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Original Article

Identification of a functional *FADS1* 3′UTR variant associated with erythrocyte n-6 polyunsaturated fatty acids levels

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BACKGROUND: Blood polyunsaturated fatty acid (PUFA) levels are determined by diet and by endogenous synthesis via Δ5- and Δ6-desaturases (encoded by the *FADS1* and *FADS2* genes, respectively). Genome-wide association studies have reported associations between *FADS1-FADS2* polymorphisms and the plasma concentrations of PUFAs, HDL- and LDL-cholesterol, and triglycerides. However, much remains unknown regarding the molecular mechanisms explaining how variants affect the function of *FADS1-FADS2* genes.

OBJECTIVE: Here, we sought to identify the functional variant(s) within the *FADS* gene cluster.

METHODS: To address this question, we (1) genotyped individuals (n = 540) for the rs174547 polymorphism to confirm associations with PUFA levels used as surrogate estimates of desaturase activities and (2) examined the functionality of variants in linkage disequilibrium with rs174547 using bioinformatics and luciferase reporter assays.

RESULTS: The rs174547 minor allele was associated with higher erythrocyte levels of dihomo-γ-linolenic acid and lower levels of arachidonic acid, suggesting a lower Δ5-desaturase activity. In silico analyses suggested that rs174545 and rs174546, in perfect linkage disequilibrium with rs174547, might

Declarations of interest: None.

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alter miRNA binding sites in the *FADS1* 3′UTR. In HuH7 and HepG2 cells transfected with *FADS1* 3′UTR luciferase vectors, the haplotype constructs bearing the rs174546T minor allele showed 30% less luciferase activity. This relative decrease reached 60% in the presence of miR-149-5p and was partly abolished by cotransfection with an miR-149-5p inhibitor.

CONCLUSION: This study identifies *FADS1* rs174546 as a functional variant that may explain the associations between *FADS1-FADS2* polymorphisms and lipid-related phenotypes.
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Introduction

Polyunsaturated fatty acids (PUFAs) are involved in many pathophysiological processes including cardiovascular,[1,2] immune,[3] and nervous systems.[4] Blood and tissue PUFA concentrations are determined by both dietary intake and endogenous synthesis via the successive elongation and desaturation of the dietary precursors linoleic acid (18:2n-6, LA) and α-linolenic acid (18:3n-3, ALA). The Δ5-desaturase (D5D) and Δ6-desaturase (D6D) are involved in this enzymatic process[5] and are encoded by the *FADS1* and *FADS2* genes, respectively. Liver is a primary organ involved in PUFA biosynthesis and is a major site for expression of *FADS1* and *FADS2*.[6,7]

The *FADS1* and *FADS2* genes are located in a cluster on chromosome 11q12-13.1, together with the *FADS3* gene. This gene cluster comprises 91.9 kb with a head-to-head orientation of *FADS1* and *FADS2* and a tail-to-tail orientation of *FADS2* and *FADS3*. 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*. The biological function of *FADS3* is still unknown.[8] Many association studies have associated minor alleles of single-nucleotide polymorphisms (SNPs) in the *FADS1* or *FADS2* genes with lower blood concentrations of n-3 and n-6 long-chain PUFAs (LCPUFAs), indicating potential decreased desaturase activities.[9,10] The linkage disequilibrium (LD) of SNPs across *FADS1* and *FADS2* is high (Supplementary Fig. 1), making it difficult to test individual SNP effect. Of note, SNPs located in the *FADS1* and *FADS2* genes, but not in the *FADS3* gene, have been consistently associated with serum lipids composition in genome-wide association study (GWAS),[11-13] and none of the *FADS3* SNPs are in strong LD ($r^2 > 0.8$) with the associated SNPs. By combining data from a GWAS and a metabolomic study, Suhre *et al.* showed that the rs174547 SNP (minor allele frequency ∼0.30), located in *FADS1* intron 9, explained 30% of the variance of metabolites resulting from the *FADS1* activity.[14] Meta-analyses of GWAS have also reported associations between *FADS* variants (in strong LD with rs174547) and the plasma concentrations of triglycerides and total-, HDL-, and LDL-cholesterol.[15,16] However, much remains unknown regarding the molecular mechanisms explaining how variants affect *FADS* gene function.

In the present study, we sought to identify the functional variant(s) within the *FADS* gene cluster that may explain the lower desaturase activity observed in individuals carrying the *FADS* minor alleles. To address this question, we (1) genotyped the rs174547 SNP in 3 population-based studies (n = 540) to assess the impact of this SNP on n-3 and n-6 PUFA levels in French individuals from 3 different regions of France (North, East, and South) and (2) examined the functionality of SNPs within the rs174547 LD block using bioinformatics and luciferase reporter assays.

Materials and methods

The MONA LISA study

Details of the MONA LISA study have been reported previously.[17] The MONA LISA study is an epidemiological, cross-sectional, population-based study performed in the Lille Urban Community (northern France), the Bas-Rhin county (eastern France), and the Haute-Garonne county (southern France). Inhabitants aged 35 to 74 years were randomly sampled from electoral rolls after stratification by town size, gender and 10-year age groups (n = 4644). This study received ethical approval from the appropriate ethics committee for the protection of people participating in biomedical research, and all participants provided written informed consent. Measurements of the erythrocyte fatty acid composition were performed for 540 participants (30 men and 30 women per 10-year age group and per center, as detailed elsewhere).[18] Briefly, a fasting blood sample was drawn into EDTA tubes and centrifuged at 4°C to separate the cells from the plasma. The RBCs were frozen at −80°C until the phospholipids were extracted as described by Folch *et al.*[19] Fatty acid methyl esters were prepared as previously described[20] and then purified on activated magnesium silicate (Chromabond Florisil; Macherey-Nagel, Düren, Germany). The fatty acid composition of the RBC membranes was measured by gas chromatography with a CP-Sil88 capillary column (Trace; Thermo Electron Corp., Madison, WI). PUFAs were expressed as a percentage area by integrating the area under the peak and dividing it by the total area for all PUFAs. Commercial standards were used to calibrate retention time of each PUFA.

SNP selection and genotyping

The rs174547 SNP located in the *FADS* gene cluster was selected because it is consistently associated with PUFA levels in GWAS.[12-14] The MONA LISA sample was

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