

FADS Gene Cluster Polymorphisms: Important Modulators of Fatty Acid Levels and Their Impact on Atopic Diseases

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Key Words

Arachidonic acid · Atopy · Δ^5 desaturase · Δ^6 desaturase · Single nucleotide polymorphism

Abstract

Long-chain polyunsaturated fatty acids (LC-PUFAs) play an important role in several physiological processes and their concentration in phospholipids has been associated with several complex diseases, such as atopic disease. The level and composition of LC-PUFAs in the human body is highly dependent on their intake in the diet or on the intake of fatty acid precursors, which are endogenously elongated and desaturated to physiologically active LC-PUFAs. The most important enzymes in this reaction cascade are the Δ^5 and Δ^6 desaturase. Several studies in the last few years have revealed that single nucleotide polymorphisms (SNPs) in the 2 desaturase encoding genes (*FADS1* and *FADS2*) are highly associated with the concentration of ω -6 and ω -3 fatty acids, showing that beside nutrition, genetic factors also play an important role in the regulation of LC-PUFAs. This review focuses on current knowledge of the impact of genetic polymorphisms on LC-PUFA metabolism and on their potential role in the development of atopic diseases.

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Introduction

Polyunsaturated fatty acids (PUFAs) have a major impact on human health. The composition of PUFAs in phospholipids has, among other complex diseases, been associated with the development of allergies [1–3]. The biological effects of PUFAs are thought to be mediated by the availability of long-chain polyunsaturated fatty acids (LC-PUFAs) with ≥ 20 carbon atoms and ≥ 3 double bonds, such as the ω -6 LC-PUFA arachidonic acid (20:4 ω -6), the ω -3 LC-PUFAs eicosapentaenoic acid (20:5 ω -3) and docosahexaenoic acid (22:6 ω -3). These fatty acids have several important functions in human metabolism. The composition of LC-PUFAs such as arachidonic acid and docosahexaenoic acid in phospholipids influences the integrity and fluidity of cell membranes. On the molecular level, LC-PUFAs fulfill several other central functions, such as acting as second messengers in intracellular signaling pathways or regulating transcription. In addition, LC-PUFAs are precursors for eicosanoids (leukotrienes and prostaglandins) which play an important role in inflammatory processes [4].

Sufficient dietary supply with LC-PUFAs is therefore pivotal in every stage of human life because it has a significant effect on blood and tissue LC-PUFA contents [5]. Besides its predominant endogenous synthesis from ω -6 fatty acid precursors, arachidonic acid is contained in meats and eggs, whereas marine foods are an important

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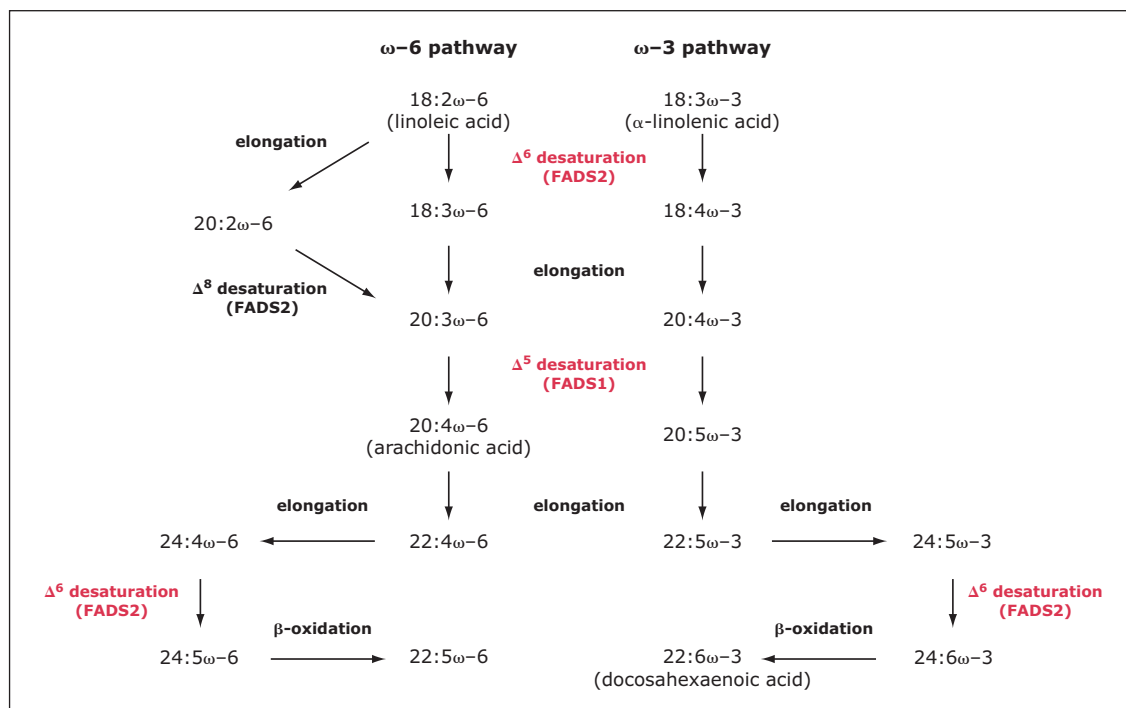


Fig. 1. Metabolic pathway of ω -6 and ω -3 LC-PUFAs in humans.

source for eicosapentaenoic acid and docosahexaenoic acid [6]. 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. The supply of LC-PUFA to the fetus by the mother is mediated by maternal-fetal placental transfer during pregnancy [7, 8]. Sufficient LC-PUFA can be supplied to the neonate by breastfeeding, which supplies preformed LC-PUFA for the child, and is therefore regarded as the preferred method of feeding during the first 6 months of life [9]. Besides the positive effects of LC-PUFAs on visual and cognitive development, a protective effect of LC-PUFAs on allergy development in children was shown in 2 different studies, assuming that early LC-PUFA provision may modulate immune responses [10, 11].

The Important Role of Δ^5 and Δ^6 Desaturase, Encoded by *FADS1* and *FADS2*, in Endogenous Production of LC-PUFAs

In addition to the dietary supply of preformed LC-PUFAs, they can also be derived by human metabolism from the precursor essential fatty acids, linoleic acid (18:2 ω -6)

and α -linolenic acid (18:3 ω -3), by consecutive desaturation and chain-elongation. The rate-limiting enzymes in this reaction cascade are the Δ^5 and Δ^6 desaturase. These enzymes are membrane-bound proteins with amino-terminal cytochrome *b5* domains carrying heme-binding motifs, 2 membrane-spanning domains, 3 His-box motifs and consist of 444 amino acids [12]. The human Δ^5 desaturase shares 61% amino acid identity and 75% similarity to the human Δ^6 desaturase [13]. The 2 enzymes catalyze the conversion of both ω -3 and ω -6 precursors to their respective LC-PUFA products in a reaction cascade [14]. In the first step, Δ^6 desaturase converts linoleic acid (18:2 ω -6) to γ -linolenic acid (18:3 ω -6) in the ω -6 pathway and α -linolenic acid (18:3 ω -3) to stearidonic acid (18:4 ω -3) in the ω -3 pathway by inserting an additional *cis* double bond at position 6 of the fatty acid chain. After an elongation step [resulting in dihomogamma-linolenic acid (20:3 ω -6) and eicosatetraenoic acid (20:4 ω -3), respectively], Δ^5 desaturase catalyzes the formation of arachidonic acid (20:4 ω -6) and eicosapentaenoic acid (20:5 ω -3). These molecules are either converted into eicosanoids or further elongated and desaturated, again with the help of a Δ^6 desaturase [4, 15], resulting in several important LC-PUFAs, among them docosahexaenoic acid

(22:6 ω -3). Several studies have shown that the final desaturation step in 22:6 ω -3 synthesis is catalyzed by the same Δ^6 desaturase that acts also on 18-carbon PUFA substrates [15–17]. The fact that fibroblasts from a human case of Δ^6 desaturase deficiency were unable to desaturate 18:2 ω -6 or 24:5 ω -3 support this hypothesis [18]. Although studies with human malignant cell lines suggested that separate Δ^6 desaturases may act on these substrates [19, 20], no Δ^6 desaturase isozyme specific to 24-carbon fatty acids is known at this time. An overview of the desaturation pathway is given in figure 1. The human desaturases were first cloned in 1999 [12, 13] and the corresponding genes (*FADS1* for Δ^5 desaturase and *FADS2* for Δ^6 desaturase) were mapped to chromosome 11q12–13.1 of the human genome in 2000 [21], which shows conserved synteny to the mouse genomic region containing the murine *fads1* and *fads2* genes on chromosome 19 [4]. The 2 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, due to their similar exon/intron organization (12 exons and 11 introns) and a high degree of sequence homology, these 3 genes have arisen evolutionary from gene duplication [21]. While the function of the Δ^5 and Δ^6 desaturase, encoded by *FADS1* and *FADS2*, respectively, is well characterized, the function of the protein product of the *FADS3* gene is still unknown. Park et al. [22] identified several alternative splice forms of *FADS3* that are expressed in many tissues showing changes in abundance in response to human neuronal cell differentiation, and hypothesized a tissue- or PUFA-specific role of *FADS3* in LC-PUFA synthesis. Δ^5 and Δ^6 desaturase are expressed in the majority of human tissues, with highest levels in liver and, to a lesser extent, in the brain, heart and lung [12, 13]. Studies on substrate specificity and enzyme kinetics of both enzymes exist, which have identified at least 5 substrates for the mammalian Δ^6 desaturase so far (18:2 ω -6, 18:3 ω -3, 24:5 ω -3, 24:4 ω -6 and 16:0) [14, 17, 23–25] and showed an additional Δ^8 desaturase activity of this enzyme on 20:2 ω -6 and 20:3 ω -3 [26]. For the Δ^5 desaturase, no other substrates beside 20:3 ω -6 and 20:4 ω -3 are known to date, which speaks for a stricter substrate specificity of this enzyme.

The importance of Δ^6 desaturase for the formation of LC-PUFAs and their influence on membrane integrity and fluidity was shown in a recent study by Stoffel et al. [27] who generated a *fads2*^{-/-} mouse. In this animal model, the membrane polarity of Sertoli and ovarian follicle cells was completely disturbed due to the lack of LC-PUFAs in knockout mice caused by the Δ^6 desaturase defi-

ciency. 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 ω -6 or C20:5 ω -3/C22:6 ω -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, eicosanoid synthesis was restored by administration of arachidonic acid. Similar effects were observed in another *fads2*^{-/-} mouse by Stroud et al. [28], who additionally reported ulcerative dermatitis and ulceration of the small intestine in their mice. A *fads1* knockout mouse has not been described until now.

A female patient with reduced Δ^6 desaturase activity, probably due to an inherited deficiency in Δ^6 desaturase, was described by Williard et al. [18]. 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 blackcurrant seed oil, fish oil capsules and a vitamin A supplement as fatty acid therapy, which was subsequently switched to a mixture of 20:4 ω -6 and docosahexaenoic acid. This therapy led to growth acceleration until normal height for her age, and an improvement of skin abnormalities.

Candidate Gene Studies Reveal an Association between *FADS* Gene Cluster Polymorphisms and LC-PUFA Levels in Different Tissues

The important function of the Δ^5 and Δ^6 desaturase in the synthesis of LC-PUFAs made it a perfect candidate gene for association studies of *FADS* gene cluster polymorphisms with PUFA and LC-PUFA levels in human tissues, to investigate the role of DNA variants on desaturase activity. 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 3 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* an 18.0-kb region. Introns 1 of *FADS1* and *FADS2* are separated by an 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

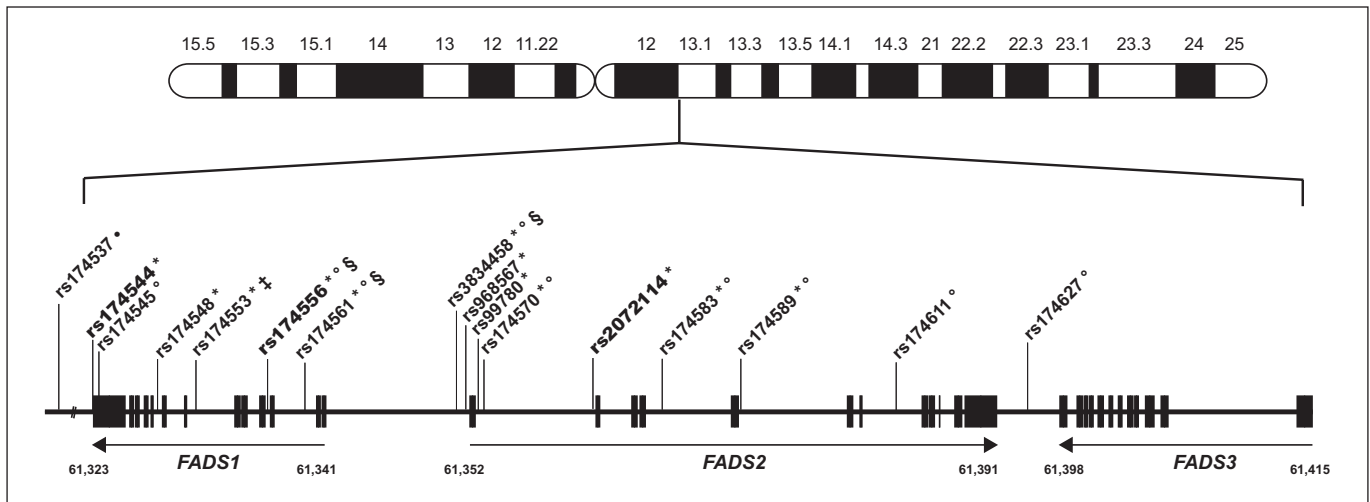


Fig. 2. Schematic structure of the human *FADS* gene cluster located on chromosome 11 and location of SNPs showing highest associations with fatty acid levels in different studies. SNPs with highest associations with fatty acids in the study of Schaeffer et al. [29] are marked with an asterisk (*). Most significant SNPs in all association studies which found a significant association of *FADS* gene cluster SNPs and fatty acid levels are indicated as follows:

° Malerba et al. [30]; § Rzehak et al. [31]; ‡ Xie and Innis [32]; • Tanaka et al. [33]. SNPs that were most significantly associated with allergic rhinitis and atopic eczema in the study of Schaeffer et al. [29] are marked in bold. The genomic position is given for each of the 3 *FADS* genes (numbers below gene names). SNP rs174537 is located 8.6 kb upstream of the *FADS1* gene (the change in scaling is indicated by //).

annotated in the NCBI database (dbSNP build 130). Schaeffer et al. [29] analyzed 18 selected SNPs in and around the *FADS1* and *FADS2* genes for association with fatty acids in serum phospholipids for the first time in 727 German probands of the European Community Respiratory Health Survey I. After calculation of linkage disequilibrium (LD) between the analyzed SNPs, which revealed a highly preserved LD block in the 48-kb genomic region from SNP rs174544 to SNP rs174589 (fig. 2), 3 redundant markers ($r^2 > 0.99$) were excluded from the analysis. Association analysis of the 15 remaining SNPs with 11 ω -6 and ω -3 fatty acids showed highly significant results for 11 SNPs, which are all located in the identified LD block, and all fatty acids (all $p < 1.0 \times 10^{-13}$) except for docosapentaenoic acid (22:5 ω -6) and docosahexaenoic acid (22:6 ω -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 ω -6, 20:2 ω -6, 20:3 ω -6, and 18:3 ω -3 and decreased levels of 18:3 ω -6, 20:4 ω -6, 22:4 ω -6, 20:5 ω -3, and 22:5 ω -3. Fatty acids belonging to other pathways, such as oleic acid (18:1 ω -9) and docosahexaenoic acid (22:6 ω -3), the source of which is mainly nutritional, did not show significant associations with the genetic variants. For the 4

SNPs beyond the LD block, the association with fatty acid levels weakened or vanished completely. Haplotype analysis was performed using all 11 associated SNPs as well as a subgroup of the first 5 strongly correlated SNPs in the gene cluster, and association results 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 ω -6 and 22:6 ω -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. Speculation about the possible causal SNPs revealed rs174561 and rs3834458 as the most interesting variants, because these are located in CpG islands as well as in hypothetical promoter regions. However, due to the highly preserved LD structure in the gene cluster region, statements about the causal SNP can hardly be made, because 1 causal SNP is sufficient to cause associations in all others. Furthermore, it is also possible that the

functionally relevant variant was not among the ones that were directly analyzed, but is just highly correlated with the analyzed SNPs. Another interesting issue in this paper was the association of *FADS* gene cluster polymorphisms with IgE levels, allergic rhinitis and atopic eczema. No significant association was observed for genotypes or haplotypes with total or specific IgE levels. For allergic rhinitis and atopic eczema, protective odds ratios for carriers of the minor alleles of several SNPs were obtained; however, after correction for multiple testing, the association did not remain significant. Although statistical significance concerning atopic diseases could not be proved in this study, it suggests a role of the desaturases in the development of atopy. The role of *FADS* genes in atopic diseases will be discussed again later in this review.

The association between *FADS* gene cluster polymorphisms and fatty acids was replicated in 3 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 [30–32]. 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 [30] analyzed 13 SNPs spanning the complete *FADS* gene cluster (including *FADS3*) in 658 north Italian subjects of the Verona Heart Project. 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 ($p < 1.0 \times 10^{-4}$). Up- or down-regulation of all analyzed fatty acids dependent on the allele was in accordance with the results shown by Schaeffer et al. [29], although only 18:3 ω -3 and 20:2 ω -6 in serum phospholipids and 18:2 ω -6 and 20:2 ω -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 ($p < 8.9 \times 10^{-4}$), but no significant association with any other fatty acid. 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 mem-

branes. The second replication study, which was performed at the same time by Rzehak et al. [31], confirmed the results from the 2 previous studies by analyzing the association of 3 *FADS* SNPs (rs174556, rs174561, and rs334458) with plasma and erythrocyte membrane fatty acids in 535 subjects (for plasma) and 163 subjects (for erythrocyte membranes) of the Bavarian Nutrition Survey II. To address the question whether *FADS* gene cluster polymorphisms have an influence on the fatty acid composition of human breast milk, Xie and Innis [32] genotyped 4 SNPs in *FADS1* and *FADS2* in 54 women, for whom data on fatty acid concentrations in breast milk 1 month postpartum were available. The authors found significant associations between SNP rs174553 and the medium-chain fatty acid 14:0 (p value = 4.6×10^{-2}), which is the end product of the de novo fatty acid synthase complex in the mammary gland and 18:1 ω -7 ($p = 1.0 \times 10^{-3}$), which is the Δ^9 desaturation-elongation product of 16:0. Medium-chain fatty acids (10:0, 12:0, 14:0) had decreased levels for the G allele of rs174553, whereas only 14:0 was statistically significant. Δ^9 desaturase products (16:1 ω -7, 18:1 ω -7 and 18:1 ω -9) increased in case of the G allele with significance only observed for 18:1 ω -7. For SNP rs174553, significant differences in PUFA concentration were obtained for 20:2 ω -6, 20:4 ω -6, 20:5 ω -3 and 22:5 ω -3 ($p < 2.6 \times 10^{-2}$). For SNP rs174575, concentrations of 20:4 ω -6, 22:5 ω -6, 20:5 ω -3 and also, interestingly, 22:5 ω -3 and 22:6 ω -3 differed significantly depending on the allele ($p < 4.4 \times 10^{-2}$). 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 fatty acids in 69 pregnant women at week 16 of gestation. They found significant associations between rs174553 and plasma 18:2 ω -6, 20:4 ω -6, 22:5 ω -3 and 22:5 ω -6 ($p < 5.0 \times 10^{-3}$). An association with plasma 20:4 ω -6 was also found for SNP rs174575 ($p = 3.0 \times 10^{-3}$). For erythrocyte ethanolamine phosphoglyceride fatty acids, an association between rs174553 and levels of 18:2 ω -6, 20:3 ω -6, 20:4 ω -6 and 22:4 ω -6 ($p < 3.8 \times 10^{-2}$) was observed, whereas no association with ω -3 fatty acids was reported.

The results of these association studies have been strengthened by a genome-wide association study published by Tanaka et al. in 2009 [33], where the authors analyzed plasma levels of 6 ω -3 and ω -6 fatty acids in 1,075 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

Table 1. Genetic association studies with associations between *FADS* polymorphisms and fatty acid concentrations

Study	n	Most significant SNP(s)	SNP location	Associated metabolites	p (most significant metabolite)
Schaeffer et al. (2006) [29]	727	rs174544, rs174553, rs174556, rs174561, rs3834458, rs968567, rs99780, rs174570, rs2072114, rs174583, rs174589	<i>FADS1/2</i>	fatty acids in serum phospholipids	single analysis: $<1.0 \times 10^{-13}$ (20:4 ω -6) haplotype analysis: 3.7×10^{-15} (20:4 ω -6)
Malerba et al. (2008) [30]	658	rs174545, rs174556, rs174561, rs3834458, rs174570, rs174583, rs174589, rs174611, rs174627	<i>FADS1/2/3</i>	fatty acids in serum phospholipids and erythrocyte membranes	single analysis: $<1 \times 10^{-4}$ (20:4 ω -6 in serum and erythrocytes) haplotype analysis: $<1 \times 10^{-4}$ (20:4 ω -6 in serum) and $<2 \times 10^{-4}$ (20:4 ω -6 in erythrocytes)
Rzehak et al. (2008) [31]	163 (plasma) 535 (erythrocytes)	rs174556, rs174561, rs3834458	<i>FADS1/2</i>	fatty acids in plasma phospholipids and erythrocyte membranes	single analysis: 7.9×10^{-10} (20:3 ω -6 in erythrocytes), plasma: data not shown haplotype analysis: 2.7×10^{-9} (20:3 ω -6 in erythrocytes) and 7.8×10^{-11} (20:4 ω -6 in plasma)
Xie et al. (2008) [32]	69 (plasma/ erythrocytes) 54 (breast milk)	rs174553	<i>FADS1</i>	fatty acids in plasma and erythrocyte phospholipids and in breast milk	plasma: $<1 \times 10^{-3}$ (18:2 ω -6, 20:2 ω -6 and 20:4 ω -6), erythrocytes: $<1 \times 10^{-3}$ (20:3 ω -6), breast milk: 1×10^{-3} (18:1 ω -7)
Tanaka et al. (2009) [33]	1,210 plus 1,076 (replication)	rs174537	5' upstream region of <i>FADS1</i>	plasma fatty acids	initial study: 5.95×10^{-46} (20:4 ω -6) replication study: $<1.0 \times 10^{-3}$ (20:4 ω -6)

in the 14.4 kb 5' region of *FADS1*, showed the most significant association with arachidonic acid ($p = 5.95 \times 10^{-46}$). Individuals homozygous for the minor allele of rs174537 had lower concentrations of 20:4 ω -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 ω -6. Furthermore, the SNP was associated with altered levels of 20:2 ω -6 ($p = 6.78 \times 10^{-9}$) and 20:5 ω -3 ($p = 1.04 \times 10^{-14}$). The association with 18:2 ω -6 and 18:3 ω -3 did not reach genome-wide significance ($p = 5.58 \times 10^{-7}$ and 2.76×10^{-5} , respectively) and there was no association with 22:6 ω -3 at all. The effects of rs174537 were confirmed in an independent sample of 1,076 participants of the GOLDN study in erythrocyte membranes. A summary of all association studies on *FADS* polymorphisms and fatty acid levels is given in table 1.

***FADS* Gene Cluster Polymorphisms May Modulate the Development of Atopic Diseases**

The levels and composition of various PUFAs and LC-PUFAs has been associated with the development of atopic diseases in numerous studies, although the direction of association differs among reports. Higher plasma levels

of 18:2 ω -6, and lower levels of 18:3 ω -6, 20:3 ω -6 and 20:4 ω -6 in adults and children with atopic eczema have been reported very early [34]. Several, but not all, following studies on diverse atopic disorders observed disturbances in ω -6 and ω -3 fatty acids [35–43]. A good review on the results of these studies is provided by Duchén and Björkstén [1]. Analyses of the phospholipid PUFA composition in chord blood and early infancy resulted in partly contrary findings about the prediction of atopic development during the first months of life, based on the fatty acid composition [44–48]. Despite small controversies in the different studies, it is widely assumed that atopy is related to a disturbed metabolism of ω -6 and ω -3 LC-PUFA [1]. A defect in the enzyme activity of the Δ^6 desaturase has been suggested to be present early in atopic disease patients [49], due to the higher levels of 18:2 ω -6 and lower levels of its longer metabolites in atopic children and adults. This defect in enzyme activity would explain the proposed dysregulation of arachidonic acid metabolism in atopy [50]. Arachidonic acid is one of the main precursors of prostaglandins and leukotrienes [51] and activates allergic immune responses via its products, prostaglandin E2 and leukotriene B4 [52–56]. For ω -3 fatty acids like eicosapentaenoic acid and docosahexaenoic acid, modulation of cytokine responses has also been

shown [57], whereas dietary treatment with these ω -3 fatty acids in inflammatory disorders suggests rather anti-inflammatory properties in contrast to the products of arachidonic acid [58].

One reason for the proposed defect in enzyme activity of the Δ^6 desaturase, possibly leading to an imbalance between ω -6 and ω -3 LC-PUFAs and therefore to disturbed inflammatory processes, could be mutations or polymorphisms in the Δ^6 desaturase encoding the *FADS2* gene. First concrete indications of a relation between the *FADS* genes and atopic diseases were provided by linkage studies [59, 60]. In the first candidate gene study on the association of *FADS* polymorphisms with fatty acid composition in serum phospholipids, Schaeffer et al. [29] reported a lower prevalence of allergic rhinitis and atopic eczema in carriers of the rare alleles of several SNPs for the first time, although these associations were not significant after correction for multiple testing. No association was found for total and specific IgE levels.

An association between breastfeeding and the development of atopic manifestations early in life has been suggested repeatedly [61]. Several studies exist, which investigated the PUFA composition of human milk in relation to the atopic status of the mother [62, 63] and the development of atopic disease in children [47, 64, 65]. Although differences exist in the composition of colostrum, transitional and mature milk [62, 66], most studies agree on altered levels of ω -6 and ω -3 LC-PUFAs in milk of atopic mothers compared to non-atopic mothers [47, 62, 63]. The LC-PUFA composition of transitional human milk agrees especially well with the reported low levels of ω -6 and ω -3 LC-PUFAs in plasma [35, 36, 40] and membrane phospholipids [37, 43, 67] in atopic children and adults. The composition of LC-PUFAs in maternal milk affects the PUFA status of the child, as has been shown by several studies [68–70]. Those few studies dealing with the relationship between maternal milk PUFA composition and development of atopy in the children [47, 64, 65] found contrary results on the influence of maternal milk ω -6 LC-PUFA composition on the children's atopic status, but low levels of the ω -3 LC-PUFAs 20:5 ω -3, 22:5 ω -3 and 22:6 ω -3 in maternal milk seemed to be related to early childhood development of allergies. The ratio of 20:4 ω -6 to 20:5 ω -3 is consistently higher in milk from mothers of atopic children, suggesting a disturbed balance in the arachidonic acid and eicosapentaenoic acid metabolism in atopic patients, and corroborates the relationship between ω -3 LC-PUFA metabolism and atopy [1, 47]. Besides the influence of maternal diet and lifestyle on the fatty acid composition in human breast milk [71,

72], maternal *FADS* genotypes have a great influence as well, as was shown by Xie et al. [32]. It would therefore be very interesting if *FADS* polymorphisms modulate the development of atopic diseases in early childhood by regulating the fatty acid composition in breast milk. To our knowledge, not a single study has investigated a possible modulation of the relationship between LC-PUFAs in breast milk and atopic status of the child by *FADS* genotypes, although a Δ^6 desaturase deficiency has been proposed in atopic subjects [1, 49]. The inclusion of *FADS* genotypes in future cohort and intervention studies might help to understand the role of LC-PUFAs in allergy development better and may also lead to enhanced sensitivity and precision of such studies [6].

Conclusion and Outlook

This review focused on current knowledge of the effects of *FADS* gene cluster polymorphisms on fatty acid levels in different human tissues as well as the effect of these polymorphisms on the development of atopic diseases. *FADS* genotypes account for up to 28% of the variability observed for fatty acid levels [29]. Besides the regulation by dietary fatty acids and hormonal signals, *FADS* genotypes are important regulators of desaturase activity and thus the balance of ω -6 and ω -3 PUFAs and LC-PUFAs in the human body.

The integration of *FADS* genotypes into future studies on fatty acid-related phenotypes will help us to better understand the biological effects of PUFAs and LC-PUFAs. Large gene-nutrition-interaction studies on complex disorders like atopic diseases 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 and to understand the complex regulation mechanisms of desaturase activity by dietary and endogenous fatty acids as well as individual *FADS* genotypes. Based on the results of such studies, individualized dietary recommendations dependent on the *FADS* genotype might help to balance PUFA/LC-PUFA intake and availability and by this contribute to the prevention of the onset of fatty acid-related diseases such as atopy later in life.

Furthermore, the identification of the regulatory functional *FADS* variants will improve our understanding of the regulation pathways and might better explain the contribution of polymorphisms in the biological effects of LC-PUFAs. First results on functional effects of a *FADS2* promoter polymorphism exist, where we were

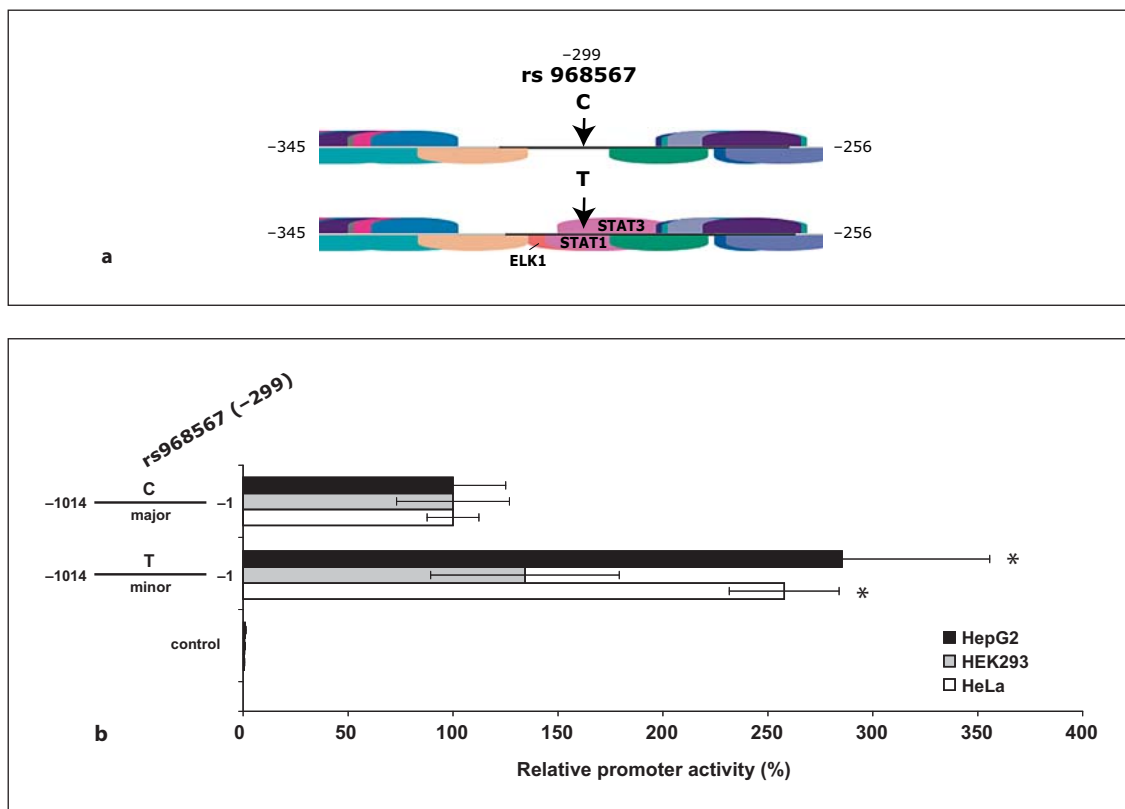


Fig. 3. Results on functional studies of *FADS2* promoter SNP rs968567 [73], showing a regulatory function of this SNP. **a** Prediction of transcription factors binding sites by Genomatix MatInspector suggested allele-specific binding affinity of 3 transcription factors (ELK1, STAT1 and STAT3). A higher binding affinity of the ELK1 protein to this promoter region when the minor T allele of rs968567 is present could be verified by DNA affinity chromatography and immunoblotting (not shown). Numbers indicate the sequence position relative to the translation start site. **b** Rela-

tive promoter activity of constructs of the human *FADS2* gene promoter in 3 different human cell lines increased when the major C allele of rs968567 was replaced by the minor T allele, possibly due to increased binding of ELK1 transcription factor. Values represent the mean of 3 independent experiments performed in triplicates. Promoter activity of the construct with the major allele was used as reference and set at 100%. Numbers indicate the relative position to the *FADS2* translation start site. * Statistically significant, t test.

able to show an increase in promoter activity and higher binding affinity of a specific transcription factor to the SNP-containing promoter region when the major allele was replaced by the minor allele in in vitro experiments [73] (fig. 3). The consequence of the observed effects on Δ^6 desaturase expression or activity and its biological importance has to be analyzed in further studies.

In summary, the knowledge that *FADS* gene polymorphisms influence fatty acid levels in human tissues and by this might modulate the characteristics of fatty acid-related phenotypes, is hopefully just the starting shot for further studies on the interaction of nutritional and genetic factors and their concerted effect on complex phenotypes like atopic diseases.

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