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DNA Polymorphisms in *SREBF1* and *FASN* Genes Affect Fatty Acid Composition in Korean Cattle (Hanwoo)

M. S. A. Bhuiyan, S. L. Yu, J. T. Jeon[1], D. Yoon[2], Y. M. Cho[2], E. W. Park[3]
N. K. Kim[2], K. S. Kim[4] and J. H. Lee*
Division of Animal Science and Resources, College of Agriculture and Life Sciences,
Chungnam National University, Daejeon 305-764, Korea

ABSTRACT : Sterol regulatory element binding factor 1 (*SREBF1*) and fatty acid synthase (*FASN*) genes play an important role in the biosynthesis of fatty acids and cholesterol, and in lipid metabolism. This study used polymorphisms in the intron 5 of bovine *SREBF1* and in the thioesterase (TE) domain of *FASN* genes to evaluate their associations with beef fatty acid composition. A previously identified 84-bp indel (L: insertion/long type and S: deletion/short type) of the *SREBF1* gene in Korean cattle had significant associations with the concentration of stearic (C18:0), linoleic (C18:2) and polyunsaturated fatty acids (PUFA). The stearic acid concentration was 6.30% lower in the SS than the LL genotype ($p<0.05$), but the linoleic and PUFA contents were 11.06% and 12.20% higher in SS compared to LL ($p<0.05$). Based on the sequence analysis, five single nucleotide polymorphisms (SNPs) g.17924G>A, g.18043C>T, g.18440G>A, g.18529G>A and g.18663C>T in the TE domain of the *FASN* gene were identified among the different cattle breeds studied. Among these, only g.17924 G>A and g.18663C>T SNPs were segregating in the Hanwoo population. The g.17924G>A SNP is a non-synonymous mutation (thr2264ala) and was significantly associated with the contents of palmitic (C16:0) and oleic acid (C18:1). The oleic acid concentration was 3.18% and 2.79% higher in Hanwoo with the GG genotype than the AA and AG genotypes, respectively ($p<0.05$), whereas the GG genotype had 3.8% and 4.01% lower palmitic acid than in those cattle with genotype AA and AG, respectively ($p<0.05$). Tissue expression data showed that *SREBFI* and *FASN* genes were expressed in a variety of tissues though they were expressed preferentially in different muscle tissues. In conclusion, the 84-bp indel of *SREBF1* and g.17924G>A SNP of the *FASN* gene can be used as DNA markers to select Hanwoo breeding stock for fatty acid composition. (**Key Words :** *SREBF1*, *FASN*, Fatty Acid, Polymorphism, Gene Expression, Hanwoo)

INTRODUCTION

In the beef industry, the amount and distribution of fat within muscle are directly associated with quality and value of the meat. Fatty acid composition of beef is of great interest because of its implications for human health. Saturated fatty acids (SFA) are the main fatty acids found in meat and about 80% of the fatty acid in beef is composed of palmitic, stearic and oleic acid and the remaining 20% is distributed among 30 different fatty acids (Whetsell et al., 2003). Among the SFA, lauric, myristic and palmitic acid are considered to have the most harmful cardiovascular effects (Mozaffarian et al., 2005), whereas stearic acid is not detrimental to human health (Bonanome and Grundy, 1988). On the contrary, monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids increase hepatic low density lipoprotein (LDL) receptor activity, thereby decreasing the circulating concentration of LDL-cholesterol (Woollett et al., 1992). In addition to marbling, the fatty acid composition is the main factor defining meat quality parameters such as texture and taste and can be improved by an increased ratio of MUFA to SFA (Yang et al., 1999).

During the past few decades, advances in molecular genetics have lead to the identification of genes or markers affecting meat quality traits. DNA polymorphisms in some

* Corresponding Author: J. H. Lee. Tel: +82-42-821-5779, Fax: +82-42-825-9754, E-mail: junheon@cnu.ac.kr
[1] Division of Applied Life Science, Gyeongsang National University, Jinju, 660-701, Korea.
[2] Division of Animal Genomics and Bioinformatics, National Institute of Animal Science, Suwon 441-706, Korea.
[3] Division of Animal Biotechnology, National Institute of Animal Science, Suwon 441-706, Korea.
[4] Department of Animal Science, Chungbuk National University, Cheongju 361-763, Korea.
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candidate genes have been reported for their association with beef quality traits. Polymorphisms in μ-calpain, lysyl oxidase and calpastatin genes are associated with beef tenderness trait (Casas et al., 2006). The genes encoding leptin (Shin and Chung, 2007a); *DGAT1* (Thaller et al., 2003); *TG* (Shin and Chung, 2007b) and *GH1* (Barendse et al., 2006) have been associated with beef marbling. Recent studies have reported associations between fatty acid composition of beef and polymorphisms in candidate genes like *SREBF1* (Hoashi et al., 2007) and *FASN* (Zhang et al., 2008).

SREBF1 is a member of the basic helix-loop-helix-leucine zipper family of transcription factors that is involved in adipocyte differentiation, biosynthesis of cholesterol and fatty acids (Brown and Goldstein, 1997) as well as playing an essential role in energy homeostasis. *SREBF1* is predominantly expressed in adipose tissue and liver (Kim and Spiegelman, 1996). Its expression has been observed in human and mouse muscles (Shimomura et al., 1997) and in a wide variety of chicken tissues (Assaf et al., 2003). *FASN* is a multifunctional enzyme complex that regulates *de novo* biosynthesis of long chain fatty acids (Roy et al., 2001). Bovine *FASN* gene has been mapped on BTA19 where several QTL affecting beef fatty acid composition (Zhang et al., 2008), adipose fat and milk fat content (Roy et al., 2006; Morris et al., 2007) were found. Whole genome shotgun sequence from NCBI shows that *SREBF1* is also located on the same chromosome (http://www.ncbi.nlm.nih.gov/projects/mapview). The four exons (39 to 42) in the *FASN* complex that encode for the TE domain are responsible for fatty acid synthesis, mainly C16:0, by hydrolyzing the acyl-S-phosphopantetheine thioester. Therefore, the TE domain determines the product chain length of *FASN* and variation in the TE domain among individuals would be a candidate for heritable differences in fatty acid composition (Zhang et al., 2008). Bovine *FASN* expression was higher in brain, testis and adipose tissue than in liver and heart (Roy et al., 2005). Knockout or transgenic mice experiments demonstrated that *SREBP* transcription factors play key roles in the regulation of *FASN* transcription (Shimano et al., 1999).

An 84-bp indel in intron 5 of the *SREBF1* gene had significant association with MUFA content in Japanese Black cattle (Hoashi et al., 2007) and polymorphisms in the TE domain of the *FASN* gene affect fatty acid composition in Angus cattle (Zhang et al., 2008). Kim et al. (2002) reported that SFA (C16:0 and C18:0) and MUFA (C16:1 and C18:1) comprised nearly 90% of Hanwoo muscle lipid and C18:1 concentration was nearly 90% of the total MUFA. However, to date, no information on *SREBF1* and *FASN* gene polymorphisms in Hanwoo and their corresponding effects on fatty acid composition are available. Therefore, the objectives of this study were to investigate mRNA expression profiles of *SREBF1* and *FASN* genes and to evaluate SNPs within *SREBF1* and *FASN* genes for their association with fatty acid composition in Hanwoo.

MATERIALS AND METHODS

Sampling and phenotypic data

The genomic DNA and meat samples were collected from the National Institute of Animal Science (NIAS), Suwon, Korea. DNA samples were obtained from 90 Hanwoo individuals having fatty acid measurement data and these animals were reared under a progeny testing program at NIAS. They were the half-sib progenies of 22 sires and the number of progeny varied from 2 to 7 per sire. Their feeding condition, concentration and forage intake and fattening period were controlled. Genomic DNA samples of Limousine, Angus, Simmental, Brahman and Red Chittagong (Bangladeshi native) cattle were obtained from the University of Adelaide, Australia and Bangladesh Agricultural University, Bangladesh and used to determine allele frequencies of *SREBF1* and *FASN* gene polymorphisms. For cDNA synthesis, three different types of skeletal muscles; longissimus muscle (LM), round muscle (RM) and lean meat of short rib (SRM) and four organ tissue (heart, spleen, liver and lung) samples were also collected within 30 min of slaughter. The samples were then cut into small pieces and immediately transferred into liquid nitrogen and stored at -70°C until RNA preparation. Fatty acid components were measured from muscle tissues by a direct trans-esterification method according to Lepage and Roy (1986) using a gas chromatograph (GC; Hewlett Packard, model 5972).

SNP discovery and genotyping

Primers were constructed based on the sequences obtained from GenBank using Primer 3 software (Rozen and Skaletsky, 2000) to detect the 84 bp indel in intron 5 of *SREBF1* (NC_007317) and TE domain sequence of *FASN* (AF285607) gene. The oligonucleotide sequences, annealing temperatures and PCR product sizes are presented in Table 1. Eighteen animals from five different cattle breeds were used for initial polymorphism screening in the *FASN* gene. The PCR amplification was performed using a GeneAmp 2700 (Applied Biosystems, USA) thermal cycler in a 25 μl reaction volume containing 50 ng of genomic DNA, 1×PCR gold buffer (50 mM KCl, 10 mM Tris-HCl, pH 8.3), 1.5 mM $MgCl_2$, 200 μM dNTPs, 0.4 pmol of each primer and 1 U *Taq* polymerase (Ampli Tag Gold™, Applied Biosystems, USA). The thermal cycling conditions were as follows: 94°C for 10 min for initial denaturation, followed by 35 cycles of 30 sec at 94°C, 30 sec at 59-60°C, 30 sec at 72°C and a final extension step at

Table 1. Primer sequences, annealing temperatures and sizes of PCR products for *SERBF1* and *FASN* genes

Gene	Primer sequences (5′ to 3′)	Annealing temp (°C)	Product size (bp)
SREBF1	Forward: AACGCTACCGCTCTTCCATC	60	760
	Reverse: TCTGGTTGCTGTGCTGAAGG		
	Forward$_{int5}$: AACGCTACCGCTCTTCCATC	60	447
	Reverse $_{int5}$: GCCTCTGTTCACACATCGCA		
FASN	Forward: TCTTCACAGAGCTGACGGAC	60	624
	Reverse: GGAGGAAGAGCTGTTGCAGT		
	Forward: CTTCGTGATGGCCTACACTC	59	558
	Reverse: TGTACAGACACCTTGCCGT		
	Forward: CGCTCACTGTCCTGTCCTAC	60	373
	Reverse: GCTGTGAATAATACTAAGGATGGA		

72°C for 10 min. For the *SREBF1* gene, PCR products were cloned using the pGEM®-T easy vector systems (Promega, USA). Recombinant plasmid DNAs were purified using a QIAprep miniprep (Qiagen, USA). For the *FASN* gene, PCR products were purified using an Accuprep® PCR purification kit (Bioneer, Korea) and sequencing was performed on a 3100 automated DNA sequencer (Applied Biosystems, USA). The SNPs were discovered by comparing sequences of 18 individuals using Chromas ver. 2.01 (www.technelysium.com.au); ClustalW (http://www.ebi.ac.uk/tools/clustalw); MEGA ver. 4.0 (Tamura et al., 2007) and GENSCAN software (Burge and Karlin, 1997). The SNP genotyping was carried out in a total reaction volume of 20 μl using PCR-RFLP technique. The resulting fragments were analyzed in 3% agarose gel with ethidium bromide in 1×TBE buffer. An additional primer pair was designed for genotyping of the indel in intron 5 (Table 1).

Total RNA extraction and complementary DNA (cDNA) synthesis

A 250 mg sample of each tissue was ground in liquid nitrogen and total RNA was extracted from LM, RM, SRM, heart, liver, spleen and lung using RNeasy fibrous tissue and RNeasy midi kit (Qiagen, CA, USA). Following RNA isolation, the concentration and purity of the extracted RNA were quantified by spectrophotometer (Ultrospec 3100 ^{pro}, Biochrom Ltd., England) at 260/280 nm. Single-stranded cDNA was synthesized from 1 μg of total RNA by incubation at 42°C for 1 h using a reverse transcription system (Promega, USA). The reaction was performed by PTC-200 programmable thermocycler (MJ research, USA) in a 20 μl mixture consisting of 15 U avian myeloblastosis virus (AMV) reverse transcriptase, 20 U of RNasin ribonuclease inhibitor, 25 ng random primers, 5 mM $MgCl_2$, 1 mM deoxynucleoside triphosphate (dNTP) mix and 1X reverse transcription buffer (10 mM Tris-HCl at pH 8.8), 50 mM KCl and 0.1% Triton® X-100). The reaction was terminated by heating at 95°C for 5 min and rapidly cooling on ice. The reaction mixture was diluted 10 times and 2 μl of the diluted cDNA sample was used as a template for the mRNA expression experiment.

mRNA expression analysis by semi-quantitative real time PCR

Amplification of *SREBF1* and *FASN* gene cDNA was carried out by semi-quantitative real time PCR (Bioneer corp., Korea) for mRNA expression analysis. The PCR mixture contained 0.5 U HS Prime Taq DNA polymerase, 2.5 mM $MgCl_2$, 1.0 mM dNTPs, 1× SYBR green fluorescent dye, 1× buffer (10 mM Tris-HCl; pH 8.0, 50 mM KCl, 0.25 mM EDTA, 0.05 mM DTT, 0.25% Tween 20, 0.25% Nonidet P-40 and 25% glycerol) and 1.0 pmol of each primer in a 20 μl reaction volume. The amplification parameters consisted of initial denaturation at 94°C for 5 min, followed by 40 cycles at 94°C for 30 sec, 58-60°C for 30 sec and 72°C for 30 sec and a final extension at 72°C for 1 min. Each reaction was carried out in triplicate and each expression analysis was also performed in two independent experiments. For normalization of expression data, β-actin gene was used as an internal control. The normalized target gene expression level for each tissue was expressed in

Table 2. Oligonucleotide sequence information and PCR conditions for quantitative real time PCR

Gene	Nucleotide sequences (5′ to 3′)	Amplicon size (bp)	Annealing temp (°C)
SREBF1	Forward: ACCACACCAGCTGACAGCTC	209	58
	Reverse: ATTCTGTGCCAGACGCCTC		
FASN	Forward: GCTGGCGGCATCTACATC	190	60
	Reverse: TGGTCTGCAGTGCCTGTG		
β-actin	Forward: CACGGCATCGTCACCAACTG	226	58
	Reverse: ACCGGAGTCCATCACGATGC		

Table 3. Polymorphisms detected in *SREBF1* and *FASN* genes from five cattle breeds

Gene	GenBank accession no.	SNP position[a]	Location	Amino acid change	Restriction enzyme
SREBF1	NC_007317	84 bp indel	Intron 5	Not applicable	None
FASN	AF285607	g.17924G>A	Exon 39	Missense (Thr>Ala)	*Msc*I
		g.18043C>T	Intron 39	Not applicable	*Nla*III
		g.18440G>A	Exon 41	Missense (Glu>Lys)	*Hpy188*III
		g.18529G>A	Intron 41	Not applicable	None
		g.18663C>T	Exon 42	Synonymous	None

[a] Represent position of each SNP relative to the published cattle sequence as it appears in GenBank.

comparison to heart, chosen to be the reference tissue. The relative gene expression level was calculated by the ΔΔCt method (Livak and Schmittgen, 2001) using exicycler 3 software (Bioneer, Korea).

Statistical analysis

The genotype and allele frequencies were calculated according to Falconer and Mackay (1996). A Chi-square test was performed to test the Hardy-Weinberg equilibrium (HWE) at each locus by comparing expected and observed genotype frequencies (Falconer and Mackay, 1996). Associations between individual SNP and fatty acid measurements were evaluated using the mixed model of SAS for windows 9.1.3. The linear model used was as follows:

$$Y_{ijk} = \mu + YS_i + G_j + bD_{ijk} + e_{ijk}$$

Where Y_{ijk} is the observation of the fatty acid measurement traits; μ is the overall mean, YS_i is the effect of i_{th} year and season of calving, G_j is the fixed effect of j_{th} genotype, b is the regression co-efficient for slaughter age in days, D is the slaughter age in days and e_{ijk} is the random residual effect. In addition, sire is considered as a random effect.

RESULTS

Identification of sequence polymorphisms and genotyping

In the current study, *SREBF1* and *FASN* genes were chosen based on their biological functions to evaluate association between polymorphisms and beef fatty acid composition. We identified one 84-bp indel and five SNPs in the *SREBF1* and *FASN* genes, respectively. The details of all polymorphisms, mutation type and corresponding restriction enzymes are shown in Table 3. The 84-bp length polymorphism in intron 5 of the *SREBF1* gene was detected directly from amplified products. Genotyping was carried out based on the different length of PCR products (Figure 1) and detected as S (deletion/short type, 363 bp) and L (insertion/long type, 447 bp) types. The genotype frequencies were 0.52 (LL type), 0.40 (LS type) and 0.08 (SS type), and estimated allele frequencies were 0.72 and 0.28 for L and S allele, respectively. We also investigated a total of 188 cattle from 5 different breeds to determine minor allele frequency and the estimated S allele frequencies were 0.22, 0.00, 0.00, 0.05 and 0.01 for Limousine (n = 36), Angus (n = 34), Simmental (n = 49), Brahman (n = 20) and Red Chittagong cattle (n = 49), respectively (data not shown).

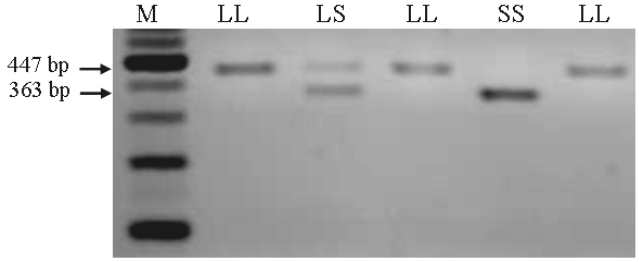

Figure 1. Genotyping of *SREBF1* 84-bp indel polymorphism in intron 5. The arrows show the DNA fragment size (bp) for L and S genotypes which were fractionated by 2% agarose gel.

Three sets of primers were used to amplify the TE domain of the *FASN* gene and the sequencing results revealed five nucleotide substitutions in different cattle breeds (Table 3); three in coding exons (g.17924 G>A, g.18440G>A and g.18663C>T) and two in introns (g.18043C>T and g.18529G>A). Among the identified SNPs, g.17924G>A and g.18440G>A were non-synonymous type which replaced amino acid from alanine (GCC) to threonine (ACC), and glutamic acid (GAG) to lysine (AAG), respectively. In our study, a *Bos indicus* specific novel mutation 18440G>A was identified and was found only in Brahman and Red Chittagong cattle (Figure 2B). The SNP was genotyped using restriction enzyme *Hpy*188III, with A allele resulting in 247/222,175 and 85, 47 bp bands of the 554 bp amplicon and the G allele resulting in 247, 222 and 85 bp bands. The mutant A allele frequency was 0.93 (20) and 0.84 (46), respectively, in Brahman and Red Chittagong cattle, whereas this allele frequency was 0 in 112 *Bos taurus* individuals genotyped from Hanwoo, Angus, Hereford, Simmental and Shorthorn breeds. This missense mutation might play a role for beef fatty acid content differentiation between zebu and taurine cattle, and could be used as a marker with further investigation for breed discrimination. However, we

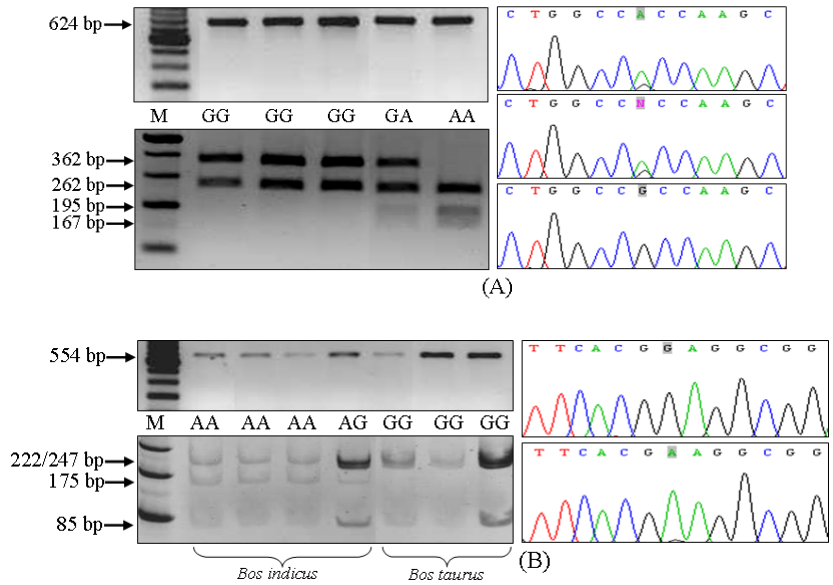

Figure 2. Genotyping of the g.17924G>A (A) and g.18440G>A (B) polymorphisms in the *FASN* gene. SNP positions are shown as shaded letters in chromatograms. The arrowheads show the size of DNA fragment (bp) and M is the 100 bp size marker.

identified only g.17924 G>A and g.18663C>T polymorphisms in the TE domain of Hanwoo cattle, whereas the later polymorphism had no restriction site. The genotypes of g.17924G>A SNP were determined by the PCR-RFLP method using *Msc*I restriction enzyme (Figure 2A). The G allele represented two fragments of 362 and 262 bp, while the A allele showed three fragments of 262, 195 and 167 bp. The frequencies of GG, GA and AA genotypes were 0.73, 0.22 and 0.04, respectively. The G and A allele frequencies were 0.84 and 0.16, respectively (Table 4). The genotype frequencies were in agreement with HWE for each polymorphism (p>0.05).

Table 4. Genotype and allele frequencies of *SREBF1* and *FASN* genes in Hanwoo

Genes	SNP/indel	*N*	Genotype frequency			Allele frequency		x^2 test[a]
SREBF1	84 bp indel	88	LL	LS	SS	L	S	p>0.05
			0.52 (46)	0.40 (35)	0.08 (07)	0.72	0.28	
FASN	g.17924G>A	90	GG	GA	AA	G	A	p>0.05
			0.73 (66)	0.22 (20)	0.04 (04)	0.84	0.16	

[a] Hardy-Weinberg equilibrium test for each locus according to Falconer and Mackay (1996).
N denotes total number of animals investigated and values in the parentheses indicate the number of observation in each genotype.

Table 5. Effect of 84-bp indel in intron 5 of *SREBF1* gene on fatty acid composition in Hanwoo[1]

Traits	84 bp indel			p-value
	LL (n = 46)	LS (n = 35)	SS (n = 7)	
Myristic acid (C14:0)	2.89±0.11	3.12±0.12	2.90±0.28	0.36
Palmitic acid (C16:0)	28.55±0.26	28.04±0.30	28.07±0.68	0.42
Stearic acid (C18:0)	10.95^{a}±0.16	10.33^{b}±0.18	10.26^{b}±0.42	0.03
Oleic acid (C18:1)	49.21±0.33	49.79±0.38	50.07±0.88	0.42
Linoleic acid (C18:2)	2.09^{b}±0.07	2.33^{a}±0.08	2.35^{a}±0.18	0.04
γ-linoleic acid (C18:3)	0.11±0.02	0.06±0.02	0.12±0.04	0.08
SFA[2]	42.39±0.33	41.49±0.38	41.24±0.86	0.15
UFA[3]	57.60±0.33	58.50±0.38	59.75±0.87	0.15
MUFA[4]	55.09±0.34	55.72±0.38	55.88±0.87	0.39
PUFA[5]	2.52^{b}±0.07	2.78^{ab}±0.08	2.87^{a}±0.19	0.04

[1] Values are expressed as LSM±SE, fatty acid contents are expressed as g/100 g of total fatty acids.
[2] Total saturated fatty acids, [3]Total unsaturated fatty acids, [4]Total monounsaturated fatty acids and [5]Total polyunsaturated fatty acids.
[a, b] Least square means within a row without a common superscript differ significantly (p<0.05).

Table 6. Effect of g.17924A>G mutation of *FASN* gene on beef fatty acid composition in Hanwoo[1]

Traits	g.17924A>G			p-value
	GG (n = 65)	GA (n = 19)	AA (n = 4)	
Myristic acid (C14:0)	3.02±0.09	2.83±0.18	3.05±0.38	0.63
Palmitic acid (C16:0)	27.99[b]±0.22	29.16[a]±0.41	29.10[a]±0.89	0.03
Stearic acid (C18:0)	10.58±0.14	10.86±0.25	10.72±0.55	0.62
Oleic acid (C18:1)	49.90[a]±0.28	48.51[b]±0.52	48.31[b]±1.12	0.04
Linoleic acid (C18:2)	2.23±0.06	2.16±0.11	2.08±0.24	0.73
γ-linoleic acid (C18:3)	0.08±0.01	0.12±0.03	0.16±0.05	0.21
SFA[2]	41.60±0.28	42.85±0.52	42.86±1.14	0.08
UFA[3]	58.40±0.28	57.14±0.52	57.14±1.14	0.08
MUFA[4]	55.72±0.28	54.55±0.51	54.69±1.11	0.11
PUFA[5]	2.68±0.07	2.59±0.12	2.45±0.27	0.62

[1] Values are expressed as LSM±SE, Fatty acid contents are expressed as g/100 g of total fatty acids.
[2] Total saturated fatty acids, [3] Total unsaturated fatty acids, [4] Total monounsaturated fatty acids and [5] Total polyunsaturated fatty acids.
[a, b] Least square means within a row without a common superscript differ significantly (p<0.05).

Association analysis with fatty acid composition

Among the identified polymorphisms, the 84 bp length polymorphisms in *SREBF1* and g.17924G>A SNP in the *FASN* gene were investigated for association with beef fatty acid composition. The fatty acid concentration profiles for *SREBF1* and *FASN* genes are shown in Table 5 and 6, respectively. The *SREBF1* genotypes were associated with the concentration of stearic (C18:0), linoleic (C18:2) and PUFA in Hanwoo (Table 5). The C18:2 and PUFA contents were 11.06% and 12.20% higher in the SS genotype as compared to LL (p<0.05), while the concentration of C18:0 was 6.30% lower in SS than LL genotype (p<0.05). Moreover, no significant differences were observed between SS and LS genotypes for C18:0, C18:2 and PUFA contents. The γ-linoleic acid content also tended to be greater (p = 0.08) in the SS genotype than in LL and LS genotypes. However, no significant association was found between the *SREBF1* genotypes and total SFA, UFA and MUFA content. In the *FASN* gene, significant association was observed between g.17924G>A SNP genotypes and the concentrations of palmitic (C16:0) and oleic acid (C18:1) (Table 6). The C18:1 concentration was 3.18% and 2.79% higher in Hanwoo with GG genotype than in AA and AG genotypes, respectively (p<0.05). In addition, cattle with genotype GG had 3.81% and 4.01% lower C16:0 than in those with genotype AA and AG, respectively (p<0.05). Total UFA tended to be greater (p = 0.08) and a lower trend for SFA concentrations was also observed in the GG genotype than in AG and AA genotypes (p = 0.08). However, no significant association was observed between g.17924A>G genotypes and other considered fatty acid concentrations.

Expression analysis of *SREBF1* and *FASN* genes

The mRNA expression profiles of bovine *SREBF1* and *FASN* genes were investigated using semi-quantitative real time PCR (qPCR) from LM, RM, SRM, heart, liver, spleen and lung samples. The oligonucleotides used in qPCR are shown in Table 2. Results of expression patterns in seven different tissues revealed that *SREBF1* and *FASN* genes were ubiquitously expressed. *SREBF1* gene was highly expressed in different muscle tissues, while moderate expression was observed in heart and liver, and lower expression in spleen and lung (Figure 3A). For the *FASN* gene, the highest mRNA expression was found in longissimus muscle, with lower expression in round muscle, short rib muscle, heart, liver and lung, and expression was lowest in spleen (Figure 3B).

DISCUSSION

SREBF1 belongs to the family of basic helix-loop-helix-leucine zipper transcription factors that bind to the sterol regulatory element DNA sequence TCACNCCAC and acts as a key regulatory element for fatty acid biosynthesis and cholesterol homeostasis (Felder et al., 2005). Like other mammals, there were no splice variants detected in the bovine *SREBF1* gene and it has the most similarity with the human *SREBF1a* sequence (Hoashi et al., 2007). Human *SREBF1a* is a more potential transcriptional activator than the other isoform *SREBF1c*, due to its longer NH2-terminal trans-activation domain (Shimano et al., 1997). The 84 bp indel in Hanwoo illustrated three different genotypes which are in accordance with previous results reported by Hoashi et al. (2007) in Japanese Black cattle. Furthermore, this length polymorphism was only found in the Limousine breed among the five different breeds studied suggesting that it might be a breed specific phenomenon and may not be segregating widely in different cattle populations. Our study demonstrated that the length polymorphism in the *SREBF1* gene is significantly associated with the content of several beef fatty acids. The SS genotype contributes to higher unsaturated fatty acid but lower saturated fatty acid

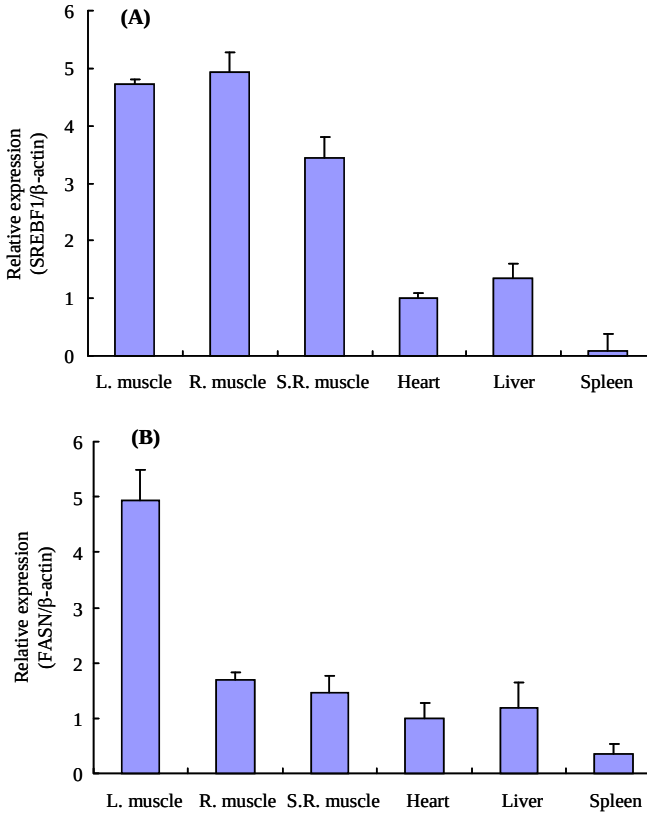

Figure 3. Analysis of mRNA expression patterns in different tissues using quantitative real time PCR for *SREBF1* (A) and *FASN* (B) genes. The numbers on the Y axis indicate fold differences. The obtained gene expression values of each gene were normalized to the expression level of β-actin as controls for the same sample. Each reaction was carried out in triplicate and experiments were repeated at least two times. Bars indicate standard deviation.

content in meat than the LL and LS genotypes. This agrees with the previous findings of Hoashi et al. (2007). They found that the SS type contributed to 1.3% higher MUFA proportion in intramuscular fat ($p<0.05$). Two silent mutations in the *SREBF1* gene were significantly associated ($p<0.05$) with intramuscular fat content in pigs (Chen et al., 2008) which supports our present findings. Recent studies showed that SNPs in the intron region had significant association with marbling score (Cheong et al., 2007), carcass and meat quality traits (Di Stasio et al., 2005; Shin and Chung, 2007c). Although the 84 bp indel of *SREBF1* gene is not located in the coding sequence, it is possible that this length polymorphism may affect mRNA expression level/translation efficiency and thereby indirectly contributes to fat quality characteristics (Kennes et al., 2001).

FASN is an enzymatic system composed of a 272 kDa multifunctional protein that is involved in the synthesis of fatty acids and plays a central role in *de novo* lipogenesis in mammals. Recently, a significant QTL for fatty acid composition was mapped on BTA19 and a candidate gene in this region, *FASN*, was identified (Roy et al., 2001; Morris et al., 2007). In the current study, we focused on the polymorphisms in the TE domain of the *FASN* gene and five SNPs were identified. Among them, two SNPs g.17924G>A and g.18663C>T were reported previously by Zhang et al. (2008) in Angus cattle and the former one was also reported in Jersey and Limousine cattle breeds by Morris et al. (2007). We independently identified 3 other SNPs g.18043C>T, g.18440G>A and g.18529G>A in our study. We found that SNP g.17924G>A was significantly associated with C16:0 and C18:1 which accords with the previous findings of Morris et al. (2007) and Zhang et al. (2008). They reported that MUFA and SFA contents had significant association with g.17924G>A polymorphism and the GG genotype contained higher amount of UFA and relatively lower SFA content than AG and AA genotypes. In another investigation, polymorphisms g.763G>C and g.16009A>G associated with milk fat content in a Holstein-Friesian population was reported by Roy et al. (2006) which supports our study. It is mentioned here that the favorable GG genotype frequency was only 13% in the Angus population (Zhang et al., 2008) whereas the estimated frequency for this genotype was 73% in the Hanwoo population. This might have occurred naturally in the Hanwoo population through long term selective breeding for other associated traits. The product of mammalian *FASN* is mainly C16:0, with minor amounts of C14:0 and it also

contributes to the contents of other fatty acids. In the human, the TE domain has a hydrophobic groove that constitutes the fatty acyl substrate binding site (Chakravarty et al., 2004) with high specific activity towards C16-acyl ACP, but not C14-acyl ACP. The amino acid substitution in the TE domain predicted by the SNP g.17924A>G may influence the structure of the substrate binding site and consequently affect the specific activity of the TE domain (Zhang et al., 2008).

We observed a higher mRNA expression in three different muscles for both *SREBF1* and *FASN* genes which indicates the potential influence of these genes on cattle muscle compared with the other tissues studied. In the human, *SREBF1* isoforms were expressed in all tissues studied, but higher expression was found in adipose tissue, brain, liver and testis (Felder et al., 2005). *SREBF1* is predominantly expressed in liver and adipose tissue of the mouse (Kim and Spiegelman, 1996). The *SREBF1* was expressed preferentially in the liver and uropygial gland in chicken compared with other tissues examined, adipose tissue, lung, kidney, intestine, muscle, brain and testis (Assaf et al., 2003). This discrepancy might be due to physiological differences between the two species. Roy et al. (2005) reported that *FASN* expression was higher in bovine brain, testis and adipose tissue while lower expression was observed in liver and heart. However, we did not include adipose tissue samples in our experiment. In our results, *FASN* gene had the highest expression in LM among the three different muscles which supports that this gene may contribute remarkably to adipogenesis in LM.

In conclusion, we identified an 84-bp indel in *SREBF1* and two SNPs in the *FASN* gene in Korean Hanwoo cattle. We found that the polymorphisms in these genes had significant association with composition of several fatty acids of beef. The exact molecular and physiological mechanisms underlying the association of SNP with fatty acid content reported in this study are unknown. The use of these polymorphisms as genetic markers can be a useful tool for selection of animals and improve meat quality in Korean cattle.

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REFERENCES

Assaf, S., D. Hazard, F. Pitel, M. Morisson, M. Alizadeh, F. Gondret, C. Diot, A. Vignal, M. Douaire and S. Lagarrigue. 2003. Cloning of cDNA encoding the nuclear form of chicken sterol response element binding protein-2 (SREBP-2), chromosomal localization and tissue expression of chicken SREBP-1and -2 genes. Poult. Sci. 82:54-61.

Barendse, W., R. J. Bunch, B. E. Harrison and M. B. Thomas. 2006. The growth hormone GH1: c.457C>G mutation is associated with relative fat distribution in intra-muscular and rump fat in a large sample of Australian feedlot cattle. Anim. Genet. 37:211-214.

Bonanome, A. and S. Grundy. 1988. Effect of dietary stearic acid on plasma cholesterol and lipoprotein concentrations. N. Engl. J. Med. 318:1244-1248.

Brown, M. S. and J. L. Goldstein. 1997. The SREBP pathway: Regulation of cholesterol metabolism by proteolysis of a membrane bound transcription factor. Cell. 89:331-340.

Burge, C. and S. Karlin. 1997. Prediction of complete gene structures in human genomic DNA. J. Mol. Biol. 268:78-94.

Casas, E., S. N. White, T. L. Wheeler, S. D. Shackelford, M. Koohmaraie, D. G. Riley, C. C. Chase, D. D. Johnson and T. P. L. Smith. 2006. Effects of calpastatin and mu-calpain markers in beef cattle on tenderness traits. J. Anim. Sci. 84:520-525.

Chakravarty, B., Z. Gu, S. S. Chirala, S. J. Wakil and F. A. Quiocho. 2004. Human fatty acid synthase: structure and substrate selectivity of the thioesterase domain. Proc. Natl. Acad. Sci., USA. 101:15567-15572.

Chen, J., X. J. Yang, D. Xia, J. Chen, J. Wegner, Z. Jiang and R. Q. Zhao. 2008. Sterol regulatory element binding transcription factor 1 expression and genetic polymorphism significantly affect intramuscular fat deposition in the longissimus muscle of Erhualian and Sutai pigs. J. Anim. Sci. 86:57-63.

Cheong, H. S., D. Yoon, L. H. Kim, B. L. Park, H. W. Lee, C. S. Han, E. M. Kim, H. Cho, E. R. Chung, I. Cheong and H. D. Shin. 2007. Titin-cap (TCAP) polymorphisms associated with marbling score of beef. Meat Sci. 77:257-263.

Di Statio, L., G. Destefanis, A. Brugiapaglia, A. Albera and A. Ronaldo. 2005. Polymorphism of the GHR gene in cattle and relationship with meat production and quality. Anim. Genet. 36:138-140.

Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to quantitative genetics (4th edn.). Longman, England.

Felder, T. K., K. Klein, W. Patsch and H. Oberkofler. 2005. A novel SREBP-1 splice variant: Tissue abundance and trans-activation potency. Biochem. Biophys. Acta. 1731:41-47.

Hoashi, S., N. Ashida, H. Ohsaki, T. Utsugi, S. Sasazaki, M. Taniguchi, K. Oyama, F. Mukai and H. Mannen. 2007. Genotype of bovine sterol regulatory element binding protein-1 (SREBP-1) is associated with fatty acid composition in Japanese Black cattle. Mamm. Genom. 18:880-886.

Kennes, Y. M., B. D. Murphy, F. Pothier and M. F. Palin. 2001. Characterization of swine leptin (LEP) polymorphisms and their association with production traits. Anim. Genet. 32:215-218.

Kim, J. B. and B. M. Spiegelman.1996. ADD1/SREBP1 promotes adipocyte differentiation and gene expression linked to fatty acid metabolism. Genes. Dev. 10:1096-1107.

Kim, Y. M., J. H. Kim, S. C. Kim, H. M. Ha, Y. D. Ko and C.H. Kim. 2002. Influence of dietary addition of dried wormwood (Artemisia sp.) of the performance, carcass characteristics and fatty acid composition of muscle tissues of Hanwoo Heifers. Asian-Aust. J. Anim. Sci. 15:549-554.

Lepage, G. and C. C. Roy. 1986. Notes on methodology: Direct transesterification of all classes of lipids in a one-step reaction. J. Lip. Res. 27:114-120.

Livak, K. J. and T. D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}{}_T$ method. Methods. 25:402-408.

Morris, C. A., N. G. Cullen, B. G. Glass, D. L. Hyndman, T. R. Manley, S. M. Hickey, J. C. McEwan, W. S. Pitchford, C. D. K. Bottema and M. A. H. Lee. 2007. Fatty acid synthase effects on bovine adipose fat and milk fat. Mamm. Genom. 18:64-74.

Mozaffarian, D., A. Ascherio, F. B. Hu, M. J. Stampfer and W. C. Willett. 2005. Interplay between different polyunsaturated fatty acids and risk of coronary heart disease in man. Circulation 111:257-164.

Roy, R., L. Ordovas, P. Zaragoza, A. Romero, C. Moreno, J. Altarriba and C. Rodellar. 2006. Association of polymorphisms in the bovine FASN gene with milk fat content. Anim. Genet. 37:215-218.

Roy, R., M. Gautier, H. Hayes, P. Laurent and R. Osta. 2001. Assignment of the fatty acid synthase (FASN) gene to bovine chromosome 19 (19q22) by in sito hybridization and confirmation by somatic cell hybrid mapping. Cytogenet. Cell. Genet. 93:141-142.

Roy, R., S. Taourit, P. Zaragoza, A. Eggen and C. Rodellar. 2005. Genomic structure and alternative transcript of bovine fatty acid synthase gene (FASN): comparative analysis of the FASN gene between monogastric and ruminant species. Cytogenet. Genom. Res. 111:65-73.

Rozen, S. and H. J. Skaletsky. 2000. Primer 3 on the WWW for general users and for biologist programmers. In: Bioinformatics methods and protocols: Methods in molecular biology (Ed. S. Krawetz and S. Misener). Humana Press, Totowa, NJ. pp. 365-386.

Shimano, H., N. Yahagi, M. Amemiya-Kudo, A. H. Hasty, J. Osuga, Y. Tamura, F. Shionoiri, Y. Iizuka, K. Ohashi, K. Harada, T. Gotoda, S. Ishibashi, and N. Yamada. 1999. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. J. Biol. Chem. 274:35832-35839.

Shimomura, I., H. Shimano, J. D. Horton, J. L. Goldstein and M. S. Brown. 1997. Differential expression of exons 1a and 1c in mRNA for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. J. Clin. Invest. 99:838-845.

Shin, S. C. and E. R. Chung. 2007a. Association of SNP marker in the leptin gene with carcass and meat quality traits in Korean cattle. Asian-Aust. J. Anim. Sci. 20:1-6.

Shin, S. C. and E. R. Chung. 2007b. Association of SNP marker in the thyroglobulin gene with carcass and meat quality traits in Korean cattle. Asian-Aust. J. Anim. Sci. 20:172-177.

Shin, S. C. and E. R. Chung. 2007c. SNP detection of carboxypeptidase E gene and its association with meat quality and carcass traits in Korean cattle. Asian-Aust. J. Anim. Sci. 20:328-333.

Tamura, K., J. Dudley, M. Nei and S. Kumar. 2007. MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24:1596-1599.

Thaller, G., C. Kuhn, A. Winter, A. Ewald, O. Bellmann, J.Wegner, H. Zuhlke and R. Fries. 2003. DGAT1, a new positional and functional candidate gene for intramuscular fat deposition in cattle. Anim. Genet. 34:354-357.

Whetsell, M. S., E. B. Rayburn and J. D. Lozier. 2003. Human health effects of fatty acids in beef. A report on "Pasture based beef systems for Appalachia," West Virginia University, USA.

Woollett, L. A., D. K. Spady and J. M. Dietschy. 1992. Saturated and unsaturated fatty acid independently regulate low density lipoprotein receptor activity and production rate. J. Lip. Res. 33:77-78.

Yang, A., T. W. Larsen, S. B. Smith and R. K. Tume. 1999. Δ^9-desaturase activity in bovine subcutaneous adipose tissue of different fatty acid composition. Lipids. 34:971-978.

Zhang, S., T. J. Knight, J. M. Reecy and D. C. Beitz. 2008. DNA polymorphisms in bovine fatty acid synthase are associated with beef fatty acid composition. Anim. Genet. 39:62-70.