Effects of bovine fatty acid synthase, stearoyl-coenzyme A desaturase, sterol regulatory element-binding protein 1, and growth hormone gene polymorphisms on fatty acid composition and carcass traits in Japanese Black cattle

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Effects of bovine fatty acid synthase, stearoyl-coenzyme A desaturase, sterol regulatory element-binding protein 1, and growth hormone gene polymorphisms on fatty acid composition and carcass traits in Japanese Black cattle[1]

T. Matsuhashi,*[2] S. Maruyama,* Y. Uemoto,† N. Kobayashi,* H. Mannen,‡ T. Abe,† S. Sakaguchi,* and E. Kobayashi†

*Gifu Prefectural Livestock Research Institute, Kiyomi, Takayama, Gifu 506-0101, Japan;
†National Livestock Breeding Center, Nishigo, Fukushima 961-8511, Japan;
and ‡Graduate School of Agricultural Science, Kobe University, Rokkodai, Kobe 657-8501, Japan

ABSTRACT: The quality of fat is an important factor in defining the quality of meat. Fat quality is determined by the composition of fatty acids. Among lipid metabolism-related genes, including fatty acid synthesis genes, several genetic variations have been reported in the bovine fatty acid synthase (*FASN*), stearoyl-CoA desaturase (*SCD*), sterol regulatory element-binding protein 1 (*SREBP1*), and *GH* genes. In the present study, we evaluated the single and epistatic effects of 5 genetic variations (4 SNP and 1 insertion/deletion) in 4 genes (*FASN*, *SCD*, *SREBP1*, and *GH*) on the fatty acid composition of the longissimus thoracis muscle and carcass and meat quality traits in 480 commercial Japanese Black cattle. Significant single effects of *FASN*, *SCD*, and GH^{L127V} polymorphisms on the fatty acid composition of the longissimus thoracis muscle were detected. The A293V polymorphism of *SCD* had the largest effect on myristic acid (C14:0, $P < 0.001$), myristoleic acid (C14:1, $P < 0.001$), stearic acid (C18:0, $P < 0.001$), oleic acid (C18:1, $P < 0.001$), and MUFA ($P < 0.001$). Polymorphisms in the *FASN*, *SCD*, and *SREBP1* genes showed no effect on any meat yield trait. There were no significant epistatic effects on fatty acid composition among pairs of the 3 genes (*FASN*, *SCD*, and *SREBP1*) involved in fatty acid synthesis. No epistatic interactions ($P > 0.1$) were detected between *FASN* and *SCD* for any carcass trait. When the genotypes of 3 markers (*FASN*, *SCD*, and GH^{L127V}) were substituted from the lesser effect allele to the greater effect allele, the proportion of C18:1 increased by 4.46%. More than 20% of the genetic variance in the C18:1 level could be accounted for by these 3 genetic markers. The present results revealed that polymorphisms in 2 fatty acid synthesis genes (*FASN* and *SCD*) independently influenced fatty acid composition in the longissimus thoracis muscle. These results suggest that SNP in the *FASN* and *SCD* genes are useful markers for the improvement of fatty acid composition in commercial Japanese Black cattle.

Key words: bovine, carcass trait, fatty acid, fatty acid synthase, growth hormone, stearoyl-coenzyme A desaturase

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INTRODUCTION

Wagyu beef is highly rated because of its abundant marbling and desirable tenderness. Recently, the quality of the fat has become an important factor in defining the quality of the meat in the Japanese beef market. The quality of the fat is determined by its fatty acid composition, and fat tissue containing MUFA possesses a lower melting point, which contributes positively to favorable beef flavor and tenderness (Westerling and Hedrick, 1979; Melton et al., 1982). Oleic acid (C18:1) is one of the major MUFA in beef fat, and C18:1 is thought to be the source of the aroma of the cooked beef (Melton et al., 1982; Mandell et al., 1998). For

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[2]Corresponding author: matsuhashi-tamako@pref.gifu.lg.jp

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optimal beef, it is important to improve the fatty acid composition, especially the proportion of C18:1.

The fatty acid composition is a heritable trait with heritability ranging between 0.31 and 0.73 (Inoue et al., 2008). Several genetic variations have been reported in the bovine 3 fatty acid synthesis genes: fatty acid synthase (***FASN***; Abe et al., 2009), stearoyl-CoA desaturase (***SCD***; Taniguchi et al., 2004), and sterol regulatory element-binding protein 1 (***SREBP1***; Hoashi et al., 2007). In addition to these fatty acid synthesis genes, it has been reported that polymorphisms in the bovine *GH* gene are related to the fatty acid composition in adipose tissue (Ardiyanti et al., 2009).

Considering the strong functional relationships among these factors, it is important not only to reveal the independent effects of each factor, but also to analyze their combined effects. However, previous studies have been sporadic in their reporting of the single-factor effects, and have not always considered independent and combined effects. Additionally, for the beef cattle breeding program, it is important to ensure that there are no negative influences on carcass traits when they are used as genetic markers for the improvement of fatty acid composition in meat.

In the present study, we evaluated the independent and epistatic effects of 5 polymorphisms [4 SNP and 1 insertion/deletion (**ins/del**)] in 4 lipid metabolism-related genes [3 fatty acid synthesis genes (*FASN*, *SCD*, *SREBP1*, and *GH*) on the fatty acid composition of the longissimus thoracis muscle and carcass traits in the commercial Japanese Black cattle population.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not obtained for this study because data were collected from beef cattle shipped to a meat processing plant in Gifu, Japan.

Animals and Carcass Traits

The present study used a commercial Wagyu population produced in Gifu, Japan. Animals were the progeny of 84 sires and were fattened at 38 farms. A total of 2,390 animals, traced back for 3 generations, were used to generate pedigree information in this study. Adipose tissue and longissimus thoracis muscle fat were collected from 480 Japanese Black cattle slaughtered at 24 to 34 mo of age (mean ± SD: 28.05 ± 1.34 mo), with over 300 kg of carcass weight (**CW**). The samples were collected a few days after the cattle were slaughtered during 2005 to 2008. Each carcass was graded at the 6th- to 7th-rib interface by official Japanese graders in accordance with the Japanese Grading Standards (JMGA, 1989). Analyzed traits were as follows: CW (kg), rib eye area (**REA**, cm^2), rib thickness (**RT**, cm), subcutaneous fat thickness (**SFT**, cm), carcass yield estimate (**YE**), beef marbling standard [**BMS**, ranging from 1 (poor) to 12 (very abundant)], beef color standard [ranging from 1 (light) to 7 (dark)], luster [ranging from 1 (debased) to 5 (excellent)], firmness [ranging from 1 (debased) to 5 (excellent)], and texture [ranging from 1 (debased) to 5 (excellent)]. Carcass yield estimate was calculated using CW, REA, RT, and SFT as the estimated percentage of salable meat [YE (%) = 69.419 + 0.130REA + 0.667RT − 0.025CW − 0.896SFT]. Adipose tissues and longissimus thoracis muscle samples were harvested from sliced meat 1 cm thick between the 6th and 7th ribs. The moisture content of the longissimus thoracis muscle was calculated from the weight difference between wet and dried samples. Quantitative analysis of crude fat in the longissimus thoracis muscle was determined by the standard Soxhlet extraction technique.

Fatty Acid Analysis

We used the longissimus thoracis muscle in this study because this muscle has the most influence on the market price in Japan. Fatty acid content was analyzed by HPLC using the method by Borch (1975) with some modifications. Approximately 500 mg of minced longissimus thoracis muscle samples were saponified in 5 mL of 25% KOH/EtOH (1:1, vol/vol) at 90°C for 2 h. After the addition of an equal amount of H_2O and 15 mL of hexane for washing, 2 *N* HCl was added for acidification, and the hydrolysate was extracted twice with 5 mL of hexane. The hexane extracts were dehydrated with anhydrous sodium sulfate, and dried with a nitrogen purge at 60°C. The pellets were converted to phenacyl esters by adding 100 μL of 50 m*M* phenacyl bromide and 100 μL of triethylamine at 60°C for 2 h. Prepared fatty acid phenacyl esters (**FAPE**) were separated by HPLC using a Shim-pack FC-ODS column (75 mm × 4.6 mm inner diameter; Shimazu, Kyoto, Japan), with elution (1 mL/min, 45°C) by a linear gradient of acetonitrile/10 m*M* sodium phosphate (pH 2.6), started at 80/20 (vol/vol) for 20 min, increased to 90:10 (vol/vol) for 7 min, and returned to 80:20 (vol/vol) for 3 min. Elution of FAPE was monitored by absorbance at 254 nm.

DNA Isolation and Genotyping

In this study, we genotyped the polymorphisms of 4 lipid metabolism-related genes, *FASN*, *SCD*, *SREBP1*, and *GH*, as described by Abe et al. (2009), Taniguchi et al. (2004), Hoashi et al. (2007), and Chikuni et al. (1997), respectively. The polymorphisms of these genes analyzed in this study were g.16024A > G (T1950A) and g.16039T > C (W1955R) in exon 34 of *FASN* gene, c.878T > C (A293V) in exon 5 of *SCD* gene, 84-bp insertion (allele L) or deletion (allele S) in intron 5 of *SREBP1* gene, and L127V and T172M in exon 5 of *GH* gene. Two SNP in the *GH* gene were analyzed individually in the present study because they had independent effects on fatty acid composition and carcass traits.

Total DNA was prepared from adipose tissue using a NucleoSpin Food kit (Macherey-Nagel, Düren, Germa-

ny). The PCR-RFLP, amplified fragment-length polymorphism (**AFLP**), and quenching probe (**QP**) systems (Kurata et al., 2001; Matsumoto et al., 2007) were applied to detect the 5 nucleotide substitutions in the 4 genes. The PCR amplification was performed with 5- to 20-ng genomic DNA, a primer set, and Animal Taq (ABgene, Epsom, UK). Primers and PCR conditions for genotyping are shown in Table 1. Flanking genome sequences of each mutation site are shown in Supplemental Table 1 (http://jas.fass.org/content/vol89/issue1/). Amplification was performed using a TaKaRa PCR Thermal Cycler MP (TP-3000; Takara Bio, Shiga, Japan). Because SNP g.16024A > G and g.16039T > C in the *FASN* gene are completely linked (Abe et al., 2009), either SNP was selected to genotype the *FASN* gene. Similarly, SNP c.702G > A and c. 878T > C in the *SCD* gene are completely linked (Taniguchi et al., 2004), so c.702G > A only was genotyped in place of c. 878T > C. The *FASN*, *SCD*, and *SREBP1* genotypes of some samples were identified simultaneously using the QP system. A ready-made QP Genotyping Probe/Primer Set for the bovine *SCD* gene (Prescribe Genomics, Ibaraki, Japan), and custom QP Genotyping Probe/Primer Sets for the bovine *FASN* gene and the bovine *SREBP1* gene (J-Bio21, Tokyo, Japan) were used. Genotypes of markers GH^{L127V} and GH^{T172M} were determined with the QP system for all samples, using a bovine *GH* 2plex QP Genotyping Probe/Primer Set (Prescribe Genomics).

Statistical Analyses

A mixed-inheritance animal model was used to evaluate the genotypic effects on the traits under study. The snp_ad option of Qxpak software (Pérez-Enciso and Misztal, 2004) was used for individual SNP. The ins/del polymorphism in *SREBP1* was treated as a SNP. The assumed model for the phenotypic data of each trait was as follows:

$$y_{ijklm} = sex_i + year_j + month_k + farm_l + bx_{ijklm} + SNP + u_{ijklm} + e_{ijklm}, \quad [1]$$

where y_{ijklm} is the observation *ijklm* for the traits, sex_i is the fixed effect of sex i (2 classes), $year_j$ is the fixed effect of slaughter year j (4 classes, 2005 to 2008), $month_k$ is the fixed effect of slaughter month k (12 classes), $farm_l$ is the fixed effect of farm l (38 classes), b is a covariate coefficient with x_{ijklm} being slaughter age, u_{ijklm} is the infinitesimal genetic effect of animal *ijklm*, which is distributed as $N(0, \mathbf{A}\sigma_u^2)$ (**A** is the numerator relationship matrix), and e_{ijklm} is the residual effect. The *SNP* is the single-locus SNP genotypic effect, which was partitioned into additive (a) and dominance (d) effects. For carcass traits, we also included parity effect (55 classes) as a fixed effect in this model (Eq. [1]). Pedigrees of the base population animals were traced back for 3 generations to create the numerator relationship

Table 1. Oligonucleotide primers and genotyping conditions

Marker[1]	Genotyping method[2]	Primer sequence (5′ to 3′)	Annealing temperature, °C	Substitution site	Enzyme	Source
FASN	PCR-RFLP	TTAAGGGCACCTTAGGCTTG	55	T1950A (g.16024A > G)	*Hha*I	Abe et al. (2009)
		GCCTTTGGAGGGCTTCTTAG		W1955R (g.16039T > C)	*Nci*I	
	QP	—[3]				
SCD	PCR-RFLP	GTGTCCTGTTGTTGTGCTTCATCCTGCC	63	V293A (c.702G > A)	*Nco*I	Taniguchi et al. (2004)
		AATATTCTCTCGGGGGTTGATGGTCTTG				
	QP	—[3]				
SREBP1	AFLP	CCACAACGCCATCGAGAAACGCTAC	60			Hoashi et al. (2007)
		GGCCTTCCCTGACCACCCAACTTAG				
	QP	—[3]				
GH^{L127V}	QP	—[3]		L127V		
GH^{T172M}	QP	—[3]		T172M		

[1] *FASN* = fatty acid synthase; *SCD* = stearoyl-CoA desaturase; *SREBP1* = sterol regulatory element-binding protein 1.
[2] QP = quenching probe method; AFLP = amplified fragment-length polymorphism.
[3] Sequence information for QP probes and primers was not disclosed. Instead, the flanking sequence of each mutation site is provided in Supplemental Table 1 (http://jas.fass.org/content/vol89/issue1/).

matrix, and 2,390 animals were included in the pedigree analysis. Likelihood ratio tests were performed by removing the single locus SNP genotypic effects in the model (Eq. [1]), and nominal *P*-values were obtained assuming a χ^2 distribution of the likelihood ratio test. The proportion of additive genetic variance accounted for by the single locus SNP genotypic effect was calculated as

$$\text{variance percentage} = \{2pq[a + d(q - p)]^2\} / V_A,$$

where p and q were the allelic frequencies at the SNP locus, and V_A was the additive genetic variance of the trait obtained from an animal model analysis ignoring the single locus SNP genotypic effects (Falconer, 1989).

For epistasis analysis, the epi_snp option of Qxpak software (Pérez-Enciso and Misztal, 2004) was used. The statistical model for testing epistasis effects was as follows:

$$\begin{aligned} y_{ijklm} &= sex_i + year_j + month_k + farm_l + bx_{ijklm} \\ &+ SNP1 + SNP2 + (SNP1 \times SNP2) + u_{ijklm} + e_{ijklm}, \end{aligned} \quad [2]$$

where *SNP1* and *SNP2* are the 2 single-locus genotypic effects, and *SNP1* × *SNP2* is the 2-locus interaction effect. The 2 locus interaction effect was partitioned into 4 individual epistasis effects (additive × additive, additive × dominance, dominance × additive, and dominance × dominance) using the extended Cockerham model (Cockerham, 1954). Likelihood ratio tests were performed by removing the 2-locus interaction effect in the model (Eq. [2]), and nominal *P*-values were obtained assuming a χ^2 distribution of the likelihood ratio test.

To account for multiple testing, we considered the false discovery rate (**FDR**) and calculated *q*-values with the R procedure (http://www.r-project.org/) using the method of Benjamini and Hochberg (1995). A *q*-value of a test measures the proportion of false positive incurred (referred to as FDR) when a particular test is called significant. The FDR procedure was run separately for single effects analyses (105 tests) and for epistatic effects analyses (210 tests).

RESULTS

Summary of the Animals and Allele Frequencies

Statistical data for the fatty acid composition of intramuscular fat in the longissimus thoracis muscle, and carcass traits, are shown in Table 2. Genotypic and allelic frequencies for the 5 polymorphic genetic markers (4 SNP and 1 ins/del) in 4 genes, *FASN*, *SCD*, *SREBP1*, and *GH*, are shown in Table 3. The major alleles of each marker were as follows: allele TW in *FASN*, allele A in *SCD*, allele V in GH^{L127V}, and allele T in GH^{T172M}. The allele frequencies for *FASN* were substantially biased toward the allele TW. There were only 4 animals with the genotype AR/AR among the analyzed samples (Table 3). There was little bias in the allele frequencies for *SREBP1*.

Single Effects on Fatty Acid Composition

There are 2 groups of fatty acids: the SFA and the unsaturated fatty acids. Saturated fatty acids in beef fat are mainly composed of myristic (C14:0), palmitic (C16:0), and stearic (C18:0) acids. Unsaturated fatty acids are mainly composed of myristoleic (C14:1), palmitoleic (C16:1), oleic (C18:1), linoleic (C18:2), and linolenic (C18:3) acids.

Independent effects on fatty acid composition are shown in Table 4. In this study, we detected significant effects of *FASN*, *SCD*, and GH^{L127V} polymorphisms on fatty acid composition of the longissimus thoracis muscle. When allele AR was substituted for allele TW in *FASN*, the proportion of 16-C or shorter fatty acids (C14:0, $P < 0.001$; C14:1, $P < 0.05$; C16:0, $P < 0.05$; C16:1, $P < 0.05$) was decreased, and the proportion of C18:1 ($P < 0.001$) was increased. The *q*-values of C14:0 ($q < 0.001$) and C18:1 ($q < 0.01$) were also significant. In *SCD*, when allele V was substituted for allele A, the proportion of SFA (C14:0, $P < 0.001$; C18:0 $P < 0.001$) was decreased and the proportion of MUFA (C14:1, $P < 0.001$; C18:1, $P < 0.001$; MUFA, $P < 0.001$) was increased. The *q*-values of all effects against these fatty acids remained significant ($q < 0.001$). When allele L was substituted for allele V in GH^{L127V}, the proportion of 16-C or shorter fatty acids (C14:0, $P < 0.001$; C14:1, $P < 0.05$; C16:0, $P < 0.05$; C16:1, $P < 0.01$) were decreased and the proportion of C18:1 ($P < 0.05$) was increased. The *q*-values of C14:0 ($q < 0.01$) and C16:1 ($q < 0.01$) remained significant. No significant single effect ($P > 0.1$) on any fatty acid composition was detected for the *SREBP1* or GH^{T172M} polymorphisms. No effects ($P > 0.1$) on C18:2 or C18:3 were detected among these 5 genetic markers.

For C18:1, when the genotypes of 3 markers (*FASN*, *SCD*, and GH^{L127V}) were substituted from the lesser effect allele to the greater effect allele, the proportion of C18:1 would rise by 4.46% [= (0.79% × 2) + (1.12% × 2) + (0.32% × 2)]. More than 20% of the genetic variance in C18:1 level could be accounted for by these 3 markers: 7.55% from *FASN*, 11.72% from *SCD*, and 3.84% from GH^{L127V}.

Single Effects on Carcass Traits

Independent effects on carcass traits are shown in Table 4. Carcass traits were classified into 2 groups. The meat yield traits were CW, REA, RT, SFT, and YE. The meat quality traits were BMS, beef color standard, luster, firmness, texture, moisture, and crude fat. Polymorphisms of the 3 fatty acid synthesis genes (*FASN*, *SCD*, and *SREBP1*) showed no effect on any

Table 2. Number of records, means, SD, and genetic variance of traits[1]

Trait	Abbrev	Records	Mean	SD	V_A
Fatty acid composition, %					
Myristic acid	C14:0	480	3.01	0.53	0.11
Myristoleic acid	C14:1	480	0.90	0.32	0.04
Palmitic acid	C16:0	480	26.12	1.91	1.48
Palmitoleic acid	C16:1	480	4.47	0.71	0.20
Stearic acid	C18:0	480	10.74	1.53	0.90
Oleic acid	C18:1	480	50.94	2.73	3.02
Linoleic acid	C18:2	480	2.91	0.77	0.23
Linolenic acid	C18:3	480	0.29	0.33	0.02
MUFA	MUFA	480	56.31	2.84	3.22
Carcass trait					
Carcass weight, kg	CW	480	409.4	42.5	693.3
Rib eye area, cm^2	REA	480	53.90	6.90	18.70
Rib thickness, cm	RT	480	7.56	0.76	0.22
Subcutaneous fat thickness, cm	SFT	480	2.29	0.66	0.17
Yield estimate[2]	YE	480	74.30	1.10	0.50
Beef marbling standard[3]	BMS	480	5.88	1.92	1.43
Beef color standard[4]		480	3.75	0.55	0.12
Luster[5]		480	4.09	0.73	0.21
Firmness[5]		480	4.02	0.77	0.23
Texture[5]		480	4.08	0.74	0.22
Moisture, %		471	48.30	4.70	8.59
Crude fat, %		471	36.20	6.20	15.50

[1]Abbrev = abbreviation of traits; V_A = additive genetic variance.
[2]YE (%) = 69.419 + 0.130REA + 0.667RT – 0.025CW – 0.896SFT.
[3]BMS was scored from 1 to 12.
[4]Beef color standard was scored from 1 to 7.
[5]Luster, firmness, and texture were scored from 1 to 5.

meat yield trait. Gene effects of *SCD* were detected for 3 meat quality traits (luster, $P < 0.05$; firmness, $P < 0.05$; and texture, $P < 0.05$). However, none of the effects remained significant at the FDR threshold ($q < 0.05$). A significant effect of GH^{L127V} on SFT ($P < 0.01$) was detected, and it remained significant after correcting for FDR. Gene effects of GH^{T172M} were detected for both types of carcass traits (CW, $P < 0.05$; RT, $P < 0.01$; SFT, $P < 0.01$; and firmness, $P < 0.05$). However, none was significant after multiple testing corrections.

Epistatic Effects on Fatty Acid Composition

Epistatic effects between all pairs of genes affecting fatty acid composition are shown in Table 5. There were no significant epistatic effects on fatty acid composition among pairs of the 3 fatty acid synthesis genes (*FASN*, *SCD*, and *SREBP1*). Epistatic interactions were detected between *SCD* and GH^{L127V} for C14:0 ($P < 0.05$), C18:2 ($P < 0.05$), and C18:3 ($P < 0.001$) levels and between *FASN* and GH^{L127V} for C18:0 levels ($P < 0.05$). However, none of the effects remained significant after correcting for FDR, except for the effect between *SCD* and GH^{L127V} on C18:3.

Epistatic Effects on Carcass Traits

Epistatic effects between all pairs of genes affecting carcass traits are shown in Table 5. No epistatic interactions ($P > 0.1$) were detected between *FASN* and *SCD* for any carcass trait. Although an independent effect of *SREBP1* was not detected on carcass traits, epistatic interactions were detected between *SREBP1* and other markers. Epistatic effects were detected be-

Table 3. Genotypic and allelic frequencies of markers

		Genotypic frequencies (freq.)						Allelic freq.			
		pp		pq		qq		p		q	
Marker[1]	Records	Genotype	Freq.	Genotype	Freq.	Genotype	Freq.	Allele	Freq.	Allele	Freq.
FASN	480	TW/TW	0.71	TW/AR	0.28	AR/AR	0.01	TW	0.85	AR	0.15
SCD	480	AA	0.50	VA	0.42	VV	0.08	A	0.71	V	0.29
SREBP1	480	SS	0.27	LS	0.49	LL	0.24	S	0.51	L	0.49
GH^{L127V}	441	VV	0.56	LV	0.35	LL	0.09	V	0.74	L	0.26
GH^{T172M}	473	TT	0.71	MT	0.27	MM	0.02	T	0.85	M	0.16

[1]*FASN* = fatty acid synthase; *SCD* = stearoyl-CoA desaturase; *SREBP1* = sterol regulatory element-binding protein 1.

Table 4. Single gene effects on fatty acid composition and carcass traits[1]

Trait[2]	Marker[3]	LRT	*P*-value	*q*-value[4]	a ± SE	d ± SE	%VA due to marker
Fatty acid composition, %							
C14:0	*FASN*	23.8	<0.001	<0.001	−0.07 ± 0.12	0.19 ± 0.13	9.08
	SCD	25.3	<0.001	<0.001	−0.23 ± 0.05	−0.07 ± 0.06	14.22
	GH^{L127V}	18.1	<0.001	0.01	−0.14 ± 0.05	0.06 ± 0.06	9.86
C14:1	*FASN*	8.3	0.02	0.12	−0.06 ± 0.08	0.03 ± 0.08	4.29
	SCD	35.0	<0.001	<0.001	0.15 ± 0.03	0.01 ± 0.03	20.42
	GH^{L127V}	8.2	0.02	0.12	−0.05 ± 0.03	0.03 ± 0.03	4.35
C16:0	*FASN*	7.5	0.02	0.13	−0.43 ± 0.46	0.08 ± 0.47	4.02
	GH^{L127V}	7.6	0.02	0.13	−0.11 ± 0.17	0.42 ± 0.21	2.48
C16:1	*FASN*	8.5	0.01	0.12	−0.05 ± 0.17	0.16 ± 0.18	3.29
	GH^{L127V}	13.3	0.01	0.02	−0.18 ± 0.06	0.04 ± 0.08	7.35
C18:0	*SCD*	24.7	<0.001	<0.001	−0.60 ± 0.14	−0.04 ± 0.16	15.45
C18:1	*FASN*	14.7	<0.001	0.01	0.79 ± 0.65	−0.24 ± 0.67	7.55
	SCD	21.9	<0.001	<0.001	1.12 ± 0.24	0.45 ± 0.28	11.72
	GH^{L127V}	8.8	0.01	0.11	0.32 ± 0.24	−0.47 ± 0.30	3.84
MUFA	*FASN*	6.9	0.03	0.15	0.67 ± 0.68	−0.06 ± 0.70	3.89
	SCD	25.5	<0.001	<0.001	1.25 ± 0.25	0.48 ± 0.30	13.96
Carcass trait							
CW, kg	GH^{T172M}	6.8	0.03	0.16	16.33 ± 6.27	15.20 ± 7.03	1.29
RT, cm	GH^{T172M}	9.8	0.01	0.07	0.34 ± 0.12	0.24 ± 0.13	3.63
SFT, cm	GH^{L127V}	11.3	0.01	0.04	−0.20 ± 0.06	−0.15 ± 0.07	3.86
	GH^{T172M}	8.0	0.02	0.12	0.28 ± 0.10	0.19 ± 0.12	3.42
Luster	*SCD*	7.0	0.03	0.15	0.12 ± 0.06	−0.05 ± 0.08	3.68
Firmness	*SCD*	7.2	0.03	0.14	0.12 ± 0.07	−0.05 ± 0.08	3.7
	GH^{T172M}	6.3	0.04	0.19	0.13 ± 0.12	−0.05 ± 0.13	3.09
Texture	*SCD*	7.5	0.02	0.13	0.12 ± 0.07	−0.06 ± 0.08	3.78

[1]Only those for which statistically significant ($P < 0.05$) gene effects were detected are listed for each trait. LRT = likelihood ratio test; a = additive effect; d = dominance effect; %VA = proportion of additive genetic variance.

[2]C14:0 = myristic acid; C14:1 = myristoleic acid; C16:0 = palmitic acid; C16:1 = palmitoleic acid; C18:0 = stearic acid; C18:1 = oleic acid; CW = carcass weight; RT = rib thickness; SFT = subcutaneous fat thickness.

[3]*FASN* = fatty acid synthase; *SCD* = stearoyl-CoA desaturase.

[4]The *q*-value of a test measures the proportion of false positives incurred when that particular test is called significant.

Table 5. Epistatic effects between pairs of 4 genes on fatty acid composition and carcass traits[1]

	Marker[3]				
Trait[2]	SNP1	SNP2	LRT	*P*-value	*q*-value[4]
Fatty acid composition, %					
C14:0	*SCD*	GH^{L127V}	4.11	0.04	0.63
C18:0	*FASN*	GH^{L127V}	4.24	0.04	0.63
C18:2	*SCD*	GH^{L127V}	4.23	0.04	0.63
C18:3	*SCD*	GH^{L127V}	14.11	<0.001	0.04
Carcass trait					
CW, kg	*FASN*	GH^{L127V}	4.73	0.03	0.63
	SREBP1	GH^{L127V}	3.99	0.05	0.63
REA, cm^2	*SREBP1*	GH^{L127V}	5.82	0.02	0.63
Beef color standard	*SCD*	*SREBP1*	5.99	0.01	0.63
Firmness	*FASN*	*SREBP1*	4.05	0.04	0.63
	SREBP1	GH^{T172M}	4.85	0.03	0.63
Texture	*SREBP1*	GH^{T172M}	4.77	0.03	0.63
Moisture, %	*SREBP1*	GH^{T172M}	4.22	0.04	0.63
Crude fat, %	*SCD*	GH^{L127V}	4.02	0.05	0.63

[1]Only those pairs for which statistically significant ($P < 0.05$) gene effects were detected are listed for each trait. LRT = likelihood ratio test statistic.

[2]C14:0 = myristic acid; C18:0 = stearic acid; C18:2 = linoleic acid; C18:3 = linolenic acid; CW = carcass weight; REA = rib eye area.

[3]*SCD* = stearoyl-CoA desaturase; *FASN* = fatty acid synthase; *SREBP1* = sterol regulatory element-binding protein 1.

[4]The *q*-value of a test measures the proportion of false positives incurred when that particular test is called significant.

tween *SREBP1* and GH^{L127V} for CW ($P < 0.05$) and REA ($P < 0.05$), between *SREBP1* and *SCD* for beef color standard ($P < 0.05$), between *SREBP1* and *FASN* or GH^{T172M} for firmness ($P < 0.05$ each), and between *SREBP1* and GH^{T172M} for texture ($P < 0.05$) and moisture ($P < 0.05$). The GH^{L127V} was involved in epistatic effects on meat yield traits (CW, $P < 0.05$; and REA, $P < 0.05$) and crude fat ratio. The GH^{T172M} was involved in some epistatic effects on meat quality traits (firmness, texture, and moisture). However, those significances were generally low, and significant q-values were not detected between any pair of genes.

DISCUSSION

Population and Phenotypic Measurement

In previous studies, half-sib families or experimental populations were used to detect the effects of polymorphisms in lipid metabolism-related genes (Ge et al., 2003; Morris et al., 2007; Oka et al., 2007; Abe et al., 2009). To use genetic markers in breeding programs by marker-assisted selection, it is necessary to estimate the actual effects in field data. In this study, we used the commercial Wagyu beef cattle population, produced in a representative Japanese Black cattle-producing area in Japan, to clarify the actual effects of these gene polymorphisms.

The fatty acid composition in the longissimus thoracis muscle is very important in Wagyu beef because the quality of the beef is mainly evaluated in this muscle. The longissimus thoracis muscle commands increased prices because it is regarded as the most palatable cut. Therefore, it is important to investigate the relationship between the effects of these genes and the fatty acid composition of the longissimus thoracis muscle. In previous studies, discarded tissues, such as the perinephric fat and the backfat, were used to analyze the relationship between gene polymorphisms and fatty acid composition (Taniguchi et al., 2004; Morris et al., 2007; Abe et al., 2008, 2009; Hoashi et al., 2008). However, we used samples from graded sections of the longissimus thoracis muscle itself to ensure that any gene effects detected were valid in this tissue.

Allele Frequencies

In a previous study, Abe et al. (2009) reported that the frequency of allele TW of the *FASN* gene was greater in Japanese Black cattle than in Angus, Hereford, and Holstein. In the present study, the frequency of allele TW was much greater (0.85) than in the previous report (0.67; Abe et al., 2009). The effective population size of Japanese Black cattle has been reported to be very small as the result of the side-effects of animal improvement (Nomura et al., 2001; Honda et al., 2005). A small effective population size could easily bias allele frequencies in local populations.

In the present study, the frequencies of allele A in the *SCD* gene and allele S in the *SREBP1* gene were almost similar to those previously reported for Japanese Black cattle (Taniguchi et al., 2004; Hoashi et al., 2007; Ohsaki et al., 2009). A comparable polymorphism of *SCD* has been reported in other cattle breeds, such as Holstein or Jersey (Mele et al., 2007; Moioli et al., 2007; Komisarek and Dorynek, 2009). These results might indicate that the *SCD* polymorphism analyzed in the present study had little effect on the traits selected for the breeding of cows or beef cattle.

Three *GH* haplotypes (haplotype A, Leu127/Thr172; haplotype B, Val127/Thr172; haplotype C, Val127/Met172), based on the combination of 2 nonsynonymous SNP (L127V and T172M), have been observed in Japanese Black cattle (Chikuni et al., 1994, 1997). Allele frequencies of GH^{L127V} and GH^{T172M} in Japanese Black cattle can be calculated from previous reports: the frequencies of alleles V and L of GH^{L127V} were 0.5 each (Chikuni et al., 1994), or 0.732 and 0.269, respectively (Tatsuda et al., 2008). The frequencies of alleles T and M of GH^{T172M} were 0.644 and 0.356 (Chikuni et al., 1994) or 0.690 and 0.311 (Tatsuda et al., 2008), respectively. The frequencies of allele V of GH^{L127V} and allele T of GH^{T172M} in the present study were similar to those in previous results.

Single Effects on Fatty Acid Composition

For *FASN* gene polymorphisms, Abe et al. (2009) reported significant effects of the *FASN* genotype on the C14:0, C14:1, C16:0, C16:1, and C18:1 content of the trapezius muscle intramuscular fat in Japanese Black populations. In the present study, the same effect of the *FASN* marker was detected for the intramuscular fat of the longissimus thoracis muscle. Detection of same effect in different breeding populations increases its reliability. Fatty acid synthase produces 16-C or shorter-chain SFA from acetyl-CoA and malonyl-CoA. The present result, that the composition of C16 and shorter-chain fatty acids were decreased by the AR > TW substitution, might indicate that the analyzed mutation was related to the function of FASN. Because these mutations are located in the β-ketoacyl reductase domain in the *FASN* gene (Abe et al., 2009), they might influence that function.

For *SCD* gene polymorphisms, a previous report showed the effects of the *SCD* genotype on C14:1, C18:0, C18:1, and MUFA abundance (Ohsaki et al., 2009). The present results corroborate these effects. When allele V was changed to allele A in *SCD*, the proportion of SFA (C14:0 and C18:0) was decreased, and the proportion of MUFA (C14:1 and C18:1) was increased. Stearoyl-CoA desaturase introduces a double bond between carbon atoms 9 and 10 of a SFA to produce C14:1, C16:1, and C18:1. The mutations analyzed in present study were located in the transmembrane region. The present result reinforces the possibility that the analyzed

mutation is related to the function of SCD. The results might imply that SCD generated from an *SCD* gene with the allele A has greater functionality than that generated from an *SCD* gene with the allele V. Analysis of gene function is necessary for the evaluation of these polymorphisms.

For *GH* gene polymorphisms, in a previous study, the group with genotype LL for the GH^{L127V} marker had a greater percentage of C14:1 and C16:1 and a reduced percentage of C18:0 in LM lipid, compared with the group with genotype VV (Oka et al., 2007). In the present study, similar effects were detected at the same mutation site. In addition to the effect of a decreased ratio of 16-C or shorter fatty acids, the presence of allele V of the GH^{L127V} marker resulted in an increased proportion of C18:1, though q-value was not significant.

Epistatic Effects on Fatty Acid Composition

None of the expected interactions were significant for fatty acid composition except for *SCD* and GH^{L127V}. For fatty acid synthesis genes, no epistatic effects were detected between *FASN* and *SCD* markers, or between *FASN* and *SREBP1* markers. Because the function of *FASN* is to synthesize SFA from acetyl-CoA, *FASN* cannot increase C18:1 abundance by itself (Chakravarty et al., 2004). Considering this, it was expected that the effect of the *FASN* marker on C18:1 would be influenced by the function of *SCD* and *SREBP1*. However, no epistatic effects for C18:1 abundance were found in the present study. This could indicate that the effect of *FASN* on C18:1 abundance is influenced by fatty acid synthesis factors other than SCD or SREBP1. Epistatic effects were expected between *SREBP1* and other genes because *SREBP1* is a major transcription factor that regulates the expression of several fatty acid synthesis genes including *SCD* and *FASN* (Magaña and Osborne, 1996; Magaña et al., 1997; Shimano et al., 1999; Tabor et al., 1999; Horton et al., 2003; Zhang et al., 2003; Miyazaki et al., 2004; Matsuzaka et al., 2007). However, in the present study, no epistatic effects were detected between *SREBP1* and these genes. That the *SREBP1* marker did not show direct or indirect effects on fatty acid composition might indicate that the analyzed polymorphism in *SREBP1* is not relevant to variation in fatty acid composition.

Polymorphism in the regulatory factor, *GH*, was expected to influence the function of downstream genes because it has been reported that *GH* influences the expression of *FASN*, *SCD*, and *SREBP1* (Beswick and Kennelly, 2000; Frick et al., 2002; Louveau and Gondret, 2004). In this study epistatic effects were detected between GH^{L127V} and *SCD* on C14:0, C18:2, and C18:3 levels and between GH^{L127V} and *FASN* on C18:0 levels. However, none of the effects, except for that between GH^{L127V} and *SCD* on C18:3, remained significant after correcting for FDR. It has been reported that GH enhances the expression of *SREBP1*, *SCD*, and *FASN* in the rat liver, but not in adipose tissue (Frick et al., 2002). The present results imply that, although *GH* polymorphisms are related to lipolysis (Asada et al., 2000a,b), the effects of *GH* on fatty acid synthesis might be limited in adipose tissue. In this study, a significant epistatic effect for C18:3 between *SCD* and GH^{L127V} was detected, and further study must be necessary to confirm the epistatic effect of these loci in other commercial population.

Single Effects on Carcass Traits

For the fatty acid synthesis genes, the gene effects of *SCD* and *SREBP1* polymorphisms on carcass traits have been studied previously. Ohsaki et al. (2009) reported that the *SREBP1* polymorphism was related to CW and that no significant effect on carcass traits was detected for the *SCD* polymorphism. The present study indicated that 3 markers in the 3 fatty acid synthesis genes (*FASN*, *SCD*, and *SREBP1*) were not related to meat yield traits. These results imply that if the genotypes of *FASN*, *SCD*, and *SREBP1* were used as markers to improve fatty acid composition, there would be no effect on the meat yield traits. Among those 3 genes, only the *SCD* marker showed significant effect on meat quality traits (luster, firmness, and texture of the meat). However, their significances were generally small, and the effects on these traits did not remain significant after correcting for FDR. In present study, significant relations were detected between those meat quality traits and fatty acid composition (data not shown). Animals with decreased C18:0, increased C14:1, and increased MUFA abundance tended to have greater scores of luster, firmness, and texture. The effect of *SCD* polymorphism on fatty acid composition might influence those meat quality traits. The present study suggests that the SNP in the *FASN* and *SCD* genes analyzed in this study are excellent markers for the improvement of fatty acid composition.

For *GH* gene polymorphisms, previous studies reported that animals possessing allele L of the GH^{L127V} marker showed a faster growth rate, greater CW, smaller BMS, and greater fat thickness than those possessing allele V (Oprządek et al., 2005; Barendse et al., 2006; Oka et al., 2007; Tatsuda et al., 2008). On the other hand, other studies detected no significant effects on carcass traits (Yao et al., 1996; Di Stasio et al., 2002; Han et al., 2010). In the present study, animals possessing allele V of the GH^{L127V} marker showed a thinner SFT than those possessing allele L. However, no significant effect of the GH^{L127V} marker was detected on CW or BMS. The discordance among the results on carcass traits implies that the analyzed SNP in *GH* gene might not be the polymorphisms responsible for variations in those carcass traits. Further analysis is required for the evaluation of *GH* polymorphisms on carcass traits. The GH^{T172M} polymorphism has not been detected in breeds other than Japanese Black and Japanese Brown cattle (Chikuni et al., 1994). A previous study reported that animals possessing allele M (haplo-

type C) of the GH^{T172M} marker showed a greater BMS than those possessing allele T (Tatsuda et al., 2008). However, no effect of the GH^{T172M} marker was detected on BMS in the present study. Although effects of the GH^{T172M} polymorphism on CW, RT, SFT, and firmness were detected, none of those effects remained significant correcting for FDR.

Epistatic Effects on Carcass Traits

Epistatic effects between fatty acid synthesis genes were detected on some meat quality traits. Considering the fact that single effects of *SCD* were observed on the luster, firmness, and texture of the meat, and that SREBP1 is a transcription factor affecting the expression of the *FASN* and *SCD* genes, there might be relations between fatty acid synthesis genes and those meat quality traits. However, their significance was generally small, and none of the associations remained significant after correcting for FDR.

Epistatic effects of the *GH* markers were detected for meat yield and quality traits. These effects might occur because GH stimulates the expression of particular downstream factors. It has been reported that GH regulates the expression of *FASN*, *SCD*, and *SREBP1* in the liver (Frick et al., 2002) and that GH stimulates lipolysis in adipocytes (Asada et al., 2000a,b). The epistatic effects of GH^{L127V} on crude fat content might reflect these functions of GH. However, none of the effects remained significant after correcting for FDR. Further study in a larger number of animals is necessary to reveal the relation between GH and other genes to carcass traits.

Other Candidate Genes Affecting Fatty Acid Composition

The present results revealed that SNP from the *FASN* and *SCD* genes independently influenced fatty acid composition in the longissimus thoracis muscle. Other polymorphisms in these or other genes must also affect fatty acid composition because these 5 gene polymorphisms contributed only approximately 25% of the total genetic variation in C18:1 abundance. In previous studies, several other polymorphisms were reported in the *FASN* gene, and some of those polymorphisms were related to fatty acid composition in adipose fat and milk fat (Roy et al., 2006; Zhang et al., 2008). One of those polymorphisms has also been analyzed in a Japanese Black half-sibling population, although no variation was detected (Abe et al., 2009). However, it is not clear whether this lack of variation for that polymorphism extends to all the Japanese Black cattle, and whether these polymorphisms, including the polymorphism described above, have effects on the fatty acid composition in Wagyu beef. Several other enzymes, such as acetyl-CoA carboxylase and the elongation of very long chain fatty acid proteins, are related to fatty acid synthesis. There are also many other factors involved in triglyceride synthesis, lipolysis, or β-oxidation of fatty acids. Other proteins control the gene expression of lipid metabolism-related factors, like *SREBP1*. Many fat-metabolizing hormones and factors exist that function as adipocyte-stimulating factors, such as GH. It is important to consider the effect of these factors and their interactions when planning the improvement of fatty acid composition in livestock.

In this study, we analyzed 5 gene polymorphisms in lipid metabolism-related genes (*FASN*, *SCD*, *SREBP1*, and *GH*) for their effects on fatty acid composition and carcass traits in the commercial Wagyu beef cattle population. The present study is the first report to assess the epistatic effects among pairs of lipid metabolism-related genes. The results revealed that polymorphisms in 2 fatty acid synthesis genes (*FASN* and *SCD*) separately influence fatty acid composition in the longissimus thoracis muscle. This suggests that the analyzed SNP in the *FASN* and *SCD* genes are appropriate markers for the improvement of fatty acid composition. If particular genotypes of these genes were selected to increase the C18:1 content, there would be no adverse effects on the carcass traits. To use these gene polymorphisms directly for marker-assisted selection, the additive and dominance effect values should be estimated more exactly in a larger number of animals. We are currently performing candidate gene analysis of these genes in a large number of animals to confirm the effects of these polymorphisms.

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