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Identification of the SNP (Single Necleotide Polymorphism) of the Stearoyl-CoA Desaturase (SCD) Associated with Unsaturated Fatty Acid in Hanwoo (Korean Cattle)*

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ABSTRACT : Fatty acid composition of beef intramuscular tissue is an important trait because high proportions of mono-unsaturated fatty acid are related to favorable beef flavor. In this study, we investigated the effects of genetic factors, such as stearoyl-CoA desaturase (SCD), on beef carcass traits, including fatty acid composition, in the Hanwoo. Analysis of fatty acids in Hanwoo was performed using a breed raised in Gyeonbuk province (n = 395). Compared to the homozygote, the GA, CT, and CT genotypes of exon 5 in the SCD polymorphism showed a higher content of oleic acid ($p<0.05$) and higher contents of mono-unsaturated fatty acid ($p<0.05$) and marbling scores ($p<0.05$) in intramuscular fat. Results of haplotype analysis showed a significant presence of unsaturated fatty acids and marbling score in the ht1*ht2 and ht2*ht2 groups ($p<0.05$). Furthermore, haplotype effects more powerful than a single gene were also observed. These ht1 and ht2 types also showed a significant difference in unsaturated fatty acids and marbling score, affecting beef flavor in the Hanwoo groups. Therefore, it can be inferred that the ht1 and ht2 types might be valuable new markers for use in improvement of Hanwoo. (**Key Words :** Hanwoo, Unsaturated Fatty Acid, Stearoyl-CoA Desaturase(SCD), Haplotype, Beef Flavor)

INTRODUCTION

According to changes in consumer preference, fatty acid composition of dietary fat has been receiving a lot of attention recently due to its influence on human health. Recently, the quality of the fat has become an important factor in defining the quality of the meat in the Hanwoo beef market. It has been reported that major factors of beef flavor include marbling degree, colors of meat and fat, tissue texture of meat. These factors influence tenderness, juiciness, and aroma. (Lee et al., 1994; Vander Wal et al., 1997; Robins et al., 2003; Monson et al., 2005).

Fatty acid composition of intramuscular tissue in cattle has become important in the beef industry because fat tissue containing abundant mono-unsaturated fatty acid (MUFA) reflects a lower melting point, which contributes positively to favorable beef flavor and tenderness (Dryden and Marchello, 1970; Melton et al., 1982; Studivant and Lunt et al., 1992; Jeremiah, 1996). And oleic acid (C18:1), one of the major MUFAs in beef fat, has been identified as a primary factor for control of beef flavor (Dryden and Marchello, 1970; Yoshimura and Namikawa, 1983; Tsuji, 2008). In addition, Zembayashi et al. (1995) demonstrated that adipose tissue of Japanese Black contains a higher proportion of MUFA than that of Holstein, Japanese Brown, or Charolais. In general, marbling score in Japanese Black cattle showed absolute correlation with beef flavor (Ibi et al., 2005). Juicier of beef has been reported to show higher unsaturated fatty acid content and higher beef quality, and it was estimated that a higher content of oleic acid suggested an already deep relation of several factors, along with unsaturated fatty acid, that influence beef flavor (Waldman et al., 1965).

Stearoyl-CoA desaturase (SCD), an enzyme for chemical conversion of saturated fatty acid to unsaturated fatty acid, has been reported as a main factor for change of stearic acid (C18:0) and palmitic acid (C16:0) to oleic acid (C18:1) on unsaturated fatty acid of w-9; and interest in the increase of unsaturated fatty acid for improvement of meat flavor and for the health of human beings has been the focus of important research (Kuchel et al., 2004; Scollan et

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al., 2006; Alexander et al., 2007; Zhang and Knight, 2007; Erkkila et al., 2008; Webb and O'Neill, 2008).

The bovine SCD gene is approximately 17,088 bp in size and consists of 6 exons and 5 introns. Discovery of 8 single nucleotide polymorphisms (SNP) of SCD has been reported; 3 of these SNPs were found within the open reading frame (ORF) of exon 5 in Wagyu (Taniguchi et al., 2003; Tsuji, 2008; Barton et al., 2009). Existence of the same 3 SNPs in the ORF was reported in Canadian Holstein and Jersey breeds, with an additional SNP in exon 3 found only in Holsteins (Kgwatalala et al., 2007; Milanesi et al., 2008). The exon5 within SCD was also shown to be associated with the unsaturated fatty acid in Wagyu and Fleckvieh bulls (Taniguchi et al., 2003; Tsuji, 2008; Barton et al., 2009; Ohsaki et al., 2009). And SNPs within the UTR region of SCD also showed significant association with carcass traits and beef traits without evaluation of fatty acid in Hanwoo (Shin et al., 2006).

As in the above description, the SCD gene, which was deeply associated with oleic acid of unsaturated fatty acid as an important factor associated with beef flavor, was considered a valuable factor for use in improvement of beef quality. Therefore, this study was conducted for detection of genetic information on SNPs at exon 5 of SCD, which was associated with unsaturated fatty acid as an important factor in higher beef quality and for application of these factors for improvement of beef flavor in Hanwoo.

MATERIALS AND METHODS

Animals and phenotype data

The present study used a commercial Hanwoo population (n = 395) produced in Gyeungbuk, Korea. Animals were bred from 14 places in Gyeonbuk and born during 2006-2008. They were the progeny of 37 sires. The pedigree records of 395 steers were collected by the Research Institution of Charmpoom Hanwoo (Gyeungbuk, Korea). All steers were slaughtered at 941 days of age (mean±SD: 941.36±71.51), with an average carcass weight of 427 kg. Steers in Gyeonbuk province were fed according to the feeding program of each farmer. In general, they were weaned at 6 months of age, castrated at 6 months of age, fed with growth stage feed for 18 months, and given a high concentration diet in the last 6 months. Twenty-four hours after slaughter, carcass weight was measured and the carcasses were dissected at the last rib and the first lumber vertebra according to the Animal Product Grading System of Korea for measurement of carcass traits. Samples were collected from the longissimus muscle at the level of the 13^{th} thoracic and lumbar.

Carcass traits included in this study were backfat thickness, marbling score, and carcass weight. The marbling score was numbered as 1 thorough 9 according to the Korean Beef Marbling Standard (1 = trace, 9 = very abundant). And data on fatty acid composition, saturated fatty acid at myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), and MUFA (mono-unsaturated fatty acid) at myristoleic acid (C14:1), palmitoleic acid (C16:1), oleic acid (C18:1), and M/S (Mono unsaturated fatty acid/Saturated fatty acid), C14 index ([C14:1/(C14:0+ C14:1)]×100), C16 index ([C16:1/(C16:0+ C16:1)]×100), and C18 index ([C18:1/(C18:0+C18:1)]×100), were measured. Table 1 shows the average phenotypic value and standard deviation for the observed carcass traits and fatty acid composition from steers of the Hanwoo populations.

Fatty acid analysis

For each breed, loin eye muscle was extracted at an amount of 0.5 g using methanol and chloroform solutions (1:2, v/v), based on the methods of Folch (Folch et al., 1957). It was then filtered through a filter paper in a water bath (40°C). The filtrate was mixed with distilled water, from which a layer of methanol and water was removed. Then, following removal of the chloroform and lipid layers using nitrogen gas, the sample was treated with BF3-methanol (14%) and then subjected to transmethylation at a temperature of 65°C. Total compositions of fatty acids were analyzed using gas-chromatography (Perkin-Elmer CO., USA).

Genomic DNA and SNP genotyping

Total genomic DNA was prepared from longissimus muscle using the COSMO GENETECH kit (COSMO co, Ltd. Korea). In this study, we genotyped the polymorphisms of SCD, as described in a report (Taniguchi et al., 2003; Barton et al., 2009; Ohsaki et al., 2009). The polymorphisms analyzed in this study included g.10153 A>G, g.10213 T>C, and g.10329 C>T (A10329V) in exon 5 of the SCD gene.

For genotyping of 3 SNPs, primers for amplification and extension were designed for single-base extension (SBE) (Vreeland et al., 2002) using primer 3 input programs. The primer sequences used were as follows- : forward sequence (5'-CAGAAAATTTCCTTGCCCATT-3'), reversed sequence (5'-TGTTGCTTAACTTTCAAGGGTT T-3'), extension sequence (g.10153 A>G 5'-ATCC TGCCCACACTCGTGCC-3') (g.10213 T>C 5'-TTTGCCACCTTATTCCGTTA-3') (g.10329 C>T 5'-TCTGGTTTCCCTGGGAGCTG-3'). Reactions of primer extension were performed using the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, CA). To purify the reactions of primer extension, SAP (shrimp alkaline phosphatase) mixtures were added to the reaction mixtures. Samples were cultured at a temperature of 37°C for one hour and then inactivated at a temperature of 72°C for 15 minutes. PCR products, which were finally

Table 1. The means and standard deviation for the traits and fatty acid composition in Hanwoo populations

Trait	Mean±SD	Min	Max
Carcass weight (kg)	427.93±43.56	321.00	573.00
Backfat thickness (cm)	13.33±5.46	3.00	42.00
MS (marbling score)	5.37±2.01	1.00	9.00
Fatty acid composition (%)			
C14:0	3.62±0.59	2.28	5.52
C16:0	25.27±1.80	18.86	29.54
C18:0	10.36±1.31	6.74	15.10
C14:1	1.29±0.38	0.51	2.73
C16:1	6.63±0.92	4.25	10.29
C18:1	44.36±2.63	36.85	53.69
C18:2	3.20±0.47	1.26	4.93
SFA[1]	40.33±2.74	30.36	48.44
MUFA[2]	53.43±2.84	45.82	63.46
M/S[3]	1.34±0.16	0.99	2.07
C14 index[4]	25.78±5.16	11.98	39.80
C16 index[5]	20.79±2.62	13.73	31.18
C18 index[6]	81.04±2.27	74.60	87.34

[1] SFA = Saturated fatty acid. [2] MUFA = Mono unsaturated fatty acid. [3] M/S = Mono unsaturated fatty acid/saturated fatty acid.
[4] C14 index = [C14:1/(C14:0+C14:1)]×100. [5] C16 index = [C16:1/(C16:0+C16:1)]×100. [6] C18 index = [C18:1/(C18:0+C18:1)]×100.

prepared, were well mixed by addition of Genescan 120 Liz standard and HiDi formamide (Applied Biosystems, Foster City, CA), followed by denaturation at a temperature of 95°C for five minutes. Electrophoresis was then performed using an ABI PRISM 3130XL Genetic Analyzer, followed by an assay of electrophoresis products using GeneMapper v.4.0 software (Applied Biosystems, Foster City, CA).

Statistical analysis

For statistical analysis, Heterozygosity (H), minor allele frequency (MAF) and haplotype block were tested using a Haploviewer v4.2 (Barrett et al., 2005). Methods defined by Gabriel et al. (2002), based on the D' values of pairwise SNP, which were calculated in the specific domain of exon 5 of chromosome 26, were used. No pedigrees were associated with the breed used for the analysis. Here, estimated values of linkage disequillibrium were based on those of the frequency of haplotype, which was determined without genetic information on parents using the algorithm reported by Qin et al. (2002), based on the methods of the Haploviewer v4.2 program. And, according to Gabriel et al. (2002), any breed whose D' values had an upper limit of >0.98 and a lower limit of >0.7 at a 95% confidence interval corresponded to strong linkage disequilibrium. Accordingly, the haplotype block was selected as the domain where the confidence interval between the pairwise SNP exceeded 95%. In the absence of information on pedigree, composition and frequency of haplotype in each breed were calculated using an algorithm developed by Scheet et al. (2006). And, phase probabilities for each site were calculated for each individual using this software (PHASE) (input option-: ignoring families). Using this software, phase probabilities of all polymorphic sites for haplotypes were calculated for each individual. Associations between individual SNPs and carcass weight, backfat thickness, marbling score, and fatty acid composition were determined by the mixed effect model, treating "place at the calving (14 classes)" as a fixed effect and "sire" as a random effect; "age" at slaughter was also included in the model as a covariate in the SPSS statistics v18.0 package. Other covariates were not available for this analysis. We used a single SNP model. Single SNP/haplotype effects were tested in the mixed effect model. For haplotype analyses, we fitted the model with the same covariates in a similar manner.

RESULTS AND DISCUSSION

SNP selection of the stearyol-CoA desaturase gene and haplotype analysis in Hanwoo

Observation of a powerful relationship between the SNP in exon 5 of the SCD gene and association of the unsaturated fatty acids with beef flavor in Japanese black cattle and Fleckvieh bulls have been reported (Taniguchi et al., 2003; Tsuji, 2008; Barton et al., 2009; Ohsaki et al., 2009). Based on these reports, three candidate SNPs of exon 5 in the SCD gene have statistical analysis genotypic and allelic frequencies, heterozygote and MAF (minor allele frequency), which are shown in Table 2.

The genetic frequencies of the three polymorphic SNPs were calculated based on Mendel's law of segregation, and were found to be 0.495-0.500. These results confirmed the

Table 2. Genotype and frequency of 3 polymorphic SNPs of the SCD gene in Hanwoo

SNP	Region	Genotype (No. of head) Frequency				H^1	MAF^2
g.10153 A>G	Exon 5	AA (94)	GA (177)	GG (124)	N^3 (395)	0.497	0.462
		0.238	0.448	0.314	1		
g.10213 T>C	Exon 5	CC (84)	CT (232)	TT (79)	N (395)	0.500	0.494
		0.213	0.587	0.200	1		
g.10329 C>T	Exon 5	CC (56)	CT (237)	TT (101)	N (394)	0.443	0.443
		0.142	0.602	0.256	1		

[1] Heterozygosity. [2] Minor allele frequency. [3] Total number.

polymorphism based on estimation of heterozygote. Also, minor allele frequency was lower than 0.100, and segregation was not well observed following analysis of linkage disequilibrium. This leads to decreased accuracy of analysis (Eberle et al., 2006; Lee et al., 2006). Based on this, all three of the candidate SNPs were found to have values of 0.462, 0.494, and 0.443, higher than 0.100. Therefore, the possibility that analytical accuracy might be lowered could be minimized.

Thereafter, three polymorphic SNPs located in exon 5, which showed a significant difference in content of unsaturated fatty acids and the important function of genetics, were analysis of linkage disequilibrium. Thus, the haplotype block was prepared and the results are shown in Figure 1. According to Gabriel et al. (2002), the lower limit of a 95% confidence interval exceeded 0.7, and the status of linkage disequilibrium was reached. Based on reports, Figure 1 shows that three SNPs (g.10153 A>G, g.10213 T>C, and g.10329 C>T) located in exon 5 have a powerful degree of linkage disequilibrium. Therefore, three SNPs of exon 5 in the SCD gene have a linkage disequilibrium indicating involvement of a multitude of variations, rather than a single gene.

In this research study, the exon 5 region was located at 10,099-10,331 bp among genomic DNAs of the 17,088 bp SCD gene. Among them, the 10,153 nucleotide was replaced by CCG in the base sequence of CCA. Nevertheless, there was no change in the amino acid as Proline. Similarly, TAT was replaced by TAC in the 10213 nucleotide and there was no change in the amino acid as Tyrosine. However, the 10,329 nucleotide is a nonsynonymous SNP whose amino acid sequences of Valine are replaced by Alanine due to replacement of GCG by GTG. Taniguchi et al. (2003) also reported that expression of nonsynonymous SNP genes, where Valine is replaced by Alanine, was observed in SNPs of the ORF domain of SCD genes in Japanese black cattle. And the SCD genotype has been reported to show effects on C18:1 and MUFA levels (Barton et al., 2009; Ohsaki et al., 2009). The present results corroborate these effects. When the V (G C T) type allele was changed to the A (A T C) type in the SCD gene, the proportion of saturated fatty acids (C14:0 and C18:0) was decreased, and the proportion of MUFA (C14:1 and C18:1) was increased. As shown in this study, the content of unsaturated fatty acids and marbling score appears higher when we have the Ht2 allele; we should be more interested in the functions of the g.10329 C>T SNP of exon 5 in Hanwoo.

Based on linkage disequilibrium of the haplotype block, three representative SNPs located within exon 5 in the SCD

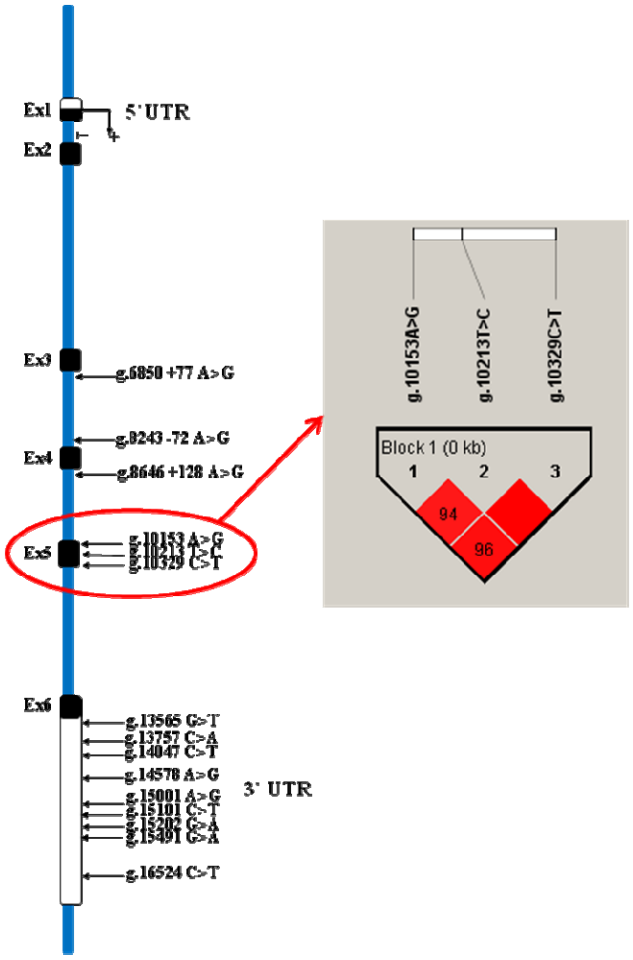

Figure 1. SNP within the SCD gene and linkage disequilibrium between 3 SNP pairs of exon 5 in SCD. The color code on the Haploview plot follow the standard color scheme: red(|D'|<1, |D'|=1, LOD≥2). Numbers in cells are D' value. However, the D' values of 1.0 are not shown (empty).

Table 3. Haplotype blocks of 3 SNPs within the SCD gene and their frequencies

Haplotype	g.10153 A>G	g.10213 T>C	g.10329 C>T	Frequency
Ht1	G	C	T	0.437678
Ht2	A	T	C	0.374776
Ht3	G	T	C	0.045483
	A	C	T	0.040318
	G	T	T	0.039073
	A	T	T	0.036899
	A	C	C	0.014088
	G	C	C	0.011686

gene using the Haploviewer v4.2 program were determined by frequency analysis, as shown in Table 3.

As shown in Table 3, the frequency of Ht1 and Ht2 in Hanwoo accounted for almost 80%; however, that of Ht3 was 20%. It has been reported that combinations of Type A (A T C) and Type V (G C T) accounted for almost 100% and the frequency of Ht3 was close to 0% in Japanese black cattle, unlike that observed in Hanwoo (Taniguchi et al., 2003; Hoashi et al., 2007; Tsuji, 2008; Ohsaki et al., 2009). Based on these results, genetic variations associated with beef flavor between Hanwoo and Japanese black cattle could be demonstrated. It can also be inferred that raising the genetic frequency leaves something to be desired for improvement of beef flavor.

Effect of SNP genotype in carcass traits and fatty acid composition

In a previous report, unsaturated fatty acids affect beef flavor (Alexander et al., 2008) and higher content of MUFA (mono-unsaturated fatty acid) of unsaturated fatty acids greatly affects beef flavor (Hoashi and Hinenoya, 2008; Tsuji, 2008). Based on these reports, we analysis composition of fatty acids and the carcass phenotype depending on a single genotype in Hanwoo. The results in Table 4 show significant differences depending on the type of SNP gene.

In palmitic acid (C16:0), palmitoleic acid (C16:1), and linoleic acid (C18:2), with a high proportion of fatty acids, there were no association with SNPs in the SCD gene. However, myristic acid (C14:0) and stearic acid (C18:0) showed a higher proportion of the GG genotype of the g.10153 A>G SNP, the CC genotype of the g.10213 T>C SNP, and the TT genotype of the g.10329 C>T SNP.

SFA (saturated fatty acid) in the g.10213 T>C SNP showed no genetic effects. However, the g.10153 A>G and g.10329 C>T SNPs were observed at a higher content of

Table 4. Effect of the polymorphism in 3SNPs of the SCD gene on carcass trait and fatty acid composition of intramuscular fat

Trait	g.10153 A>G				g.10213 T>C				g.10329 C>T(A10329V)				
	AA (N = 94) LSmean±SE	GA (N = 177) LSmean±SE	GG (N = 124) LSmean±SE		CC (N = 84) LSmean±SE	CT (N = 232) LSmean±SE	TT (N = 79) LSmean±SE		CC (N = 56) LSmean±SE	CT (N = 237) LSmean±SE	TT (N = 101) LSmean±SE	Total (N = 394) LSmean±SE	
Carcass weight (kg)	426.49±4.17	428.49±3.21	428.23±4.22	ns	426.02±4.77	429.37±2.93	425.72±4.50	ns	424.50±5.03	428.81±2.87	427.42±4.52	427.936±2.19	ns
Back fat Thickness (cm)	13.70±0.56	13.23±0.41	13.19±0.49	ns	13.30±0.59	13.28±0.35	13.52±5.78	ns	13.34±0.75	13.27±0.35	13.46±0.53	13.33±0.27	ns
MS (marbling score)	4.90±0.21^{a}	5.85±0.14^{b}	5.02±0.19^{a}	***	5.23±0.22^{ab}	5.58±0.13^{b}	4.89±0.24^{a}	*	4.77±0.27^{a}	5.62±0.13^{b}	5.11±0.19^{ab}	5.37±0.10	**
Fatty acid composition (%)													
C14:0	3.47±0.06^{a}	3.64±0.04^{b}	3.72±0.06^{b}	**	3.77±0.08^{b}	3.64±0.04^{b}	3.42±0.06^{a}	**	3.38±0.08^{a}	3.65±0.04^{b}	3.70±0.07^{b}	3.62±0.03	**
C16:0	25.29±0.17	25.25±0.14	25.28±0.17	ns	25.33±0.21	25.27±0.12	25.21±0.19	ns	25.19±0.23	25.27±0.12	25.32±0.19	25.27±0.09	ns
C18:0	10.06±0.13^{a}	10.23±0.09^{a}	10.77±0.13^{b}	***	10.74±0.16^{b}	10.27±0.08^{a}	10.21±0.14^{a}	**	10.30±0.17^{a}	10.20±0.08^{a}	10.80±0.15^{b}	10.36±0.07	***
C14:1	1.48±0.04^{c}	1.32±0.03^{b}	1.08±0.03^{a}	***	1.11±0.04^{a}	1.29±0.03^{b}	1.44±0.04^{c}	***	1.44±0.04^{b}	1.33±0.02^{b}	1.07±0.04^{a}	1.29±0.02	***
C16:1	6.55±0.09	6.65±0.07	6.66±0.09	ns	6.70±0.11	6.67±0.06	6.42±0.10	ns	6.30±0.11	6.69±0.06	6.65±0.11	6.63±0.05	ns
C18:1	44.43±0.26^{b}	44.81±0.18^{b}	43.64±0.25^{a}	**	43.72±0.31^{a}	44.54±0.17^{b}	44.48±0.29^{b}	*	44.48±0.34^{b}	44.60±0.16^{b}	43.71±0.29^{a}	44.36±0.13	*
C18:2	3.33±0.05	3.16±0.04	3.14±0.05	ns	3.17±0.07	3.16±0.04	3.34±0.06	ns	3.36±0.07	3.17±0.04	3.16±0.06	3.20±0.03	Ns
SFA[1]	39.92±0.27^{a}	40.13±0.19^{a}	40.97±0.27^{b}	**	40.95±0.33	40.23±0.17	40.02±0.32	ns	40.07±0.38^{a}	40.15±0.17^{a}	40.95±0.30^{b}	40.33±0.14	*
MUFA[2]	53.52±0.27^{b}	53.99±0.20^{b}	52.56±0.26^{a}	***	52.64±0.32^{a}	53.71±0.18^{b}	53.43±0.30^{b}	*	53.29±0.36^{ab}	53.83±0.18^{b}	52.58±0.30^{a}	53.43±0.14	**
M/S[3]	1.36±0.02^{b}	1.35±0.01^{b}	1.30±0.01^{a}	**	1.30±0.02^{a}	1.34±0.01^{b}	1.36±0.02^{b}	*	1.35±0.02^{b}	1.35±0.01^{b}	1.30±0.02^{a}	1.34±0.01	*
C14 index[4]	29.74±0.44^{c}	26.31±0.36^{b}	22.03±0.47^{a}	***	22.22±0.56^{a}	25.80±0.35^{b}	29.51±0.50^{c}	***	29.72±0.58^{c}	26.40±0.32^{b}	22.00±0.54^{a}	25.78±0.28	***
C16 index[5]	20.59±0.27	20.86±0.19	20.85±0.25	ns	20.92±0.31	20.91±0.17	20.32±0.30	ns	20.03±0.35	20.95±0.16	20.80±0.29	20.79±0.13	ns
C18 index[6]	81.45±0.23^{b}	81.41±0.15^{b}	80.20±0.22^{a}	***	80.24±0.27^{a}	81.24±0.14^{b}	81.30±0.26^{b}	**	81.18±0.31^{b}	81.39±0.14^{b}	80.15±0.25^{a}	81.04±0.11	***

[1] SFA = Saturated fatty acid. [2] MUFA = Mono unsaturated fatty acid. [3] M/S = Mono unsaturated fatty acid/Saturated fatty acid.

[4] C14 index = [C14:1/(C14:0+C14:1)]×100. [5] C16 index = [C16:1/(C16:0+C16:1)]×100. [6] C18 index = [C18:1/(C18:0+C18:1)]×100.

MS = Marbling score(1-9). [a, b, c] Means with different superscripts within the same column are significantly different ($p<0.05$).

* $p<0.05$, ** $p<0.01$, **** $p<0.001$. ns = Non-significant.

40.97% and 40.95% in the genotypes of GG and TT ($p<0.05$).

However, in unsaturated fatty acids, such as oleic acid (C18:1) and MUFA, the g.10153 A>G SNP showed that genotypes of AA and GA had an oleic acid content of 44.43%, 44.81%, and an MUFA content of 53.52%, 53.99%, diametrical to the content of SFA. These results indicate that the content of unsaturated fatty acids was significantly higher than with the GG genotype ($p<0.01$). The g.10213 T>C SNP showed that genotypes of CT and TT had an oleic acid content of 44.54%, 44.48%, and an MUFA content of 53.71%, 53.43%. That is, it was found to be higher than the oleic acid content of 43.72%, and the MUFA content of 52.64%, as seen in the genotype of CC ($p<0.05$). In the g.10329 C>T SNP, genotypes of CC and CT showed an oleic acid content of 44.48%, 44.60%, and MUFA of 53.83%. And these results were higher than the content of the genotype of TT (43.71% had oleic acid and 52.58% had MUFA) ($p<0.05$). These results were diametrical to the content of saturated fatty acids. Also, as an unsaturated fatty acid, oleic acid (C18:1) generally accounted for over 80% of the whole contents of unsaturated fatty acids. Each SNP genotype of oleic acid (C18:1) showed a similar tendency to MUFA's content.

Mono-unsaturated fatty acid/saturated fatty acid, C14 index, and C18 index showed that the AA and GA genotypes in the g.10153 A>G SNP, the CT and TT genotypes in the g.10213 T>C SNP, and the CC and CT genotypes in the g.10329 C>T SNP were associatied with a higher content of unsaturated fatty acids ($p<0.001$). In previous studies, oleic acid (C18:1) and MUFA showed similar trends.

Following a comparison between these results for composition of fatty acids in Hanwoo and other breeds, Angus had an oleic acid (C18:1) content of 42.65%, which was found to be lower, compared with Hanwoo. Also, MUFA had a content of 49.27%, which was found to be lower (May et al., 1993; Chio et al., 2008; Smith et al., 2009). In Australian beef, the content of oleic acid (C18:1) was found to be 39.80% and MUFA had a content of 44.80% (Smith et al., 2009). Following comparison of the content of oleic acid (C18:1) and MUFA, the content was found to be lower by 4.56% and 8.62%, compared with Hanwoo. These results were also lower than those seen in Angus. However, in Japanese black cattle, unlike Angus or Australian beef, the content of oleic acid (C18:1) was found to be 52.90%, which was higher than that of Hanwoo. It was also found to be 60.59% in MUFA, which was higher, compared with Hanwoo. Based on these results for unsaturated fatty acids in Hanwoo, the content of oleic acid (C18:1) and MUFA was lower by 8.54% and 7.14%, compared with the results seen in Japanese black cattle. (Oka et al., 2002; Taniguchi et al., 2003; Chung et al., 2007; Tsuji, 2008; Ohsaki et al., 2009; Smith et al., 2009).

Considering the content of unsaturated fatty acids similar in direction to that of Japanese black cattle, improvement of Hanwoo will also be necessary (Taniguchi et al., 2003; Tsuji, 2008; Ohsaki et al., 2009; Smith et al., 2009). Thus, following a comparison between breeds of cattle, the contents of oleic acid (C18:1) and MUFA were significantly higher in Japanese Black cattle, compared with Australian beef, other Wagyu breeds, and Angus (Zembayashi et al., 1995). This suggests that the difference in fatty acid between the breed is in agreement with production of a high-quality beef.

Following an analysis of the relationship between carcass traits and SCD genes, there were no effects on carcass weight and Backfat thickness in Hanwoo. However, there was a significantly higher association with intramuscular marbling scores. In the GA genotype of the g.10153 A>G SNP and the CT genotype of the g.10213 T>C SNP and the g.10329 C>T SNP, intramuscular marbling scores showed significant associations compared with other genotypes ($p<0.05$). In association with these results, as shown in Table 4, illustrating the relationships between unsaturated fatty acids and three SNPs, heterozygote genotypes of GA, CT, and CT on the 3 different SNPs showed the highest degree of marbling score. However, the homozygote did not show a definite relationship. Definite results in this study show that the content of both oleic acid (C18:1) and MUFA was significantly increased as the beef grades of Hanwoo. This indicates that the content of unsaturated fatty acids in Hanwoo beef had a significantly higher association with intramuscular marbling scores.

In general, fatty acids affected beef flavor and were associated with beef grades and marbling score in Japanese black cattle (Taniguchi et al., 2003; Ibi et al., 2005). Association of higher content of unsaturated fatty acids with a higher degree of beef grade and beef flavor has been reported (Jeremiah, 1996; Oka et al., 2001). As with the previous results, we have identified a relationship between beef grade and unsaturated fatty acid in Hanwoo.

Effect of haplotype on carcass traits and fatty acid composition

A powerful degree of linkage disequilibrium was observed between the g.10153 A>G SNP, the g.10213 T>C SNP, and the g.10329 C>T SNP, which were located in exon 5 of SCD genes. A strong linkage disequilibrium between SNPs was attributed to an association between genes that effect a combination of multiple variations, rather than a single variation. Therefore, there are demerits, in that the phenotypes are influenced by a complex number of genes, as shown in the composition of fatty acids and intramuscular marbling scores that are associated with beef

flavor using a single SNP. Table 5 shows these demerits were compensated the through haplotype analysis.

As shown in Table 5, the genotype of the ht1*ht2 group showed a significantly higher effect for intramuscular marbling scores, compared with those of other genotypes. The genotype of the ht3*ht3 group was found to be 4.14, which was the lowest significant effected. Compared with the lowest value due to the effects seen in a single genotype, it was found to be lower by approximately 0.63. These results corresponded with the findings shown in Table 4, showing that the heterozygote had the highest significant effect for intramuscular marbling scores and haplotype effects were more powerful than single gene effects.

Analysis of the relationship between composition of fatty acids and combinations of haplotype showed that palmitic acid (C16:0), palmitoleic acid (C16:1), and linoleic acid (C18:2) had nothing to do with the gene. In such saturated fatty acids as myristic acid (C14:0) and stearic acid (C18:0), the proportion of genotypes of ht1*ht2 and ht2*ht2 was relatively lower than in those with other genotypes. By contrast, the C14 index, indicating the ratio of unsaturated fatty acids, such as myristoleic acid (C14:1) and myristoleic acid (C14:1), was relatively higher in the genotype of the ht2*ht2 group (1.46% and 30.01%), and this was a statistically significant association ($p<0.001$).

The content of oleic acid (C18:1) was the highest in genotypes of the ht1*ht2 and ht2*ht2 groups ($p<0.01$). Content of MUFA was also the highest in genotypes of ht1*ht2. On the other hand, content of saturated fatty acids (SFA) that were diametrical to that of unsaturated fatty acids, was relatively lower in genotypes of the ht1*ht2 group and the ht2*ht2 group ($p<0.05$). And, M/S, indicating the proportion of a mono-unsaturated fatty acid, and C18 index, indicating the proportion of unsaturated fatty acids of oleic acid (C18:1), also showed the same trends as that of the content of MUFA.

These results comparing the effects of single SNPs are shown in Table 4; the highest composition of oleic acid (C18:1) also showed a content of 44.81% in the GA genotype of the g.10153 A>G SNP. On the other hand, genotypes of the haplotype ht1*ht2 group showed an oleic acid content of 44.92%, which corresponded to a higher content of 0.11%. Following comparison between a single genotype, having the lowest content in the GG genotype of the g.10153 A>G SNP, and those with a genotype of the ht3*ht3 group, there was a difference of 0.90%. Also, following a comparison of the content of MUFA, there was a difference of 0.15% between a genotype of the ht1*ht2 group and those with a single genotype at GA of the g.10153 A>G SNP. And, following a comparison of the content of fatty acids between a genotype of the ht3*ht3 group having the lowest content of fatty acids and those with a single genotype, there was a difference of 0.32%. That is, these results also showed similar trends to those

Table 5. Effect of the polymorphism in haplotypes of the SCD gene on carcass trait and fatty acid composition of intramuscular fat

Trait	Haplotype							
	ht1*ht1 (N = 74) LSmean±SE	ht1*ht2 (N = 170) LSmean±SE	ht1*ht3 (N = 51) LSmean±SE	ht2*ht2 (N = 49) LSmean±SE	ht2*ht3 (N = 44) LSmean±SE	ht3*ht3 (N = 7) LSmean±SE	Total (N = 395) LSmean±SE	
Carcass weight (kg)	425.15±5.22	428.70±3.37	433.78±6.72	425.57±5.23	425.66±6.53	426.71±16.0	427.93±2.19	ns
Backfat thickness (cm)	13.45±0.65	13.39±0.43	12.90±0.72	13.53±0.79	13.70±0.78	9.86±1.35	13.33±0.27	ns
MS(marbling score)	5.18±0.22^{ab}	5.86±0.15^{b}	5.00±0.31^{ab}	4.80±0.30^{ab}	5.05±0.30^{ab}	4.14±0.70^{a}	5.37±0.10	**
Fatty acid composition (%)								
C14:0	3.73±0.08^{bc}	3.63±0.04^{abc}	3.76±0.08^{c}	3.39±0.08^{ab}	3.56±0.07^{abc}	3.37±0.21^{a}	3.62±0.03	**
C16:0	25.22±0.23	25.14±0.14	25.51±0.26	25.21±0.24	25.74±0.21	24.56±0.66	25.27±0.09	ns
C18:0	10.84±0.17^{bc}	10.19±0.10^{ab}	10.59±0.18^{abc}	10.22±0.18^{ab}	10.06±0.15^{a}	11.03±0.37^{c}	10.36±0.07	**
C14:1	1.07±0.04^{a}	1.33±0.03^{b}	1.08±0.05^{a}	1.46±0.05^{b}	1.44±0.05^{b}	1.42±0.21^{b}	1.29±0.02	***
C16:1	6.64±0.13	6.67±0.07	6.73±0.13	6.29±0.13	6.69±0.11	6.79±0.32	6.63±0.05	ns
C18:1	43.77±0.34^{ab}	44.92±0.19^{b}	43.61±0.36^{ab}	44.67±0.38^{b}	43.90±0.35^{ab}	42.74±0.70^{a}	44.36±0.13	**
C18:2	3.21±0.07	3.17±0.05	3.02±0.07	3.34±0.07	3.29±0.07	3.31±0.029	3.20±0.03	ns
SFA[1]	40.88±0.36^{ab}	39.95±0.20^{a}	41.00±0.38^{ab}	39.81±0.41^{a}	40.54±0.32^{ab}	41.79±0.50^{b}	40.33±0.14	*
MUFA[2]	52.58±0.35^{ab}	54.14±0.21^{b}	52.64±0.38^{ab}	53.48±0.39^{ab}	53.12±0.35^{ab}	52.24±0.85^{a}	53.43±0.14	***
M/S[3]	1.29±0.02^{ab}	1.36±0.01^{b}	1.29±0.01^{ab}	1.36±0.02^{b}	1.31±0.01^{ab}	1.25±0.03^{a}	1.34±0.01	**
C14 index[4]	21.95±0.59^{a}	26.50±0.38^{b}	21.96±0.66^{a}	30.01±0.57^{c}	28.62±0.61^{bc}	29.04±2.90^{bc}	25.78±0.28	***
C16 index[5]	20.83±0.34	20.99±0.20	20.87±0.36	19.98±0.39	20.64±0.28	21.69±1.03	20.79±0.13	ns
C18 index[6]	80.14±0.29^{ab}	81.50±0.16^{b}	80.47±0.29^{ab}	81.36±0.33^{b}	81.32±0.29^{ab}	79.48±0.65^{a}	81.04±0.11	***

[1] SFA = Saturated fatty acid. [2] MUFA = Mono unsaturated fatty acid. [3] M/S = Mono unsaturated fatty acid/Saturated fatty acid.
[4] C14 index = [C14:1/(C14:0+C14:1)]×100. [5] C16 index = [C16:1/(C16:0+C16:1)]×100. [6] C18 index = [C18:1/(C18:0+C18:1)]×100.
MS = Marbling score(1-9). [a, b, c] Means with different superscripts within the same column are significantly different ($p<0.05$).
* $p<0.05$, ** $p<0.01$, *** $p<0.001$. ns = Non-significant.

seen in oleic acid (C18:1). Based on these results, as shown in the composition of fatty acids, the effects of a haplotype genotype were more powerful than the effects of a single genotype.

Following analysis of the composition of unsaturated fatty acids depending on the SNP genotype of the SCD gene in Japanese Black cattle, phenotypes with an A/A type (A T C) among the open reading frame (ORF) of exon 5 have been reported to show a higher degree of composition of unsaturated fatty acids (Taniguchi et al., 2003; Tsuji, 2008; Ohsaki et al., 2009). In Fleckvieh bulls, breeds with an A/A type (A T C)/(A T C) and an A/V type (A T C)/(G C T) were found to have a high-significant association with oleic acid (C18:1) and unsaturated fatty acids (Barton et al., 2010). These results indicate that genotypes of the ht1*ht1 and ht1*ht2 groups, having a higher content of unsaturated fatty acids, had a positive effect on beef flavor, as shown in this study. That is, these findings are also based on the genetic characteristics of Hanwoo. Therefore, as shown in this study, haplotypes of the g.10153 A>G SNP, the g.10213 T>C SNP, and the g.10329 C>>T SNP, which were located in exon 5, showed a significantly positive relationship with the composition of unsaturated fatty acids and intramuscular marbling scores. This might contribute to an increase in the content of unsaturated fatty acids associated with beef flavor.

Therefore, based on results showing that the exon 5 haplotype with such genotypes as those of the ht1*ht2 and ht2*ht2 groups having a higher content of unsaturated fatty acids and intramuscular marbling scores associated with beef flavor, it can be inferred that there might be a further applicability as an indicator for unsaturated fatty acids in determination of beef flavor of Hanwoo and the marbling score associated with the quality of beef. Thus, these results are presumed to be the baseline data contributing to development of the Hanwoo-related industry.

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