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## Fatty Acid Desaturase 1 (FADS1) Gene Polymorphisms Control Human Hepatic Lipid Composition

Libo Wang<sup>1,\*</sup>, Shaminie Athinarayanan<sup>2,\*</sup>, Guanglong Jiang<sup>3</sup>, Naga Chalasani<sup>4</sup>, Min Zhang<sup>1,5</sup>, and Wanqing Liu<sup>2</sup>

Libo Wang: wang220@purdue.edu; Shaminie Athinarayanan: sathinar@purdue.edu; Guanglong Jiang: ggjiang@iu.edu; Naga Chalasani: nchalasa@iu.edu; Min Zhang: minzhang@purdue.edu; Wanqing Liu: liu781@purdue.edu

<sup>1</sup>Department of Statistics, College of Science, Purdue University, West Lafayette, IN 47907

<sup>2</sup>Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue University, West Lafayette, IN 47907

<sup>3</sup>Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202

<sup>4</sup>Division of Gastroenterology and Hepatology, Indiana University School of Medicine, Indianapolis, IN 46202

<sup>5</sup>Beijing Institute for Brain Disorders, Capital Medical University, Beijing 100069, P. R. China

### Abstract

*Fatty Acid Desaturase (FADS)* genes and their variants have been associated with multiple metabolic phenotypes including liver enzymes and hepatic fat accumulation but the detailed mechanism remains unclear. We aimed to delineate the role of *FADS*s in modulating lipid composition in human liver. We performed a targeted lipidomic analysis of a variety of phospholipids, sphingolipids and ceramides among 154 human liver tissue samples. The associations between previously Genome-wide Association Studies (GWAS)-identified six *FADS* single nucleotide polymorphisms (SNPs) and these lipid levels as well as total hepatic fat content (HFC) were tested. The potential function of these SNPs in regulating transcription of 3 *FADS* genes (*FADS1*, *FADS2* and *FADS3*) in the locus was also investigated. We found that while these SNPs were in high linkage disequilibrium ( $r^2 > 0.8$ ), the rare alleles of these SNPs were consistently and significantly associated with the accumulation of multiple very-long-chain fatty acids (VLCFAs), with C47H85O13P (C36:4), a phosphatidylinositol (PI) and C43H80O8PN (C38:3), a phosphatidylethanolamine (PE) reached the Bonferroni corrected significance ( $p < 3 \times 10^{-4}$ ). Meanwhile, these SNPs were significantly associated with increased ratios between the more saturated and relatively less saturated forms of VLCFAs, especially between PEs, PIs and phosphatidylcholines (PCs) ( $p = 3.5 \times 10^{-6}$ ). These alleles were also associated with increased total HFC ( $p < 0.05$ ). Further analyses revealed that these alleles were associated with decreased hepatic expression of *FADS1* ( $p = 0.0018$  for rs174556), but not *FADS2* or *FADS3* ( $p > 0.05$ ).

Corresponding author: Wanqing Liu, Ph.D., Department of Medicinal Chemistry and Molecular Pharmacology, Purdue University, RHPH 224B, 575 Stadium Mall Drive, West Lafayette, IN 47907, Phone: (765) 494-6389, Fax: 765-494-1414, liu781@purdue.edu.  
\*LW and AS contributed equally to the study.

### CONFLICT OF INTEREST

The authors declared no conflict of interest.

**Conclusion**—Our findings revealed critical insight into the mechanism underlying *FADS1* and its polymorphisms in modulating hepatic lipid deposition by altering gene transcription and controlling lipid composition in human livers.

### Keywords

FADS1; polymorphism; hepatic fat content; hepatic lipid accumulation; lipidomics

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## INTRODUCTION

It is widely recognized that status of polyunsaturated fatty acid (PUFA), especially long-chain PUFA (LC-PUFA) has a major impact on human health and has been demonstrated to be associated with clinical outcomes in various physiological processes including cardiovascular, immunology, peripheral and central nervous systems (1). Encoded respectively by the *fatty acid desaturase 1 (FADS1)* and *2 (FADS2)* genes, the delta-5 desaturase (D5D) and delta-6 desaturase (D6D) are membrane-bound enzymes that catalyze the rate-limiting formation of endogenous long-chain PUFA (1–5). The *FADS1* and *FADS2* genes are clustered on human chromosome 11 (11q12-q13.1), which also includes the *FADS3* gene with unclear function (6, 7). Previous candidate gene-based association studies identified correlation between *FADS* polymorphisms and complex diseases such as coronary artery disease (CAD) (8, 9), metabolic syndrome (10), allergic rhinitis and atopic eczema (2, 11), attention deficit/hyperactivity disorder (ADHD) (12) and intelligence development (13, 14). Significant associations were also reported in several recent genome-wide associations studies (GWAS) that revealed multiple polymorphisms at the *FADS* locus associated with various metabolic phenotypes especially serum lipid compositions and metabolic perturbations (See Table S1 for summary). These findings strongly suggest a functional linkage between genetic variations in *FADS* locus and multiple human diseases via altering the LC-PUFA metabolism (4, 5). However, a few important questions remain to be addressed. First, given the high linkage disequilibrium (LD) level across the *FADS* gene cluster (please see supplemental Fig S1), there thus far lacks a clear validation which *FADS* gene(s) mediates these genotype-phenotype correlations. Second, the regulation of lipid metabolism is a complex systematic process involving a large number of genes in multiple tissues/organs throughout the organism. However, a mechanistic linkage between genetic variations within *FADS* genes, PUFA metabolism and functional implications in critical tissues/organs has not been established.

The liver is the most important organ involved in lipid synthesis and metabolism. Homeostasis of circulating PUFA levels is significantly controlled by the dietary intake and hepatic metabolism (15). Therefore, the variability in lipid perturbations as well as the underlying disorders may be largely attributed to the variation in the function of *FADS* genes in the liver. Unfortunately, a complete delineation of the role of polymorphisms in modulating *FADS* genes as well as lipid profile in liver tissue has not been achieved to date. Collectively, filling this knowledge gap will not only reveal the mechanism that *FADS* genes involved in hepatic fat deposition, which is highly relevant to certain chronic liver disorders [e.g. nonalcoholic fatty liver disease (NAFLD)]; but will also help establish the causal pathways underlying the dysregulation of lipid metabolism in other human diseases

mentioned above. We therefore conducted this study to explore the mechanistic pathway linking genetic polymorphisms at the *FADS* locus, *FADS* gene expression, lipid profile and metabolism as well as total fat accumulation in human livers, using an integrated multi-omics approach including genomic, transcriptomic and lipidomic data obtained from human liver samples.

## MATERIALS AND METHODS

### Samples and Associated Genomic and Transcriptomic Data

Liver tissue samples (n=206) collected from liver transplantation donors were used in this study. The sample procurement procedure and related information have been described in our previous publication (16). The use of these livers and the performance of this study were approved by the Institutional Review Board (IRB) of Purdue University. These samples have been previously analyzed for SNP genotypes and mRNA expression using Illumina quad 610 SNP array and Agilent expression arrays (Agilent-014850 4×44k arrays, GPL4133). After removing samples with incomplete demographic and co-factors (age, gender, race and BMI) as well as samples with record of heavy drinking, 154 remaining samples were used to conduct the subsequent analyses.

### Measurement of Hepatic Fat Content (HFC)

Total lipid fraction was extracted from ~50mg liver tissue and measured using the method described previously (17). An aliquot of liver homogenate (20µl) was set aside for subsequent measurement of total protein concentration using the standard Bradford method. The extracted total lipid fraction was then used to perform lipidomic analysis. The hepatic contents of total fat and individual lipids were normalized to the total protein concentration for further analysis.

### Lipidomic Analysis

Targeted lipidomic analysis of 357 lipids (Table S2) was conducted using an automated electrospray ionization (ESI)-tandem mass spectrometry approach according to a protocol described previously (18). More technical details were included in the supplemental materials.

### Western Blotting

The protein expression of *FADS1* was examined with Western blotting. Detailed protocol was included in the supplemental materials.

### Genotyping

We genotyped rs738409 of the *Patatin-like phospholipase domain-containing protein 3* (*PNPLA3*) gene using a Taqman assay according to the manufacturer instructions (Life Technologies, Forster City, CA, USA).

## Data Processing and Statistical Analysis

Six SNPs (rs174576, rs1535, rs174546, rs102275, rs174537 and rs174556) from the *FADS* gene cluster were investigated in this study. These SNPs were available on the Illumina quad 610 SNP array platform, and were also either identified in previous GWAS and/or in LD ( $r^2 > 0.8$ ) with them (Table S1 listed all previous studies identifying the associations with these SNPs). SNP genotype and gene expression information were extracted from the previously published data (16). The analysis of the HapMap data revealed that these 6 SNPs are in strong LD ( $r^2 > 0.8$ ) (Fig S1).

We performed an integrated analysis to understand the role of *FADS* polymorphisms as well as *FADS* genes in regulating hepatic lipid composition, using various omics data. The detailed analysis plan was included in the supplemental materials. A flowchart describing study design along with key observations was also shown in Fig S2.

## RESULTS

### Measurement of Hepatic Fat Content and Lipid Profile

Among the liver samples, the median total hepatic fat content (mg fat/mg total protein) was 0.093 (range 0.04–0.58). The frequency distribution of the hepatic fat content among all samples was plotted in Fig S3. The targeted lipidomic profiling was conducted focusing on 357 individual lipid species. After profiling, 169 lipids with detectable levels in the liver were included in the data analyses and each individual lipid level was normalized to total protein content.

### Association between *FADS* SNPs and Hepatic Content of Individual Lipids, Lipid Ratios and Total HFC

In order to test whether 6 *FADS* SNPs were associated with the hepatic lipid profile, these SNPs were correlated with the hepatic content of 169 individual lipids. We found that the hepatic content of two phospholipids, C47H85O13P (C36:4), a phosphatidylinositol (PI) and C43H80O8PN (C38:3), a phosphatidylethanolamine (PE) were significantly associated with genotype of most SNPs (5 out of all 6 SNPs reached the Bonferroni corrected significance  $p < 3 \times 10^{-4}$ ) (Table 1). Compared to the PE, the PI lipid level was more strongly associated with all the SNPs. SNP rs174576 possessed the highest correlation with the two lipids levels ( $p = 5.3 \times 10^{-8}$  and  $1.9 \times 10^{-5}$ , respectively), with its minor allele associated with increased hepatic lipid level (Fig 1A).

Previous studies have suggested that the use of metabolite concentration ratios as a surrogate measurement of enzymatic activity helps reduce non-genetic variation and yielded stronger statistical associations between genetic alleles and metabolite profile (19, 20). We therefore conducted the association between these 6 SNPs and the 14,196 pair-wise ratios of all 169 lipids. We observed that these SNPs were significantly associated with a number of lipid ratios even after Bonferroni correction ( $p < 3.5 \times 10^{-6}$ ). These lipids were particularly very long chain fatty acids (VLCFA) especially PEs, PIs and phosphatidylcholines (PCs). Interestingly, the results consistently suggested that the minor allele of each SNP was associated with higher ratios of more saturated over less saturated lipid forms, reflecting the

functional variability in fatty acid desaturase (Table S3). The strongest association observed was between rs102275 and the ratio of a pair of PI C47H83O13P (C38:4) and (C47H85O13P, C38:3) ( $p=4.3\times 10^{-10}$ ) (Table S3).

To test whether this modulation also alter total fat accumulation in the liver, SNPs were associated with the total HFC measured by biochemical extraction method. We found that all SNPs were consistently associated with the total HFC ( $p<0.04$  for all tests) (Table 2). Consistently, the minor alleles were associated with increased hepatic fat deposition.

### Potential Effect of the *PNPLA3* Polymorphism rs738409 on the Association between *FADS* SNPs and Lipids Phenotypes

The *PNPLA3* polymorphism rs738409 is thus far the single strongest genetic risk factor for hepatic lipid accumulation and NAFLD (21). To evaluate whether this polymorphism may confound the associations observed between *FADS* polymorphisms and hepatic lipid traits, we conducted a conditional analysis by controlling the rs738409 genotype. After this, associations between the *FADS* SNPs and HFC or individual lipids remained significant (Table 1 and 2). In our dataset, rs738409 was marginally associated with total HFC ( $p=0.08$ , data not shown) but not with individual lipids ( $p>0.1$  for both lipids, data not shown).

### Genome-wide Association Analyses of Hepatic Contents of Lipids and Lipid Ratios Associated with the *FADS* SNPs

We next analyzed to see whether the *FADS1* SNPs were the only polymorphisms affecting the hepatic contents of the PI C36:4 as well as the lipid ratios identified above. After the GWAS analyses, the 6 *FADS* polymorphisms were still the most significant genome-wide associated SNPs with the level of PI C36:4 (Fig 2A). Similarly, the *FADS* polymorphisms were also the most significant SNPs genome-wide associated with most of the lipid ratios identified above. (See Fig 2B–C with C47H85O13P/C49H85O13P and C47H83O13P/C47H85O13P as examples).

### Association between *FADS* SNPs and Hepatic mRNA levels of *FADS* Genes

We further set out to understand the mechanism underlying this genotype-lipid association. Given the multiple *FADS* gene clustered in the region as well as their overlapping functions, we first focused on the question which *FADS* gene (s) was potentially involved in the genotype-phenotype correlations that were identified in the previous studies (Table S1). We found that all 6 SNPs were consistently and significantly associated with *FADS1* gene expression, but not with *FADS2* or *FADS3* (Table 3). The rs174556 exerted the highest correlation with *FADS1* gene expression ( $p=1.8\times 10^{-3}$ ), which remains significant after Bonferroni correction ( $p<0.017$ ) (Fig 1B).

To further screen whether additional SNPs located in the locus were also associated with *FADS* gene expression, we performed a locus-wide *cis*-eQTL mapping for the three *FADS* genes. We included all genotyped and imputed SNPs [ $k=2,177$  after quality control (QC) filtering] in the region spanning from 500kb upstream and downstream of the *FADS* cluster (hg19, Chr11: 61,071,142–62,159,006), which is far beyond the LD block harboring the 6 SNPs (Fig S1). We found that the SNPs in the LD block were consistently associated with

*FADS1* mRNA expression (most significant SNPs  $10^{-3} < p < 10^{-2}$ ) but not with *FADS2* or *FADS3* ( $p > 0.05$  for both), albeit the results did not reach statistical significance after Bonferroni correction ( $p < 2.3 \times 10^{-5}$ ). Meanwhile, although a few other SNPs were associated with each of the three *FADS* genes at the level of  $p = 0.01$ , they were in weak LD ( $r^2 < 0.2$ ) with the SNPs in the LD block (Fig S4A–C). In case that nonsynonymous or splicing variants in *FADS2* and *FADS3* that may be in LD with the 6 SNPs but do not alter gene expression, we manually screened the nonsynonymous SNPs and the SNPs located within  $\pm 50$ bp region of all exon-intron boundaries in these two genes in 1000 Genome data. No such a SNP with significant LD ( $r^2 > 0.5$ ) with the 6 core SNPs was found.

Given the non-significant association after Bonferroni correction for the locus-wide expression quantitative traits loci (eQTL) mapping, we further validated the results in an additional hepatic eQTL dataset published recently (22). Again, imputed and genotyped SNP data ( $k = 1,619$  after QC) in the same region were used. We found that in this population, the SNPs in the LD block were strongly associated with the expression of *FADS1* ( $10^{-8} < p < 10^{-6}$ ) but much weakly with *FADS2* ( $10^{-4} < p < 10^{-2}$ ) and no association with *FADS3* ( $p > 0.05$ ) (Fig S5A–E). After Bonferroni correction, only the results for *FADS1* remained significant (corrected significance  $p = 3 \times 10^{-5}$ ). It should be noted that *FADS1* and *FADS2* mRNA levels in this population were highly correlated ( $p = 1.2 \times 10^{-48}$ ) (Fig S5F), possibly explained the weak but consistent associations between these SNPs and *FADS2* expression. Collectively, these data strongly suggested that the SNPs in the LD block are *cis*-regulators for *FADS1* gene transcription.

### Association between FADS SNPs and FADS1 Protein Expression

We also set out to test whether the *FADS* SNPs determine FADS1 protein level in the livers. We performed immunoblotting of the FADS1 protein in the livers with enough protein samples available ( $n = 29$ ). We found that these SNPs (represented by rs174556) were significantly associated with FADS1 protein level ( $R^2 = 0.22$ ,  $p = 0.01$ ) (Fig 3A–B).

### Interaction between FADS SNPs and Transcription Factors (TFs)

To further elucidate the potential mechanism underlying the regulation of *FADS1* gene transcription by the SNPs, we hypothesized that the 6 SNPs and/or their LD proxies ( $r^2 > 0.8$ ) may be more likely to interact with *trans*-regulatory factors, e.g. TFs, than other SNPs in the locus. To test this, we investigated the distribution of these SNPs and their LD proxies in the TF binding sites (TFBS) identified by the Encyclopedia of DNA Elements (ENCODE) project, and compared this data to that of the SNPs that are not in the LD block but with similar minor allele frequency (MAF) ( $> 0.25$ ). We found that among 42 highly linked SNPs, 29 (69%) are located in TFBS. This ratio was significantly higher than that of the SNPs out of the LD block [132 (43%) out of 309 SNPs; Chi-square test,  $p = 0.001$ ], suggesting that indeed the 6 SNPs and their LD proxies are more likely to interact with TFs.

## DISCUSSION

SNPs located at the *FADS* locus have been consistently associated with multiple phenotypes related to lipid metabolism, especially serum lipids composition. Our study for the first time

observed that these SNPs are also significantly associated with lipid composition and deposition in the liver tissue. While the detailed intermediate mechanism underlying these *FADS1* genotype-phenotype associations remains unexplored thus far, our study demonstrated that 1) specific hepatic lipids are affected by altered *FADS1* gene expression/function, and 2) *FADS1* but not *FADS2* or *FADS3* is the gene mediating these associations. Our study established the role of *FADS1* in lipid composition in the liver as a central organ, and revealed a causal pathway encompassing DNA variation to gene/protein expression/function, followed by intermediate lipid metabolism, and eventually leading to hepatic fat accumulation. Combined with previous observations between these polymorphisms and metabolism of specific lipids e.g. in blood and breast milk (see Table S1 for summary) as peripheral tissues, our findings completed the picture showing the central role of *FADS1* in lipid homeostasis in human body and underlying health and disease (Fig 4).

Our findings suggested a role of *FADS1* and its polymorphisms involved in hepatic total fat accumulation by modulating the accumulation of VLCFAs, especially PEs, PCs and PIs. The associations between the SNPs and HFC were weaker compared to their associations with individual lipids and lipid ratios. This further reflects the greater effect size of genetic alleles on intermediate phenotypes compared to the endpoint phenotypes as pointed out previously (19) (Fig 4), especially when the sample size is limited. Our results were consistent with our previous study where a strong negative association between *FADS1* mRNA expression and total hepatic fat content in human livers was demonstrated (23). The involvement of *FADS1* in hepatic fat accumulation is also supported by the strong association between the same set of SNPs and liver enzyme levels in a previous GWAS (24). Liver function has been negatively correlated with fat accumulation, and the variability in hepatic fat content and hepatic function share similar genetic basis, e.g. genetic variations in *PNPLA3* gene (21, 25–27), although our data suggest that the influence of the *FADS1* polymorphism on hepatic lipids accumulation is likely independent of *PNPLA3* variant. In addition, fat accumulation among hepatocytes is highly correlated with various metabolic perturbations including NAFLD, insulin resistance, hyperlipidemia, obesity and cardiovascular diseases. Our findings thus are also in concert with the associations between *FADS* SNPs and these perturbations in previous studies (8–10, 28–33). For examples, a study by Araya et al showed significant reductions in the activities of D5 and D6 desaturases in the liver of obese NAFLD samples which was postulated to play a role in altering lipid homeostasis and causing hepatic lipid accumulation (32). Others also showed association between decreased D5 desaturase activity with adverse metabolic risk profiles and in the pathogenesis of atherosclerosis (29, 30).

Our study also identified specific hepatic lipids significantly affected by *FADS1* and its polymorphism. First, among 169 hepatic lipids, we identified two phospholipids whose content in the liver was significantly associated with *FADS1* SNPs. Furthermore, this association stayed to be the most significant association in the subsequent genome-wide association study (lipid-QTL mapping), suggesting a highly specific linkage between these SNPs, *FADS1* mRNA/protein expression and these two individual lipids. This linkage was further confirmed by the strong correlation between *FADS1* SNPs and pair-wise lipid ratios. In particular, the minor allele carriers demonstrated to be associated with higher ratios of

more saturated over less saturated lipids, suggesting that these SNPs are associated with reduced D5 desaturase enzyme activity. Indeed, the minor allele was also associated with reduced FADS1 protein level. This finding is consistent with previous studies showing that *FADS* polymorphisms are associated with increased desaturation precursor while decreased desaturation products of fatty acid, PUFA and LC-PUFA in serum, plasma, erythrocyte and breast milk in both candidate gene based and genome-wide studies (2, 19, 20, 28, 34–38). In particular, several lipids [e.g. PC (36:4), PC (36:5), PC (38:4) and PI (38:4)] in the liver which were significantly associated with these SNPs in our study were the same serum lipids showing significant association with the same SNPs in previous GWAS (19, 37), suggesting a highly specific role of FADS1 on the metabolism of these lipids. It is possible that hepatic FADS1 may determine the levels of these lipids in the liver, and these lipids are subsequently secreted into blood.

Given the clustering of three *FADS* genes and the high LD level across the locus, as well as the similarity in the function of *FADS1/2*, it remains to be unclear which gene is exactly causal to these phenotypes. Our study confirmed that *FADS1* is the causal gene involved in the previously observed genotype-phenotype associations. This notion is supported by 1) the correlation between the core SNPs and transcription level of *FADS1* but not *FADS2* or *FADS3*, even in different populations; 2) the lack of other functional variants (missense or splicing) in *FADS2* or *FADS3* that are in LD with the core SNPs but do not alter gene transcription; and 3) the enrichment of the SNPs in the LD block at the TFBS. Our data thus warrants further investigation of the role of FADS1 in regulating PUFA metabolism and affecting susceptibility to various metabolic perturbations.

Our study fostered strong rationale for new hypotheses to be tested in the next step. First, although we observed a strong correlation between those SNPs, *FADS1* gene/protein expression and hepatic lipid metabolism, the SNPs studied here are in high LD. It is unclear which SNP(s) is causal for the altered *FADS1* gene function, since at least 29 SNPs in the LD block demonstrated to interfere with TFs (Table S4). How these TFs interplay with the polymorphisms and lead to the altered gene transcription remain to be investigated. Second, how altered modulation of the specific hepatic lipids identified in our study is involved in total hepatic fat accumulation represents another interesting but unaddressed question, especially many of these lipids were also associated with the same SNPs in other tissue and fluids. Given the intrinsic linkage between hepatic lipid accumulation and metabolic disorders, it is very possible that these lipids may be critical bioactive molecules involved in energy metabolism and homeostasis. It is thus warranted to further explore their roles in lipids homeostasis, e.g. whether blocking their synthesis or increase the intake of its desaturated substrates may improve/prevent hepatic fat accumulation and/or other metabolic comorbidities. Recent studies have demonstrated encouraging results in taking LC-PUFA such as prolonged n-3 polyunsaturated fatty acid for treating NAFLD (39–43). One study has also shown the potential protective effect of dietary PIs supplement on the development of NAFLD, although a detailed composition of the dietary PIs was unknown (44).

In conclusion, our study for the first time delineated the detailed mechanism in human livers that *FADS* polymorphisms significantly control the hepatic lipid composition, which is further involved in hepatic fat accumulation. The findings provided clues in understanding



the general role of *FADS1* gene in conferring susceptibility to other metabolic perturbations as well. Our work highlights the power of multi-level integrated omics (systems biology) approach to better understand causal mechanisms behind the genotype-phenotype associations noted by GWAS.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGEMENT

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## LIST OF ABBREVIATIONS

<b>FADS</b>	Fatty Acid Desaturase
<b>GWAS</b>	Genome-wide Association Study
<b>SNP</b>	Single Nucleotide Polymorphism
<b>HFC</b>	Hepatic Fat Content
<b>VLCFA</b>	Very Long Chain Fatty Acid
<b>PI</b>	Phosphatidylinositol
<b>PE</b>	Phosphatidylethanolamine
<b>PC</b>	Phosphatidylcholines
<b>PUFA</b>	Polyunsaturated Fatty Acid
<b>LC-PUFA</b>	Long Chain Polyunsaturated Fatty Acid
<b>D5D</b>	Delta-5 desaturase
<b>D6D</b>	Delta-6 desaturase
<b>CAD</b>	Coronary Artery Disease
<b>ADHD</b>	Attention Deficit/Hyperactivity Disorder
<b>LD</b>	Linkage Disequilibrium
<b>NAFLD</b>	Nonalcoholic Fatty Liver Disease
<b>BMI</b>	Body Mass Index
<b>ESI</b>	Electrospray Ionization
<b>PNPLA3</b>	Patatin-like Phospholipase Domain-containing Protein 3 gene
<b>VLCFA</b>	Very Long Chain Fatty Acids

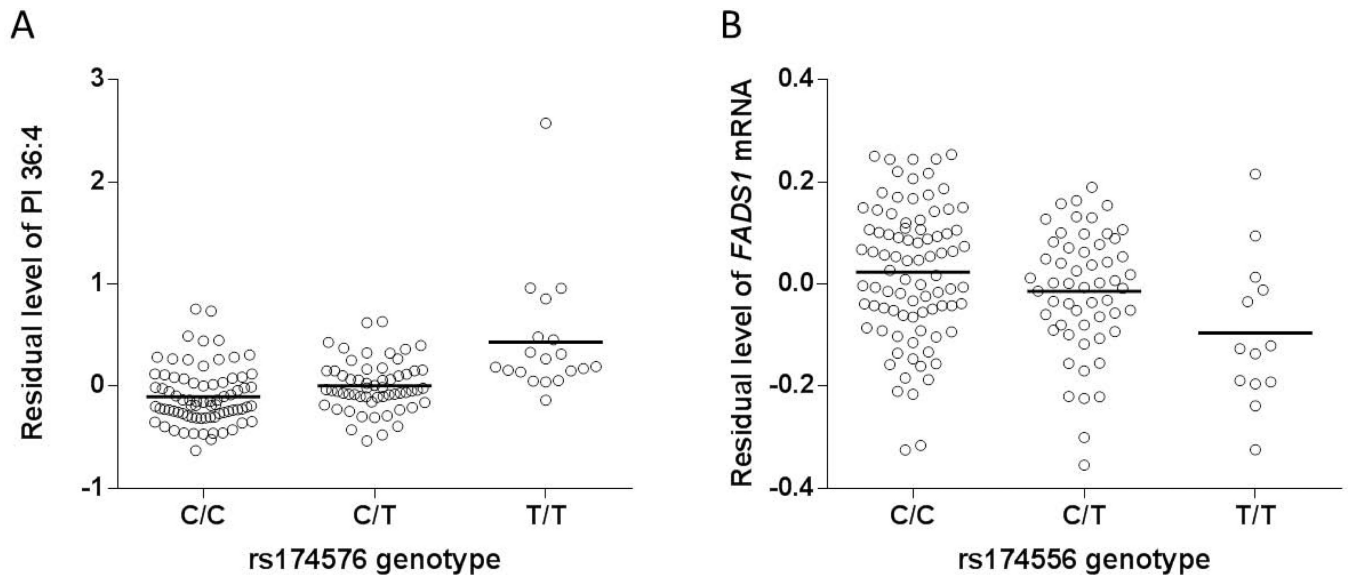
<b>eQTL</b>	Expression Quantitative Traits Loci
<b>QC</b>	Quality Control
<b>TF</b>	Transcription Factor
<b>TFBS</b>	Transcription Factor Binding Site
<b>ENCODE</b>	The Encyclopedia of DNA Elements
<b>MAF</b>	minor allele frequency (MAF)

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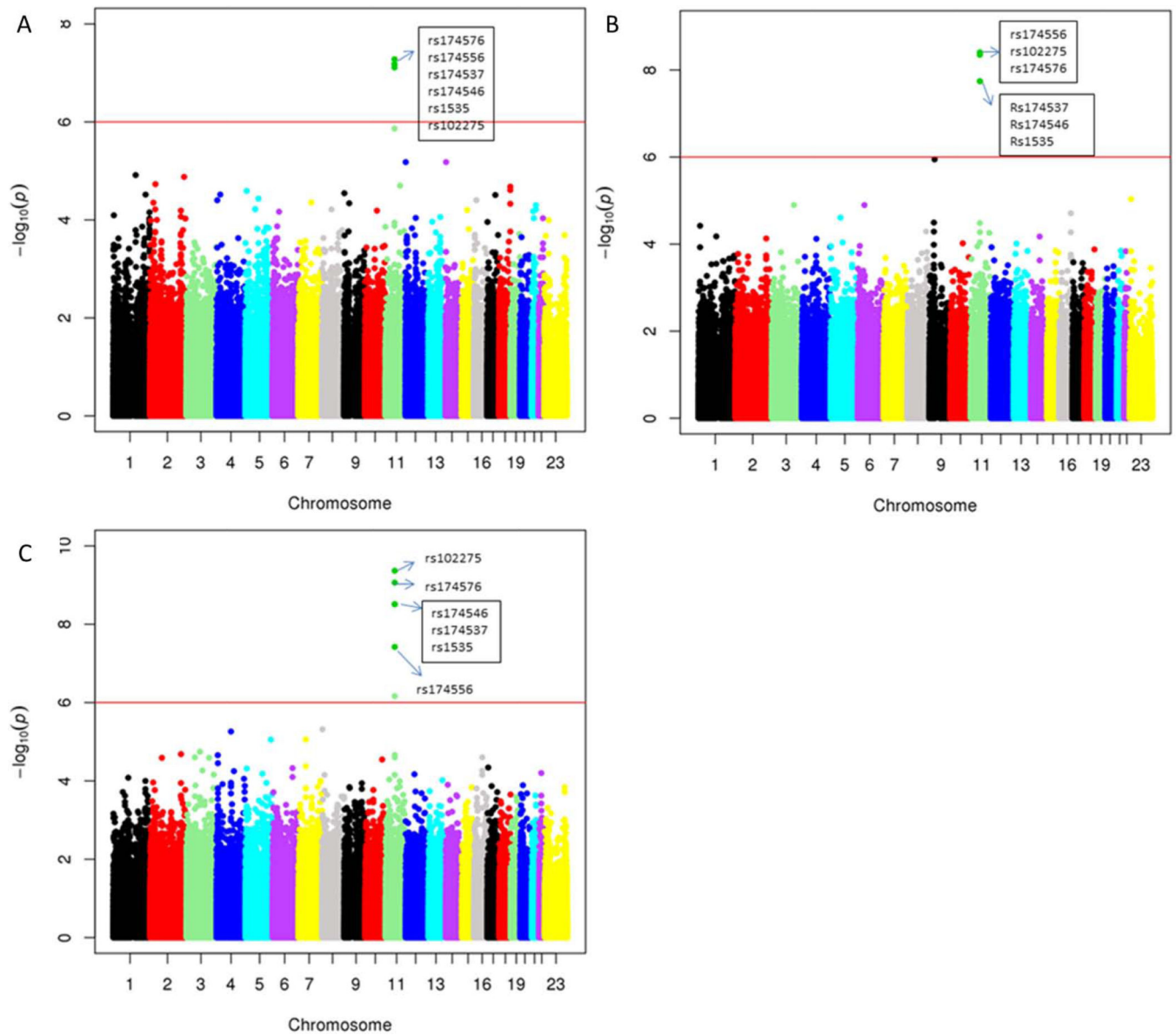
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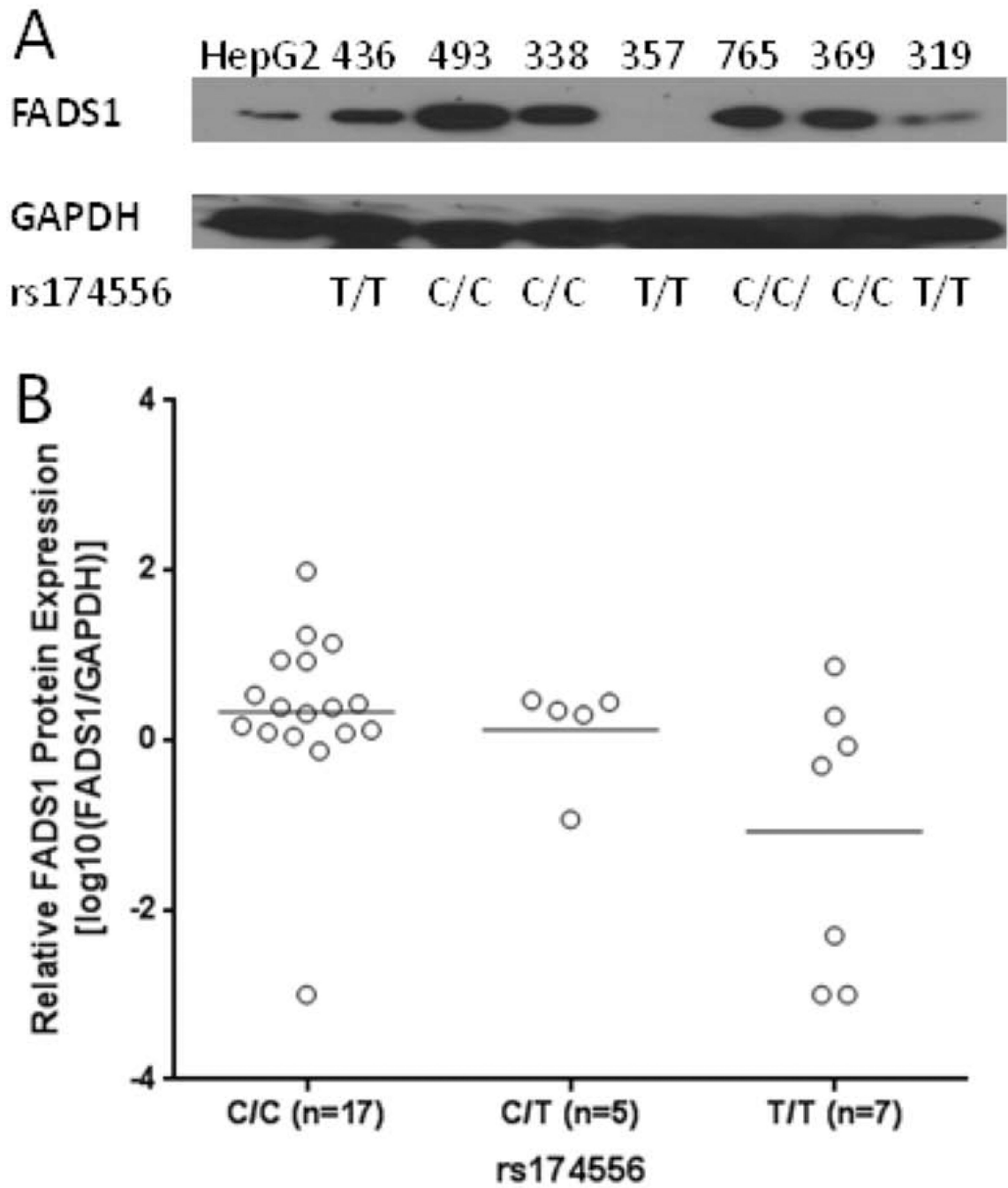


**Fig 1.**

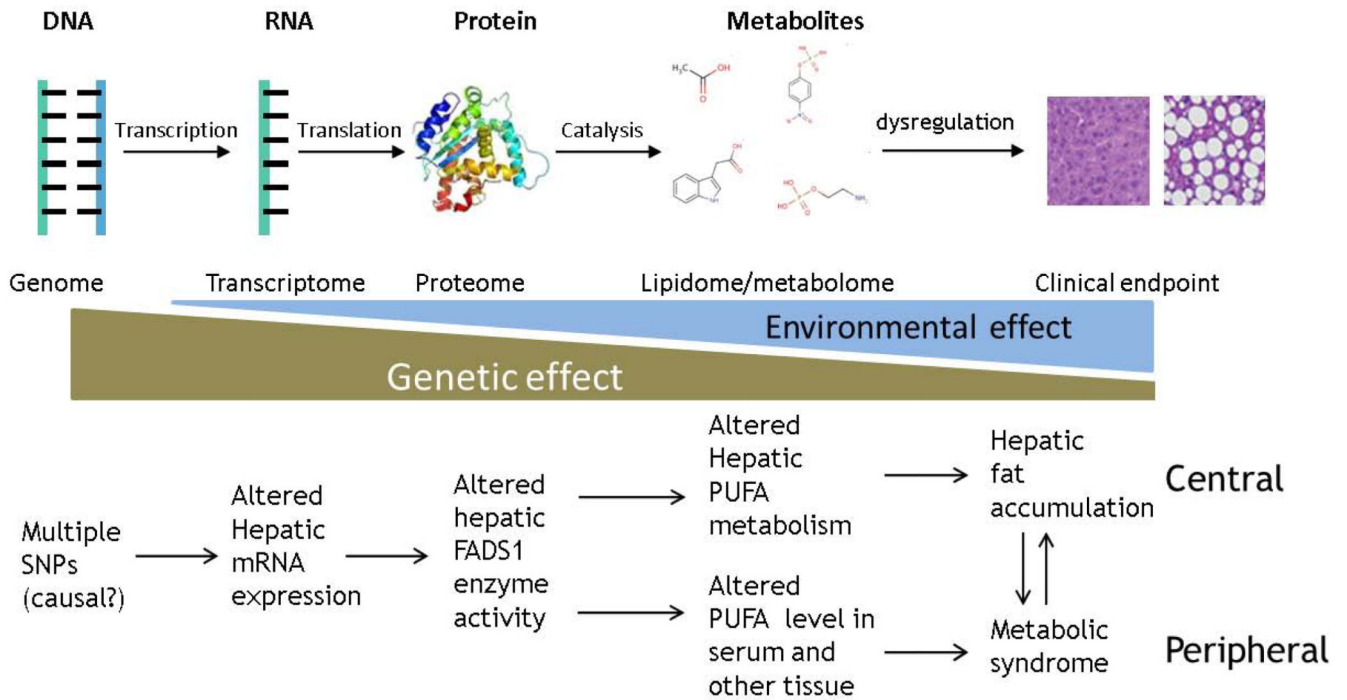
Association between SNP genotype (rs174556 as an example) and hepatic lipid level and FADS1 mRNA expression. **A)** Association between rs174576 and the hepatic level of PI 36:4. **B)** Association between *FADS1* polymorphism rs174556 as an example and *FADS1* mRNA expression. Plots were demonstrated as the correlation between the genotypes and the residual hepatic *FADS1* mRNA expression or PI 36:4 levels after adjusted for covariates. Horizontal bars indicate mean values.



**Fig 2.** Manhattan plots of quantitative traits loci (QTL) mapping. A) PI (36:4) (C47H85O13P); B) lipid ratio C47H85O13P/C49H85O13P and C) lipid ratio C47H83O13P/C47H85O13P. Each dot represented the  $-\log(p)$  value for one SNP. The significance level ( $10^{-6}$ ) based on Bonferroni correction was indicated in each plot.



**Fig 3.** Association between *FADS* polymorphisms (rs174556 as an example) and FADS1 protein level. **A)** The protein levels of FADS1 and GAPDH (as a loading control) determined with Western blotting. **B)** Correlation between rs174556 and FADS1/GAPDH ratios (+log<sub>10</sub> transformed) ( $R^2=0.22$ ,  $p=0.01$ ). Samples with non-detectable FADS1 but normal GAPDH level were assigned a ratio of 0.001 to facilitate the log transformation.

**Fig 4.**

Schematic illustration of the findings in our and previous studies. The upper panel demonstrated the general intermediate molecular mechanism underlying the genotype-phenotype association. The effect size of genetic factors on various intermediate phenotypes decreases in general along with the increased distance from DNA, while environmental effect may increase accordingly, underscoring the power of the integration of intermediate phenotypes with different omics approaches in understanding complex endpoint phenotypes. The lower panel showed the intermediate mechanism underlying the association between *FADS1* alleles and various metabolic phenotypes. The genetic effect of these polymorphisms on *FADS1* mRNA expression and enzyme function is conserved in both central (liver) and peripheral tissues/organs (breast, blood, etc.) in humans, which together composes the complete role of the *FADS1* biology in metabolic perturbations. The importance of systems approach was therefore highlighted.



Table 1

Association between *FADS* SNPs and individual lipid levels.

SNP	Physical Position	C47H85O13P PI(36:4)				C43H80O8PN PE(38:3)			
		Beta	R <sup>2</sup>	P-value	Adjusted P*	Beta	R <sup>2</sup>	P-value	Adjusted P
rs174537	61309256	0.218	0.175	6.6E-08	2.9E-08	0.111	0.109	3.0E-05	3.3E-05
rs102275	61314379	0.215	0.174	7.7E-08	3.3E-08	0.112	0.114	1.9E-05	1.8E-05
rs174546	61326406	0.218	0.175	6.6E-08	2.9E-08	0.111	0.109	3.0E-05	3.3E-05
rs174556	61337211	0.235	0.175	6.6E-08	2.7E-08	0.102	0.079	4.1E-04	5.1E-04
rs1535	61354548	0.218	0.175	6.6E-08	2.9E-08	0.111	0.109	3.0E-05	3.3E-05
rs174576	61360086	0.221	0.178	5.3E-08	2.3E-08	0.114	0.114	1.9E-05	2.1E-05

\* Adjusted p refers to the p values after adjusting for rs738409 genotype.

Table 2

Association between *FADS* polymorphisms and hepatic fat content.

SNP	Physical Position	HFC			
		Beta	R <sup>2</sup>	P-value	Adjusted P*
rs174537	61309256	0.116	0.034	0.023	0.021
rs102275	61314379	0.095	0.023	0.061	0.052
rs174546	61326406	0.116	0.034	0.023	0.021
rs174556	61337211	0.115	0.028	0.037	0.037
rs1535	61354548	0.116	0.034	0.023	0.021
rs174576	61360086	0.112	0.031	0.029	0.027

\* Adjusted p refers to the p values after adjusting for rs738409 genotype.

Table 3

Association between SNPs and *FADS* mRNA expression.

SNP	MAF	Physical Position	<i>FADS1</i>			<i>FADS2</i>			<i>FADS3</i>		
			Beta	R <sup>2</sup>	P-value	Beta	R <sup>2</sup>	P-value	Beta	R <sup>2</sup>	P-value
rs174537	0.31	61309256	-0.035	0.035	0.021	0.017	0.009	0.2468	-3.8E-16	5.5E-03	0.36
rs102275	0.35	61314379	-0.032	0.030	0.032	0.020	0.013	0.1660	-3.7E-16	5.5E-03	0.36
rs174546	0.31	61326406	-0.035	0.035	0.021	0.017	0.009	0.2468	-3.8E-16	5.5E-03	0.36
rs174556	0.26	61337211	-0.051	0.062	0.0018	0.016	0.007	0.2880	-4.1E-16	5.7E-03	0.35
rs1535	0.31	61354548	-0.035	0.035	0.021	0.017	0.009	0.2468	-3.8E-16	5.5E-03	0.36
rs174576	0.31	61360086	-0.033	0.031	0.029	0.016	0.008	0.2719	-3.9E-16	5.9E-03	0.34