Identification of Exonic Nucleotide Variants of the *Thyroid Hormone Responsive Protein* Gene Associated with Carcass Traits and Fatty Acid Composition in Korean Cattle

Dong-yep Oh[a], Yoon-seok Lee[1,a], Boo-mi La[2], Jea-young Lee[3], Yong-soo Park[4], Ji-hong Lee[5],
Jae-jung Ha, Jun-koo Yi, Byung-ki Kim, and Jung-sou Yeo[2,*]

Livestock Research institute, Yeongju 750-871, Korea

ABSTRACT: The *thyroid hormone responsive protein* (*THRSP*) gene is a functional gene that can be used to indicate the fatty acid compositions. This study investigates the relationships of exonic single nucleotide polymorphisms (SNPs) in the *THRSP* gene and fatty acid composition of muscle fat and marbling score in the 612 Korean cattle. The relationships between fatty acid composition and eight SNPs in the *THRSP* gene (g.78 G>A, g.173 C>T, g.184 C>T, g.190 C>A, g.194 C>T, g.277 C>G, g.283 T>G and g.290 T>G) were investigated, and according to the results, two SNPs (g.78 G>A and g.184 C>T) in exon 1 were associated with fatty acid composition. The GG and CC genotypes of g.78 G>A and g.184 C>T had higher unsaturated fatty acid (UFA) and monounsaturated fatty acid (MUFA) content ($p<0.05$). In addition, the ht1*ht1 group (Val/Ala haplotype) in a linkage disequilibrium increased MUFAs and marbling scores for carcass traits ($p<0.05$). As a result, g.78 G>A and g.184 C>T had significantly relationships with UFAs and MUFAs. Two SNPs in the *THRSP* gene affected fatty acid composition, suggesting that GG and CC genotypes and the ht1*ht1 group (Val/Ala haplotype) can be markers to genetically improve the quality and flavor of beef. (**Key Words:** *Thyroid Hormone Responsive Protein* (*THRSP*) Gene, Unsaturated Fatty Acid, SNP, Beef Flavor, Korean Cattle)

INTRODUCTION

Beef quality depends not only on the marbling score but also on the fatty acid composition. The fatty acid composition of adipose tissue in livestock has been recognized as an important carcass trait influencing meat quality because a higher monounsaturated fatty acid (MUFA) concentration leads to a lower fat melting point, which affects the softness of fat and beef flavor (Melton et al., 1982; Yang et al., 1999). Beef with the most desirable flavor has lower percentages of saturated fatty acids (SFAs) and polyunsaturated fatty acids (PUFAs) and higher percentages of MUFAs in carcass fat (Dryden and Marchello, 1970; Westerling and Hedrick, 1979; Melton et al., 1982). In addition, the MUFA concentration has important implications for human health (Rudel et al., 1995).

Stearic acid (C18:0) is a primary determinant of fat hardness (Smith et al., 1998; Wood et al., 2004; Chung et al., 2006). Oleic acid (C18:1) is a major MUFA, accounting for the highest percentage of fatty acids in bovine adipose tissue, and is positively correlated with the marbling, flavor, and tenderness of beef and the softness of fat (Westerling and Hedrick, 1979; Kim et al., 2002; Lee et al., 2004; Smith

* Corresponding Author: Jung-sou Yeo. Tel: +82-53-810-3021, Fax: +82-53-813-2936, E-mail: jsyeo@ynu.ac.kr
[1] Institute of Green Bio Science and Technology, Seoul National University, Pyeongchang 232-916, Korea.
[2] School of Biotechnology, Yeungnam University, Gyeongsan 712-749, Korea.
[3] Department of Statistics, Yeungnam University, Gyeongsan 712-749, Korea.
[4] Department of Equine Industry, Korea National College of Agriculture and Fisheries, Hwaseong 445-760, Korea.
[5] Gyeongbuk Provincial College, Yecheon 750-767, Korea.
[a] Dong-yep Oh and Yoon-seok Lee are contributed equally to this work.

et al., 2009). Therefore, genetic factors that enhance the conversion of stearic acid (C18:0) into oleic acid (C18:1) enhance the tenderness, marbling, and flavor of beef (Yang et al., 1999). As shown in Jenkins (1993), a majority of dietary fatty acids are chemically reduced by microorganisms in the rumen and absorbed as SFAs. However, it is known that diet also influences bovine fatty acid composition (Cabezas et al., 1965; Edwards et al., 1961). Zembayashi et al. (1995) demonstrated that the adipose tissue of Japanese Black cattle contains a higher proportion of MUFAs than that of Holstein, Japanese Brown cattle and Charolais. These results suggest that fatty acid composition may be controlled by genetic factors such as genes closely related to lipid synthesis and fatty acid metabolism (Narukami et al., 2011).

The *thyroid hormone responsive protein* (*THRSP*) gene encodes a nuclear protein, closely associated with the regulation of fatty acid synthesis in lipogenic tissue based on several studies of its tissue distribution and correlation with lipogenesis (Jump et al., 1985; Freake et al., 1986; Perez-Castillo et al., 1987). The *THRSP* has been reported to act at the transcription level to regulate genes that encode enzymes required for the synthesis of long-chain fatty acids (Cunningham et al., 1998). Knockout studies have reported *THRSP* null mice to show a marked deficiency in de novo lipogenesis and revealed that the *THRSP* may work in the cytoplasm by tethering the fatty acid synthase to the microtubule (Lafave et al., 2006).

Noteworthy is that the *THRSP* gene encodes a protein called Spot14 or S14. The adipose tissue and liver are predominant sites of S14 expression. In addition, the *THRSP* gene has been identified to be associated with *de novo* lipogenesis (Zhu et al., 2005). S14 is a protein with 150 amino acids, and it is abundantly expressed in lipogenic tissue and is regulated in a manner similar to that for other enzymes involved in fatty acid synthesis (Colbert et al., 2010). Therefore, this study was aimed to evaluate the associations between single nucleotide polymorphisms in the *THRSP* gene carcass traits and fatty acid composition in Korean cattle.

MATERIALS AND METHODS

Animals and phenotypic data

A total of 612 Korean cattle (known as Hanwoo in Korea), bred in Gyeungbuk, Korea, were considered in this study for data on genotypes and carcass traits. All feeding conditions for steers were controlled under the livestock management of the calving place (n = 14). The steers were the progeny of 10 sires. Carcass phenotypes and marbling scores were measured 24 h after slaughter. To measure carcass traits, the steers were dissected and samples were collected at the last rib and the first lumber vertebra

according to the Animal Product Grading System of Korea. The steers were slaughtered at 941 ± 72 days of age, and average carcass weight was 427.25 kg. Backfat thickness and marbling scores were measured or scored in the left carcass cut across the vertebra between the last thoracic vertebra and the first lumbar vertebra. The degree of marbling was scored from 1 (trace) to 9 (very abundant) according to the Korean Beef Marbling Standard, in which the higher the score, the more abundant the intramuscular fat, and the mean was 5.39 (Table 1). Genomic DNA was extracted from the *m. longissimus dorsi muscle* by using the COSMO GENETECH kit (COSMO Co, Ltd., Seoul, Korea).

According to Folch et al. (1957), total lipids were extracted from approximately 500 mg of the *m. longissimus dorsi* muscle by using chloroform: methanol (2:1, vol/vol) and then methylated with sodium methylate by following O'Keefe et al. (1968). Samples were filtered through a filter paper in a water bath (40°C). The filtrate was mixed with distilled water, from which the layer of methanol and water was removed. Chloroform and lipid layers were removed using nitrogen gas. The samples were then treated with BF-3 methanol (14%) and subjected to trans-methylation at 65°C. Fatty acid contents was analyzed by gas-chromatography (PerkinElmer, Inc., Waltham, MA, USA), and fatty acids that were analyzed included myristic acid, palmitic acid, stearic acid, myristoleic acid, palmitoleic acid, oleic acid, linoleic acid, and linolenic acid. The compositions and functions of fatty acids were used as phenotypes in the analysis of genetic relationship (Table 1).

Single nucleotide polymorphism genotyping

Exonic SNPs in the *THRSP* gene were selected based on the nucleotide sequence of the bovine *THRSP* gene in GenBank (Accession No. AC_000186.1), and selected SNPs were subjected to a preliminary analysis. For the eight SNPs, primers for amplification and extension were designed for single-base extension by using PRIMER 3.0, a web-based software package (Supplementary Table 1).

Primer extension reactions were performed using the SNaPshot dNTP Primer Extension Kit (Applied Biosystems, Foster City, CA, USA). For primer extension, exonuclase 1 and shrimp alkaline phosphatase were added to the reaction mixtures. Samples were cultured at 37°C for 1 h and then inactivated at 72°C for 15 min. Polymerase chain reaction products were analyzed based on the Genescan 120 LIZ standard and HiDi formamide (Applied Biosystems, Foster City, CA, USA), followed by denaturation at 95°C for 5 min. Electrophoresis was performed using the ABI PRISM 3130XL Genetic Analyzer and analyzed using GeneMapper v.4.0 (Applied Biosystems, USA).

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) was tested for

Table 1. Means and standard deviations for carcass traits and fatty acid composition in Korean cattle

Trait	Description	Mean±SD	Min	Max
Carcass trait				
CW (kg)	Carcass weight	427.25±43.89	321.00	576.00
BFT (cm)	Backfat thickness	13.18 ±5.20	3.00	42.00
MS	Marbling score	5.39±1.93	1.00	9.00
Fatty acid composition (%)				
C14:0	Myristic acid	3.58±0.65	1.98	5.52
C16:0	Palmitic acid	25.83±1.99	18.86	32.23
C18:0	Stearic acid	10.35±1.50	6.54	15.34
C14:1	Myristoleic acid	1.21±0.39	0.47	2.73
C16:1	Palmitoleic acid	6.42±1.16	3.87	10.29
C18:1	Oleic acid	44.20±2.65	36.14	53.69
C18:2n6	Linoleic acid	3.01±0.79	0.05	5.59
C18:3n3	Linolenic acid	0.35±0.21	0.04	1.03
SFA	Saturated fatty acid	40.46±2.87	30.36	48.44
MUFA	Monounsaturated fatty acid	52.98±3.30	45.65	63.46
Fatty acid index				
M/S	MUFA/SFA	1.32±0.16	0.97	2.07
I_{C14}	[C14:1/(C14:0+C14:1)]×100	25.06±6.31	10.82	39.80
I_{C16}	[C16:1/(C16:0+C16:1)]×100	19.91±3.34	8.91	31.18
I_{C18}	[C18:1/(C18:0+C18:1)]×100	81.03±2.59	72.16	94.55

SD, standard deviation.

Supplementary Table 1. Information of primer sequences of SNPs on the *THRSP* gene in Korean cattle

SNP		Sequences	Product size
g.78 G>A	F	ATCATCACCACCTGCTCCAT	252
	R	AGCCCTAGGATTGGCCTAAA	
	E	CCAAGAACTGCCTGCTGACC	
g.173 C>T	F	GCCTTGAGCATGGTGAAGTAG	118
	R	ATGGAGCAGGTGGTGATGAT	
	E	GAGGCGCCCACCTGGGCATG	
g.184 C>T	F	GCTCTCCTCCTCAGCCTCTT	242
	R	ATGGAGCAGGTGGTGATGAT	
	E	ACACGGGTACCATGCCCAGG	
g.190 C>A	F	GCCTTGAGCATGGTGAAGTAG	118
	R	ATGGAGCAGGTGGTGATGAT	
	E	GCAGGAGTGGCAGGCCAAGG	
g.194 C>T	F	GCCTTGAGCATGGTGAAGTAG	118
	R	ATGGAGCAGGTGGTGATGAT	
	E	GTGAAGTAGTTGTAGAGATC	
g.277 C>G	F	GCTCTCCTCCTCAGCCTCTT	165
	R	CGCCTCCGATCTCTACAACT	
	E	GCCCCGGCAGGAGTGGCAGG	
g.283 T>G	F	GCTCTCCTCCTCAGCCTCTT	165
	R	CGCCTCCGATCTCTACAACT	
	E	GTACCATGCCCAGGTGGGCG	
g.290 T>G	F	GCTCTCCTCCTCAGCCTCTT	126
	R	CATCCGCATGGATATGGAG	
	E	TCAGCTCCCTCGGCTTTGCC	

SNPs, single nucleotide polymorphisms; THRSP, thyroid hormone responsive protein; F, forward sequence; R, reverse sequence; E, extension sequence.

each locus by comparing expected and observed genotype frequencies through the Chi-square statistic. Associations between phenotypes (carcass traits and fatty acid composition) and individual SNPs in the 612 samples were analyzed based on a mixed analysis of covariance linear model using SPSS v19.0 (SPSS Inc., Chicago, IL, USA). The linear model used was as follows:

$$Y_{ijk} = \mu + S_i + P_j + G_k + \beta \times age + e_{ijk}$$

where Y_{ijk} is the phenotypic observations, μ is the overall mean of each trait, S_i is the random effect of a sire, P_j is the fixed effect of the calving place (14 classes), G_k is the fixed effect of the SNP genotype, β is a regression coefficient, age is a covariate for age in days at slaughter, and e_{ijk} is a random residual. Significant differences in the mean value between different genotypes were calculated using Duncan's multiple range test at $p<0.05$, and additive and dominance effects were estimated using the regression procedure in SAS 9.2.

This genetic association model was used for the analysis of haplotypes as well as individual SNPs by replacing the genotype effect with the haplotype effect. For the haplotype analysis, the linkage disequilibrium (LD) between SNP pairs was measured using D' and r^2. The HaploView software package was used in the LD analysis. Haplotypes were constructed using Haploviewer v4.2, and associations between haplotypes and phenotypes in a population of Korean cattle were analyzed. With this software package, the phase probability of each polymorphic site for all

haplotypes was calculated for each individual.

RESULTS

All eight SNPs (g.78 G>A, g.173 C>T, g.184 C>T, g.190 C>A, g.194 C>T, g.277 C>G, g.283 T>G and g.290 T>G) located at the exon suggested by the National Center for Biotechnology Information were collected to identify association between SNPs in the *THRSP* gene and fatty acid composition. The exon 1 region was located at 33-485 bp in the genomic DNA of the 5,907 bp *THRSP* gene. According to a pair-wise LD analysis with the g.78 G>A and g.184 C>T SNPs, the *THRSP* gene could be conducted in the LD block, the 107 bp region spanning from exon 1.

According to a preliminary analysis of the genotype frequency of the eight SNPs in the exon of the *THRSP* gene, there were only two polymorphic loci in Korean cattle. This outcome was statistically analyzed to determine genotypic and allelic frequency, heterozygote, Minor allele frequency (MAF) and HWE. The genetic frequency of two polymorphic SNPs (g.78 G>A and g.184 C>T) were calculated based on Mendel's law of segregation, and the resulting values were 0.361 and 0.481, respectively. The genotype frequency (%) of g.78 G>A was 59.3, 34.0, and 6.7 for GG, GA, and AA, respectively, and that of g.184 C>T was 17.3, 46.0, and 36.7 for CC, CT, and TT, respectively. MAF was less than 0.100 and segregation was not well observed based on the LD analysis, which reduced analysis accuracy (Eberle et al., 2006; Lee et al., 2006). Based on this analysis, these two selected SNPs had values of 0.237 and 0.404 respectively, exceeding 0.100, and their

genotypes did not deviate from the HWE (p>0.05).

Previous studies have demonstrated significant effect of unsaturated fatty acid (UFA) (Alexander et al., 2007) and MUFA (Hoashi et al., 2008) content on the flavor of beef. Based on these studies, the composition of fatty acids and the carcass phenotype were analyzed for a single genotype in Korean cattle (Table 2). This analysis revealed significant differences depending on the SNP of the *THRSP* gene.

Carcass traits and two SNPs (g.78 G>A and g.184 C>T) were not related in this sample of Korean cattle. In addition, stearic acid (C18:0), myristoleic acid (C14:1) and palmitoleic acid (C16:1) were not related to the SNPs. However, for UFAs, cattle with the GG genotype of g.78 G>A and the CC genotype of g.184 C>T had the highest oleic acid content of 44.47% and 45.60% respectively, and MUFA content of 53.23% and 54.46%, respectively, in contrast to the content of SFA (p<0.001).

Based on the LD of the haplotype block, two representative SNPs located within exon 1 in the *THRSP* gene based on Haploviewer v4.2 were determined through a frequency analysis. The haplotype frequency of Ht1 (Valnine [GTC] of g.78 G>A and Alanine [GCG] of the g.184 C>T combination) was 0.404012; that of Ht2 (Valnine [GTC] of g.78 G>A and Valnine [GTG] of the g.184 C>T combination) was 0.359007; and that of Ht3 (Isoleucine [ATC] of g.78 G>A and Valnine [GTG] of the g.184 C>T combination) was 0.236981.

The haplotype block included g.78 G>A and g.184 C>T in exon 1. Haplotypic associations of blocks were found in fatty acid composition (p<0.05, Table 3). In addition, the haplotypes were further associated with marbling score

Table 2. Effects of two polymorphic SNPs in the *THRSP* gene on carcass traits and fatty acids composition

SNP	g.78 G>A						g.184 C>T					
Genotype	GG	GA	AA	p-value	Genetic effect		CC	CT	TT	p-value	Genetic effect	
N	363	208	41		Additive	Dominance	106	282	224		Additive	Dominance
Carcass trait												
CW (kg)	426.95±2.22	427.85±3.29	426.88±6.28	0.971	−0.979±8.061	−3.212±10.343	431.39±3.98	425.16±2.64	427.94±2.98	0.441	6.235±5.476	6.076±7.922
BFT (mm)	13.04±0.25	13.50±0.40	12.71±0.83	0.504	−0.792±0.955	−0.934±1.226	13.11±0.54	13.00±0.31	13.43±0.33	0.649	−0.437±0.649	0.760±0.940
MS	5.51±0.11	5.42±0.15	4.63±0.28	0.053	−0.888±0.359*	−0.710±0.460	5.80±0.22	5.46±0.13	5.21±0.13	0.054	0.586±0.244	0.088±0.353
Fatty acid composition (%)												
C14:0	3.52±0.03[a]	3.62±0.04[a]	3.92±0.11[b]	0.001	0.419±0.120***	0.259±0.154	3.39±0.07[a]	3.55±0.04[b]	3.71±0.04[c]	0.000	−0.321±0.081***	−0.099±0.117
C16:0	25.71±0.11[a]	25.81±0.13[a]	26.98±0.27[b]	0.001	1.276±0.362**	1.042±1.465	25.18±0.21[a]	25.91±0.12[b]	26.04±0.12[b]	0.001	−0.865±0.246**	−0.541±0.356
C18:0	10.34±0.08	10.37±0.09	10.30±0.20	0.941	−0.072±0.059	−0.011±0.033	10.38±0.16	10.43±0.09	10.22±0.09	0.289	0.219±0.176	−0.324±0.254
C14:1	1.21±0.02	1.19±0.03	1.27±0.07	0.459	0.089±0.061	0.128±0.091	1.14±0.03[a]	1.20±0.02[ab]	1.25±0.03[b]	0.049	−0.111±0.048*	−0.036±0.070
C16:1	6.41±0.06	6.42±0.07	6.53±0.18	0.808	0.128±0.091	0.277±0.233	6.39±0.10	6.32±0.07	6.56±0.08	0.067	−0.241±0.123	0.108±0.178
C18:1	44.47±0.14[b]	44.19±0.16[b]	41.87±0.37[a]	0.000	−2.616±0.484***	−1.982±0.621**	45.60±0.29[b]	44.10±0.15[a]	43.66±0.16[a]	0.000	1.946±0.327***	0.859±0.473
C18:2n6	2.93±0.04[a]	3.05±0.05[ab]	3.49±0.12[b]	0.000	0.589±0.139***	0.439±0.179	2.74±0.08[a]	2.99±0.05[b]	3.14±0.05[b]	0.000	−0.458±0.095***	−0.095±0.138
C18:3n3	0.36±0.01[b]	0.34±0.01[b]	0.26±0.03[a]	0.018	−0.097±0.038*	−0.072±0.049	0.41±0.02[b]	0.34±0.01[a]	0.32±0.01[a]	0.001	0.094±0.025***	0.038±0.037
SFA	40.22±0.16[a]	40.60±0.18[a]	41.84±0.40[b]	0.002	1.629±0.528**	0.903±0.678	39.53±0.32[a]	40.61±0.16[b]	40.71±0.18[b]	0.001	−1.184±0.357***	−1.206±0.157
MUFA	53.23±0.17[b]	52.96±0.21[b]	50.72±0.54[a]	0.000	−2.518±0.544***	−1.881±0.699	54.46±0.32[b]	52.70±0.19[a]	52.63±0.21[a]	0.000	1.838±0.369***	1.059±0.534
Fatty acid index												
M/S	1.34±0.01[b]	1.32±0.01[b]	1.24±0.02[a]	0.002	-	-	1.40±0.02[b]	1.32±0.01[a]	1.31±0.01[a]	0.000	-	-
I_{C14}[1]	25.78±0.36	24.74±0.46	25.05±1.41	0.217	-	-	25.88±0.75	25.38±0.36	25.12±0.50	0.647	-	-
I_{C16}[2]	20.55±0.17	20.44±0.20	20.39±0.40	0.894	-	-	20.87±0.29	20.30±0.18	20.70±0.21	0.348	-	-
I_{C18}[3]	80.92±0.15	80.85±0.16	80.24±0.39	0.318	-	-	81.22±0.28	80.68±0.15	80.86±0.11	0.195	-	-

SNPs, single nucleotide polymorphisms; THRSP, thyroid hormone responsive protein; CW, carcass weight; BFT, backfat thickness; SFA, saturated fatty acid; MUFA, mono unsaturated fatty acid; M/S, MUFA/SFA.

[1] $I_{C14} = [C14:1/(C14:0+C14:1)] \times 100$. [2] $I_{C16} [C16:1/(C16:0+C16:1)] \times 100$. [3] $I_{C18} [C18:1/(C18:0+C18:1)] \times 100$.

Means with different superscripts in the same row for each SNP indicate significance at p<0.05, * p<0.05, ** p<0.01, *** p<0.001.

Table 3. Haplotype effects of polymorphic SNPs in the *THRSP* gene on carcass traits and fatty acids composition

Trait	LD Block						p-value
	ht1*ht1 (n = 101)	ht1*ht2 (n = 148)	ht1*ht3 (n = 131)	ht2*ht2 (n = 109)	ht2*ht3 (n = 79)	ht3*ht3 (n = 44)	
Carcass trait							
CW (kg)	432.15±4.23	423.15±3.50	428.04±4.04	427.83±4.12	428.20±5.55	424.34±5.45	0.773
BFT (mm)	12.87±0.53	12.27±0.36	13.82±0.53	13.85±0.45	12.95±0.59	13.75±0.86	0.516
MS	5.99±0.20[c]	5.33±0.15[b]	5.43±0.17[bc]	5.42±0.18[bc]	5.33±0.21[b]	4.18±0.26[a]	0.025
Fatty acid composition (%)							
C14:0	3.32±0.07[a]	3.55±0.05[b]	3.55±0.05[b]	3.67±0.06[b]	3.71±0.07[bc]	3.90±0.11[c]	0.000
C16:0	25.19±0.22[a]	26.16±0.17[bc]	25.63±0.16[ab]	25.68±0.17[ab]	26.15±0.22[bc]	26.63±0.28[c]	0.001
C18:0	10.40±0.16	10.38±0.14	10.52±0.11	10.17±0.14	10.10±0.15	10.47±0.20	0.588
C14:1	1.17±0.03	1.19±0.03	1.20±0.03	1.19±0.04	1.18±0.05	1.23±0.07	0.103
C16:1	6.39±0.11	6.29±0.11	6.33±0.09	6.65±0.11	6.53±0.13	6.44±0.18	0.293
C18:1	45.80±0.28[c]	44.05±0.22[b]	44.12±0.20[b]	44.00±0.22[b]	44.30±0.26[b]	41.59±0.30[a]	0.000
C18:2n6	2.70±0.08[a]	2.93±0.06[ab]	3.09±0.07[b]	3.07±0.07[b]	2.97±0.08[ab]	3.63±0.11[c]	0.000
C18:3n3	0.42±0.02[c]	0.35±0.02[b]	0.33±0.02[b]	0.32±0.02[ab]	0.35±0.02[b]	0.26±0.02[a]	0.001
SFA	39.47±0.34[a]	40.61±0.24[b]	40.66±0.22[b]	40.42±0.25[b]	40.48±0.31[b]	41.71±0.40[c]	0.002
MUFA	54.65±0.33[c]	52.57±0.29[b]	52.79±0.26[b]	53.11±0.27[b]	53.17±0.37[b]	50.40±0.43[a]	0.000
Fatty acid index							
M/S	1.40±0.02[c]	1.32±0.01[b]	1.31±0.01[b]	1.32±0.01[b]	1.33±0.02[b]	1.23±0.02[a]	0.000
I_{C14}	26.06±0.78	25.57±0.56	25.19±0.47	25.93±0.60	23.90±0.98	24.52±1.22	0.316
I_{C16}	20.58±0.29	20.26±0.28	20.35±0.23	20.85±0.30	20.61±0.38	20.37±0.40	0.709
I_{C18}	81.23±0.28	80.64±0.25	80.73±0.19	80.97±0.24	81.07±0.30	80.21±0.38	0.270

SNPs, single nucleotide polymorphisms; THRSP, thyroid hormone responsive protein; LD, linkage disequilibrium; CW, carcass weight; BFT, backfat thickness; MS, marbling score from 1 to 9 such that the larger score, the more abundant the intramuscular fat; SFA, saturated fatty acid; MUFA, mono unsaturated fatty acid; M/S, MUFA/SFA.

[1] $I_{C14} = [C14:1/(C14:0+C14:1)]\times100$. [2] $I_{C16} = [C16:1/(C16:0+C16:1)]\times100$. [3] $I_{C18} [C18:1/(C18:0+C18:1)]\times100$.

Means with different superscripts in the same row for each linkage disequilibrium block indicate significance at p<0.05.

(p<0.025), but not with carcass weight and back fat thickness (p>0.05, Table 3) as shown in the single locus analysis.

DISCUSSION

In this study, the relationships between exonic SNPs of *THRSP* gene, fatty acids, and carcass traits were examined for Korean cattle. As shown in Table 2, the two selected SNPs had significant relationships with oleic acid and MUFAs. In addition, the C187T (A51V) SNP was related to the tenderness and water holding capacity (WHC) of Qinchuan cattle. According to the multi-comparison results, tenderness was significantly higher for the CC genotype than for CT (p<0.05), and the WHC of TT was significantly higher than that of CT (p<0.05) (Zhang et al., 2009). The *THRSP* gene has consistently been found to be highly-correlated with marbling content in breeds widely known to possess an extremely high level of capacity for marbling such as Japanese Black or Wagyu (Wang et al., 2005; Wang et al., 2009).

On the other hand, the C187T SNP associated with increased fat yield as was shown by the T allele of *THRSP* in Italian Brown cattle (Mancini et al., 2013). The inconsistency between these findings and the results of the present study can be explained by the use of different breeds. Also, Saatchi et al. (2013) reported that *THRSP* genotypes of Illumina 54K bovine SNPchip significantly affected the C14:0, C14:1, C16:0, C16:1, C18:0 and cis-9 C18:1 fatty acids in Angus. The SNPs in Saatchi et al. (2013) studied are located in the part of the intron in the *THRSP* gene, which is different from the positions of the two SNPs in the present study, and this may explain the slightly inconsistent results. However, there is some consistency in that each SNP in the *THRSP* gene had a relationship not only with fatty acids but also with the marbling score.

The LD coefficients (r^2) between individual SNPs, which reached the 5% level of genome-wide significance for single-marker associations, was calculated to assess the LD, and haplotype blocks were identified using the so-called "four-gamete rule" (Wang et al., 2002). In the four-gamete rule approach, the population frequency of four possible two-marker haplotypes is computed. If all four are observed with the frequency of at least 0.01, then recombination is deemed to have taken place. Blocks are then formed by consecutive markers where only three gametes are observed. In this study, this analysis was

conducted using Haploviewer v4.2 (Barret et al., 2005).

A powerful LD was observed between g.78 G>A and g.184 C>T. As shown in Table 2, the two SNPs had no effect on the marbling score (p>0.05) based on the single locus analysis. However, according to Table 3, the haplotype had a significantly effect on the marbling score (p<0.05), and the contents were higher than the single locus analysis. The genotype of the ht1*ht1 group (Val/Ala haplotype) had a significantly greater effect on the marbling score than other genotypes. The genotype of the ht3*ht3 group (Iso/Val haplotype) was found to be 4.18, which had the lowest significant effect on the marbling score. No haplotypic effects (p>0.05) were detected on the contents of stearic acid (C18:0), myristoleic acid (C14:1) and palmitoleic acid (C16:1), as were the single SNP genotypes.

The highest oleic acid (C18:1) content was found for the genotype of the ht1*ht1 group (Val/Ala haplotype) (45.80%, p<0.001). In addition, the highest MUFA and linolenic acid (C18:2n3; omega3) content was found for the genotype of the ht1*ht1 group (Val/Ala haplotype) (54.65% and 0.42%, respectively). On the other hand, SFA content was relatively low in the genotype of the ht1*ht1 group (Val/Ala haplotype) and higher in genotype of the ht3*ht3 group (Iso/Val haplotype) (p<0.05).

Table 2 compares the effects of single genotypes. The highest (45.60%) oleic acid (C18:1) content was observed in the CC genotype of g.184 C>T, and the lowest (41.87%) content, in the AA genotype of g.78 G>A. On the other hand, the genotypes of the ht1*ht1 group (Val/Ala haplotype) and ht3*ht3 group (Iso/Val haplotype) groups showed oleic acid (C18:0) content of 45.80% and 41.59%, respectively. In addition, the largest difference in MUFA content was 0.19% between the genotype of the ht1*ht1 group (Val/Ala haplotype) and the CC genotype of g.184 C>T for the single genotype. Further, the smallest difference in MUFA content was 0.32% between the ht3*ht3 group (Iso/Val haplotype) and the AA genotype of g.78 G>A for the single genotype. The results for linolenic acid (C18:2n3; omega-3) content are similar to those for oleic acid (C18:0) and MUFA content. However, linoleic acid (C18:2n6; omega-6) content provided opposite results. These results imply that the strong LD between SNPs can be attributed to relationships between genes that can influence a combination of multiple variations, not a single variation.

Previous studies have found that the *THRSP* gene encodes a protein called Spot14 or S14 (Zhu et al., 2005). S14 is expressed only in lipogenic tissue, and the S14 mRNA levels are strongly correlated the lipogenic rate for each type of tissue (Jump et al., 1985). The SNPs may change their function by producing missense codons. The S14 region is located in the 43 to 495 bp coding sequence of the 1,404 bp *THRSP* gene. In this study, two selected

SNPs were nonsynonymous. The sequence of g.78 G>A, was substituted by A̲TC, which changed the codon for valine to isoleucine. In addition, the SNP g.184 C>T changed the GC̲G codon to GT̲G, which changed the amino acid from valine to alanine. Therefore, the selected SNPs were associated with fatty acid synthesis and composition and located in the *THRSP* gene sequence.

Harvatine and Bauman. (2006) reported that conjugated linoleic acid (omega-6) inhibits S14 gene expression in bovine mammary glands. Trans-10, cis-12 CLA is often referred to as having antiobesity effects because of its ability to reduce body fat accretion in several species (Wang et al., 2004). In addition, clinical studies have shown that long-chain omega-3 PUFAs (n-3 PUFA) have beneficial effects on human health and that high levels of n-6 PUFAs in humans are closely related to certain diseases such as cancer, cardiovascular disease, and various mental disorders (Reidiger et al., 2009). As shown in Table 3, the lowest linoleic acid content and the highest oleic acid and MUFA content in the ht1*ht1 group (Val/Ala haplotype). This indicates that the selected SNPs were associated with fatty acid synthesis and composition because the SNPs led to codon changes in the *THRSP* gene, altering the S14 protein.

The results suggest that an increase in the homozygous genotype with the ht1*ht1 group (Val/Ala haplotype) at g.78 G>A and g.184 C>T may genetically improve the quality of beef by increasing UFA content and intramuscular marbling scores. The simultaneous use of multiple markers may accelerate genetic improvements through additive and/or epistatic effects, as demonstrated in Ryu et al. (2012).

In conclusion, Korean cattle with the individual genotype of GG or CC at the exonic SNP of g.78 G>A and g.184 C>T in the *THRSP* gene showed high MUFA content and low SFA content. In addition, this genotype showed higher marbling scores. These individual SNPs and their haplotypes may be important genetic targets for improving beef quality, which should benefit the Korean cattle industry.

REFERENCES

Alexander, L. J., M. D. MacNeil, T. W. Geary, W. M. Snelling, D. C. Rule, and J. A. Scanga. 2007. Quantitative trait loci with additive effects on palatability and fatty acid composition of meat in a Wagyu-Limousin F_2 population. Anim. Genet. 38:506-513.

Barret, J. C., B. Fry, J. Maller, and M. J. Daly. 2005. Haploview: Anaylsis and visualization of LD and haplotype maps. Bioinformatics 21:263-265.

Cabezas, M. T., J. F. Hentges, J. E. Moore, and J. A. Olson. 1965. Effect of diet on fatty acid composition of body fat in steers. J. Anim. Sci. 24:57-61.

Colbert, C. L., C. W. Kim, Y. A. Moon, L. Henry, M. Palnitkar, W. B. McKean, K. Fitzgerald, J. Deisenhofer, J. D. Horton, and H. J. Kwon. 2010. Crystal structure of Spot 14, a modulator of fatty acid synthesis. Proc. Natl. Acad. Sci. USA 107:18820-18825.

Chung, K. Y., D. K. Lunt, C. B. Choi, S. H. Chae, R. D. Rhoades, T. H. Adams, B. Booren and S. M. Smith. 2006. Lipid characteristics of subcutaneous adipose tissue and *M. longissimus thoracis* of Angus and Wagyu steers fed to US and Japanese endpoints. Meat Sci 73:432-441.

Cunningham, B. A., J. T. Moncur, J. T. Huntington, and W. B. Kinlaw. 1998. "Spot 14" protein: A metabolic integrator in normal and neoplastic cells. Thyroid 8:815-825.

Dryden, F. D and J. A. Marchello. 1970. Influence of total lipid and fatty acid composition upon the palatability of three bovine muscles. J. Anim. Sci. 31:36-41.

Eberle, M. A., M. J. Rieder, L. Kruglyak, and D. A. Nickerson. 2006. Allele frequency matching between SNPs reveals an excess of linkage disequilibrium in genic regions of the human genome. PLoS Genetics 9:1319-1327.

Edwards, R., S. B. Tove, T. N. Blumer, and E. R. Barrick. 1961. Effects of added dietary fat on fatty acid composition and carcass characteristics of fatting steers. J. Anim. Sci. 20:712-717.

Folch, J., M. Lee, and G. H. S. Stanley. 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226:487-509.

Freake, H. C. and J. H. Oppenheimer. 1987. Stimulation of S14 mRNA and lipogenesis in brown fat by hypothyroidism, cold exposure, and cafeteria feeding: evidence supporting a general role for S14 in lipogenesis and lipogenesis in the maintenance of thermogenesis. Proc. Natl. Acad. Sci. USA 84:3070-3074.

Harvatine, K. J. and D. E. Bauman. 2006. SREBP1 and thyroid hormone responsive spot 14 (S14) are involved in the regulation of bovine mammary lipid synthesis during diet-induced milk fat depression and treatment with CLA. J. Nutr. 136:2468-2474.

Hoashi, S., T. Hinenoya, A. Tanaka, H. Ohsaki, H. Sasazaki, M. Taniguchi, K. Oyama, F. Mukai, and H. Mannen. 2008. Association between fatty acid compositions and genotypes of *FABP4* and *LXR-alpha* in Japanese Black cattle. BMC Genet. 9:84.

Jump, D. B. and J. H. Oppenheimer. 1985. High basal expression and 3,5,3'-triiodothyronine regulation of messenger ribonucleic acid S14 in lipogenic tissues. Endocrinology 117:2259-2266.

Kim, J. H., C. H. Kim, and Y. D. Ko. 2002. Influence of dietary addition of dried wormwood (*Artemisia* sp.) of the performance and carcass characteristics of Hanwoo steers and the nutrient digestibility of sheep. Asian Australas. J. Anim. Sci. 15:390-395.

LaFave, L. T., L. B. Augustin, and C. N. Mariash. 2006. S14: Insights from knockout mice. Endocrinology 147:4044-4047.

Lee, S. H., D. H. Yoon, S. H. Hwang, E. Y. Cheong, O. H. Kim, and C. S. Lee. 2004. Relationship between monounsaturated fatty acid composition and stearoyl-CoA desaturase mRNA level in Hanwoo liver and loin muscle. J. Anim. Sci. Technol. (Kor) 46:7-14.

Lee, M. A., O. M. Keane, B. C. Glass, T. R. Manley, N. G. Cullen, K. G. Dodds, A. F. McCulloch, C. A. Morris, M. Schreiber, J. Warren, A. Zadissa, T. Wilson, and J. C. McEwan. 2006. Establishment of a pipeline to analyse non-synonymous SNPs in *Bos Taurus*. BMC Genom. 7:298.

Mancini, G., E. L. Nicolazzi, A. Valentini, G. Chillemi, P. A. Marsan, E. Santus, and L. Pariset. 2013. Association between single nucleotide polymorphisms (SNPs) and milk production traits in Italian Brown cattle. Livest. Sci. 157:93-99.

Melton, S. L., M. Amiri, G. W. Davis, and W. R. Backus. 1982. Flavor and chemical characteristics of ground beef from grass-, forage-grain- and grain-finished steers. J. Anim. Sci. 55:77-87.

Narukami, T., S. Sasazaki, K. Oyama, T. Nogi, M. Taniguchi, and H. Mannen. 2011. Effect of DNA polymorphism related to fatty acid composition in adipose tissue of Holstein cattle. Anim. Sci. J. 82:406-411.

O'Keefe, P. W., G. H. Wellington, L. R. Mattick, and J. R. Stouffer. 1968. Composition of bovine muscle lipids at various carcass locations. J. Food Sci. 33:188-192.

Perez-Castillo, A., H. L. Schwartz, and J. H. Oppenheimer. 1987. Rat hepatic mRNA-S14 and lipogenic enzymes during weaning: role of S14 in lipogenesis. Am. J. Physiol. Endocrinol. Metab. 253:E536-E542.

Reidiger, N. D., R. A. Othman, M. Suh, and M. H. Moghadasian. 2009. A systemic review of the roles of n-3 fatty acids in health and disease. J. Am. Diet. Assoc. 109:668-679.

Rudel, L. L., J. S. Park, and J. K. Sawyer. 1995. Compared with dietary monounsaturated and saturated fat, polyunsaturated fat protects African green monkeys from coronary artery atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 15:2101-2110.

Ryu, J., Y. Kim, C. Kim, J. Kim, and C. Lee. 2012. Association of bovine carcass phenotypes with genes in an adaptive thermogenesis pathway. Mol. Biol. Rep. 39:1441-1445.

Saatchi, M., D. J. Garrick, R. G. Tait Jr, M. S. Mayes, M. Drewnoski, J. Schoonmaker, C. Diaz, D. C. Beitz, and J. M. Reecy. 2013. Genome-wide association and prediction of direct genomic breeding values for composition of fatty acids in Angus beef cattle. BMC Genom. 14:730.

Smith, S. B., A. Yang, T. W. Larsen, and R. K. Tume. 1998. Positional analysis of triacylglycerols from bovine adipose tissue lipids varying in degree of unsaturation. Lipids 33:197-207.

Smith, S. B., C. A. Gill, D. K. Lunt, and M. A. Brooks. 2009. Regulation of fat and fatty acid composition in beef cattle. Asian Australas. J. Anim. Sci. 22:1225-1233.

Wang, N., J. M. Akey, K. Zhang, R. Chakraborty, and L. Jin. 2002. Distribution of recombination crossovers and the origin of haplotype blocks: The interplay of population history, recombination, and mutation. Am. J. Hum. Genet. 71:1227-1234.

Wang, Y. H., K. A. Byrne, A. Reverter, G. S. Harper, M. Taniguchi, S. M. McWilliam, H. Mannen, K. Oyama, and S. A. Lehnert. 2005. Transcriptional profiling of skeletal muscle tissue from two breeds of cattle. Mamm. Genome 16:201-210.

Wang, Y. H., N. I. Bower, A. Reverter, S. H. Tan, N. De Jager, R. Wang, S. M. McWilliam, L. M. Cafe, P. L. Greenwood, and S.

A. Lehnert. 2009. Gene expression patterns during intra-muscular fat development in cattle. J. Anim. Sci. 87:119-130.

Wang, Y. W. and P. J. Jones. 2004. Conjugated linoleic acid and obesity control: Efficacy and mechanisms. Int. J. Oves. Relat. Metab. Disord. 28:941-955.

Westerling, D. B. and H. B. Hedrick. 1979. Fatty acid composition of bovine lipids as influenced by diet, sex and anatomical location and relationship to sensory characteristics. J. Anim. Sci. 48:1343-1348.

Wood, J. D., R. I. Richardson, G. R. Nute, A. V. Fisher, M. M. Campo, E. Kasapidou, P. R. Sheard, and M. Enser. 2004. Effects of fatty acids on meat quality: A review. Meat Sci. 66: 21-32.

Yang, A., T. W. Larsen, V. H. Powell, and R. K. Tume. 1999. A comparison of fat composition of Japanese and long-termgrain-fed Australian steers. Meat Sci. 51:1-9.

Zhang, X. B., L. Zan, H. Wang, R. Hao, and Y. Yang. 2009. Correlation of C184T mutation in *THRSP* gene with meat traits in the Qinchuan cattle. Scientia Agricultura Sinica 42:4058-4063.

Zembayashi, M., K. Nishimura, D. K. Lunt, and S. B. Smith. 1995. Effect of breed type and sex on the fatty acid composition of subcutaneous and intramuscular lipids of finishing steers and heifers. J. Anim. Sci. 73:3325-3332.

Zhu, Q., G. W. Anderson, G. T. Mucha, E. J. Parks, J. K. Metkowski, and C. N. Mariash. 2005. The spot 14 protein is required for *de novo* lipid synthesis in the lactating mammary gland. Endocrinology 146:3343-3350.