47.1	25.2	0.170
rs174578	61605499	FADS2 intron	T/A	50	45.0	40.0	60.0	44	39.6	56.8	43.2	17	15.3	52.9	47.1	25.2	0.170
rs174579a	61605613	FADS2 intragenic	C/T	78	70.9	48.7	51.3	26	23.6	46.2	53.8	6	5.5	50.0	50.0	11.4	0.070
rs526126	61624885	FADS2 intron	C/G	80	72.1	47.5	52.5	28	25.2	50.0	50.0	3	2.7	66.7	33.3	21.0	0.772
rs174449	61640379	FADS3 intron	A/G	54	48.6	55.6	44.4	41	36.9	41.5	58.5	16	14.4	43.8	56.3	9.0	0.086
rs174455	61656117	FADS3 intragenic	A/G	50	45.0	54.0	46.0	45	40.5	44.4	55.6	16	14.4	43.8	56.3	23.7	0.268

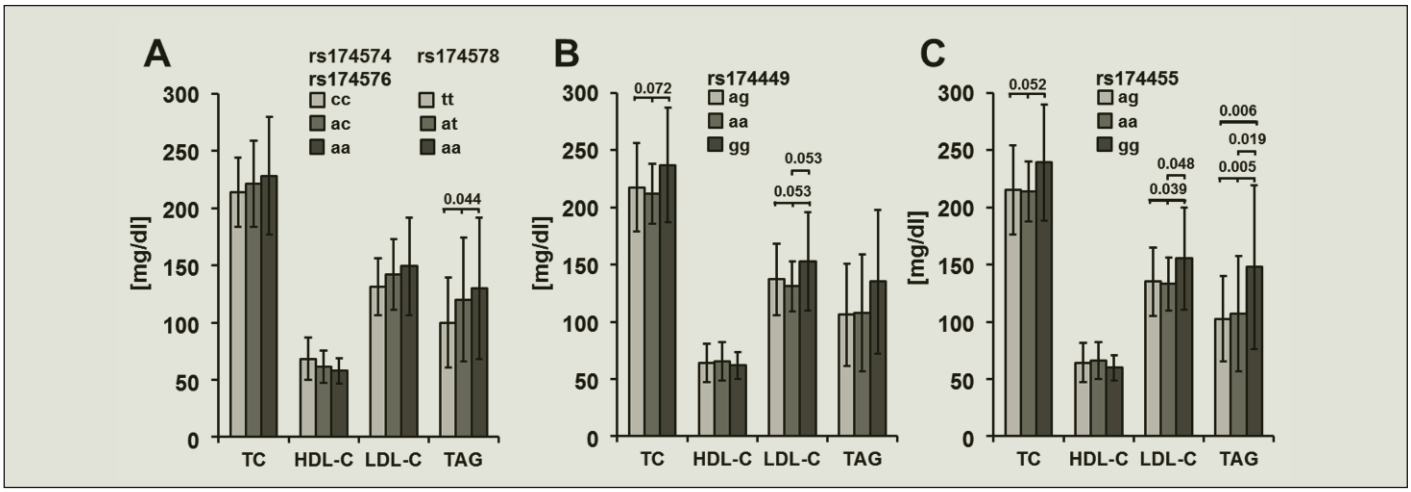
* derived from Pearson's chi-square test for Hardy-Weinberg Equilibrium. a n=110. Abbreviations: SNPs, single nucleotide polymorphism; FADS, fatty acid desaturase; H-W, Hardy-Weinberg Equilibrium; MAF, Minor allele frequency.

Figure 1
Major n-6 and n-3 PUFA levels in erythrocyte membranes classified by rs1535 (FADS2) genotype in MCI patients (n=111)



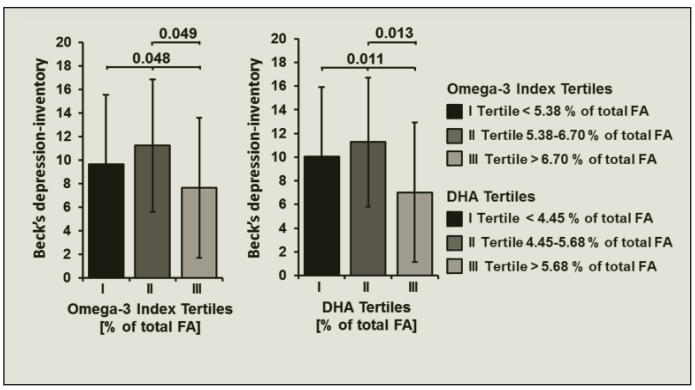
Total study population was classified by rs1535 gene type into major and minor allele groups. Relative LC PUFA levels in erythrocyte membranes were compared among major and minor allele groups. All results are shown as the mean+SD. Differences between groups were calculated by univariate ANOVA and Scheffé post hoc test with $p \leq 0.05$. >C20 n-6 PUFA includes C20:2, C20:3, C20:4, C22:4, and C22:5n-6. < C20 n-6 PUFA includes C18:2 and C18:3n-6. >C20 n-3 PUFA includes C20:5, C22:5n-3 and C22:6; Abbreviations: FADS, fatty acid desaturase

Figure 2
Serum lipid levels classified by genotypes in FADS2 and FADS3 gene clusters in MCI patients (n=111)



Total study population was classified by A) rs174574 (rs174576 and rs174578) (all three FADS2), as well as B) rs174449 and C) rs174455 (both FADS3) gene type into major and minor allele groups. The major and minor alleles of rs174574, rs174576 and rs174578 were present in the same patients without exception. Serum lipid levels were compared among major and minor allele groups. All results are shown as the mean+SD. Differences between groups were calculated by univariate ANOVA and Scheffé post hoc test for differences among individual groups with $p \leq 0.05$. Abbreviations: FADS, fatty acid desaturase; TC, total cholesterol; TG, triacylglycerol.

Figure 3
Association between Beck's depression-inventory and omega-3 index and docosahexaenoic acid (DHA, C22:6n-3) status in MCI patients



Total study population was classified by A) rs174574 (rs174576 and rs174578) (all three FADS2), as well as B) rs174449 and C) rs174455 (both FADS3) gene type into major and minor allele groups. The major and minor alleles of rs174574, rs174576 and rs174578 were present in the same patients without exception. Serum lipid levels were compared among major and minor allele groups. All results are shown as the mean+SD. Differences between groups were calculated by univariate ANOVA and Scheffé post hoc test for differences among individual groups with $p \leq 0.05$. Abbreviations: FADS, fatty acid desaturase; TC, total cholesterol; TG, triacylglycerol.

Associations of FADS1, FADS2 and FADS3 genotype with LC PUFA status

The genotypes in the investigated FADS1, FADS2 and FADS3 gene clusters were associated with the erythrocyte membrane levels of LA, ALA, and AA. Significant higher levels of the precursor PUFAs LA and ALA and lower AA levels in minor allele carriers compared to major allele carriers were observed for rs174546, rs174548, rs3834458, rs1535, rs174574 (rs174576 and rs174578), and rs174575 (table 5; figure 1, table S1-S5). Characteristic erythrocyte n-6 and n-3 PUFA patterns in major and minor allele carriers of MCI patients are exemplarily shown for rs174548 (FADS1, table 5) and rs1535 (FADS2, figure 1) genotype. For rs174579 (FADS2, table S6) as well as rs174449 and rs174455 (both FADS3, table S8/9) differences in erythrocytes levels were only observed for AA and ALA, while only AA levels differed significantly between major and minor allele carriers classified by rs526126 (table S7). For rs174546, rs3834458, rs1535, rs174575, rs174574 (rs174576 and rs174578), rs174579 and rs526126, levels of >C20 FAs – especially DHA – were higher in major compared to minor allele carriers. However, differences were not significant except for rs174548, where erythrocytes levels of DHA ($p = 0.014$) and the omega-3 index ($p = 0.024$) differed significantly in addition to LA ($p < 0.001$) and ALA levels ($p < 0.001$) (table 5). Noteworthy, in rs174548 only the heterozygotes showed lower DHA levels, while the DHA levels in C/C and G/G were almost equally high.

Besides, ratios of the Δ-5 and Δ-6 desaturase products to their LA and ALA precursors in erythrocyte membranes were calculated as indices of potential differences in desaturase activities among the MCI patients grouped by FADS genotypes. The n-6 and n-3 FA product/precursor ratios (AA/LA, EPA/ALA, DHA/ALA and sum n-6 or n-3 PUFA >C20/<C20 ratios) were significantly lower in the minor allele carriers compared to the major allele carriers for rs174546, rs3834458, rs1535, rs174575, and rs174574 (rs174576 and rs174578) (figure 1, table S1-S5). The relationships were similar, but less pronounced for rs174579 (table S6), rs174449 (table S8), and rs174455 (table S9). For rs526126, no differences in these PUFA product/precursor ratios were found between genotypes.

Table 4
Pairwise Pearson's correlations between the 12 FADS SNPs among the MCI patients

Pearson's r	rs174546	rs 174548	rs3834458	rs1535	rs174574	rs174575	rs174576	rs174578	rs174579	rs526126	rs174449
rs174546	-	-	-	-	-	-	-	-	-	-	-
rs174548	0.894 b	-	-	-	-	-	-	-	-	-	-
rs3834458	0.958 b	0.842 b	-	-	-	-	-	-	-	-	-
rs1535	0.974 b	0.861 b	0.983 b	-	-	-	-	-	-	-	-
rs174574	0.965 b	0.876 b	0.958 b	0.974 b	-	-	-	-	-	-	-
rs174575	0.711 b	0.577 b	0.716 b	0.702 b	0.711 b	-	-	-	-	-	-
rs174576	0.965 b	0.876 b	0.958 b	0.974 b	1.0 a	0.711 b	-	-	-	-	-
rs174578	0.965 b	0.876 b	0.958 b	0.974 b	1.0 a	0.711 b	1.0 a	-	-	-	-
rs174579	0.613 b	0.444 b	0.653 b	0.642 b	0.613 b	0.859 b	0.613 b	0.613 b	-	-	-
rs526126	0.367 b	0.286 c	0.405 b	0.393 b	0.392 b	0.571 b	0.392 b	0.392 b	0.689 b	-	-
rs174449	0.152	0.232 d	0.193 d	0.171	0.170	0.078	0.170	0.170	0.083	0.064	-
rs174455	0.690	0.173	0.133	0.089	0.087	0.210	0.087	0.087	0.013	0.088	0.735 b

a $p<0.0001$; b $p<0.001$; c $p<0.005$; d $p<0.05$

Associations of FADS1, FADS2 and FADS3 genotype with serum lipid levels

SNPs in the FADS2 and FADS3 gene clusters were associated with serum lipid levels (figure 2). In the minor allele carriers of rs174574 (rs174576 and rs174578) (FADS2) and rs174455 (FADS3) TG levels were significantly higher compared to major allele carriers (figure 2A, C), whereas minor allele carriers for rs174449 and rs174455 (FADS3) exhibited significantly higher TC and LDL-C levels compared to major allele carriers (figure 2B, C).

Associations of PUFA status with depression

We observed associations between the DHA status as well as the omega-3 index with the BDI (figure 3). BDI values differed significantly between DHA and omega-3 index tertiles (ANOVA). In each case Scheffé post hoc tests revealed significant BDI differences between tertile III (highest DHA and omega-3 index status group) and tertile II (intermediate DHA and omega-3 index status group). However, there were no differences in BDI values between tertile I and III and correlations between BDI and DHA levels in erythrocyte membranes (r -0.170, $p = 0.090$) as well as the omega-3 index (r -0.176, $p = 0.078$) were weak and only tended towards significance.

Discussion

Little is known about the complex interplay between different FADS gene cluster polymorphisms, the PUFA metabolism and the neuropsychological functions in MCI patients. This study aimed to observe associations between genotypes of the FADS1, FADS2, and FADS3 gene clusters and the endogenous LC PUFA status with a specific focus on the levels of n-6 and n-3 precursor FAs LA and ALA as well as the biologically more relevant LC PUFAs AA, EPA and DHA in older adults with MCI (primary question). Secondary questions were potential relations between FADS polymorphisms and LC PUFA levels with serum lipid levels and depressive symptoms as well as the assessment of the LC n-3 PUFA supply status of the MCI collective by using the omega-3 index.

Our results indicate a robust association between the several FADS genotypes (rs174546, rs174548 (FADS1), rs3834458, rs1535, rs174574, rs174575, rs174576, and rs174578 (FADS2)) for higher n-6 and n-3 precursor PUFA (LA and ALA) levels and lower AA levels in erythrocyte membranes in minor allele carriers compared to the major allele carriers. Differences in erythrocyte levels of the physiologic important FAs EPA and DHA showed the same trend between minor and major allele carriers but were not significant (except for DHA and the omega-3 index in rs174548, $p \leq 0.05$), possibly due to high interindividual variability in EPA and DHA levels. However, from the substrate/product ratios of n-3 PUFAs (EPA/ALA, DHA/ALA, >C20/ALA) it can be seen that minor allele carriers of investigated FADS genotypes, likewise, show lower EPA and especially DHA levels in erythrocyte membranes compared to the major allele carriers. These results thus indicate a lower conversion rate of precursor PUFAs to longer chain derivatives dependent on FADS genotype. Hence, our findings are consistent with other populations, where associations of diverse FADS genotypes with PUFA profiles in serum phospholipids (28–30), plasma phospholipids (31), erythrocyte membranes (32), erythrocyte ethanolamine phosphoglyceride and breast milk (31) were investigated.

Table 5
Major n-6 and n-3 PUFA levels and omega-3 index in erythrocyte membranes classified by rs174548 (FADS1) genotype in MCI patients (n=111)

Total study population was classified by rs174548 gene type into major and minor allele groups. Relative LC n-6 and n-3 PUFA levels in erythrocyte membranes as well as ratios were compared among major and minor allele groups. >C20 n-6 PUFA includes C20:2, C20:3, C20:4, C22:4, and C22:5n-6. < C20 n-6 PUFA includes C18:2 and C18:3n-6. >C20 n-3 PUFA includes C20:5, C22:5n-3 and C22:6.

	C/C, n=58 mean ± SD	C/G, n=39 mean ± SD	G/G, n=14 mean ± SD	P*
n-6 PUFAs				
C18:2n-6 (LA)	11.53 ± 1.92	13.10 ± 2.13	13.43 ± 1.71	<0.001
C18:3n-6 (GLA)	0.11 ± 0.04	0.09 ± 0.04	0.08 ± 0.03	n.s.
C20:4n-6 (AA)	15.80 ± 1.38	14.79 ± 1.34	13.83 ± 1.33	<0.001
C22:5n-6 (DPA)	0.47 ± 0.16	0.52 ± 0.15	0.54 ± 0.24	n.s.
Ratio AA/LA	1.41 ± 0.30	1.17 ± 0.27	1.05 ± 0.20	<0.001
Ratio >C20/<C20	1.78 ± 0.36	1.52 ± 0.35	1.39 ± 0.30	<0.001
n-3 PUFAs				
C18:3n-3 (ALA)	0.20 ± 0.07	0.27 ± 0.18	0.38 ± 0.24	<0.001
C20:5n-3 (EPA)	1.12 ± 0.47	0.99 ± 0.44	0.99 ± 0.59	n.s.
C22:5n-3 (DPA)	2.60 ± 0.33	2.55 ± 0.37	2.42 ± 0.31	n.s.
C22:6n-3 (DHA)	5.42 ± 1.27	4.68 ± 1.05	5.20 ± 1.31	0.014
Omega-3 index	6.54 ± 1.62	5.67 ± 1.22	6.19 ± 1.73	0.024
Ratio EPA/ALA	6.11 ± 2.86	4.33 ± 2.48	3.14 ± 1.76	<0.001
Ratio DHA/ALA	30.44 ± 12.82	21.04 ± 9.34	18.41 ± 9.50	<0.001
Ratio >C20/ALA	51.15 ± 19.86	36.91 ± 14.84	30.40 ± 15.14	<0.001

* calculated by univariate ANOVA with $p \leq 0.05$. Results of post hoc test for differences among groups are not shown.

Furthermore, several FADS2 and 3 genotypes were associated with serum lipid levels. Carriers of minor alleles of rs174574 (rs174576, rs174578) (FADS2) and rs174455 (FADS3) had significantly higher TG levels compared to major allele carriers, whereas minor allele carriers for rs174449 (FADS3) exhibited significantly higher TC and LDL-C levels compared to major allele carriers. It should be noted that the TG levels of the MCI collective were normal (111 ± 51 mg/dl), while TC were slightly elevated (218 ± 37 mg/dl). Similar associations of FADS genotypes with blood lipid levels have been described recently for serum LDL-C levels (rs174547, [33]) and plasma TG levels (rs174546, [34]). The TG lowering effect of LC n-3 PUFAs is viewed as an important physiological effect of LC n-3 PUFAs to mediate their cardiovascular benefits [35]. Summarizing, SNPs in FADS2 and 3 genotypes impair desaturase function resulting in a lower conversion of ALA to EPA and DHA and therewith a lower inhibitory effect on TG synthesis in hepatic cells. Indeed, we observed associations of the same SNPs with ALA levels in erythrocytes as well as EPA/ALA, DHA/ALA, >C20/ALA ratios. Hence, a potential pathophysiological link between FADS genotypes or the LC PUFA status and cognitive impairment may be CVD (21), which is a risk factor for both vascular and degenerative dementia (36, 37).

Another potential relationship of impaired LC n-3 PUFA levels with memory and cognitive functions may be depression. Depression is often accompanied with cognitive dysfunction (38). Vice versa, cognitive deficits in major depressive disorder are viewed as a principal mediator of psychosocial impairment (39). Epidemiological studies reported a significant inverse correlation between the intake of LC n-3 PUFA-rich oily fish and depression (40, 41). Here, we showed that a lower DHA status and omega-3 index was associated with depressive symptoms. Hence, these findings support the finding that depression is associated with a reduced omega-3 index (42). It has also been shown that EPA+DHA supplementation of MCI patients over six month results in reduced depressive symptoms (11). However, the MCI patients were selected with regard to their cognitive performance, not on depressive symptoms. Hence, the association between the LC PUFA status, depression and cognitive decline in MCI patients' needs to be further investigated in more suitable cohorts.

Up to now only small populations of MCI patients were investigated with regard to the LC n-3 PUFA status (9, 11). The present MCI cohort of 111 patients exhibited an average omega-3 index of 6.19 ± 1.55 %, slightly higher as compared to an MCI cohort (n = 50) from an Australian study (5.49 ± 0.13 % SEM) (9), but lower as compared to a cohort (n = 65) of cognitively healthy older people (8.0 ± 2.5) (43). An omega-3 index target range of 8 to 11 % has been suggested for optimal cardiovascular health (44). If this range is also applicable for optimal cognitive health needs to be investigated in future trials. However, a low omega-3 index is associated with major depression (45), and suboptimal neurocognitive performance with regard to executive function (46). In the present study, more than 85 % of the MCI patients showed an omega-3 index below 8 %, and 23 % even below 5 %. In view of the beneficial effects of LC n-3 PUFAs for brain functions and mood, the EPA and DHA status of the present MCI cohort can therefore be classified as insufficient.

Strength and Limitations

The strength of the study is the comprehensive phenotyping of the patients including several specific molecular as well as numerous classical clinical parameters. In general, effects of SNPs on complex phenotypes are low (47). Hence, with 111 patients the amount is possibly too little to observe strong associations. A limitation of the study is the lack of a healthy control group, which would enable to investigate the influence of FADS genotypes or the LC PUFA status on cognitive performance.

Conclusion

This is the first study that investigated associations between FADS genotypes and the LC PUFA status in MCI patients. Our results confirm that genetic variation in FADS1, FADS2 and FADS3 genotypes are associated with erythrocyte levels of LA, ALA and AA as well as Δ-5 and Δ-6 desaturase substrate/product ratios (AA/LA, >C20n-6/<C20n-6, EPA/ALA, DHA/ALA, >C20n-3/ALA). To what extent FADS genotypes and a lower conversion of LA and ALA to biologically important LC PUFAs such as AA, EPA and DHA contributes to cognitive decline should be investigated in future trials.

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Ethical Standards: The study was conducted in accordance with the German law. The Ethics Committee of the Charité – University of Medicine, Berlin, Germany, approved the study. Written informed consent was obtained from all participants.

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