Evidence for an association between genetic variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and the fatty acid composition of erythrocyte membranes

Peter Rzehak1,2,*, Joachim Heinrich1, Norman Klopp1, Linda Schaeffer1, Sebastian Hoff3, Günter Wolfram4, Thomas Illig1 and Jakob Linseisen3,5

1Institute of Epidemiology, Helmholtz Zentrum München, German Research Centre for Environmental Health, Neuherberg 85764, Germany
2Ludwig-Maximilian University Munich, IBE Chair of Epidemiology, Oberschleißheim 85758, Germany
3Human Nutrition and Cancer Prevention, Technical University of Munich, Freising 85350, Germany
4Department of Food and Nutrition, Technical University of Munich, Freising 85350, Germany
5Division of Cancer Epidemiology, German Cancer Research Centre, Heidelberg 69120, Germany

The present study gives further evidence for the recently found association between variants of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster and PUFA in blood phospholipids and explores this association for cellular fatty acids in erythrocyte membranes. In a subgroup of adults participating in the Bavarian Nutrition Survey II, a cross-sectional population-based study conducted in Bavaria, Germany, allelic variation in three selected loci of the FADS1 FADS2 gene cluster was analysed and used for haplotype construction. Associations with plasma phospholipid PUFA (n=163) and PUFA in erythrocyte membranes (n=535) were investigated by regression analysis. All haplotypes of the original five-loci haplotypes of our previous study could be replicated. In addition, associations with serum phospholipid PUFA were confirmed in the present data set. Although less pronounced, associations between FADS1 FADS2 haplotypes and PUFA in erythrocyte membranes, particularly arachidonic and dihomo-γ-linolenic acid, could be established. We provide the first replication of the association of the FADS1 FADS2 gene cluster with PUFA in blood phospholipids. For the first time, such associations were also shown for PUFA in cell membranes.

FADS1 FADS2 gene cluster: Polynsaturated fatty acids: Blood phospholipids: Erythrocyte membranes: Bavarian Nutrition Survey II

Recently, strong associations between variants in the human Δ-5 and Δ-6 desaturase genes fatty acid desaturase 1 (FADS1) and fatty acid desaturase 2 (FADS2) on chromosome 11q12-11q13.1 and fatty acid composition in serum phospholipids have been detected by our group1. In particular, the all-minor haploype (which is composed of the minor allele at each locus) reconstructed from five loci (rs174544, rs174553, rs174556, rs174561, rs3834458) showed very strong associations with the n-6 fatty acid arachidonic acid (20:4n-6), which often functions as a precursor of inflammatory eicosanoids (4-series leucotrienes and 2-series prostaglandins), depending on the metabolic situation. Further strong associations of the all-minor haploype were observed with other n-6 fatty acids (18:2n-6, 18:3n-6, 20:2n-6 and 20:3n-6) and to a lesser extent with the n-3 fatty acids 18:3n-3, 20:5n-3 and 22:5n-3. These results are important as they have been shown in several studies that this region on chromosome 11q12-11q13.1 is linked with atopy2–6. In fact, in our previous study, it was shown that carriers of the rare alleles of several single nucleotide polymorphisms (SNP) and their respective haplotypes had a lower prevalence of allergic rhinitis and atopic eczema. As such associations may be mediated by products of the arachidonic acid pathway, however, it is crucial to demonstrate that these haplotypes are also associated with the substrates, i.e. membrane PUFA.

In the present study we aimed (i) to replicate the reconstructed haplotypes and their associations with fatty acids in blood phospholipids as observed in the previously studied population of the European Community Respiratory Health Survey (ECRHS) in Erfurt, East Germany, using an independent population sample7. In addition we (ii) wanted to test for effects of these haplotypes at the cellular level, using erythrocyte membranes as proxy membranes. The latter is an interesting study question, since the proof of an effect would have implications for other cell types with no or low desaturase activities, including leucocyte sub-populations.

Abbreviations: BVS-II, Bavarian Nutrition Survey II; ECRHS, European Community Respiratory Health Survey; FADS1, fatty acid desaturase 1; FADS2, fatty acid desaturase 2; LD, linkage disequilibrium; SNP, single nucleotide polymorphism.

* Corresponding author: Dr Peter Rzehak, fax +49 89 3187 3380, email peter.rzehak@helmholtz-muenchen.de
Materials and methods

Study design and population

The Bavarian Nutrition Survey II (BVS-II) was designed as a representative study of the Bavarian population to investigate dietary and lifestyle habits. Between September 2002 and June 2003, about 1000 subjects of the German-speaking population in Bavaria (Germany) aged 13–80 years were recruited by a three-stage sampling procedure. At baseline, the subjects’ lifestyle and socio-economic characteristics as well as their health status were assessed by means of a computer-aided personal interview. The overall participation rate in the study was 71% (n 1050). In the following weeks, the participants were contacted three times by phone in order to collect information on dietary intake and physical activity (24 h recalls). Within 6 weeks after recruitment, adults (age ≥ 18 years) who completed at least one 24 h dietary recall (n 879) were invited to their nearest health office for blood sampling and standardised anthropometric measurements. Of these, 65% (n 568) provided blood samples, with one participant denying the use of DNA for further studies. Information on SNP and fatty acid composition of erythrocyte membranes as well as plasma phospholipids and SNP data were available for 535 and 163 participants, respectively. Note that the latter 163 participants are a subset of the 535 participants of the BVS-II study for whom PUFA was determined both in plasma and in erythrocytes. Only marginal differences by age, socio-economic status or smoking status were observed between study participants invited for blood sampling and those finally providing blood samples. All participants gave their written informed consent. The study was approved by the local ethics committee.

Blood sampling

Venous blood was drawn into EDTA tubes (1·6 mg/l blood), chilled at 4°C, and processed subsequently. Plasma and buffy coat (leucocytes) were separated from erythrocytes by centrifugation at 2000 g for 20 min at 4°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min for forty-five cycles, and finally incubation at 72°C for 10 min. After PCR a shrimp alkaline phosphate- and primer extension reaction was carried out according to the iPLEX™ reaction protocol (Sequenom). All reactions (PCR amplification, base extension) were carried out in a Tetrad PCR thermal cycler (Bio-Rad, Hercules, CA, USA).

Genotyping

Calculating the linkage disequilibrium (LD) structure in the ECRHS, we realised that three SNP (rs174556, rs174561, rs3834458) are sufficient to cover most of the genetic information regarding common SNP. Therefore we decided to restrict genotyping to these three SNP in the BVS-II study.

Genomic DNA was extracted from ‘buffy coat’ using the FlexiGene DNA kit (Qiagen GmbH, Hilden, Germany). The samples were genotyped with the MassARRAY system using the iPLEX™ chemistry as suggested by the manufacturer (Sequenom, San Diego, CA, USA).

Briefly, genomic DNA was amplified by PCR using HotStarTaq DNA Polymerase (Qiagen). Genotyping assays were carried out by using 5 ng genomic DNA. PCR primers were used at 167 nM final concentrations for a PCR volume of 6 μl. The PCR conditions were 95°C for 15 min for a hot start, followed by denaturing at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min for forty-five cycles, and finally incubation at 72°C for 10 min. After PCR a shrimp alkaline phosphate- and primer extension reaction was carried out according to the iPLEX™ reaction protocol (Sequenom). All reactions (PCR amplification, base extension) were carried out in a Tetrad PCR thermal cycler (Bio-Rad, Hercules, CA, USA). The final base extension products were treated with SpectroCLEAN resin (Sequenom) to remove salts in the reaction buffer. This step was carried out with a Multimek 96-channel autopipette (Beckman Coulter, Inc., Fullerton, CA, USA), and 16 μl resin–water suspension was added into each base extension reaction, making the total volume 26 μl. After a quick centrifugation (2000 rpm, 3 min) in an Eppendorf centrifuge 5810, 30 nl of reaction solution was dispensed onto a 384 format SpectroCHIP (Sequenom) prespotted with a matrix of 3-hydroxypicolinic acid by using a SpectroPoint nanodispenser (Sequenom). A modified Bruker Autoflex matrix-assisted laser desorption ionisation-time-of-flight mass spectrometer (Sequenom) was used for data acquisitions from the SpectroCHIP. The resulting mass spectra were analysed automatically for peak identification using the SpectroTYPE R T 3.4 software (Sequenom). For quality reasons 10% of the spectra were checked by two independent trained individuals. No differences in spectra calling could be detected.
Statistical analysis

Allele frequencies, Hardy–Weinberg equilibrium and tests on LD for the BVS-II study data were performed with procedures ‘proc allele’ and ‘proc haplotype’ of the statistical software module SAS/Genetics of SAS version 9.1.3 (SAS Institute, Inc., Cary, NC, USA) and with the special-purpose software JLIN\(^{(11)}\). Hardy–Weinberg equilibriums were tested by Fisher’s exact test and LD by the likelihood ratio test of allelic association and pairwise LD measures Lewontin’s \(D^l\) and pairwise squared correlation \(r^2\). The software JLIN was used to calculate pairwise LD measures \(D^l\) and \(r^2\) and to show LD blocks graphically.

Both single SNP and haplotype regression analyses for the nine PUFA were conducted applying an additive model. Haplotype reconstruction was conducted with the procedure haplo.glm of the module haplo.stats of the R-software (R Foundation for Statistical Computing, Vienna, Austria; http://www.r-project.org). Haplo.glm is based on a special expectation-maximisation (EM)-based algorithm for estimation of haplotypes described by Schaid et al.\(^{(12)}\) and was also used to estimate the associations of haplotypes on fatty acids by linear regression. Haplotypes below 1% of haplotype frequency were combined in a rare-haplotype category. The reference category of all association analyses was the major allele frequency proportion above 1% (see Table 2). Haplotypes were constructed from participants with no missing data on the three selected SNP and data available on the fatty acid composition (phospholipids or erythrocytes respectively).

The three-loci haplotypes reflected the five-loci haplotypes (rs174544, rs174553, rs174556, rs174561, rs3834458) as estimated in the data of the ECRHS in Erfurt very well. An all-major allele haplotype (MaA, with alleles C-T-T), an all-minor allele haplotype (MiA, with alleles T-C-del), and a haplotype of major alleles except for the last locus (rs3834458; haplo.1, with alleles C-T-del) were estimated from the three SNP (rs174556, rs174561, rs3834458). In addition, the haplotype frequency proportions were about the same in the BVS-II and the ECRHS. It should be noted that the estimation of haplotypes from the available three of the five SNP resulted in a haplotype pattern at the common loci identical to that estimated in the ECRHS.

Fatty acid composition of plasma phospholipids and erythrocyte membranes and the FADS1 FADS2 gene cluster

Table 3 shows the mean PUFA content in erythrocytes and phospholipids in carriers of two copies of the MaA haplotype and those with two copies of the MaA haplotype. In carriers of the MiA haplotype, significantly elevated proportions of dihomo-\(\gamma\)-linolenic acid (20:3\,-6) and EPA (20:5\,-3) were log-transformed. To keep the nominal level of type 1 error to 5% despite numerous tests of associations, \(P\) values of the primary analyses were conservatively corrected by multiplying the uncorrected \(P\) value by the number of analysed outcomes (fatty acids) times the effective number of loci (SNP) according to Nyholt’s method\(^{(13)}\). Effective loci were calculated using the spectral decomposition method software SNPSpD (Queensland Institute of Medical Research, Herston, QLD, Australia; http://genepi.qimr.edu.au/general/daleN/SNPSpD/).

Results

Single nucleotide polymorphism associations with fatty acids

As a first step – before conducting haplotype analyses – we performed single SNP analyses with each PUFA derived from erythrocytes and from plasma phospholipids in the 535 and 163 participants of the BVS-II study respectively. From Table 1, it can be seen that for PUFA from erythrocytes each of the three SNP (rs174556, rs174561 and rs3834458) is highly significantly associated with dihomo-\(\gamma\)-linolenic and arachidonic acid, even after correction for multiple testing. Associations for arachidonic acid are significant for rs174556 and rs174561, but not for rs3834458, when correction of multiple testing is accounted for. The associations of the SNP with EPA and n-3 docosapentaenoic acid were only significant before correction for multiple testing. Similar results as found for PUFA in erythrocytes were observed for PUFA in plasma phospholipids in the BVS-II study (data not shown).

Estimated haplotypes

Prerequisites of valid haplotype analyses were satisfied. All three SNP (rs174556, rs174561, rs3834458) of the BVS-II study used for haplotype construction were in Hardy–Weinberg equilibrium. Minimal \(P\) value for the exact test of violation of Hardy–Weinberg equilibrium was 0.38 for rs3834458. All three SNP showed very strong LD as shown diagrammatically by pairwise Lewontin’s \(D^l\) and pairwise squared correlation \(r^2\) values in Fig. 1. Strong LD over a block of these and further SNP has also been shown previously in the ECRHS\(^{(1)}\).

Haplotypic construction for the participants in the BVS-II study with PUFA in plasma phospholipids as well as with erythrocyte membranes resulted in three haplotypes with a haplotype frequency proportion above 1% (see Table 2). Haplotypes were constructed from participants with no missing data on the fatty acid composition (phospholipids or erythrocytes respectively). The three-loci haplotypes reflected the five-loci haplotypes (rs174544, rs174553, rs174556, rs174561, rs3834458) as estimated in the data of the ECRHS in Erfurt very well. An all-major allele haplotype (MaA, with alleles C-T-T), an all-minor allele haplotype (MiA, with alleles T-C-del), and a haplotype of major alleles except for the last locus (rs3834458; haplo.1, with alleles C-T-del) were estimated from the three SNP (rs174556, rs174561, rs3834458). In addition, the haplotype frequency proportions were about the same in the BVS-II and the ECRHS. It should be noted that the estimation of haplotypes from the available three of the five SNP resulted in a haplotype pattern at the common loci identical to that estimated in the ECRHS.
Table 1. Association of single nucleotide polymorphisms (SNP) of the fatty acid desaturase 1 fatty acid desaturase 2 (FADS1 FADS2) gene cluster with PUFA in erythrocyte membranes in the Bavarian Nutrition Survey II (n 535)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Linoleic acid (18:2n-6)</th>
<th>γ-Linolenic acid (ln(18:3n-6))</th>
<th>Dihomo-γ-linolenic acid (20:3n-6)</th>
<th>Arachidonic acid (20:4n-6)</th>
<th>Adrenic acid (22:4n-6)</th>
<th>α-Linolenic acid (18:3n-3)</th>
<th>EPA (ln(20:5n-3))</th>
<th>n-3 Docosapentaenoic acid (22:5n-3)</th>
<th>DHA (22:6n-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs174556*</td>
<td>P value (corrected)†</td>
<td>1·000</td>
<td>0·106</td>
<td>6·7 × 10⁻³</td>
<td>1·3 × 10⁻³</td>
<td>0·015</td>
<td>0·000</td>
<td>0·360</td>
<td>0·276</td>
</tr>
<tr>
<td></td>
<td>β Coefficient‡</td>
<td>0·065</td>
<td>-0·037</td>
<td>0·224</td>
<td>-1·296</td>
<td>-0·257</td>
<td>0·001</td>
<td>-0·145</td>
<td>-0·166</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>11·044</td>
<td>-2·450</td>
<td>1·414</td>
<td>14·904</td>
<td>2·842</td>
<td>0·092</td>
<td>-0·363</td>
<td>2·317</td>
</tr>
<tr>
<td>rs174561*</td>
<td>P value (corrected)†</td>
<td>1·000</td>
<td>0·098</td>
<td>4·1 × 10⁻³</td>
<td>1·1 × 10⁻³</td>
<td>0·014</td>
<td>0·000</td>
<td>0·248</td>
<td>0·241</td>
</tr>
<tr>
<td></td>
<td>β Coefficient‡</td>
<td>0·070</td>
<td>-0·037</td>
<td>0·225</td>
<td>-1·300</td>
<td>-0·257</td>
<td>0·001</td>
<td>-0·155</td>
<td>-0·169</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>11·041</td>
<td>-2·449</td>
<td>1·412</td>
<td>14·909</td>
<td>2·842</td>
<td>0·092</td>
<td>-0·357</td>
<td>2·319</td>
</tr>
<tr>
<td>rs3834458*</td>
<td>P value (corrected)†</td>
<td>1·000</td>
<td>0·294</td>
<td>7·9 × 10⁻¹⁰</td>
<td>0·012</td>
<td>0·129</td>
<td>1·000</td>
<td>0·222</td>
<td>1·000</td>
</tr>
<tr>
<td></td>
<td>β Coefficient‡</td>
<td>0·181</td>
<td>-0·030</td>
<td>0·227</td>
<td>-1·057</td>
<td>-0·194</td>
<td>0·004</td>
<td>-0·154</td>
<td>-0·099</td>
</tr>
<tr>
<td></td>
<td>Intercept</td>
<td>10·965</td>
<td>-2·452</td>
<td>1·388</td>
<td>14·826</td>
<td>2·816</td>
<td>0·090</td>
<td>-0·349</td>
<td>2·283</td>
</tr>
</tbody>
</table>

* SNP is coded additive as 0, 1, 2. Allele frequencies (in %) for the three SNP are: rs174556, C/T = 70·5/29·5; rs174561, T/C = 70·4/29·6; rs3834458, T/deletion = 67·5/32·5.
† The P value is corrected for multiple testing by multiplying the uncorrected P value by 9 (for the nine outcomes). Note that P values exceeding 1·0 after correction for multiple testing have been marked down to 1·0.
‡ Regression coefficient β from linear regression of the fatty acid (outcome) on the respective single SNP.
is also true for the association between the MiA haplotype on PUFA in erythrocytes (regression coefficients not shown).

We repeated the regression analyses of the effect of haplotypes on PUFA in both plasma phospholipids and erythrocytes and adjusted for total energy intake (kJ/d), physical activity (categorised as amount (h) of physical exercise per week, zero or less than 1 h per week, 1–< 2 h per week, 2–< 4 h per week, 4–< 6 h per week, 6–< 8 h per week, 8–< 10 h per week and 10 + h per week) and BMI (continuous), both in separate analyses and simultaneously. We did not find any substantial differences between crude and adjusted analyses in P values, or in regression estimates (data not shown). In the 163 BVS-II participants with PUFA data on both plasma phospholipids and erythrocyte membranes, correlation coefficients for PUFA ranged from 0·05 to 0·47; for α-linolenic acid, dihomo-γ-linolenic acid and arachidonic acid correlation coefficients were 0·47 \( (P< 3·6 \times 10^{-10}) \), 0·27 \( (P< 3·9 \times 10^{-7}) \) and 0·05 \( (P<0·51) \), respectively.

**Discussion**

This is the first replication of the previously found associations of the genetic variants in the FADS1 FADS2 gene cluster with the PUFA composition of serum phospholipids\(^1\) in an independent population-based study. Moreover, this is the first study showing similar differences in PUFA composition in erythrocyte membranes by polymorphisms of the FADS1 FADS2 gene cluster. In particular, we found that the MiA haplotype is negatively associated with the arachidonic acid content in plasma phospholipids as well as in erythrocyte membranes. Additionally, distinct associations between FADS1 FADS2 haplotypes and the dihomo-γ-linolenic acid (20:3n-6) and arachidonic acid (22:4n-6) content in erythrocyte membranes were observed. Erythrocyte membranes are used in the present study as an easily obtainable model membrane

![Fig. 1. Pairwise linkage disequilibrium \( D' \) and \( r^2 \) plots of the three common single nucleotide polymorphisms (SNP) in \( r^2 \) and \( D' \) for 174556 in the Bavarian Nutrition Survey (BVS-II) constructed from common single nucleotide polymorphisms (SNP) in Turkish Community Respiratory Health Survey (ECRHS) (\( n \) 727) and the three-loci haplotypes in the Bavarian Nutrition Survey II (BVS-II) constructed from common single nucleotide polymorphisms (SNP).](image-url)
Dietary PUFA are metabolised as energy sources, redistribution of dietary PUFA to circulation via VLDL for adipose tissue (14,15). Hepatic PUFA metabolism has a key role in the PUFA intake, although its composition is already affected by phospholipids is often considered as a biomarker of dietary erythrocyte membranes. The PUFA composition of plasma and membrane lipids a major contribution of the liver desaturase–elongase gene cluster on the composition of PUFA in plasma phospholipids and erythrocyte membranes. Similar effects (with respect to the expected direction, i.e. positive β-coefficient for substrates, negative β-coefficient for products) on n-3 PUFA were noted but were less pronounced and did not reach statistical significance. Although functional data are still missing, our data may indicate a lower activity of hepatic desaturases – especially Δ-5 desaturase (FADS1) catalysing the reaction of dihom γ-linolenic acid to arachidonic acid – in carriers of the MiA haplotype v. MaA haplotype.

Arachidonic acid and EPA, incorporated in phospholipids of cell membranes, are substrates for enzymes of the arachidonic acid pathway after their release by phospholipases (20,22). It has been well documented that eicosanoids derived from arachidonic acid (n-6 PUFA) are potent pro-inflammatory mediators while the effects of eicosanoids from EPA (n-3 PUFA) or dihomo γ-linolenic acid are much less pronounced or even non-inflammatory (20,22). Thus, a lower proportion of arachidonic acid in cellular membranes as found for the carriers of the MiA haplotype could be interpreted as a metabolic advantage in terms of less pronounced inflammatory reactions, if induced by different stimuli. However, biological activities other than modulation of inflammation are described for PUFA as well as eicosanoids (and docosanoids) which could also influence disease risk (23–28).

Concerning limitations and strengths of the study, reconstruction of the five-loci haplotypes as identified in the ECRHS study with only three SNP in the BVS-II study worked well since these SNP are in very strong LD, and an identical haplotype structure was found in the BVS-II sample. Both studies are population-based studies but conducted in different geographic regions in Germany. The genotyping success rates were high in both the BVS-II study and the ECRHS at the three SNP, rs174556 (97.8 and 94.4 %), rs174561 (97.8 and 96.4 %) and rs3834458 (98.8 and 99.2 %). Due to the lower sample size of the BVS-II study (n 535 and n 163) in comparison with the ECRHS (n 727), we cannot rule out that non-significant differences in results regarding the FADS1 FADS2 gene cluster and PUFA in plasma phospholipids are due to low power. However, despite lower power, results for dihomo γ-linolenic and arachidonic acid are similar between ECRHS and BVS-II data.

Table 3. Mean levels of PUFA content (%) in erythrocyte membranes and plasma phospholipids for carriers and non-carriers of the all-minor alleles haplotype in the Bavarian Nutrition Survey II (BVS)

<table>
<thead>
<tr>
<th>Haplotype pair</th>
<th>Erythrocyte sample (n 535)</th>
<th>Phospholipid sample (n 163)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIA/MIA*</td>
<td>MIA/MIA*</td>
</tr>
<tr>
<td>Linoleic acid (18 : 2n-6)</td>
<td>11-12</td>
<td>23-95</td>
</tr>
<tr>
<td>γ-Linolenic acid (18 : 3n-6)</td>
<td>0-03</td>
<td>0-07</td>
</tr>
<tr>
<td>Dihomo-γ-linolenic acid (20 : 3n-6)</td>
<td>1-85</td>
<td>3-87</td>
</tr>
<tr>
<td>Arachidonic acid (20 : 4n-6)</td>
<td>11-81</td>
<td>9-76</td>
</tr>
<tr>
<td>EPA (20 : 5n-3)</td>
<td>0-78</td>
<td>0-61</td>
</tr>
<tr>
<td>n-3 Docosapentaenoic acid (22 : 5n-3)</td>
<td>1-88</td>
<td>0-93</td>
</tr>
<tr>
<td>DHA (22 : 6n-3)</td>
<td>4-06</td>
<td>3-97</td>
</tr>
</tbody>
</table>

* MIA/MIA = mean level of PUFA for individuals with two copies of the all-minor alleles haplotype MIA (2-2/2-2-2-2 = T-C-del/T-C-del).
† MAA/MAA = mean level of PUFA for individuals with two copies of the all-major alleles haplotype MAA (1-1-1-1-1 = C-T-C-T-C-T).
‡ P values test for the difference in PUFA levels between carriers and non-carriers of the all-minor alleles haplotype. These are based on the special multiple regression model described in the Materials and methods section. This model assesses the influence of the estimated haplotypes (reported in Table 2) on the respective outcome (i.e. PUFA).
§ P values are corrected for multiple testing according to Nyholt's procedure (19), i.e. P values are multiplied by 11 for both BVS samples (11 approximately equal to 1/191 effective loci × 9 outcomes for the BVS erythrocyte sample; 11 approximately equal to 1/163 effective loci × 9 outcomes for the BVS phospholipid sample). Note that P values exceeding 1-0 after correction for multiple testing have been marked down to 1-0.
In conclusion, in the present study we could not only replicate associations between haplotypes of the FADS1 FADS2 gene cluster and PUFA in plasma phospholipids but found also evidence for such associations in erythrocyte membranes. Especially, the association of the all-minor alleles haplotype (MiA) with a lower arachidonic acid content in cellular membrane lipids supports the hypothesis of a link between the FADS1 FADS2 gene cluster and several chronic diseases, where products of the arachidonic acid pathway are suspected to be causally involved. However, experimental data confirming the functional relevance of these genetic variants are urgently needed.

Acknowledgements

The BVS-II study was supported by funds of the Kurt-Eberhard-Bode-Stiftung and the Bavarian Ministry of Environment, Health and Consumer Protection. The present study was partly funded by the German Ministry of Education and Research (BMBF)/National Genome Research Network (NGFN) research net. Financial support by the Munich Centre of Health Sciences, which contributed to this research, is greatly acknowledged.

None of the authors has any conflict of interest.

P. R. drafted the manuscript, performed statistical analyses and was involved in interpretation. J. H., J. L. and T. I. supervised this paper and actively contributed to writing of the paper and interpretation of the results. J. L. and G. W. are principal investigators of the BVS-II and conducted together with S. H. the laboratory measurement of fatty acids in biological specimens. T. I. and N. K. did the genotyping and wrote the section on genotyping. All authors contributed to the interpretation and discussion of the results and saw and approved the final version of this manuscript.

References


