

# A genome-wide association study for the fatty acid composition of breast meat in an F2 crossbred chicken population

Eunjin Cho<sup>1</sup>, Minjun Kim<sup>2</sup>, Sunghyun Cho<sup>3</sup>, Hee-Jin So<sup>4</sup>, Ki-Teak Lee<sup>4</sup>, Jihye Cha<sup>5</sup>, Daehyeok Jin<sup>6</sup> and Jun Heon Lee<sup>1,2\*</sup>

<sup>1</sup>Department of Bio-AI Convergence, Chungnam National University, Daejeon 34134, Korea

<sup>2</sup>Division of Animal & Dairy Science, Chungnam National University, Daejeon 34134, Korea

<sup>3</sup>Research and Development Center, Insilicogen Inc., Yongin 19654, Korea

<sup>4</sup>Department of Food Science and Technology, Chungnam National University, Daejeon 34134, Korea

<sup>5</sup>Animal Genome & Bioinformatics, National Institute of Animal Science, Rural Development Administration, Wanju 55365, Korea

<sup>6</sup>Animal Genetic Resources Research Center, National Institute of Animal Science, Rural Development Administration, Hamyang 50000, Korea



Received: Oct 31, 2022

Revised: Dec 28, 2022

Accepted: Jan 2, 2023

## \*Corresponding author

Jun Heon Lee

Department of Bio-AI Convergence,  
Chungnam National University,  
Daejeon 34134, Korea.

Tel: +82-42-821-5779

E-mail: junheon@cnu.ac.kr

Copyright © 2023 Korean Society of  
Animal Sciences and Technology.

This is an Open Access article  
distributed under the terms of the  
Creative Commons Attribution  
Non-Commercial License (<http://creativecommons.org/licenses/by-nc/4.0/>) which permits unrestricted  
non-commercial use, distribution, and  
reproduction in any medium, provided  
the original work is properly cited.

## ORCID

Eunjin Cho

<https://orcid.org/0000-0003-4800-1603>

Minjun Kim

<https://orcid.org/0000-0002-8173-8431>

Sunghyun Cho

<https://orcid.org/0000-0002-2657-9159>

Hee-Jin So

<https://orcid.org/0000-0002-7230-8528>

Ki-Teak Lee

<https://orcid.org/0000-0002-7482-6775>

## Abstract

The composition of fatty acids determines the flavor and quality of meat. Flavor compounds are generated during the cooking process by the decomposition of volatile fatty acids via lipid oxidation. A number of research on candidate genes related to fatty acid content in livestock species have been published. The majority of these studies focused on pigs and cattle; the association between fatty acid composition and meat quality in chickens has rarely been reported. Therefore, this study investigated candidate genes associated with fatty acid composition in chickens. A genome-wide association study (GWAS) was performed on 767 individuals from an F2 crossbred population of Yeonsan Ogye and White Leghorn chickens. The Illumina chicken 60K significant single-nucleotide polymorphism (SNP) genotype data and 30 fatty acids (%) in the breast meat of animals slaughtered at 10 weeks of age were analyzed. SNPs were shown to be significant in 15 traits: C10:0, C14:0, C18:0, C18:1n-7, C18:1n-9, C18:2n-6, C20:0, C20:2, C20:3n-6, C20:4n-6, C20:5n-3, C24:0, C24:1n-9, monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). These SNPs were mostly located on chromosome 10 and around the following genes: *ACSS3*, *BTG1*, *MCEE*, *PPARG-C1A*, *ACSL4*, *ELOVL4*, *CYB5R4*, *ME1*, and *TRPM1*. Both oleic acid and arachidonic acid contained the candidate genes: *MCEE* and *TRPM1*. These two fatty acids are antagonistic to each other and have been identified as traits that contribute to the production of volatile fatty acids. The results of this study improve our understanding of the genetic mechanisms through which fatty acids in chicken affect the meat flavor.

**Keywords:** Genome-wide association study, Fatty acid composition, Meat flavor, Chicken

Jihye Cha  
<https://orcid.org/0000-0002-9705-2979>  
 Daehyeok Jin  
<https://orcid.org/0000-0001-5091-4271>  
 Jun Heon Lee  
<https://orcid.org/0000-0003-3996-9209>

#### Competing interests

No potential conflict of interest relevant to this article was reported.

#### Funding sources

This study was partly supported by the "Cooperative Research Program for Agriculture Science & Technology Development (No. PJ015785)" of the Rural Development Administration, Korea and also supported by Chungnam National University.

#### Acknowledgements

Not applicable.

#### Availability of data and material

Upon reasonable request, the datasets of this study can be available from the corresponding author.

#### Authors' contributions

Conceptualization: Lee KT, Cha J, Jin D, Lee JH.

Data curation: Cho E, Kim M, Cho S, So HJ, Cha J, Jin D.

Formal analysis: Cho E, Kim M.

Methodology: Cho E, Kim M, Cho S.

Software: Cho E, Kim M.

Validation: Cho E, Kim M, Lee JH.

Investigation: Cho E, Kim M, Cho S, So HJ, Lee KT, Lee JH.

Writing - original draft: Cho E.

Writing - review & editing: Cho E, Kim M, Cho S, So HJ, Lee KT, Cha J, Jin D, Lee JH.

#### Ethics approval and consent to participate

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) of Chungnam National University (202103A-CNU-061).

## INTRODUCTION

Alongside pork and beef, poultry meat is a major source of protein. According to the Organization for Economic Cooperation Development (OECD) and Food and Agricultural Organization (FAO), poultry meat is expected to account for 41% of the world's meat supply by 2030 [1]. This reflects the lower price of poultry compared to other meats and increasing consumer preference for white meat, which is recognized as a healthy food item.

In Korea, the demand for chicken is increasing due to the trend toward the consumption of high-quality food as part of a healthy lifestyle [2]. Especially, Korean native chicken (KNC) has a different texture and taste compared to commercial broilers and layers and is highly favored by consumers. KNC has a higher amount of flavor-related components such as inosine, glycine, alanine, and proline than broiler [3]. In addition, KNCs are known to be superior to commercial chickens in physicochemical properties such as water holding capacity, tenderness, and fatty acid composition. [4,5]. For these reasons, consumers are more likely to consume KNCs even though they are more expensive. Therefore, there is a need to develop poultry products satisfying the quality and functionality requirements of consumers.

The quality and flavor of meat are determined by physicochemical properties as color, water holding capacity, tenderness, and other sensory evaluation. These traits were also affected by a complex array of substances such as free amino acids, nucleic acids, lipids, and minerals [6]. Fatty acids are among the most important contributors to meat flavor. Numerous flavor characteristics arise from volatile fatty acid degradation by lipid oxidation during the cooking process [7,8]. Lipid-derived volatiles react with other flavor compounds, such as the products of thermal oxidation and Maillard reaction, to form flavors.

Several studies have been published on candidate genes related to fatty acid composition, mainly in pigs and cattle [9–12]. The major candidate genes related to fatty acids are *FASN*, *SCD*, *FABP2*, and *ELOVL7*. It has reported that these genes were involved in the synthesis, elongation, and transportation of fatty acids. On the other hand, there have been few studies of the genetic association between fatty acid composition and meat quality in chickens [13]. According to Munyaneza et al. [14], no genome-wide association study (GWAS) has been reported on fatty acid composition in chickens although it is an important determinant of healthy meat.

Therefore, the aim of this study was to determine the candidate genes associated with fatty acid composition in chickens using a crossbred population (between two breeds with opposite phenotypes).

## MATERIALS AND METHODS

### Ethical approval

This research was approved by the Institutional Animal Care and Use Committee (IACUC) of Chungnam National University (202103A-CNU-061). All experiments were conducted following relevant guidelines and regulations.

### Experimental animals

A total of 767 birds in an F2 crossbred population between Yeonsan Ogye (YO) and White Leghorn (WL) were used for this study. The YO, which is one of KNC breed, is characterized by black feathers, skin, and bones, and has a unique meat flavor, whereas WL is a well-known layer breed with the opposite phenotype of the YO. The F2 generation was created by a reciprocal cross; one WL male was mated with five YO females (B line) and one YO male was mated with five WL

females (L line). Using the F2 population for genetic association studies via reciprocal crossbreeding across breeds with opposing phenotypes has the advantage of using normalized data by increasing the variance of the phenotype. This allows for a more accurate estimation of the effect of the genetic variation associated with the phenotype.

All birds were raised on farms in the Animal Genetic Resources Research Center, National Institute of Animal Science (NIAS, Korea) under the same environmental conditions.

### Phenotypes and quality control

The breast meat of birds slaughtered at 10 weeks of age was analyzed. The chicken carcass samples were rapidly frozen at  $-35^{\circ}\text{C}$  after slaughter and then stored at  $-20^{\circ}\text{C}$ . After 2–6 weeks, samples were transported for experiments and stored at  $-80^{\circ}\text{C}$  until deboning. Then, the breast meat was separated from carcass samples, which were thawed at  $4^{\circ}\text{C}$  for 20 h. The lipid was extracted from the breast meat, and it was sequentially mixed with pyrogallol solution, triundecanoin as an internal standard, and hydrogen chloride solution. Next, diethyl ether and petroleum ether were added, respectively, and the weight difference of the total amount was checked to calculate the content of crude fat. Following methylation, the samples were subjected to gas chromatography examination on an Agilent 6890 Gas Chromatograph (Santa Clara, USA). The concentration of each fatty acid was calculated with the internal standard, and each measured fatty acid value was divided by the total fatty acid value and expressed as a percentage.

A total of 30 fatty acids (%) were classified as follows: total saturated fatty acids (SFA), total monounsaturated fatty acids (MUFA), total polyunsaturated fatty acids (PUFA), total omega-3 ( $\omega$ -3) and total omega-6 ( $\omega$ -6). The calculations for the various fatty acid groups were as follows: SFA = C10:0 + C12:0 + C14:0 + C16:0 + C18:0 + C20:0 + C22:0 + C24:0; MUFA = C14:1 + C16:1 + C17:1 + C18:1t + C18:1n-7 + C18:1n-9 + C20:1 + C22:1n-9 + C24:1n-9; PUFA = C18:2t + C18:2n-6 + C18:3t + C18:3n-3 + C18:3n-6 + C20:2 + C20:3n-3 + C20:3n-6 + C20:4n-6 + C20:5n-3 + C22:2n-6 + C22:5n-3 + C22:6n-3;  $\omega$ -3 = C18:3n-3 + C20:3n-3 + C20:5n-3 + C22:5n-3 + C22:6n-3; and  $\omega$ -6 = C18:2n-6 + C18:3n-6 + C20:3n-6 + C20:4n-6 + C22:2n-6. P/S was the ratio between PUFA and SFA, and  $\omega$ -6/ $\omega$ -3 was the ratio between  $\omega$ -6 and  $\omega$ -3.

Before conducting a GWAS, all phenotype data were processed to remove skewness and ensure a normal distribution. Data normality was analyzed using the Shapiro-Wilk test, and the following transformation methods were applied: log, square root, sign, square, and cube. The data with the highest  $p$ -values were included in the analysis.

### Genotyping and quality control

Genomic DNA was extracted from blood samples of birds at 8 weeks of age using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The DNA samples were genotyped using the Illumina chicken 60K BeadChip. PLINK 1.9 software [15] was used for quality control (QC) based on three cut-offs: genotyping rate < 90%, minor allele frequency < 1%, and Hardy-Weinberg equilibrium (HWE) at  $p < 0.000001$ . After QC, 29,175 single nucleotide polymorphism (SNP) markers were subjected to the GWAS. Furthermore, principal component analysis (PCA) was conducted to confirm genetic relatedness and the potential for population stratification prior to the GWAS.

### Genome-wide association analysis and heritability

The GWAS was conducted on all genotyped SNPs and fatty acids using a mixed linear model (MLM). The MLM was developed with sex (male or female), line (B or L), body weight (8 weeks of age), and the top two principal components as covariates. All analyses were performed using

the MLM leaving-one-chromosome-out (MLMA-LOCO) analysis option of the Genome-wide Complex Trait Analysis (GCTA) software package [16]. The model equation was as follows:

$$y = Xb + Z\mu + g^- + e$$

where  $y$  is the phenotype for fatty acids;  $X$  and  $Z$  are incidence matrices for parameters  $b$  and  $\mu$ , respectively;  $b$  is the vector of fixed effects, including covariates;  $\mu$  is the vector of SNP effects;  $g^-$  is the accumulated effect of all SNPs except those on the chromosome where the candidate SNP is located; and  $e$  is the vector of the residual effect.

Variance components were estimated using the restricted maximum likelihood (REML) option in GCTA for calculating genomic heritability.

### Identification of candidate genes

After GWAS, significant SNPs were determined based on the Bonferroni-corrected  $p$ -value ( $\alpha = 0.05$ ). We searched for candidate genes in 1 Mb regions around SNPs that were significant in the GWAS and could be involved in the observed significant associations with the phenotypes. The gene annotation process was performed by searching the Ensembl (<https://asia.ensembl.org>) and National Center for Biotechnology Information (NCBI) (<https://www.ncbi.nlm.nih.gov>) databases based on the chicken reference genome (GRCg6a).

## RESULTS

### Phenotype statistics

A statistical summary of the fatty acid composition in F2 crossbred chickens is provided in Table 1. C18:1n-9 (21.96%) showed the highest content, followed by C16:0 (19.85%) and C20:4n-6 (17.87%). This concurred with reports of oleic acid (C18:1n-9), palmitic acid (C16:0), and linoleic acid (C18:2n-6) being the principal fatty acids in KNCs [3]. In addition, the arachidonic acid (C20:4n-6) content was high, which accorded with the report of flavor-related components being higher in KNCs than general broilers by Jin et al. [17].

### Genome-wide association analysis and heritability

The GWAS identified significant SNPs in 15 traits: C10:0, C14:0, C18:0, C18:1n-7, C18:1n-9, C18:2n-6, C20:0, C20:2, C20:3n-6, C20:4n-6, C20:5n-3, C24:0, C24:1n-9, MUFA and PUFA. As shown in Fig. 1, the significant SNPs were mainly located at GGA 10.

Table 2 lists candidate genes based on the top two SNPs identified for each trait. The same SNP (GGaluGA067637) was observed in C14:0, C18:1n-9, C20:4n-6, and PUFA, and the gene located in this SNP region was also confirmed in MUFA. Gga\_rs14381780 in C20:2 and C20:3n-6, GGaluGA070911 in C20:0 and C24:0, and Gga\_rs15572763 in C18:0 and C24:1n-9 were also identified.

The candidate genes in C14:0, C18:1n-9, C20:4n-6, PUFA, and MUFA were *MCEE* and *TRPM1*, and those in C20:2 and C20:3n-6 were *ELOVL4*, *CYB5R4*, and *ME1*. We also identified the *ACSS3* and *BTG1* genes in C10:0, the *PPARGC1A* gene in C18:1n-7, and the *ACSL4* gene in C18:2n-6.

Heritability was highest (0.416) in C18:1n-7, but had a low average value (0.169) (Table 3).

**Table 1.** Statistics of the fatty acid content (%) of F2 crossbred chickens at 10 weeks of age

Trait	Max	Min	Mean	SD
C10:0	0.99	0	0.10	0.11
C12:0	0.74	0	0.08	0.05
C14:0	0.95	0.03	0.28	0.10
C14:1	0.15	0	0.04	0.03
C16:0	25.77	12.63	19.85	1.93
C16:1	5.58	0.08	1.27	0.74
C17:1	0.25	0	0.02	0.05
C18:0	20.68	9.02	13.57	1.66
C18:1t	4.15	0	0.27	0.17
C18:1n-7	5.22	0.25	2.66	0.39
C18:1n-9	38.13	10.2	21.96	4.99
C18:2t	0.71	0	0.13	0.05
C18:2n-6	26.28	0.05	13.09	1.82
C18:3t	4.54	0	0.79	0.72
C18:3n-3	5.86	0	0.26	0.28
C18:3n-6	0.97	0	0.08	0.06
C20:0	0.8	0.12	0.23	0.07
C20:1	1.59	0.08	0.26	0.10
C20:2	1.71	0.04	0.41	0.12
C20:3n-3	0.15	0	0.01	0.02
C20:3n-6	1.77	0	1.03	0.23
C20:4n-6	31.62	0.21	17.87	5.03
C22:0	1.19	0	0.42	0.18
C20:5n-3	1.09	0	0.12	0.07
C22:1n-9	1.03	0	0.10	0.05
C22:2n-6	0.54	0	0.01	0.02
C22:5n-3	3.19	0.01	1.25	0.33
C22:6n-3	5.4	0.52	2.92	0.79
C24:0	1.05	0.05	0.28	0.12
C24:1n-9	3.95	0.01	0.43	0.19
SFA	52.17	21.85	34.81	1.48
MUFA	60.05	10.62	27.02	5.75
PUFA	83.83	0.83	37.97	5.75
$\omega$ -3	15.69	0.53	4.57	1.06
$\omega$ -6	61.18	0.26	32.07	4.75
P/S ratio	1.87	0.56	1.09	0.18
$\omega$ -6/ $\omega$ -3 ratio	18.27	3.56	7.29	1.46

Each quantified fatty acid content was divided by the total fatty acid value and calculated as a percentage.

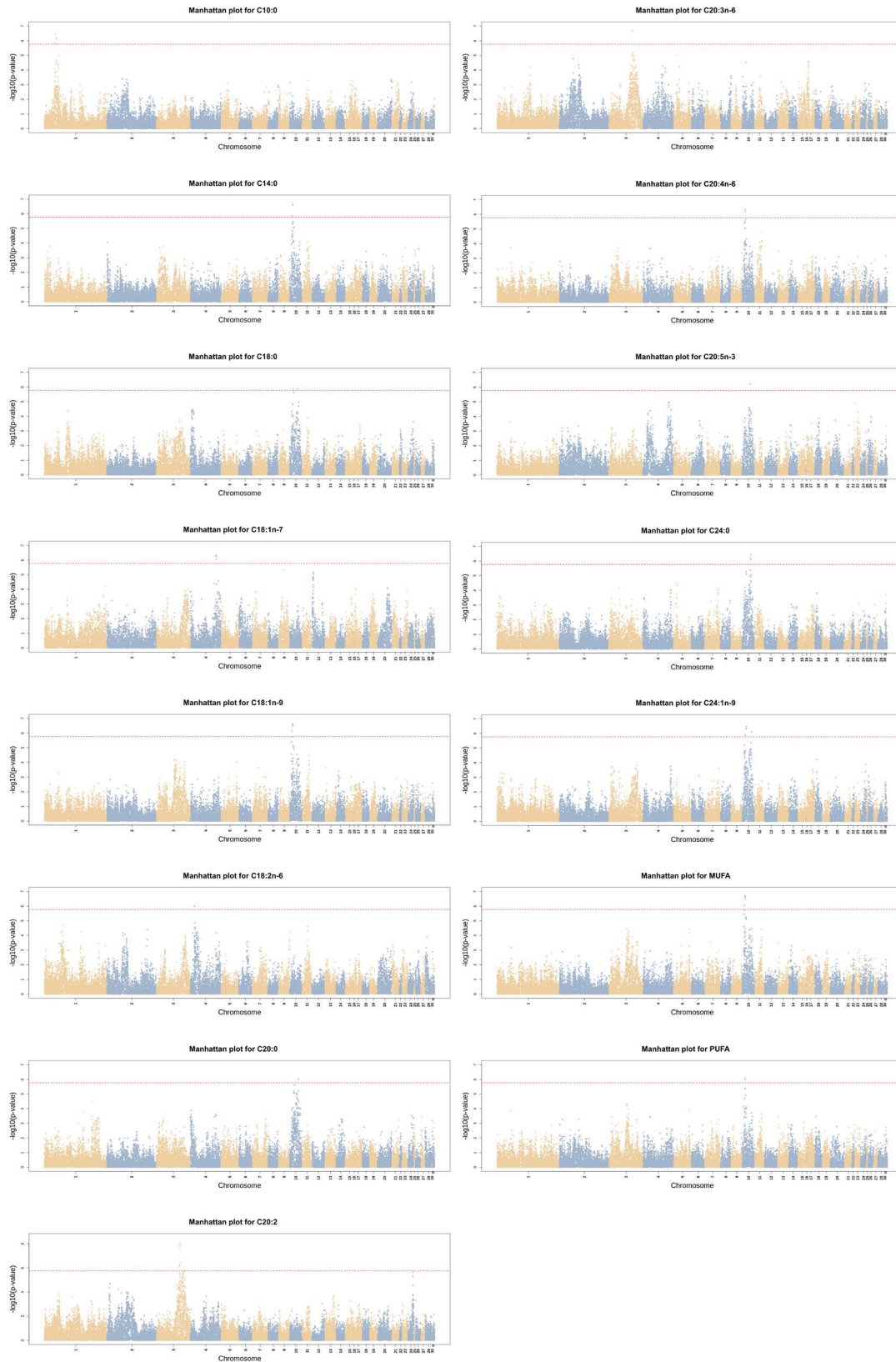
SFA, total saturated fatty acids; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids;  $\omega$ -3, total omega-3;  $\omega$ -6, total omega-6; P/S ratio, ratio between PUFA and SFA;  $\omega$ -6/ $\omega$ -3 ratio, ratio between  $\omega$ -6 and  $\omega$ -3.

## DISCUSSION

### Arachidonic acid content

High arachidonic acid content was observed in YO, a breed of KNC, in this study. A previous study





**Fig. 1.** Manhattan plots of single nucleotide polymorphism markers associated with 15 fatty acids. The red horizontal line denotes the Bonferroni-corrected threshold level of 5%.

**Table 2.** Top two single nucleotide polymorphism (SNP) markers associated with each fatty acid and possible positional candidate genes

Trait	SNP ID	Chr	Position	Allele 1	Allele 2	MAF	p-value	SNP effect	Candidate genes
C10:0	GGaluGA014205	1	40,688,299	A	G	0.02	$3.34 \times 10^{-7}$	0.14	<i>ACSS3, LUM, DCN, BTG1, zDH-HC17, NAV3</i>
	Gga_rs13857775	1	43,142,234	G	A	0.02	$7.12 \times 10^{-7}$	0.13	
C14:0	GGaluGA067637	10	6,215,383	T	C	0.29	$2.41 \times 10^{-7}$	0.10	<i>MCEE, TRPM1, APBA2, FAN1, MTMR10, KLF13</i>
	Gga_rs14003114	10	6,141,939	C	A	0.29	$2.49 \times 10^{-7}$	0.10	
C18:0	Gga_rs14008746	10	13,643,292	C	T	0.18	$1.33 \times 10^{-6}$	-0.07	<i>CGNL1, POLR2M, TCF12, ISG20, NTRK3</i>
	Gga_rs15572763	10	7,785,707	A	C	0.22	$1.47 \times 10^{-6}$	-0.06	
C18:1n-7	GGaluGA265712	4	74,285,145	A	G	0.39	$8.50 \times 10^{-7}$	-0.08	<i>PPARGC1A, ADGRA3</i>
	GGaluGA265714	4	74,314,272	G	A	0.39	$1.00 \times 10^{-6}$	-0.08	
C18:1n-9	GGaluGA067637	10	6,215,383	T	C	0.29	$2.40 \times 10^{-7}$	0.15	<i>MCEE, TRPM1, APBA2, FAN1, MTMR10, KLF13, ICE2</i>
	Gga_rs14002786	10	5,518,290	G	A	0.14	$2.52 \times 10^{-7}$	0.21	
C18:2n-6	Gga_rs14432039	4	14,106,048	T	C	0.15	$1.00 \times 10^{-6}$	0.02	<i>ACSL4, ATG4A, PSMD10, NXT2, TMEM164, KCNE5</i>
C20:0	GGaluGA070911	10	14,573,410	G	A	0.35	$1.01 \times 10^{-6}$	-0.06	<i>SV2B, KLHL25, MYO5A, LYSGM2, MAPK4</i>
	GGaluGA068440	10	9,134,046	C	T	0.12	$2.44 \times 10^{-6}$	0.09	
C20:2	Gga_rs14380944	3	77,374,297	A	G	0.44	$9.87 \times 10^{-9}$	0.07	<i>ELOVL4, CYB5R4, ME1, UBE3D, SNX14, RIPPLY2</i>
	Gga_rs14381780	3	78,062,830	C	T	0.49	$1.40 \times 10^{-8}$	-0.06	
C20:3n-6	Gga_rs14381780	3	78,062,830	C	T	0.49	$7.24 \times 10^{-7}$	-0.05	<i>CYB5R4, ME1, PRSS35, SNAP91, PGM3, TPBG</i>
C20:4n-6	GGaluGA067637	10	6,215,383	T	C	0.29	$4.75 \times 10^{-7}$	-1.44	<i>MCEE, TRPM1, APBA2, FAN1, MTMR10, KLF13</i>
	Gga_rs14003114	10	6,141,939	C	A	0.29	$6.59 \times 10^{-7}$	-1.42	
C20:5n-3	Gga_rs14008746	10	13,643,292	C	T	0.18	$6.23 \times 10^{-7}$	-0.02	<i>ISG20, NTRK3, MRPL46, AGBL1, RLBP1</i>
C24:0	GGaluGA070911	10	14,573,410	G	A	0.35	$3.73 \times 10^{-7}$	-0.10	<i>SV2B, ST8SIA2, MCTP2, KLHL25, AKAP13</i>
	GGaluGA070596	10	13,926,907	C	T	0.43	$6.58 \times 10^{-7}$	0.10	
C24:1n-9	Gga_rs15572763	10	7,785,707	A	C	0.22	$3.51 \times 10^{-7}$	-0.10	<i>MNS1, RFX7, PYGO1, RAB27A</i>
	GGaluGA068130	10	7,945,212	G	A	0.22	$4.75 \times 10^{-7}$	-0.10	
MUFA	Gga_rs14002786	10	5,518,290	G	A	0.14	$2.04 \times 10^{-7}$	0.08	<i>MCEE, TRPM1, FAN1, APBA2</i>
	GGaluGA067478	10	5,722,494	T	C	0.14	$2.04 \times 10^{-7}$	0.08	
PUFA	GGaluGA067637	10	6,215,383	T	C	0.29	$7.75 \times 10^{-7}$	-122.31	<i>MCEE, TRPM1, APBA2, FAN1, MTMR10, KLF13</i>
	Gga_rs14003114	10	6,141,939	C	A	0.29	$1.04 \times 10^{-6}$	-120.87	

Chr, chromosome; Allele 1, minor allele; Allele 2, major allele; MAF, minor allele frequency; MUFA, total monounsaturated fatty acids; PUFA, total polyunsaturated fatty acids.

reported that the arachidonic acid content of KNC breeds was significantly higher than that of broilers [18].

Chicken muscle contains more PUFAs than lamb and beef [19]. Hence, more unsaturated volatile aldehydes are produced in chicken compared to other species. These compounds are known to affect the flavor of the chicken. In chicken, the main unsaturated fatty acids are oleic acid and linoleic acid, while arachidonic acid is abundant in phospholipids. Arachidonic acid affects the flavor of meat by forming aromatic compounds such as trans-4, 5-epoxy-(E)-2-decenal, 1-octen-3-one, (E,Z)-2,4-decadienal, (E,E,Z)-2,4,7-tridecatrienal, and hexanal through oxidation [20].

In this study, the potential to explain the fatty acid content of YO using a crossbred group with WL (to enhance the GWAS) was limited. Nevertheless, considering the lack of previous reports on the fatty acid content of YO, the results should provide useful guidance for further research on the unique flavor and meat quality of YO.

**Table 3.** Heritability of the fatty acid content (%) of F2 crossbred chickens

Trait	Heritability
C10:0	0.033
C12:0	0.071
C14:0	0.189
C14:1	0.192
C16:0	0.193
C16:1	0.250
C17:1	0.077
C18:0	0.215
C18:1t	0.004
C18:1n-7	0.416
C18:1n-9	0.238
C18:2t	0.060
C18:2n-6	0.310
C18:3t	0.000
C18:3n-3	0.156
C18:3n-6	0.136
C20:0	0.238
C20:1	0.073
C20:2	0.279
C20:3n-3	0.116
C20:3n-6	0.277
C20:4n-6	0.155
C22:0	0.105
C20:5n-3	0.291
C22:1n-9	0.126
C22:2n-6	0.000
C22:5n-3	0.206
C22:6n-3	0.229
C24:0	0.213
C24:1n-9	0.238

Each quantified fatty acid content was divided by the total fatty acid value and calculated as a percentage.

### Relationship between oleic acid and arachidonic acid

In this study, the content of oleic acid, which is one of the main fatty acids in chickens, was high. Moreover, low arachidonic acid content was observed in individuals with a high oleic acid content. A negative correlation between the two fatty acids has also been reported in humans and mice [21,22].

The mechanism underlying the relationship between the two fatty acids has not yet been elucidated. One possible mechanism involves changes in the relative content of other relevant fatty acids. According to Høstmark and Haug [23],  $\alpha$ -linolenic acid content is positively related to the ratio of oleic acid to arachidonic acid. In our GWAS, the candidate gene associated with linoleic acid was *ACSL4*, which causes arachidonic acid catalysis [24].

Another possibility is that oleic acid could act as an inhibitor of Elongase-5 or Delta-5/6 desaturases, which synthesize arachidonic acid. Alternatively, arachidonic acid may act as an inhibitor of Delta-9 desaturase, which produces oleic acid. Further research is needed to validate



this hypothesis, although it is supported by previous studies showing that PUFAs of the  $\omega$ -3 group, such as  $\alpha$ -linolenic acid, inhibit this transcription [25].

The *MCEE* gene, which was common in C14:0, PUFA, and MUFA, encodes a methylmalonyl-CoA epimerase and is involved in fatty acid catabolism [26]. In addition, the *TRPM1* gene forms a protein constituting the transient receptor potential (TRP) cation channel, which plays an important role in fatty acid oxidation and signal transduction pathways [27,28]. According to Khan et al. [29], the amount of volatile compounds derived from fatty acid metabolism is increased by products formed as a result of enzymatic activity occurring during the metabolic process. Therefore, the two candidate genes identified by our GWAS may have contributed to the metabolic process of each fatty acid, and influenced the production of volatile compounds that determine the meat quality and flavor of chicken.

### Candidate genes in other fatty acids

Candidate genes that were common in C20:2 and C20:3n-6 included *ELOVL4*, *CYB5R4*, and *ME1*. The *ELOVL4* gene encodes an enzyme required to synthesize long-chain fatty acids and plays an important role in the formation of long-chain PUFAs [30]. According to Duckett and Kuber [31], oxidation products formed from long-chain unsaturated fatty acids, such as  $\omega$ -3 and 6, have a significant effect on the flavor of lamb. *CYB5R4* is an electron donor for fatty acid desaturase by stearoyl-CoA desaturase (SCD) [32]. SCD plays a key role in the formation of double bonds and contributes to the biosynthesis of unsaturated fatty acids, such as palmitoleic acid (C16:1) and oleic acid (C18:1n-9), from SFAs such as palmitic acid (C16:0) and stearic acid (C18:0). According to a study by Kawaguchi et al. [33], it was confirmed that the *CYB5R4* gene was present in the quantitative trait loci (QTL) region associated with the oleic acid percentage in Japanese Black cattle. In addition, *ME1* encodes an enzyme that generates nicotinamide adenine dinucleotide phosphate (NADPH) for fatty acid biosynthesis, and it has been reported that overexpression of endogenous ME1 promotes SFA and PUFA biosynthesis [34].

Candidate genes identified in C10:0 included *ACSS3* and *BTG1*. *ACSS3* encodes acyl-CoA synthetase and activates short-chain fatty acids. According to Dinh et al. [8], short-chain fatty acids, which are major volatile substances produced at high temperatures along with lactones, alcohols, and ketones, are among the important factors determining the flavor of meat. Because they react with other compounds during cooking, including participation in the Maillard reaction, they can produce more desirable volatiles compared to autoxidation. Also, according to Buitenhuis et al. [35], the *ACSS3* gene was identified as a candidate gene affecting the content of C10:0 in cattle milk fat composition.

The candidate gene identified in C18:1n-7 was *PPARGC1*, which encodes peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 $\alpha$ ) in humans. Nikolić et al. [36] reported that overexpression of PGC-1 $\alpha$  affects the activation of transcription factors that convert muscle cells into oxidative metabolism and increases the mRNA expression of genes regulating lipid metabolism.

### Study significance and limitations

Currently, the poultry industry in Korea relies mainly on imported foreign species due to their excellent growth ability; such species account for > 90% of the Korean poultry industry. As such, the industry would face industrial collapse if imports became difficult to obtain. Therefore, genome-based analysis of various economic traits of KNCs, as well as of meat-specific components, is urgently needed to discover physiologically functional substances. This could lay a foundation for the development of superior species for meat and enhance the international competitiveness of the

Korean poultry industry.

In this study, a GWAS was performed to determine the fatty acids contributing to the unique meat quality and flavor of a breed of KNC. However, the results may not fully explain the relationships of meat quality and flavor with fatty acid composition.

The volatile substances that affect meat quality and flavor are affected by various environmental factors. In general, it is difficult not only to generate flavor defects, but also to improve flavor during manufacturing and processing. The influence of different substances on flavor depends on the cooking method. Cooking for an extended period causes a Maillard reaction, while grilling or frying at  $\geq 100^\circ\text{C}$  results in the generation of aromatic components, such as heterocyclic compounds [37]. The nutritional conditions during poultry breeding are also very important factors affecting meat quality and safety. In particular, linoleic acid and  $\alpha$ -linolenic acid, which synthesize various fatty acids that affect flavor, are essential fatty acids not produced in the body; therefore, they are greatly affected by intake amounts. In addition, breeding conditions, storage temperature and duration, and the post-slaughter treatment process can cause many changes in meat quality and flavor. Therefore, to explain the genetic relationship between fatty acid composition and meat quality, a more detailed analysis that considers the effects of the above-mentioned environmental factors is required.

Numerous studies of the fatty acid composition and role of individual fatty acids in meat quality and taste have been conducted. However, most of the studies were conducted on ruminants or pigs; few have analyzed poultry meat. In a QTL study of fatty acid content, 1,201 QTL regions in cattle, and 6,460 in pigs, were investigated, whereas in a study on chickens only 10 QTL regions were investigated (Animal QTL Database; based on an accession date in August 2022). Therefore, the results of this study provide important genetic information on the fatty acid composition of poultry meat and help explain their influence on the quality and flavor of poultry meat.

## CONCLUSION

A GWAS was performed of the fatty acids responsible for the unique meat quality characteristics of YO, which is a breed of KNC. Significant results were obtained for 15 fatty acids, including oleic acid and arachidonic acid. The functions and mechanisms of candidate genes affecting meat quality and flavor, such as *MCEE* and *TRPM1*, were also confirmed. Additional analyses will be required before utilizing polygenic traits, such as fatty acid composition, and can be exploited in actual breeding programs for the development of high-quality breeds. Nevertheless, our study provides genetic information that could lead to the improvement of meat quality, especially the fatty acid composition of KNCs.

## REFERENCES

1. OECD [Organisation for Economic Co-operation and Development] / FAO [Food and Agricultural Organization]. OECD-FAO agricultural outlook 2021–2030. Paris: OECD; 2021.
2. Park S, Kim N, Jang Y, Lee D, Moon J. Poultry industry trends and consumer analysis in Korea: native Korean chicken and processed chicken. *Agribus Inf Manag.* 2019;11:25–34. <https://doi.org/10.14771/aim.11.2.3>
3. Choe JH, Nam KC, Jung S, Kim BN, Yun HJ, Jo CR. Differences in the quality characteristics between commercial Korean native chickens and broilers. *Korean J Food Sci Anim Resour.* 2010;30:13–9. <https://doi.org/10.5851/kosfa.2010.30.1.13>
4. Kweon YJ, Yeo JS, Sung SK. Quality characteristics of Korean native chicken meat. *Korean J*

- Poult Sci. 1995;22:223-31.
5. Lee SG, Utama DT, Baek KH, Park YH, Han JY, Lee SK. Comparison of physicochemical characteristics of the meat in four lines of Korean native chickens. *Korean J Poult Sci.* 2015;42:335-45. <https://doi.org/10.5536/KJPS.2015.42.4.335>
  6. Dashdorj D, Amna T, Hwang I. Influence of specific taste-active components on meat flavor as affected by intrinsic and extrinsic factors: an overview. *Eur Food Res Technol.* 2015;241:157-71. <https://doi.org/10.1007/s00217-015-2449-3>
  7. Arshad MS, Sohaib M, Ahmad RS, Nadeem MT, Imran A, Arshad MU, et al. Ruminant meat flavor influenced by different factors with special reference to fatty acids. *Lipids Health Dis.* 2018;17:223. <https://doi.org/10.1186/s12944-018-0860-z>
  8. Dinh TT, To KV, Schilling MW. Fatty acid composition of meat animals as flavor precursors. *Meat Muscle Biol.* 2021;5:34. <https://doi.org/10.22175/mmb.12251>
  9. Ishii A, Yamaji K, Uemoto Y, Sasago N, Kobayashi E, Kobayashi N, et al. Genome-wide association study for fatty acid composition in Japanese Black cattle. *Anim Sci J.* 2013;84:675-82. <https://doi.org/10.1111/asj.12063>
  10. Dawood M, Kramer LM, Shabbir MI, Reecy JM. Genome-wide association study for fatty acid composition in American Angus cattle. *Animals.* 2021;11:2424. <https://doi.org/10.3390/ani11082424>
  11. Yang B, Zhang W, Zhang Z, Fan Y, Xie X, Ai H, et al. Genome-wide association analyses for fatty acid composition in porcine muscle and abdominal fat tissues. *PLOS ONE.* 2013;8:e65554. <https://doi.org/10.1371/journal.pone.0065554>
  12. Zhang Y, Zhang J, Gong H, Cui L, Zhang W, Ma J, et al. Genetic correlation of fatty acid composition with growth, carcass, fat deposition and meat quality traits based on GWAS data in six pig populations. *Meat Sci.* 2019;150:47-55. <https://doi.org/10.1016/j.meatsci.2018.12.008>
  13. Jin S, Park HB, Seo D, Choi NR, Manjula P, Cahyadi M, et al. Identification of quantitative trait loci for the fatty acid composition in Korean native chicken. *Asian-Australas J Anim Sci.* 2018;31:1134-40. <https://doi.org/10.5713/ajas.17.0781>
  14. Munyaneza JP, Ediriweera TK, Kim M, Cho E, Jang A, Choo HJ, et al. Genome-wide association studies of meat quality traits in chickens: a review. *Korean J Agric Sci.* 2022;49:407-20. <https://doi.org/10.7744/kjoas.20220029>
  15. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet.* 2007;81:559-75. <https://doi.org/10.1086/519795>
  16. Yang J, Lee SH, Goddard ME, Visscher PM. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet.* 2011;88:76-82. <https://doi.org/10.1016/j.ajhg.2010.11.011>
  17. Jin S, Park HB, Jung S, Jo C, Seo DW, Choi NR, et al. The line differences and genetic parameters of linoleic and arachidonic acid contents in Korean native chicken muscles. *Korean J Poult Sci.* 2014;41:151-7. <https://doi.org/10.5536/KJPS.2014.41.3.151>
  18. Lee KH, Kim HJ, Lee HJ, Kang MG, Jo CR. A study on components related to flavor and taste in commercial broiler and Korean native chicken meat. *Korean J Food Preserv.* 2012;19:385-92. <https://doi.org/10.11002/kjfp.2012.19.3.385>
  19. Calkins CR, Hodgen JM. A fresh look at meat flavor. *Meat Sci.* 2007;77:63-80. <https://doi.org/10.1016/j.meatsci.2007.04.016>
  20. Blank I, Lin J, Vera FA, Welti DH, Fay LB. Identification of potent odorants formed by autoxidation of arachidonic acid: structure elucidation and synthesis of (E,Z,Z)-2,4,7-tridecatrienal. *J Agric Food Chem.* 2001;49:2959-65. <https://doi.org/10.1021/jf010160+>

21. Kudo N, Yamazaki T, Sakamoto T, Sunaga K, Tsuda T, Mitsumoto A, et al. Effects of perfluorinated fatty acids with different carbon chain length on fatty acid profiles of hepatic lipids in mice. *Biol Pharm Bull.* 2011;34:856-64. <https://doi.org/10.1248/bpb.34.856>
22. Høstmark AT, Haug A. Percentages of oleic acid and arachidonic acid are inversely related in phospholipids of human sera. *Lipids Health Dis.* 2013;12:106. <https://doi.org/10.1186/1476-511X-12-106>
23. Høstmark AT, Haug A. The inverse association between relative abundances of oleic acid and arachidonic acid is related to alpha-linolenic acid. *Lipids Health Dis.* 2014;13:76. <https://doi.org/10.1186/1476-511X-13-76>
24. Kuwata H, Hara S. Role of acyl-CoA synthetase ACSL4 in arachidonic acid metabolism. *Prostaglandins Other Lipid Mediat.* 2019;144:106363. <https://doi.org/10.1016/j.prostaglandins.2019.106363>
25. Ntambi JM, Bené H. Polyunsaturated fatty acid regulation of gene expression. *J Mol Neurosci.* 2001;16:273-8. <https://doi.org/10.1385/JMN:16:2-3:273>
26. Martín-Pozuelo G, Navarro-González I, González-Barrio R, Santaella M, García-Alonso J, Hidalgo N, et al. The effect of tomato juice supplementation on biomarkers and gene expression related to lipid metabolism in rats with induced hepatic steatosis. *Eur J Nutr.* 2015;54:933-44. <https://doi.org/10.1007/s00394-014-0770-4>
27. Sun W, Luo Y, Zhang F, Tang S, Zhu T. Involvement of TRP channels in adipocyte thermogenesis: an update. *Front Cell Dev Biol.* 2021;9:686173. <https://doi.org/10.3389/fcell.2021.686173>
28. Liu P, Shah BP, Croasdell S, Gilbertson TA. Transient receptor potential channel type M5 is essential for fat taste. *J Neurosci.* 2011;31:8634-42. <https://doi.org/10.1523/JNEUROSCI.6273-10.2011>
29. Khan MI, Jo C, Tariq MR. Meat flavor precursors and factors influencing flavor precursors—a systematic review. *Meat Sci.* 2015;110:278-84. <https://doi.org/10.1016/j.meatsci.2015.08.002>
30. Harkewicz R, Du H, Tong Z, Alkuraya H, Bedell M, Sun W, et al. Essential role of ELOVL4 protein in very long chain fatty acid synthesis and retinal function. *J Biol Chem.* 2012;287:11469-80. <https://doi.org/10.1074/jbc.M111.256073>
31. Duckett SK, Kuber PS. Genetic and nutritional effects on lamb flavor. *J Anim Sci.* 2001;79:E249-54. <https://doi.org/10.2527/jas2001.79E-SupplE249x>
32. Deng B, Parthasarathy S, Wang W, Gibney BR, Battaile KP, Lovell S, et al. Study of the individual cytochrome b5 and cytochrome b5 reductase domains of Ncb5or reveals a unique heme pocket and a possible role of the CS domain. *J Biol Chem.* 2010;285:30181-91. <https://doi.org/10.1074/jbc.M110.120329>
33. Kawaguchi F, Kigoshi H, Fukushima M, Iwamoto E, Kobayashi E, Oyama K, et al. Whole-genome resequencing to identify candidate genes for the QTL for oleic acid percentage in Japanese Black cattle. *Anim Sci J.* 2019;90:467-72. <https://doi.org/10.1111/asj.13179>
34. Zhu BH, Zhang RH, Lv NN, Yang GP, Wang YS, Pan KH. The role of malic enzyme on promoting total lipid and fatty acid production in *Phaeodactylum tricornutum*. *Front Plant Sci.* 2018;9:826. <https://doi.org/10.3389/fpls.2018.00826>
35. Buitenhuis B, Janss LGG, Poulsen NA, Larsen LB, Larsen MK, Sørensen P. Genome-wide association and biological pathway analysis for milk-fat composition in Danish Holstein and Danish Jersey cattle. *BMC Genomics.* 2014;15:1112. <https://doi.org/10.1186/1471-2164-15-1112>
36. Nikolić N, Rhedin M, Rustan AC, Storlien L, Thoresen GH, Strömstedt M. Overexpression

- of PGC-1 $\alpha$  increases fatty acid oxidative capacity of human skeletal muscle cells. *Biochem Res Int.* 2012;2012:714074. <https://doi.org/10.1155/2012/714074>
37. Melton SL. Current status of meat flavor. In: Xiong YL, Chi-Tang H, Shahidi F, editors. *Quality attributes of muscle foods*. Boston, MA: Springer; 1999. p. 115-33.