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## Abstract

The fatty acid composition of meat, which affects both its quality and the consumer's health, is a complex trait influenced by genetic and environmental factors. Identification of the genes influencing the fatty acid composition of meat is very important for the selection and breeding of chickens with desirable and healthier fatty acid profiles. The objective of this study was to identify functional candidate genes for fatty acid profiles of the breast meat of the Korean native chicken-red-brown line (KNC-R) through genome-wide association studies. We genotyped 382 KNC-R chickens (190 males, 192 females) using the Illumina chicken 60K SNP chip (Illumina, San Diego, CA, USA), and association tests were performed by mixed linear model in the Genome-wide Complex Trait Analysis (GCTA) software, based on mixed linear model analysis-leave-one-chromosome-out (MLMA-LOCO). We detected one SNP each on chromosomes 2 (rs13667281), 10 (rs14011157), and 22 (rs10731996) that were significantly ($p < 0.05$) associated with nervonic (C24:1), linoleic (C18:2), and eicosadienoic (C20:2) acids, respectively. We found 13 protein-coding genes related to lipid metabolism, including *IGF2BP3*, *GPNMB*, *NPY*, *OSBPL3*, *IL6*, *NR2F2*, *GPAT4*, *NKX6-3*, *ANK1*, *SFRP1*, *ERLIN2*, *STAR*, and *PPP1R3E*. Interestingly, two candidate genes (*GPNMB* and *SFRP1*) were reported to regulate the expression of genes known to be involved in fatty acid synthesis, such as the *FASN*, *ACACA*, *ACLY*, *ELOVL*, and *SCD* genes. Identification of functional candidate genes for fatty acid profiles might facilitate the selection and breeding of chickens with desirable and healthier fatty acids.



**Keywords**: Fatty acid, genome-wide association studies, Korean native chicken, meat flavor, meat quality


## Introduction

The fatty acid composition of meat has recently received more attention among meat producers, researchers, and consumers due to its effects on consumer health and

meat quality, particularly meat flavor [1]. The fat content in muscle, intramuscular fat (IMF), and the fatty acid composition greatly influence the flavor, juiciness, and tenderness of meat [2]. Fatty acids are components of fat, and are subdivided into: saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) [3]. The latter are subdivided into monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) [3]. Except for stearic acid, the consumption of SFAs has been reported to increase the content of blood cholesterol which is linked with heart disease [3]. By contrast, UFAs confer health benefits to consumers [4].

In chicken meat, palmitic acid is the predominant SFA, while oleic and linoleic acids are the most abundant MUFA and PUFA, respectively [5]. The composition of acid composition in meat affects its flavor by releasing the final flavor compounds through thermal oxidation during cooking [6]. The flavor compounds of fatty acids include alkanes, aldehydes, ketones, and organic acids [1]. For example, arachidonic acid (C20:4) is associated with better sensory characteristics of chicken meat [6, 7], oleic acid (C18:1) is a good meat flavor precursor in chicken [6], and docosahexaenoic acid (DHA; C22:6) suppresses sourness to improve the sweetness and umami taste of meat [6]. Linoleic acid (C18:2) also improves meat flavor [1].

The fatty acid composition in meat is a polygenic trait controlled by genetic and environmental factors [7]. Some fatty acids have very low heritability, and it is very difficult to improve low-heritability traits using conventional methods [8]. Genomic selection is effective for improving the performance of low- to moderate-heritability traits [9]. Different genes have been reported to influence fatty acid synthesis in chicken meat, including *DEGS1*, *ELOVL6*, *FABP3*, *FABP4*, *FASN*, and *SCD* [10]. Heritability estimates range from low for eicosenoic acid (0.025) to moderate for palmitic acid (0.290) and high

for arachidonic (0.552), oleic (0.560), and docosahexaenoic (0.510) acids in Korean native chicken (KNC) breast meat [11], suggesting that it is possible to breed chickens for favorable fatty acid composition using marker-assisted selection (MAS). Genetic methods such as genome-wide association studies (GWAS) are very effective for finding genomic regions and potential candidate genes for traits of interest [12]. For example, GWAS were used to identify the candidate genes for growth traits, disease resistance, and other important traits in chickens [13]. However, the GWAS of the fatty acid composition in chicken meat are very scarce. Therefore, this study sought potential candidate genes for the composition of fatty acid in KNC-red-brown line (KNC-R) chickens using GWAS.

## Materials and Methods

### Ethical Statement

This study referred to the guidelines established by the Institution of Animal Care and Use Committee of the National Institute of Animal Science (NIAS 20212219).

### Experimental animals

We used 382 KNC-R chickens (190 males, 192 females) from one population kept at the Poultry Research Institute's farm of NIAS in Pyeongchang, South Korea. We chose chickens from 2 generations as shown in Table 1, each generation was 52 weeks. Housing, hatching, management, feeding, slaughtering, and carcass storage conditions are described in our previous study [13]. We collected blood samples from 382 KNC-R chickens and stored at −20 ◦C until DNA extraction. At the age of 10 weeks, all 382 KNC-R chickens were slaughtered, carcasses were eviscerated, and then breast meat was separated and kept under −80 ◦C until usage.

## Phenotype measurements and Preprocessing

The fatty acid composition was analyzed from breast meat samples collected from 382 KNC-R chickens slaughtered at 10 weeks old. The fatty acid methyl ester (FAME) method was used to extract the fatty acids following all procedures used by [14]. The content of each fatty acid was expressed in percentage. We measured 24 fatty acid traits, including SFA, saturated fatty acids (SFAs): myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0); MUFA, monounsaturated fatty acids (MUFAs): palmitoleic acid (C16:1), oleic acid (C18:1), nervonic acid (C24:1); PUFA, polyunsaturated fatty acids (PUFAs): linoleic acid (C18:2), linolenic acid (C18:3), eicosadienoic acid (C20:2), mead acid (C20:3), arachidonic acid (C20:4), eicosapentaenoic acid (C20:5), docosahexaenoic acid (C22:6), UFA, ratio between UFAs and SFAs (UFA/SFA), ratio between PUFAs and SFAs (P/S), ratio between omega-6 and omega-3:n-6/n-3, omega-6: n-6, omega-3: n-3, atherogenicity index (AI), thrombogenicity index (TI). We used the Shapiro-Wilk test to normalize phenotypes and different methods including, log, cube, tri, multi, or square root scaling were used for normalizing our phenotypic data as presented in Table 2.

## Genotyping and Quality Control

Genomic DNA from blood samples of 382 KNC-R chickens was extracted by using a commercial toolkit of GeNetBio (GeNetBio, Daejeon, Korea). The Illumina chicken 60 K SNP chip (Illumina, San Diego, CA, USA) was used for genotyping 382 DNA samples. We used the PLINK1.9 version 1.90b5.2 software [15] to control of the quality of the genotypic data. We based on four criteria: low genotyping call rate (<0.9), minor allele frequency (<0.01), missing genotype call (>0.1), and low Hardy–Weinberg ($< 10^{-6}$), to

exclude SNPs from the analysis. A total of 44,573 SNPs remained and were used for GWAS.

**Analysis of genome-wide association studies and Heritability Estimates**

GWAS between SNPs from 382 genotyped samples and twenty-four fatty acid traits was conducted by the mixed linear model (MLM) leaving-one-chromosome-out (MLMA-LOCO) of genome-wide complex trait analysis (GCTA) software, version 1.93 [16]. The covariates were: sex, generation, body weight, and the top two principal components. The mathematical model used was as follows.

$$\mathbf{y = a + bx + g - + e}$$

where, **y** is the phenotypic value of each fatty acid corrected with covariates (sex, generation, body weight, and PC1 and PC2 of a principal component analysis), **a** is the mean of the phenotypic value; **b** is the additive effect of the tested SNP marker; **x** is the genotype of the SNP; **g** − is the effect of all SNPs excluding SNPs on the chromosome where the candidate SNP is mapped; and **e** is the residual effect vector. Bonferroni-corrected p-value ($\alpha = 0.05$) was used to identify the significant SNPs among the tested SNPs. We used the REML of the GCTA software to estimate the heritability of each fatty acid.

**Identification of significant SNPs and annotation of Candidate Genes**

We screened the significant SNPs by setting the Bonferroni-corrected p-value ($\alpha = 0.05$), and the candidate genes were identified within the 1 Mb (0.5 Mb upstream and downstream) region surrounding the significant SNP, and then annotated based on the GRCg6a 106 version from the Ensembl genome database. Then, candidate genes were

used to perform (KEGG) pathways and GO analyses by using the g: profiler database [17] by considering the significance level of 5%. Moreover, we searched different databases such as PubMed and NCBI to find the biological functions of the candidate genes.

## Results

### Basic statistics and heritability of the fatty acids in 10-week-old KNC-R chickens

Table 2 provides descriptive statistics for the different fatty acid profiles in 382 KNC-R chickens. The predominant fatty acids in KNC-R chickens were oleic (C18:1; average 28.252%), palmitic (C16:0; 20.895%), linoleic (C18:2; 15.975%), and arachidonic (C20:4; 10.541%) acids. Table 3 gives the heritability estimates for the fatty acid profiles of 382 KNC-R chickens. The heritability range was 0–0.438 (Table 3).

### Candidate genomic regions and annotation of potential candidate genes

We identified significant single nucleotide polymorphisms (SNPs) based on the Bonferroni-corrected *p*-value ($p < 0.05$). Significant SNPs were identified for nervonic (C24:1), linoleic (C18:2), and eicosadienoic (C20:2) acids. The top significant SNP for C24:1 was rs13667281 ($p = 5.25 \times 10^{-07}$) on chromosome 2 at bp 31215920, the most significant SNP for C18:2 was rs14011157 ($p = 7.69 \times 10^{-07}$) on chromosome 10 at bp 16289438, and the most significant SNP for C20:2 was rs10731996 ($p = 7.89 \times 10^{-07}$) on chromosome 22 at position 2910806 bp (Table 4). Here, we defined the significant genomic region for all traits as the region within 0.5 Mb upstream and downstream of the most significant SNP. The top significant SNP for nervonic acid (C24:1) was rs13667281 which is intron variant located within IGF2BP3 gene. The top significant SNP found for linoleic acid (C18:2) was rs14011157 is intergenic variant while the most significant SNP

for eicosadienoic acid (C20:2) was rs10731996 which is a missense variant located with in PPP1R3E gene (Table 4).

For nervonic (C24:1), linoleic (C18:2), and eicosadienoic (C20:2) acids, the most significant genomic regions were 30,725,920–31,715,920 bp on chromosome 2, 15,789,438–16,789,438 bp on chromosome 10, and 2,410,806–3,410,806 bp on chromosome 22, respectively. Figure 1 and Table 4 present the GWAS results for each SNP. We identified 5, 1, and 7 functional candidate genes for C24:1, C18:2, and C20:2, respectively (Table 5). There were no significant GO terms nor KEGG pathways for genes in the significant genomic regions. However, we searched the literature to identify functional candidate genes for C24:1, C18:2, and C20:2.

## Discussion

### Fatty acid profiles

Animal fatty acid composition is controlled by genetic and environmental factors [18]. The fatty acid composition of meat markedly influences meat quality and consumer health [3, 6]. We found that the predominant fatty acids in KNC-R chickens were oleic (C18:1; 28.252%), palmitic (C16:0; 20.895%), linoleic (C18:2: 15.975%), and arachidonic (C20:4; 10.541%) acids (Table 2), in agreement with previous findings [6,7]. This study also calculated the atherogenicity (AI) and thrombogenicity (TI) indexes. The AI is the ratio of all pro-atherogenic SFAs (C12:0, C14:0, and C16:0) and anti-atherogenic UFAs (MUFAs and PUFAs) [14, 19] while the TI is the ratio of pro-thrombogenic SFA (C14:0, C16:0, and C18:0) and anti-thrombogenic fatty acids (MUFAs and PUFAs: omega-6 and omega-3) [14,19]. AI and TI are good indicators of the quality of the fat in meat and milk, and lower values of AI and TI indicate that the fat contains more MUFAs and PUFAs [19, 20].

## Heritability estimates

Estimating heritability is very important for designing breeding programs and predicting the selection response [21]. We estimated heritability ranging from 0 for C14:0, C18:3, and n-3 fatty acids to 0.438 for SFAs (Table 3). Generally, the heritability estimates were lower than those reported in [11] but in the same range as those in [7]. Heritability estimates depends on the genetic background of the sampled population [21]. Here, we report the heritability estimates of AI (0.391) and TI (0.395) traits for the first time. The AI and TI traits have moderate heritability, suggesting that breeding chickens with favorable AI and TI is possible. Genetic selection is the best tool for improving meat quality traits with low or high heritability [22].

## Functional candidate fatty acid genes

The GWAS identified genes related to lipid metabolism, which influences the synthesis of different fatty acids. Genetically, the composition of fatty acid is controlled by many genes with small effects [23]. Different candidate genes that influence the fatty acid composition of chicken meat have been reported on chromosomes 1 (*ADIPOR2*, *LRP6*, and *FAR2*), 2 (*GCNT2*, *FABP4*, and *LRP12*), 3 (*FABP7* and *DEGS1*), 4 (*ELOVL6*), 6 (*SCD*), 7 (*MGAT5* and *LRP1B*), 9 (*HDLBP* and *ADIPOQ*), 10, and 18 (*FASN*) [10, 11]. Recent findings have mapped candidate genes for different fatty acid profiles on chromosomes 1, 3, 4, and 10 [7]. Here, we reported candidate genes influencing C24:1, C18:2, and C20:2 fatty acids on chromosomes 2, 10, and 22, respectively.

**Candidate genes for nervonic acid (C24:1)**

The nervonic acid (NA: C24:1**)** plays a role in brain development, brain maintenance, and memory improvement [24]. In poultry, fat is mainly synthesized in the liver [25], while *de novo* NA (C24:1) synthesis occurs in the cytoplasm and results from the elongation of oleic acid (C18:1) by ELOVL3 [26]. Exploration of the significant genomic region based on the significant SNP associated with NA revealed 16 protein-coding genes on chicken chromosome 2. However, only a few are functionally linked to lipid metabolism: the *IGF2BP3*, *GPNMB*, *NPY*, *OSBPL3*, and *IL6* genes.

Glycoprotein non-metastatic melanoma protein B (GPNMB) is encoded by the *GPNMB* gene, which is involved in melanin deposition, bone mineral deposition, regulation of inflammation, and lipid metabolism [27]. The *GPNMB* gene SNP (rs31126482) was reported to be linked with the weight of abdominal fat [27]. Additionally, *GPNMB* overexpression significantly increased the expression of the *FASN*, *SCD*, *ACACA*, *ACSL1*, *SREBP1*, and *PLIN2* genes, involved in the synthesis of different fatty acid [27]. The *GPNMG* gene might be a useful genetic marker in poultry breeding for fatty acid profiles. Insulin-like growth factor binding protein-3 (IGF2BP3) is a RNA-binding protein that controls *IGF2* expression [27]. *IGF2BP3* is also associated with IMF deposition in chickens [28].

Other candidate genes identified on chicken chromosome 2 include neuropeptide Y (NPY), which is encoded by the *NPY* gene. Its variants, such as rs16139, have been associated with obesity, high plasma LDL-cholesterol, and coronary artery disease in humans [29]. Oxysterol-binding protein-like 3 (OSBPL3), a member of the oxysterol-binding protein (OSBP) family, is encoded by the *OSBPL3* gene and has a key role in

hepatic fat accumulation [30]. Interleukin-6 (IL-6), encoded by the *IL6* gene, influences peripheral lipid metabolism [31].

**Candidate genes for linoleic acid (C18: 2)**

Linoleic acid influences meat flavor [1] and is synthetized via the desaturation of oleic acid (C18:1) by FADS2 [26]. On chromosome 10, we found one candidate gene, the *NR2F2* gene, also known as *COUP-TFII*. NR2F2 is involved in adipogenesis, lipid metabolism, and insulin secretion [32].

**Candidate genes for eicosadienoic acid (C20:2)**

Eicosadienoic acid (EDA; C20:2) is an omega-6 PUFA that is formed through the elongation of linoleic acid (C18:2) by ELOVL5 [26]. Among the 32 protein-coding genes found in the significant genomic region on chromosome 22, only 7 had functions related to lipid metabolism: the *GPAT4*, *PPP1R3E*, *NKX6-3*, *ANK1*, *SFRP1*, *ERLIN2*, and *STAR* genes. Glycerol-3-phosphate acyltransferase 4 (GPAT4) is the rate-limiting enzyme in the synthesis of glycerophospholipids (phosphoglycerides) and triacylglycerol (TAG or triglycerides) [33]. GPAT4 influences hepatic lipid accumulation [34].

Protein phosphatase 1 regulatory subunit 3E (PPP1R3E) encodes a regulatory subunit of protein phosphatase 1 (PP1), which is involved in glycogen metabolism [35]. *PPP1R3E* expression is regulated by insulin [35]. NK6 homeobox 3 (NKX6-3) is encoded by the *NKX6-3* gene and its expression is associated with increased triglyceride levels; thus, it is involved in lipid metabolism [36]. ANK1 belongs to the ankyrin family and is linked to IMF and meat quality traits, such as tenderness in pork [37].

241 Secreted frizzled-related protein 1 (SFRP1) is an SFRP protein that controls
242 adipogenesis [38]. SFRP1 regulates other genes involved in *de novo* fatty acid synthesis,
243 including *FASN*, *ACACA*, *ACLY*, *ELOVL*, and *SCD1* [38]. ER lipid raft-associated protein
244 2 (ERLIN2) is a prohibitin that regulates lipid metabolism [39]. Steroidogenic acute
245 regulatory protein (STAR) is the rate-limiting step in steroidogenesis [40]. To our
246 knowledge, this is the first report of genes on chicken chromosome 22 that are involved
247 in lipid metabolism.

248 The human body needs fatty acids for important functions, including brain
249 development. However, it is unable to synthesize fatty acids such as omega-3 (n-3) and
250 omega-6, which must be obtained from the diet [41]. Chicken, a globally popular and
251 inexpensive meat [42], and is a good source of these fatty acids. Excessive consumption
252 of some fatty acids is dangerous to humans, while other fatty acids are very beneficial.
253 Fatty acids influence the flavor of meat and can also affect consumer health. Thus,
254 selecting and producing meat with desirable fatty acids not only improves meat flavor but
255 can also improve human health. This study reported different candidate genes affecting
256 the fatty acid composition of chicken meat. The limitation of the current study was the
257 small sample size because we only used 382 samples. Therefore, these results need to be
258 validated in larger sample size. Furthermore, mapping studies are also needed to validate
259 the effects of different variants in the candidate genes.

260

261 ## Conclusion

262 Fatty acid composition is a polygenic trait influenced by many genes with small
263 effects, as well as by environmental factors including diet. These traits have low-to-
264 moderate heritability, and it is possible to improve them using genomic selection.

Through GWAS, we identified potential candidate genes affecting the fatty acid profile, such as *IGF2BP3*, *GPNMB*, *NPY*, *OSBPL3*, *IL6*, *NR2F2*, *GPAT4*, *PPP1R3E*, *NKX6-3*, *ANK1*, *SFRP1*, *ERLIN2*, and *STAR*. Interestingly, we found the *GPNMB* and *SFRP1* genes, whose expression regulates other genes related to fatty acid synthesis, including the *FASN*, *ACACA*, *ACLY*, *ELOVL*, and *SCD* genes. Our findings provide insight into the genes influencing lipid metabolism and fatty acid synthesis. Moreover, the identified SNPs might be used as biomarkers in chicken breeding.

## Conflict of interest

The authors declare no conflict of interest.

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## Tables and Figures

Table 1**.** Number of experimental chickens by generation and sex

| Generation | Male | Female | Total |
|---|---|---|---|
| Generation 1 | 98 | 92 | 190 |
| Generation 2 | 92 | 100 | 192 |
| Total | 190 | 192 | 382 |

Table 2**.** Basic statistics for fatty acids (%) in KNC-R chicken breast meat.

| Category | Trait | Mean | SD | CV (%) | Max | Min | Transf. |
|---|---|---|---|---|---|---|---|
| SFA | C14:0 | 0.326 | 0.126 | 0.016 | 2.13 | 0.16 | log |
| | C16:0 | 20.895 | 1.368 | 1.871 | 25.16 | 14.88 | tri |
| | C18:0 | 9.736 | 1.112 | 1.236 | 12.66 | 6.25 | tri |
| | SFA | 30.956 | 0.971 | 0.942 | 33.45 | 27.74 | normal |
| MUFA | C16:1 | 2.281 | 0.924 | 0.853 | 5.47 | 0.61 | sqrt |
| | C18:1 | 28.252 | 3.896 | 15.178 | 40.63 | 20.1 | log |
| | C24:1 | 1.059 | 0.312 | 0.097 | 2.12 | 0.34 | normal |
| | MUFA | 32.836 | 4.186 | 17.523 | 46.94 | 24.11 | log |
| PUFA | C18:2[2] | 15.975 | 1.789 | 3.2 | 21.43 | 3.87 | multi |
| | C18:3[1] | 0.285 | 0.68 | 0.462 | 13.5 | 0.14 | log |
| | C20:2[2] | 0.393 | 0.107 | 0.011 | 0.77 | 0.13 | normal |
| | C20:3[1] | 1.181 | 0.267 | 0.071 | 1.8 | 0.37 | normal |
| | C20:4[2] | 10.541 | 2.685 | 7.208 | 19.07 | 1.48 | multi |
| | C20:5[1] | 0.206 | 0.569 | 0.324 | 11.25 | 0.06 | log |
| | C22:6[1] | 1.059 | 0.312 | 0.097 | 2.12 | 0.34 | normal |
| | PUFA | 29.637 | 3.08 | 9.487 | 36.84 | 20.05 | multi |
| Omega-3 | n-3[1] | 2.729 | 1.32 | 1.742 | 26.51 | 1.17 | log |
| Omega-6 | n-6[2] | 26.908 | 2.95 | 8.702 | 33.72 | 5.69 | tri |
| MUFA+PUFA | UFA | 62.472 | 1.566 | 2.452 | 69.04 | 59.13 | log |
| Ratio | UFA/SFA | 2.021 | 0.099 | 0.01 | 2.41 | 1.81 | log |
| | P/S | 0.959 | 0.109 | 0.012 | 1.25 | 0.62 | normal |
| | n-6/n-3 | 10.375 | 1.845 | 3.403 | 18.84 | 0.21 | normal |
| AI | AI | 0.355 | 0.026 | 0.001 | 0.51 | 0.25 | tri |
| TI | TI | 0.814 | 0.044 | 0.002 | 0.93 | 0.3 | tri |

[1] omega (ω)-3 fatty acid, [2] omega (ω)-6 fatty acid, transf.: transformation, sqrt: square root, multi: multiple: log: logarithm, tri: triple, AI: atherogenicity index, TI: thrombogenicity index, SD: standard deviation; CV, coefficient of variation.

445 Table 3. Heritability estimates for fatty acid acids (%) in KNC-R chicken breast meat

| Category | Trait | $h^2$ |
|---|---|---|
| SFA | C14:0 | 0.000 |
| | C16:0 | 0.343 |
| | C18:0 | 0.253 |
| | SFA | 0.438 |
| MUFA | C16:1 | 0.165 |
| | C18:1 | 0.115 |
| | C24:1 | 0.115 |
| | MUFA | 0.116 |
| PUFA | C18:2[2] | 0.305 |
| | C18:3[1] | 0.000 |
| | C20:2[2] | 0.230 |
| | C20:3[1] | 0.056 |
| | C20:4[2] | 0.106 |
| | C20:5[1] | 0.192 |
| | C22:6[1] | 0.048 |
| | PUFA | 0.097 |
| Omega-3 | n-3[1] | 0.000 |
| Omega-6 | n-6[2] | 0.134 |
| MUFA+PUFA | UFA | 0.282 |
| Ratio | UFA/SFA | 0.394 |
| | P/S | 0.134 |
| | n-6/n-3 | 0.088 |
| AI | AI | 0.391 |
| TI | TI | 0.395 |

446 [1]omega (ω)-3 fatty acid, [2]omega (ω)-6 fatty acid, AI: atherogenicity index, TI:
447 thrombogenicity index.

448

449 Table 4. Significant SNPs for nervonic (C24:1), linoleic (C18:2), and eicosadienoic (C20:2) acids

| Trait | Chr | SNP | Physical position (bp) | Allele 1 | Allele 2 | SNP effect | Genomic location | P-value |
|---|---|---|---|---|---|---|---|---|
| C24:1 | 2 | rs13667281 | 31,215,920 | A | G | -0.0693569 | IGF2BP3 | $5.25 \times 10^{-07}$ |
| C18:2 | 10 | rs14011157 | 16,289,438 | A | G | 20.1483 | Intergenic | $7.69 \times 10^{-07}$ |
| C20:2 | 22 | rs10731996 | 2,910,806 | G | A | 0.0391945 | PPP1R3E | $7.89 \times 10^{-07}$ |

450 Chr: chromosome, C24:1: nervonic acid, C18:2: linoleic acid, C20:2: eicosadienoic acid.

451

452 Table 5. Candidate genes identified in the significant genomic regions

| Trait | SNP | Chr | P-value | Functional candidate genes |
|---|---|---|---|---|
| C24:1 | rs13667281 | 2 | $5.25 \times 10^{-07}$ | *IGF2BP3, GPNMB, NPY, OSBPL3, IL6* |
| C18:2 | rs14011157 | 10 | $7.69 \times 10^{-07}$ | *NR2F2* |
| C20:2 | rs10731996 | 22 | $7.89 \times 10^{-07}$ | *GPAT4, PPP1R3E, NKX6-3, ANK1, SFRP1, ERLIN2, STAR* |

453 Chr: chromosome, C24:1: nervonic acid, C18:2: linoleic acid, C20:2: eicosadienoic acid.

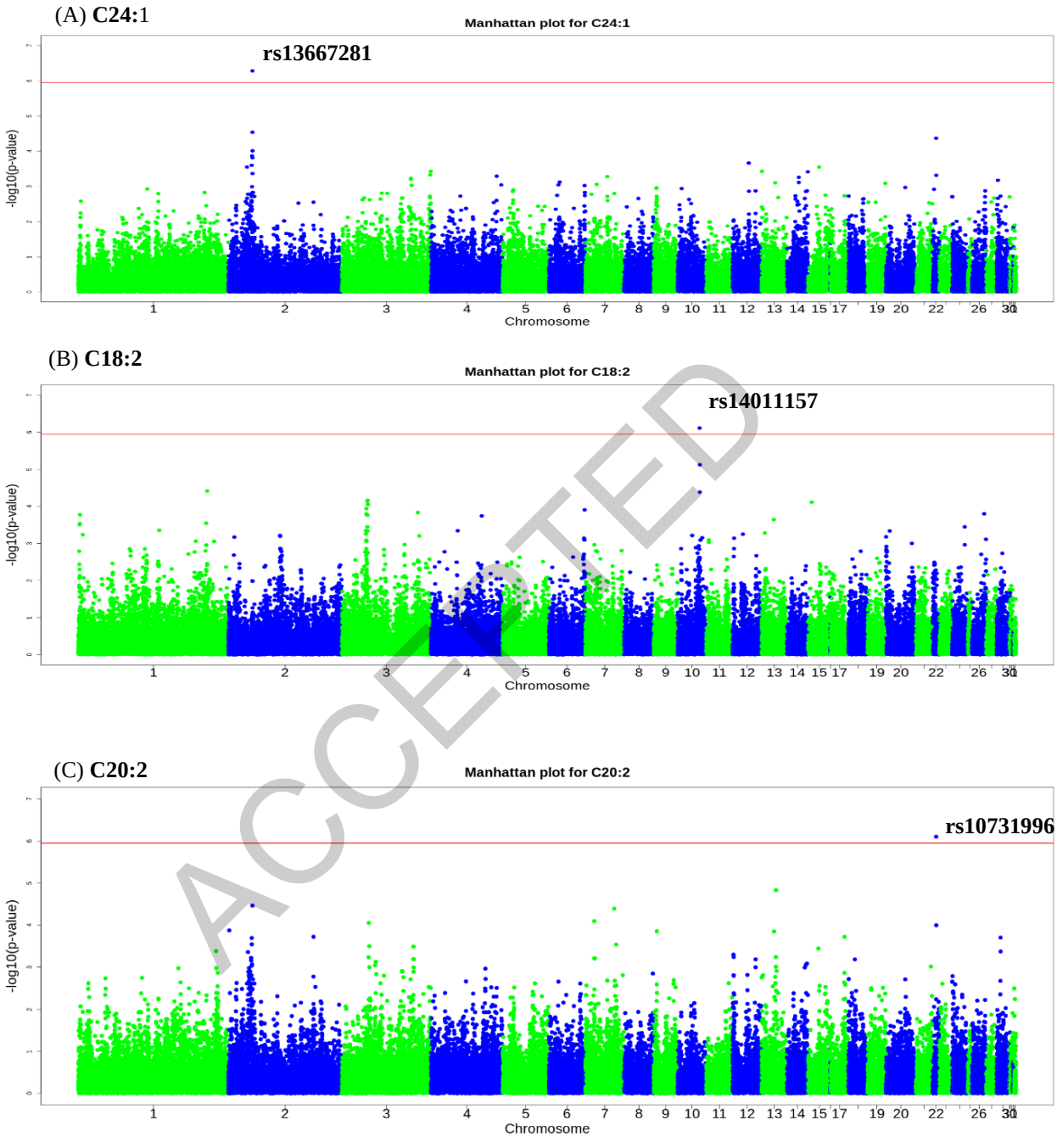




Figure 1. Manhattan plots of the genome-wide association study (GWAS) for C24:1 (A), C18:2 (B), C20:2 (C). The *x*-axis is the chromosome and the *y*-axis shows *P*-values ($-\log_{10}$). The red line indicates the Bonferroni-corrected 5% significance threshold.