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Review

Do *FADS* genotypes enhance our knowledge about fatty acid related phenotypes?

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SUMMARY

Several physiological processes, such as visual and cognitive development in early life, are dependent on the availability of long-chain polyunsaturated fatty acids (LC-PUFAs). Furthermore, the concentration of LC-PUFAs in phospholipids has been associated with numerous complex diseases like cardiovascular disease, atopic disease and metabolic syndrome. The level and composition of LC-PUFAs in the human body is mainly dependent on their dietary intake or on the intake of fatty acid precursors, which are endogenously elongated and desaturated to physiologically active LC-PUFAs. The delta-5 and delta-6 desaturase are the most important enzymes in this reaction cascade. In the last few years, several studies have reported an association between single nucleotide polymorphisms (SNPs) in the two desaturase encoding genes (*FADS1* and *FADS2*) and the concentration of omega-6 and omega-3 fatty acids. This shows that beside nutrition, genetic factors play an important role in the regulation of LC-PUFAs as well. This review focuses on current knowledge of the impact of *FADS* genotypes on LC-PUFA and lipid metabolism and discusses their influence on infant intellectual development, neurological conditions, metabolic disease as well as cardiovascular disease.

1. Introduction

The composition of polyunsaturated fatty acids (PUFAs) in phospholipids has been associated with early visual, cognitive and motor development,[1,2] mental health and psychiatric disorders,[3–5] metabolic syndrome,[6–8] cardiovascular disease mortality,[9,10] immunological and inflammatory responses[11] as well as related diseases such as allergies,[12–14] showing that PUFAs have a major impact on human health. These and other biological effects of PUFAs are assumed to be mediated by the availability of the long-chain polyunsaturated fatty acids (LC-PUFAs) with ≥20 carbon atoms and ≥3 double bonds, such as the omega-6 LC-PUFA arachidonic acid (AA; 20:4$n-6$), and the omega-3 LC-PUFAs eicosapentaenoic acid (EPA; 20:5$n-3$) and docosahexaenoic acid (DHA; 22:6$n-3$). These fatty acids have several important functions in human metabolism, such as regulating the integrity and fluidity of cell membranes. On the molecular level, LC-PUFAs act as second messengers in intracellular signaling pathways or regulate transcription. In addition, LC-PUFAs are precursors for eicosanoids (leukotrienes and prostaglandins) which play an important role in inflammatory processes.[15]

Because of its significant effect on blood and tissue LC-PUFA contents, sufficient dietary supply with LC-PUFAs is pivotal in every stage of human life.[16] Arachidonic acid is contained in meats and eggs, but mainly endogenously synthesized from $n-6$ essential fatty acid precursors. Marine foods are an important source for EPA and DHA.[17] Adequate supply with dietary LC-PUFAs is of special importance for the fetus and neonate during pregnancy and lactation to ensure optimal visual and cognitive development. Especially brain development is a critical issue in the first period of life, because brain growth accelerates during the second half of pregnancy and remains high during the first year of life.[18] It is therefore recommended that pregnant and lactating women achieve an average daily intake of at least 200 mg DHA.[19] LC-PUFA supply of the fetus by the mother is mediated by maternal–fetal placental transfer during pregnancy.[20,21] Sufficient LC-PUFA supply of the neonate can be provided by breastfeeding, which supplies preformed LC-PUFA for the child, and is therefore regarded as the preferred method of feeding during the first six months of life.[22] Besides the positive effects of LC-PUFAs on visual and cognitive development, there are also indications that early exposure to

Abbreviations: FADS, fatty acid desaturase; PUFA, polyunsaturated fatty acid; LC-PUFA, long-chain polyunsaturated fatty acid; AA or 20:4$n-6$, arachidonic acid; EPA or 20:5$n-3$, eicosapentaenoic acid; DHA or 22:6$n-3$, docosahexaenoic acid; LA or 18:2$n-6$, linoleic acid; ALA or 18:3$n-3$, alpha-linolenic acid; GLA or 18:3$n-6$, gamma-linolenic acid; SNP, single nucleotide polymorphism; LD, linkage disequilibrium; GWAS, genome-wide association study; HDL, high-density lipoprotein; LDL, low-density lipoprotein; BMI, body mass index; IQ, intelligence quotient; ADHD, attention-deficit/hyperactivity disorder; MI, myocardial infarction; CAD, coronary artery disease; hs-CRP, high-sensitivity C-reactive protein.

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dietary LC-PUFAs protects from high blood pressure and cardiovascular risk in later childhood, which is also thought to persist into adulthood.[23]

2. Endogenous production of LC-PUFAs from fatty acid precursors is mediated by delta-5 and delta-6 desaturase, encoded by *FADS1* and *FADS2*

Besides the dietary supply of preformed LC-PUFAs, they can also be derived endogenously from the precursor essential fatty acids, linoleic acid (LA or 18:2$n-6$) and alpha-linolenic acid (ALA or 18:3$n-3$), by consecutive desaturation and chain-elongation. The rate-limiting enzymes in this reaction cascade are the delta-6 and delta-5 desaturase, which are membrane-bound proteins with amino-terminal cytochrome *b*5 domains carrying heme-binding motifs, two-membrane-spanning domains, three His-box motifs, and which consist of 444 amino acids.[24] The human delta-5 desaturase shares 61% amino acid identity and 75% similarity to the human delta-6 desaturase.[25] The conversion of both omega-3 and omega-6 precursors to their respective LC-PUFA products is catalyzed by these two enzymes in a reaction cascade.[26] In the first step, delta-6 desaturase converts linoleic acid (18:2$n-6$) to gamma-linolenic acid (GLA or 18:3$n-6$) in the omega-6 pathway and alpha-linolenic acid (18:3$n-3$) to stearidonic acid (18:4$n-3$) in the omega-3 pathway by inserting an additional *cis* double bond at position 6 of the fatty acid chain. After an elongation step (resulting in dihomo-gamma-linolenic acid (20:3$n-6$) and eicosatetraenoic acid (20:4$n-3$), respectively), delta-5 desaturase catalyzes the formation of arachidonic acid (20:4$n-6$) and eicosapentaenoic acid (20:5$n-3$), which are either converted into eicosanoids or further elongated and desaturated, again with the help of a delta-6 desaturase.[15,27] Several studies have shown that the final desaturation step in docosahexaenoic acid (DHA or 22:6$n-3$) synthesis is catalyzed by the same delta-6 desaturase that acts also on 18-carbon PUFA substrates.[27–29] The fact that fibroblasts from a human case of delta-6 desaturase deficiency were unable to desaturate neither 18:2$n-6$ nor 24:5$n-3$ supports this hypothesis.[30] Although it was suggested that separate delta-6 desaturases may act on these substrates [31,32] based on studies with human malignant cell lines, no delta-6 desaturase isozyme specific to 24-carbon fatty acids is known at this time. Fig. 1 gives an overview of the desaturation pathway in humans. Cloning of the human desaturases was first reported in 1999[24,25] and the corresponding genes (*FADS1* for delta-5 desaturase and *FADS2* for delta-6 desaturase) were mapped to chromosome 11q12-13.1 of the human genome in 2000,[33] which shows conserved synteny to the mouse genomic region containing the murine *fads1* and *fads2* genes on chromosome 19.[15] The two human genes are arranged in a head-to-head orientation and build a gene cluster together with a third desaturase gene, *FADS3*. It is assumed that these three genes have arisen evolutionary from gene duplication, due to their similar exon/intron organization (12 exons and 11 introns) and a high degree of sequence homology.[33] Whereas the function of the delta-5 and delta-6 desaturase, encoded by *FADS1* and *FADS2*, respectively, is well known, the function of the protein product of the *FADS3* gene is still unrevealed. Park et al. [34] identified several alternative splice forms of *FADS3* and hypothesized a tissue- or PUFA-specific role of *FADS3* in LC-PUFA synthesis. Delta-5 and delta-6 desaturase are

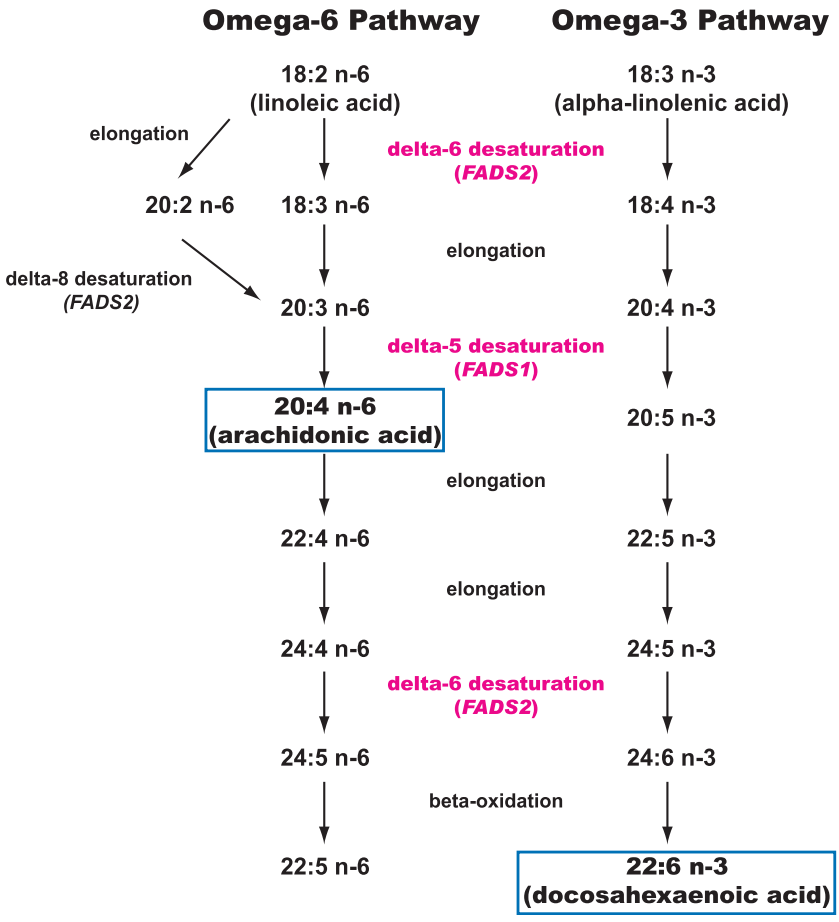

Fig. 1. Scheme of the metabolic pathway of omega-6 and omega-3 long-chain polyunsaturated fatty acids in humans.

expressed in the majority of human tissues, with highest levels in liver and to a smaller amount in brain, heart and lung.[24,25] Studies on substrate specificity and enzyme kinetics of both enzymes identified at least 5 substrates for the mammalian delta-6 desaturase so far (18:2*n* – 6, 18:3*n* – 3, 24:5*n* – 3, 24:4*n* – 6 and 16:0)[26,29,35–37] and showed an additional delta-8 desaturase activity of this enzyme on 20:2*n* – 6 and 20:3*n* – 3.[38] No other substrates beside 20:3*n* – 6 and 20:4*n* – 3 are known for the delta-5 desaturase up to date, which speaks for a stricter substrate specificity of this enzyme.

The importance of delta-6 desaturase for the formation of LC-PUFAs and their influence on membrane integrity and fluidity was substantiated in a recent study, in which an *fads2* knockout mouse was generated.[39] In this animal model, membrane polarity of Sertoli and ovarian follicle cells was completely disturbed due to the lack of LC-PUFAs in knockout mice caused by the delta-6 desaturase deficiency. Furthermore, both male and female mice were infertile and eicosanoid synthesis was disturbed. However, the administration of a LC-PUFA-rich diet (either C20:4*n* – 6 or C20:5*n* – 3/C22:6*n* – 3) enabled the *fads2*−/− mice to overcome the genetic defect, restored the fatty acid pattern in membrane lipids and rescued spermatogenesis as well as normal follicle development. Similarly, administration of arachidonic acid restored eicosanoid synthesis. Comparable effects were observed in a second *fads2*−/− mouse by Stroud et al.,[40] who additionally reported ulcerative dermatitis and ulceration of the small intestine in their mice. An *fads1* knockout mouse has not been described until now.

Williard et al.[30] described a female patient with reduced delta-6 desaturase activity, probably due to an inherited deficiency in delta-6 desaturase. This patient developed severe symptoms shortly after birth, including corneal ulceration, feeding intolerance, growth failure, skin abnormalities (cheilosis, dystrophic nails and perineal dermatitis), and photophobia. Neurological examinations were normal. Due to the abnormalities in her plasma fatty acid composition, the patient was provided with black current seed oil, fish oil capsules, and a vitamin A supplement as fatty acid therapy, which was subsequently switched to a mixture of 20:4*n* – 6 and DHA. This therapy led to an improvement of skin abnormalities as well as growth acceleration until normal height for her age.

3. *FADS* gene cluster polymorphisms are associated with LC-PUFA levels in different tissues as was shown by several candidate gene studies

The important function of the delta-5 and delta-6 desaturase in the synthesis of LC-PUFAs made the desaturase encoding genes perfect candidate genes for association studies of genetic polymorphisms with PUFA and LC-PUFA levels in human tissues. The human *FADS* gene cluster (including *FADS1*, *FADS2* and *FADS3*) comprises 91.9 kb on chromosome 11q12-13.1 with a head-to-head orientation of *FADS1* and *FADS2* and a tail-to-tail orientation of *FADS2* and *FADS3* (Fig. 2). All three genes have a similar exon/intron organization (12 exons, 11 introns), with *FADS1* spanning a 17.2 kb region, *FADS2* a 39.1 kb region and *FADS3* a 18.0 kb region. Introns 1 of *FADS1* and *FADS2* are separated by a 11.4 kb region and *FADS3* is located in the 6.0 kb telomeric side of *FADS2*. In this 91.9 kb region, around 500 single nucleotide polymorphisms (SNPs) are annotated in the NCBI database (dbSNP build 130). 18 selected SNPs in and around the *FADS1* and *FADS2* genes were for the first time analyzed by Schaeffer et al. for association with fatty acids in serum phospholipids in 727 German probands of the European Community Respiratory Health Survey I (ECRHS I).[41] Highly significant results were obtained for 11 SNPs, which are all located in the same LD block, and all analyzed fatty acids (p-values $< 1.0 \times 10^{-13}$) except for docosapentaenoic acid (22:5*n* – 6) and DHA (22:6*n* – 3). Carriers of the minor alleles of these 11 SNPs (rs174544, rs174553, rs174556, rs174561, rs3834458, rs968567, rs99780, rs174570, rs2072114, rs174583, and rs174589) had enhanced levels of 18:2*n* – 6, 20:2*n* – 6, 20:3*n* – 6, and 18:3*n* – 3 and decreased levels of 18:3*n* – 6, 20:4*n* – 6, 22:4*n* – 6, 20:5*n* – 3, and 22:5*n* – 3. Fatty acids belonging to other pathways like oleic acid (18:1*n* – 9) and DHA (22:6*n* – 3), whose source is mainly nutritional, did not show significant associations with the genetic variants. For SNPs beyond the LD block, the association with fatty acid levels weakened or vanished completely. Haplotype analyses were in line with the findings of the single SNP analysis. The variability in fatty acid levels explained by the genetic variants for the 11 analyzed SNPs varied from exceptionally high for arachidonic acid (28.5%) to low for 22:5*n* – 6 and 22:6*n* – 3 (1–3%). This first association study on *FADS* polymorphisms with fatty acid levels in serum phospholipids clearly showed a significant association with an accumulation of desaturase substrates and a decline of desaturase products due to the minor alleles of the associated SNPs. The authors concluded that this might be the case as a result of a decline in the transcriptional levels or in the conversion rates of the desaturases in subjects carrying the minor alleles.

The association between *FADS* gene cluster polymorphisms and fatty acids was replicated in three independent studies, which additionally showed an association with fatty acids in plasma, erythrocyte membrane, and breast milk phospholipids beside the already known association with serum phospholipids.[42–44] The analysis of erythrocyte membrane phospholipids is a valuable tool for the study of fatty acid metabolism, because their fatty acid composition resembles that of circulating lipoproteins, which are assembled in the liver. Furthermore, long-term effects of fatty acid regulation can be determined better in erythrocyte membranes, because the fatty acid composition of membranes is less influenced by short-term variations in dietary intake than serum/plasma phospholipids. The first replication study[42] analyzed 13 SNPs

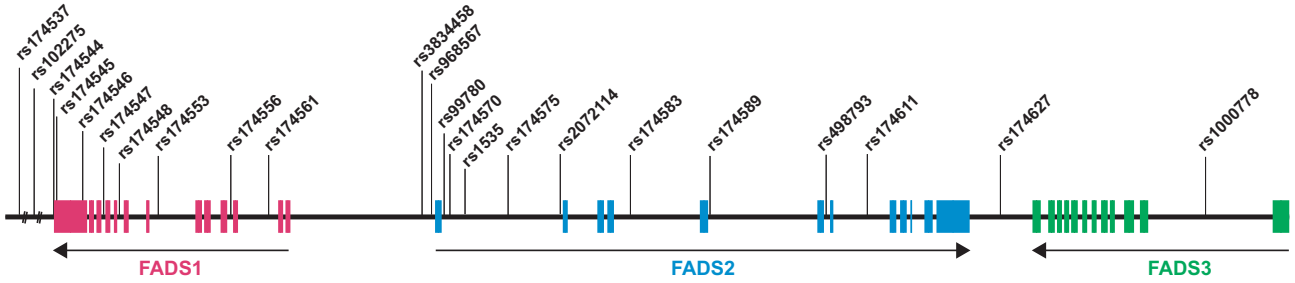

Fig. 2. Schematic structure of the human *FADS* gene cluster located on chromosome 11 with exon/intron organization and location of SNPs showing highest associations with fatty acid and lipid levels as well as complex phenotypes (compare Tables 1 and 2). SNPs rs174537 and rs102275 are located 8.6 kb and 13.7 kb upstream of the *FADS1* gene, change in scaling is indicated by //.

spanning the complete *FADS* gene cluster (including *FADS3*) in 658 Northern Italian subjects of the Verona Heart Project (VRHP). Highly significant results were observed for the majority of the analyzed *FADS1* and *FADS2* SNPs and arachidonic acid in serum as well as erythrocyte membrane phospholipids (p-value $< 1.0 \times 10^{-4}$). Up- or downregulation of all other analyzed fatty acids dependent on the allele was in accordance with the results shown by Schaeffer et al.,[41] although only 18:3$n-3$ and 20:2$n-6$ in serum phospholipids and 18:2$n-6$ and 20:2$n-6$ in erythrocyte membranes reached significance. Haplotype analysis revealed a significant association between the constructed *FADS* cluster haplotypes and the level of arachidonic acid in serum and erythrocytes (p-value $< 8.9 \times 10^{-4}$), but no significant association with all other fatty acids. This study showed evidence that *FADS* polymorphisms do not only contribute to the variability of short-term fatty acid levels in serum, but also to the variability in medium-term compartments, such as erythrocyte membranes. The second replication study, which was performed at the same time by Rzehak et al.,[43] confirmed the results from the two previous studies by analyzing the association of three *FADS* SNPs (rs174556, rs174561, and rs334458) with plasma and erythrocyte membrane fatty acids in 535 (for plasma) and 163 subjects (for erythrocyte membranes) of the Bavarian Nutrition Survey II. Xie et al.[44] genotyped four SNPs in *FADS1* and *FADS2* in 54 women for whom data on fatty acid concentrations in breast milk one month postpartum were available, to address the question whether *FADS* gene cluster polymorphisms have an influence on the fatty acid composition of human breast milk. The authors found significant associations of SNP rs174553 with the medium chain fatty acid 14:0 (p-value $= 4.6 \times 10^{-2}$), which is the end product of the de novo fatty acid synthase complex in the mammary gland and 18:1$n-7$ (p-value $= 1.0 \times 10^{-3}$), which is the delta-9 desaturation–elongation product of 16:0. For the same SNP, significant differences in PUFA concentration were obtained for 20:2$n-6$, 20:4$n-6$, 20:5$n-3$ and 22:5$n-3$ (p-value $< 2.6 \times 10^{-2}$). For SNP rs174575, concentrations of 20:4$n-6$, 22:5$n-6$, 20:5$n-3$ and also interestingly 22:5$n-3$ and 22:6$n-3$ differed significantly dependent on the allele (p-value $< 4.4 \times 10^{-2}$). The direction of change in PUFA/LC-PUFA concentrations was the same as observed in the previous studies. Simultaneously, the authors analyzed the influence of *FADS1–FADS2* polymorphisms on plasma phospholipid and erythrocyte ethanolamine phosphoglyceride (EPG) fatty acids in 69 pregnant women at week 16 of gestation, and found significant associations of rs174553 with plasma 18:2$n-6$, 20:4$n-6$, 22:5$n-3$ and 22:5$n-6$ (p-value $< 5.0 \times 10^{-3}$). An association with plasma 20:4$n-6$ was also found for SNP rs174575 (p-value $= 3.0 \times 10^{-3}$). For EPG fatty acids, an association of rs174553 with levels of 18:2$n-6$, 20:3$n-6$, 20:4$n-6$ and 22:4$n-6$ (p-value $< 3.8 \times 10^{-2}$) was observed, whereas no association with $n-3$ fatty acids was reported.

The fatty acid composition of breast milk is crucial for the sufficient supply of the breast-fed infant with important fatty acids for appropriate brain and visual development.[19,45–51] Especially for DHA (22:6$n-3$) it has been shown in a number of studies that the content in breast milk is highly dependent on the mother's dietary intake of this fatty acid.[45,52–59] It has been shown previously that both delta-6 and delta-5 desaturase are present in the lactating mammary gland.[60] However, whether the observed differences in lipogenesis and desaturase activities reflect differences in the mammary gland itself, or whether they are the consequence of altered substrate flux from other organs such as the liver remains speculative. The reason for the observed changes in saturated and monounsaturated fatty acid levels in the mammary gland due to *FADS* polymorphisms in the study of Xie et al.[44] is not understood, however, the reason could be complex feedback regulation mechanisms of the whole fatty acid synthesis pathway, activated by an accumulation of desaturase substrates due to the decreased activity of the desaturase enzymes. As with breast milk during lactation, the fatty acid composition of maternal blood during gestation influences fetal growth as well as maturation of visual and neural systems, by placental fatty acid transfer from the mother to the child.[47,61–63] Whether maternal genetic variants in the *FADS* gene cluster have a direct influence on infant fatty acid concentrations and are relevant for developmental outcomes remains an interesting question.

4. *FADS* gene cluster polymorphisms are among the most significant hits in genome-wide association studies of complex lipid traits

Since last year, several genome-wide association studies (GWAS) on complex lipid traits have been published. The first GWAS that reported a significant association of an *FADS1* polymorphism (rs174548) with altered levels of serum metabolites was published by Gieger et al. in 2008.[64] In this new approach, which combined GWAS data with metabolomics data, 187,454 SNPs were analyzed for association with 363 endogenous metabolites, including nine sugars, seven biogenic amines, seven prostaglandins, 29 acylcarnitines, 18 amino acids, 85 sphingolipids and 208 glycerophospholipids in fasting serum of 284 male participants of the KORA F3 study. SNP rs174548 (which is located in the LD block, which was previously associated with fatty acid levels in candidate gene approaches) was highly associated with several glycerophospholipid concentrations with the highest association for PC aa C36:4 (p-value $= 4.52 \times 10^{-8}$, genetically explained variance: 10.11%). PC aa C36:4 is a glycero-phosphatidylcholine with two esterified fatty acid side chains, most likely being 20:4$n-6$ and 16:0, because these represent major fatty acids in membranes. Carriers of the rs174548 minor allele variant (minor allele frequency of 27.5%) exhibited lower levels of numerous phosphatidylcholines (e.g. PC aa C34:4, PC aa C36:4, PC aa C36:5), plasmalogen/plasmenogen phosphatidylcholines (e.g. PC ae C36:4, PC ae C38:4, PC ae C38:5) and one phosphatidylinositol (PI aa C38:4) with four or more double bonds in their polyunsaturated fatty acid side chains than carriers of the major allele. Especially, arachidonic acid (20:4$n-6$) was found to be significantly reduced with increasing copy number of the minor allele. On the other hand, concentrations of glycerophospholipids with three or less double bonds in their PUFA side chains, like the phosphatidylcholines PC aa C34:2 and PC aa C36:2, the plasmalogen/plasmenogen phosphatidylcholines PC ae C34:2 and PC ae C36:2, the phosphatidylethanolamines PE aa C34:2 and PE aa C36:2 and the phosphatidylinositol PI aa C36:2, were increased in individuals carrying the minor allele of rs174548. In summary, all influences of those and some further associations could be explained by a modification in the efficiency of the fatty acid delta-5 desaturase (*FADS1*) reaction. This hypothesis was supported by the fact that by using concentration ratios of delta-5 desaturase product/substrate pairs, the association with the *FADS1* polymorphism increased by up to 14 orders of magnitude. The strongest effect size was observed for the ratio of PC aa C36:4 and PC aa C36:3 (p-value $= 2.4 \times 10^{-22}$, genetically explained variance: 28.62%). In PC aa C36:4, most likely the direct product of the delta-5 desaturase reaction 20:4$n-6$ is incorporated, whereas PC aa C36:3 contains the substrate of this reaction, 20:3$n-6$. PC aa C36:4 and PC aa C36:3 can therefore be considered as modified substrate and product of the delta-5 desaturase reaction. The association of rs174548 with the product/substrate pair of the delta-5 desaturase reaction was therefore assumed a strong indicator of the efficiency of the *FADS1* reaction, indicating that carriers of the minor allele

have decreased delta-5 desaturase activity. The authors have shown in this study that rs174548 in the *FADS1* gene strongly influences the homeostasis of glycerophospholipids in serum, which was also thought to affect cholesterol metabolism. Indeed, associations of the *FADS1* polymorphism with corresponding serum parameters, such as high-density lipoprotein (HDL), low-density lipoprotein (LDL) cholesterol and total cholesterol levels in serum were reported previously in two GWAS with up to 18,000 participants[65,66] and p-values between 1.89×10^{-4} and 6.07×10^{-5}. However, the p-values of these associations were not sufficiently small in the context of a classical GWAS study, and were therefore not included in the list of potential candidates for replication.

Final evidence that associations with HDL, LDL and total cholesterol levels in these two GWAS were true positive results despite they did not reach genome-wide significance level, came from three additional genome-wide association studies, all of them published end of 2008.[67–69] Aulchenko et al.[67] analyzed loci affecting total cholesterol, LDL and HDL cholesterol as well as triglycerides sampled randomly from 16 European population-based cohorts. This study included 17,797–22,562 participants aged 18–104 years. 22 loci were found to be associated on a genome-wide significance level (p-value $< 5.0 \times 10^{-8}$) with serum lipid levels, including SNP rs174570 which is located in intron 1 of the *FADS2* gene. This SNP showed significant associations with total cholesterol (p-value $= 1.5 \times 10^{-10}$) and LDL levels (p-value $= 4.4 \times 10^{-13}$). Carriers of the major G allele exhibited higher levels of total cholesterol (effect size $= 0.088$) and LDL (effect size $= 0.110$) than carriers of the minor A allele. A meta-analysis of seven GWAS of blood lipoprotein and lipid phenotypes was performed by Kathiresan et al.[68] including 19,840 individuals followed by replication analysis in 20,623 subjects. 30 loci were associated with lipoprotein concentrations, including 11 loci that showed genome-wide significance for the first time. One of these loci (rs174547) is located in the *FADS1* gene and was associated with HDL (p-value $= 2.0 \times 10^{-12}$) and triglyceride (p-value $= 2.0 \times 10^{-14}$) levels. Carriers of the minor C allele had lower levels of HDL (effect size $= -0.09$) and higher levels of triglycerides (effect size $= 0.06$). Interestingly, the authors also explored the influence of rs174547 on gene expression of the *FADS* genes. For this purpose, they genotyped DNA and profiled RNA expression of over 39,000 transcripts in 957 human liver tissue samples as reported previously.[70] The expression quantitative trait locus (eQTL) analyses suggested that the associated SNP rs174547 modulates gene expression of *FADS1* and *FADS3*. The major T allele was associated with higher transcript levels (p-value (*FADS1*) $= 5.0 \times 10^{-35}$, p-value (*FADS3*) $= 1.0 \times 10^{-8}$) and led to higher HDL cholesterol and lower triglyceride levels. Although it is known that $n-3$ PUFAs are able to lower plasma triglycerides, possibly by decreasing very-low-density lipoprotein secretion,[71] the exact mechanism by which genetic variants, dietary fatty acids and LC-PUFAs influence expression of the desaturase enzymes and how this is in turn connected to blood lipid levels remains unknown. Another genome-wide association study on global gene expression in lymphoblastoid cell lines[72] confirmed the results of Schadt et al.[70] by showing that those alleles that were associated with higher desaturase product levels (major alleles) lead to an increased expression level of *FADS1*, but not *FADS2*. Genotype-dependent analysis of *FADS* gene expression in other tissues does not exist up to date.

Sabatti et al.[69] analyzed nine quantitative metabolic traits (triglycerides, high-density lipoprotein, low-density lipoprotein, glucose, insulin, C-reactive protein, body mass index (BMI), and systolic and diastolic blood pressure) in 4763 individuals participating in the Northern Finland Birth Cohort 1966 (NFBC1966). The authors found a significant association of five SNPs (rs174537, rs102275, rs174546, rs174556, and rs1535) in the *FADS1–FADS2* gene cluster with LDL levels and p-values ranging between 1.30×10^{-7} and 3.65×10^{-7} after adjustment for BMI. Carriers of the minor alleles exhibited lower LDL levels with effect sizes ranging from -0.092 to -0.096, which is in agreement with the results of Aulchenko et al.[67] The latest GWAS, published by Tanaka et al. in 2009, attempted to identify additional genetic contributors to plasma PUFA concentrations.[73] The authors analyzed plasma levels of six omega-3 and omega-6 fatty acids in 1075 participants in the InCHIANTI study and found strongest associations in the *FADS* gene cluster. SNP rs174537, which is located at genomic position 61,309,256 in the 14.4 kb 5′ region of *FADS1*, showed the most significant association with arachidonic acid (20:4$n-6$) (p-value $= 5.95 \times 10^{-46}$). Individuals homozygous for the minor allele of rs174537 had lower concentrations of 20:4$n-6$ compared to the homozygous carriers of the major allele. The SNP accounted for 18.6% of the variance in the concentration of 20:4$n-6$. Furthermore, the SNP was associated with altered levels of 20:2$n-6$ (p-value $= 6.78 \times 10^{-9}$) and 20:5$n-3$ (p-value $= 1.04 \times 10^{-14}$). Interestingly, the direction of association of rs174537 with fatty acids was consistent with previous reports, with the exception of 20:2$n-6$. The reason for this discrepancy is unknown. The association with 18:2$n-6$ and 18:3$n-3$ did not reach genome-wide significance (p-values $= 5.58 \times 10^{-7}$ and 2.76×10^{-5}, respectively) and there was no association with 22:6$n-3$ at all. Individuals carrying the major allele also showed higher LDL (p-value $= 0.011$) and total cholesterol levels (p-value $= 0.027$). The effects of rs174537 were confirmed in an independent sample of 1076 participants of the GOLDN study in erythrocyte membranes. The authors also speculated about the relationship between genetic variation in *FADS* genes, endogenous level of PUFAs and its consequence on blood lipid levels. All GWAS on complex lipid traits reported decreased cholesterol levels, decreased LDL levels, decreased HDL levels and increased triglyceride levels in carriers of minor alleles of those SNPs analyzed in the individual studies (not every study analyzed the same SNP and not every study reported significance with all four lipid traits, but the results are consistent). The authors argue that the minor alleles, which are assumed to lead to a lower desaturase expression or activity, and the following observed decrease in LC-PUFAs, lead to a reduction in PPARA activation. Endogenous LC-PUFAs are natural ligands of PPARA (peroxisome proliferator activating receptor alpha),[74] whose activation has been shown to elevate HDL levels and lower triglyceride levels by inducing expression of ApoAI, Apo-AII, lipoprotein lipase and by suppressing ApoCIII.[75–78] Thus the lower amount of LC-PUFAs is supposed to result in lower PPARA activation, decreased HDL levels and increased triglyceride levels. However, because PPARA is known to enhance LDL-C clearance,[79] one would also expect higher LDL levels in minor allele carriers, but this is not the case. Other regulatory mechanisms are therefore likely. Finally, one should not forget the influence of diet and lifestyle on blood lipid levels, which make the whole story even more complicated, and the influence of the gene–diet interaction on blood lipid levels is definitely worth several more studies. Table 1 summarizes all studies on fatty acid levels and lipid traits published to date with significant associations found for the *FADS* gene cluster.

5. *FADS* gene cluster polymorphisms may modulate mental development and complex diseases

5.1. FADS polymorphisms and mental ability

Although contradictory results exist, it is widely assumed that sufficient supply of the child with $n-6$ and $n-3$ LC-PUFAs before and after birth is absolutely necessary for proper cognitive, visual and motor development, and recommendations on an average DHA

Table 1
Studies with significant associations between *FADS* polymorphisms and fatty acid/lipid concentrations.

Study	*N*	Most significant SNP(s)	Measured metabolites	Most significant metabolite
Schaeffer et al.	727	rs174544, rs174553, rs174556, rs174561, rs3834458, rs968567, rs99780, rs174570, rs2072114, rs174583 and rs174589	Fatty acids in serum phospholipids	20:4$n-6$ ($p < 1.0 \times 10^{-13}$) in serum
Malerba et al.	658	rs174545, rs174556, rs174561, rs3834458, rs174570, rs174583, rs174589, rs174611 and rs174627	Fatty acids in serum phospholipids and erythrocyte membranes	20:4$n-6$ ($p < 1.0 \times 10^{-4}$) in serum and erythrocytes
Rzehak et al.	163 (plasma) 535 (erythrocytes)	rs174556, rs174561 and rs3834458	Fatty acids in plasma phospholipids and erythrocyte membranes	20:3$n-6$ ($p = 7.9 \times 10^{-10}$) in erythrocytes; plasma: data not shown
Xie et al.	69 (plasma/ erythrocytes) 54 (breast milk)	rs174553	Fatty acids in plasma and erythrocyte phospholipids and in breast milk	18:2$n-6$, 20:2$n-6$ and 20:4$n-6$ ($p < 1.0 \times 10^{-3}$) in plasma; 20:3$n-6$ ($p < 1.0 \times 10^{-3}$) in erythrocytes; 18:1$n-7$ ($p = 1.0 \times 10^{-3}$) in breast milk
Gieger et al.	284	rs174548	363 Serum metabolites (e.g. acylcarnitines, phosphatidylcholines)	PC aa C36:4 ($p = 4.52 \times 10^{-8}$) and PC aa C36:4/PC aa C36:3 ($p = 2.4 \times 10^{-22}$)
Aulchenko et al.	17,797–22,562	rs174570	Blood lipid parameters	Total cholesterol ($p = 1.5 \times 10^{-10}$) and LDL ($p = 4.4 \times 10^{-13}$)
Kathiresan et al.	19,840 plus 20,623 (replication)	rs174547	Blood lipid parameters	Triglycerides ($p = 2.0 \times 10^{-14}$) and HDL ($p = 2.0 \times 10^{-12}$) (combined *p*-values)
Sabatti et al.	4763	rs174546, rs102275, rs174537, rs174556, rs1535	Metabolic traits (e.g. lipids, glucose)	LDL ($p = 1.3 \times 10^{-7}$–3.65×10^{-7})
Tanaka et al.	1210 plus 1,076 (replication)	rs174537	Plasma fatty acids and blood lipid parameters	20:4$n-6$ ($p = 5.95 \times 10^{-46}$), total cholesterol ($p = 2.7 \times 10^{-2}$) and LDL ($p = 1.1 \times 10^{-2}$) (*p*-values from initial study)

N = number of subjects in the study, *p*-values correspond to results from single SNP analyses.

intake of at least 200 mg per day for pregnant and lactating women exist.[19] Maternal intake of $n-6$ and $n-3$ LC-PUFAs is of special importance during pregnancy, because desaturation activity in the fetal liver was shown to be low before birth[80] and LC-PUFAs are derived predominantly through placental transfer, with the amounts in cord blood influenced by the maternal diet.[16] The effects of maternal LC-PUFA consumption during pregnancy on the child's visual and cognitive development have been investigated in numerous studies. For example, Malcolm et al. provided fish oil during pregnancy and found that the DHA status of the infants at birth was related to improved visual development at 2.5 and six months of age.[81] Another example showed, that eye-hand coordination at the age of 2.5 years was improved in infants whose mothers received high dose fish oil during pregnancy.[82] A beneficial effect of maternal fish consumption during pregnancy on the child's visual recognition memory[83] as well as on the verbal intelligence quotient up to an age of eight years[84] has been reported. In a double blind randomized trial, maternal supplementation during pregnancy and lactation with cod liver oil led to a higher intelligence scores in children tested at the age of four years.[48] Human breast milk contains $n-3$ and $n-6$ LC-PUFAs as well as precursor fatty acids, which can be further elongated and desaturated by the breast-fed child.[85–88] The amount of especially DHA in human milk, depends in turn on maternal diet and lifestyle,[52–54,57–59] and has been positively correlated to visual development in breast-fed infants in several studies.[51,89] The effect of breastfeeding with or without LC-PUFA supplementation of the mother, or feeding LC-PUFA supplemented formula, on subsequent intelligence quotient (IQ) of the child is very contradictory because of the numerous possible confounders such as duration of breastfeeding, sex, maternal age, maternal/paternal intelligence, maternal/paternal education, socioeconomic status, family size, birth order, birth weight and childhood experiences.[90] Several studies exist, where hints towards a positive association between breastfeeding and intelligence were observed, but others could not confirm these results (e.g. Refs.[48,49,91,92]). Trials that investigated the effect of direct infant LC-PUFA supplementation after birth via formulae on potential long-term benefits such as stereoacuity, vision and IQ did not report significant findings.[93–95] Numerous randomized trials with polyunsaturated fatty acid interventions in preterm and term infants, which analyzed effects on visual, neural, or developmental outcomes, exist (as reviewed in Ref.[96]), with some studies reporting significant effects, but a similar number reporting no significant effects. One reason for such inconsistent and ambiguous results could be inter-individual variations in LC-PUFA metabolism, which lead to unprecise study results. In 2007, Caspi et al. conducted a study, where they showed that a genetic variant in the *FADS2* gene (rs174575) modulated the association between breastfeeding and IQ in two birth cohorts with 1037 and 2232 subjects, respectively[97] (*p*-value of interaction = 0.035 and 0.018, see Table 2). In both cohorts, there was a difference in IQ test scores between breast-fed and not breast-fed children, but this effect was more pronounced

Table 2
Studies with associations between *FADS* polymorphisms and fatty acid related phenotypes.

Study	*N*	Most significant SNP(s)	Outcome	*p*-Value
Caspi et al.	1037 plus 2140 (replication)	rs174575	Modulation of breastfeeding effect on intelligence development	*p*-Value of interaction = 3.5×10^{-2} and 1.8×10^{-2} (replication)
Brookes et al.	360	rs498793	Attention-deficit/hyperactivity disorder	4.0×10^{-3}
Baylin et al.	3388	rs3834458	Nonfatal acute myocardial infarction	Not significant
Martinelli et al.	876	rs174545, rs174570, rs174583, rs1000778	Coronary artery disease	*p*-Value for an additive model of increasing number of risk alleles = 6.0×10^{-3}
Truong et al.	1815	rs3834458	Modulation of effect of ALA concentration on metabolic syndrome prevalence ratio	*p*-Value for interaction = 8.0×10^{-2}

N = number of subjects in the study.

and only significant in children carrying the rs174575 major C allele (IQ point advantage = 6.35 and 7.91, respectively, p-value < 0.001), whereas children with the minor G allele neither gained an advantage nor suffered a disadvantage from being fed breast milk. The child's rs174575 genotype was not related to IQ directly in either cohort. This association remained significant in both cohorts after adjustment for social class and maternal IQ as possible confounders. It was also ruled out that the child's genotype had an effect on different exposure to breastfeeding and on differences in intrauterine growth. To test the possibility of a maternal genotype effect on the child's IQ, mothers were genotyped in one of the cohort studies, but there were no significant IQ differences among children fed breast milk as a function of maternal genotype detectable. The author's therefore concluded that the rs174575 moderation of breastfeeding effects on IQ is due to genetic differences in children's LC-PUFA metabolism. They furthermore argued, as already mentioned above, that the inconsistent results obtained in studies comparing the neurodevelopment of children with degree of LC-PUFA supplementation, could be explained by the genetic heterogeneity in fatty acid metabolism and therefore suggest to include genetic analyses in future studies, which we also consider essential for future research. Although this study is an impressive example of how genetic variants can modulate environmental exposures to gain benefits for human life and health, the mechanisms by which *FADS* polymorphisms influence the regulation, concentration and composition of fatty acids are unknown and the physiological effects of LC-PUFAs on intelligence development are not understood completely. Further studies will be needed to determine the influence of the mother's genotype on transfer of fatty acids to the child during pregnancy and lactation, which has not been investigated until now. It has been shown that maternal *FADS* polymorphisms influence the fatty acid composition of breast milk,[44] but the effect on the child has not been investigated so far. The relationship between *FADS* polymorphisms, arachidonic acid levels and DHA levels needs to be examined in detail. DHA is described by many authors as the candidate fatty acid for the observed positive effects of both breastfeeding and LC-PUFA supplementation due to its presence in high quantities within the brain and retina. However, in most studies, DHA levels are not significantly associated with *FADS* genotypes, and are discussed to be more dependent on dietary intake, except in the study of Xie et al.,[44] where a significant association in breast milk was described. The question is how *FADS* polymorphisms modulate mental development if not by changing DHA levels directly. It may be assumed that the levels of arachidonic acid either obtained through endogenous conversion by the infant or provided in breast milk, which are regulated by the *FADS* genotype will alter the specific levels of DHA by yet unknown regulatory mechanisms. The elucidation of these regulatory pathways is essential to understand the biological effects of the respective fatty acids better. Also, to understand the impact of genetic variants on regulatory mechanisms, functional studies are required to identify the causal variants.

Besides the analysis of effects of *FADS* polymorphisms on intelligence, genetic variants in this region have also been associated with other mental outcomes. Brookes et al. reported an association of SNP rs498793 in the *FADS2* gene with attention-deficit/hyperactivity disorder (ADHD) with a p-value of 0.004 and an odds ratio of 1.6 in 180 ADHD cases compared to controls[98] (Table 2). This result is supported by three previous linkage scans for ADHD, two of which have identified a significant linkage peak at chromosome 11q2, which is overlapping with the location of the desaturase genes.[99–101] It has been shown that ADHD subjects have significantly decreased levels of plasma omega-3 fatty acids compared to control subjects,[102–106] which might in turn influence the regulation of dopamine concentrations in the prefrontal cortex as was observed in animal models.[107–114] The abnormal regulation of this neurotransmitter is thought to be one cause for the onset of ADHD.[115,116] An indication of an association of *FADS* gene polymorphisms with bipolar disorder was provided by a genome-wide association study of seven common diseases by the Wellcome Trust Case Control Consortium,[117] which is supported by the identification of a linkage peak in the *FADS* gene chromosomal region in a previous linkage scan on bipolar disorder,[118] although this was a very weak association. Previous randomized control trials of LC-PUFA supplementation with different combinations of $n-3$ and $n-6$ fatty acids for the treatment of ADHD, bipolar disorder and other neurological conditions have in some cases shown positive results, in others they showed no effect.[119–121] Again, the additional exploration of *FADS* genotypes in studies for the analysis of these diseases may result in more definitive findings in the future and explain the biological effects of LC-PUFAs on the onset of such diseases better.

5.2. FADS polymorphisms and coronary artery disease

Because LC-PUFAs are important precursors in the formation of prostaglandins and leukotrienes, their role in inflammatory processes has long been subject of speculation. Especially arachidonic acid (20:4$n-6$) is thought to play a role in atherosclerotic processes,[122] because high concentrations of this fatty acid in adipose tissue have been associated with a greater risk of myocardial infarction, suggesting a proatherosclerotic role of excess 20:4$n-6$.[123,124] On the other hand, $n-3$ fatty acids such as 18:3$n-3$ seem to have a protective effect on the risk of nonfatal acute myocardial infarction (MI).[125,126] Baylin et al. analyzed the association of an *FADS2* gene promoter deletion polymorphism (rs3834458) with the risk of nonfatal myocardial infarction in 1694 Costa Rican case subjects.[127] The authors were able to replicate the already known association of several fatty acids with the *FADS2* promoter variant in plasma samples, and interestingly, also in adipose tissue samples. The observed pattern of $n-6$ and $n-3$ fatty acids in adipose tissue was consistent with the plasma fatty acid pattern, and showed for the first time an influence of the *FADS2* promoter variant on fatty acid composition in adipose tissue as long-term compartment of fatty acid regulation. The effect of this polymorphism on plasma triglycerides was the same as in previous genome-wide association studies,[65,66,68] whereas total cholesterol, HDL, and LDL were not associated with the genotype. No association was observed of polymorphism rs3834458 with MI risk, and the variant did not modify the association of 18:3$n-3$ with decreased risk of MI (see Table 2). However, in a subgroup of subjects with low dietary intakes of 18:3$n-3$, the interaction between *FADS2* genotype, 18:3$n-3$ in adipose tissue and the risk of MI was significant (p-value = 0.047), suggesting that at low intakes of 18:3$n-3$, its protective effect is attenuated among carriers of the minor allele. This suggests tightly balanced regulatory mechanisms between dietary and endogenous fatty acids as well as desaturase activity and shows that appropriate dietary intake of fatty acids can obviously overcome genetic defects.

A second study dealing with *FADS* genotypes and coronary artery disease (CAD) was performed by Martinelli et al.[128] (Table 2). 13 SNPs in the *FADS1/2/3* gene cluster were genotyped in 876 subjects (610 cases and 266 controls) from the Verona Heart Study and the concentration of numerous fatty acids in plasma and erythrocyte membrane phospholipids was determined. Already known associations of *FADS* genotypes with several fatty acids in both tissues could be replicated, but no single variant was differently distributed between CAD cases and controls. By using a more complex additive model of *FADS* SNPs, *FADS* haplotypes, or both,

a clearly significant effect on high-sensitivity C-reactive protein (hs-CRP) concentrations and on a person's susceptibility to CAD was shown. With an increasing number of risk alleles (those alleles that led to increased desaturase activity), the concentrations of the inflammatory marker hs-CRP increased and carriers of these unfavorable haplotypes suffered more often from CAD than the carriers of less risk alleles. Finally, an influence of *FADS* polymorphisms in the development of CAD was supported by the findings in the WTCCC study, which reported an association of *FADS* polymorphisms with CAD.[117]

5.3. FADS polymorphisms and metabolic syndrome

Several studies have reported an association with PUFA composition in phospholipids and the development of the metabolic syndrome.[6–8] Risk factors that define the metabolic syndrome include obesity, high blood pressure, high plasma triglyceride concentrations, low HDL cholesterol, impaired fasting glucose, and others. Metabolic syndrome is a frequent precursor to type 2 diabetes mellitus and myocardial infarction.[129] It has been shown that dietary intake of $n-3$ LC-PUFAs improves certain components of the metabolic syndrome.[130–136] One possible mechanism might be the ability of these long-chain $n-3$ fatty acids to lower plasma triacylglycerols which has often been mentioned in the literature.[137–139] The essential fatty acid alpha-linolenic acid (18:3$n-3$) can be converted endogenously into $n-3$ LC-PUFAs by the desaturation–elongation pathway, and dietary intake of this fatty acid, which is predominantly found in vegetable oils such as soybean, canola and flaxseed oil, could therefore contribute to the protective effect of $n-3$ fatty acids on metabolic syndrome. Evidence for this hypothesis was not available,[140,141] until Truong et al. examined whether increased 18:3$n-3$ is associated with a lower prevalence of the metabolic syndrome and whether this association is due to a direct effect of 18:3$n-3$ or its conversion to longer chain $n-3$ fatty acids by including the *FADS2* rs3834458 genotype in the analysis in 656 metabolic syndrome cases and 1159 controls[142] (Table 2). Subjects with the metabolic syndrome had significantly lower 18:3$n-3$ concentrations and higher amounts of 20:4$n-6$ (p-value $< 1.0 \times 10^{-4}$) in adipose tissue. The *FADS2* genotype was not differentially distributed across subjects with and without the metabolic syndrome. In the interaction analysis, subjects with high 18:3$n-3$ concentrations in adipose tissue had a lower prevalence of metabolic syndrome across all three genotypes, however, subjects carrying the major T allele of rs3834458 had the lowest prevalence ratio when compared to the other genotypes (p-value for interaction $= 0.08$). Although the interaction was not significant, this observation suggests that 18:3$n-3$ exerts its protective effects by its endogenous conversion into long-chain $n-3$ fatty acids, because persons with decreased desaturase activity caused by the presence of the minor allele of rs3834458 in the *FADS2* gene, do not benefit from an increased dietary intake of 18:3$n-3$. This is another very interesting example of how *FADS* polymorphisms modulate the effect of dietary fatty acids.

6. Conclusion and outlook

This review summarized the effects of *FADS* gene cluster polymorphisms on fatty acid and lipid levels in different human tissues as well as the modulating effect of these polymorphisms on fatty acid related phenotypes. *FADS* genotypes account for up to 28% of the variability observed for fatty acid levels,[41,64] and are therefore, besides the regulation by dietary fatty acids and hormonal signals, important regulators of desaturase activity and thus the balance of PUFAs and LC-PUFAs in the human body. First data on functionally relevant variants exist,[143] which are necessary to improve our understanding of desaturase regulation pathways and which might help to explain the contribution of polymorphisms in LC-PUFA regulation better. It has been shown that *FADS* genotypes have a modulating effect on fatty acid related phenotypes such as intelligence development, risk of myocardial infarction and metabolic syndrome.[97,127,142] This might be due to altered endogenous synthesis and thus availability of LC-PUFAs dependent on the *FADS* genotype. Large gene–nutrition-interaction studies on complex phenotypes like mental development, cardiovascular disease or metabolic syndrome are urgently needed to determine the influence of *FADS* polymorphisms on the onset of such diseases in the context of individual dietary fatty acid intake. The inclusion of *FADS* genotypes as well as diet and lifestyle factors in future randomized control trials addressing biological effects of PUFAs and LC-PUFAs is strongly recommended, because this will lead to enhanced sensitivity and precision of such studies and will reveal stronger findings.

Conflict of interest

The authors do not report a potential conflict of interest.

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