

Association of Bovine CSRP3 and ACOX1 Genes with Carcass and Meat Quality Traits

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소의 도체, 육질형질과 CSRP3, ACOX1 유전자들과의 상관관계

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ABSTRACT

There is no investigation has yet been conducted for ACOX1 and CSRP3 gene polymorphisms in Korean cattle (Hanwoo), and their associations with carcass and meat quality traits. In this study, SNPs in ACOX1 and CSRP3 genes were identified and their associations with carcass and meat quality traits were investigated in 227 Hanwoo animals. Two SNPs (g.224G>A and g.19491G>A) in ACOX1 gene and one SNP (g.14859C>T) in CSRP3 gene were identified in Hanwoo and sequence analysis indicated that these SNPs were located in the coding regions. The allele frequencies of ACOX1 g.224G>A and g.19491G>A SNPs were 0.57, 0.43, and 0.56 and 0.44, respectively. For CSRP3 g.14859C>T polymorphism, the C and T allele frequencies were 0.64 and 0.36, respectively. The Hanwoo cattle were used to detect PCR-RFLP patterns for estimating the allele frequencies. Single marker association analyses were performed between genotype of each SNP, and carcass and meat quality association traits to evaluate the relationships in Hanwoo. The g.224G>A SNP genotypes of ACOX1 gene, which was significantly associated with meat quantity grade at slaughter ($P < 0.03$) and backfat thickness tended to be greater ($P = 0.06$) in Hanwoo. The previously identified g.14859C>T SNP was used in this study and the obtained genotype and allele frequencies are almost similar with the previous results reported by Bhuiyan et al. (2007). However, no significant association was found between g.19491G>A SNP in the ACOX1 and g.14859C>T SNP genotypes of CSRP3 gene and considered carcass and meat quality traits. In conclusion, the information on the identified SNPs in CSRP3 and ACOX1 genes could be useful for further association study and haplotype analysis for the development of carcass and meat quality traits in Hanwoo.

Key words: carcass, meat quality, Hanwoo, ACOX1, CSRP3

1. Introduction

In beef cattle industry, growth and meat quality traits are the most economically important traits and these are directly related with profit-loss equation of a farm. The animals that gain weight better than their counterparts are more desirable to the producers as well as to breeders. On the other hand, high quality beef fetches better price in the market which also lead the profitable beef farming. Meat quality is a generic term used to describe some attributes of meat such as marbling, tenderness, color, texture and flavor. In cattle, high levels of marbling increase the palatability and acceptability of

beef by affecting the flavor and tenderness of meat (Crouse et al., 1984). Conventional selective breeding has been practised for many years to improve carcass and meat quality traits in cattle and substantial progress has been achieved for several highly heritable traits by this time. For Hanwoo improvement program, the conventional selective breeding has been conducted since the 1959 by Ministry of Agriculture and Forestry in Korea. But it is very difficult to improve low heritable traits like meat quality by traditional breeding approach. To overcome these limitations, marker-based selection has been introduced for the development of selection criteria having better meat quality.

During the past decades, significant contribution has been made to study variation at DNA levels and to identify genes controlling quantitative traits like growth, carcass and meat quality traits. Previous studies showed DNA polymorphisms were significantly associated with meat and carcass quality traits in cattle (Li et al., 2004; Casas et al., 2003; Schenkel et al., 2005; Shin and Chung, 2007a and Cho et al., 2008). Marker assisted selection

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(MAS) defines a marker based on the genes or DNA variations which can be used for genetic determinant of a particular trait (Williams, 2005). The successful application of MAS in animal population will depend on the identification of major genes or tightly linked markers (Zuo et al., 2003). The association study of candidate genes is a step for the knowledge of the genetic basis of quantitative traits which could be efficiently implemented in breeding programs (Ovilo et al., 2006).

The cysteine and glycine-rich protein 3 (CSRP3) gene, referred to as muscle LIM protein, has putative roles in skeletal muscle for regulation of myogenic differentiation (Kong et al., 1997; Lehnert et al., 2006). This gene has mapped on BTA29 and is closely located in a QTL for hot carcass weight in cattle (Casas et al., 2003). Expression analysis revealed that CSRP3 gene expressed preferentially in bovine skeletal muscles as compared to other organ tissues (Yu et al., 2007). The Palmitoyl acyl-oA oxidase 1 (ACOX1) catalyses the beta-oxidation of very long-chain fatty acids, and thus plays an essential role in fatty acid degradation. ACOX1 has been considered as a potential candidate gene for the traits related to fat metabolism due to its involvement in the beta-oxidation pathway (Zuo et al., 2007). Expression of the ACOX1 gene was significantly increased in male mice fed a high-fat diet, compared with a low-fat diet (Kim et al., 2004). ACOX1 gene is located on BTA19, where several QTLs affecting significantly meat quality traits (Morris et al., 2007; Zhang et al., 2008) and milk fat (Roy et al., 2006). Polymorphisms in ACOX1 gene also had significant association with marbling score and meat color traits in pigs (Zuo et al., 2007).

Hanwoo is the Korean native cattle, which is famous for its superior meat quality. This breed has been originated in the Korean peninsula thousands years ago by crossing between *Bos primigenius* and *Bos indicus* cattle. Previously, Hanwoo was used as a farming animal. But today this breed has been mainly used for beef cattle. In Korea, Hanwoo meat is very popular and more expensive than the other imported cattle meat. However growth performance in Hanwoo and milk production capacity are inferior to foreign beef cattle (Kim and Lee, 2000). Therefore, efforts have been paid by the different institutions in Korea for the improvement of economically important traits of this breed. Until now, significant associations between several candidate genes polymorphisms, and carcass and meat quality traits have been

reported in Hanwoo (Kim et al., 2004; Cheong et al., 2007; Shin and Chung, 2007b). However, no investigation has yet been conducted for these two candidate genes polymorphisms and their association study with carcass and meat quality traits. Therefore, the objectives of this study were to identify SNPs in ACOX1 and CSRP3 genes and to investigate the possible association of the identified SNPs with carcass and meat quality traits in Hanwoo.

II. Materials and Methods

1. Sampling

A total of 227 Hanwoo blood samples were collected from Dong-il slaughterhouse, Bu-yeo, Korea, in a sampling tube containing heparin anti-coagulant. Genomic DNA was extracted using primeprepTM genomic DNA isolation kit (Genetbio, Korea). Phenotypic data on growth and carcass related traits were collected from the data sheet maintained by the Dong-il slaughter house according to the guidelines of animal products grading service (APGS). The following phenotypic traits were included in this study; backfat thickness (BF), carcass weight (CW), fat color (FC), longissimus muscle area (LMA), meat color (MC), marbling score (MS), meat quantity index (MQNI), meat quantity grade (MQNG), meat grade 1st (MG1), meat texture (MT), meat maturity (MM) and meat quality grade (MQLG). These phenotypic traits (LMA, BF, CW, MS, MC, FC, MQNI, MQNG, MG1,

Table 1. Overall mean, standard deviation (SD), Maximum (Max), Minimum (Min) range of traits analyzed in the experimental population (n=227).

Trait	Mean	SD	Max	Min
Carcass weight (Kg)	315.05	47.02	432	164
Backfat thickness (mm)	10.14	4.61	30	3
Longissimus muscle area (cm ²)	76.94	14.42	118	42
Meat quantity index	67.51	3.62	75.34	53.9
Meat quantity grade (1-3)	1.56	0.63	3	1
Marbling score (1-5)	4.19	2.10	9	1
1st. grade (1-5)	3.18	1.07	5	1
Meat color (1-7)	4.98	0.50	6	4
Fat color (1-7)	3.10	0.34	5	2
Meat texture (1-3)	1.39	0.49	2	1
Meat Maturity (1-9)	4.73	1.66	9	2
Meat quality grade (1-5)	3.24	1.06	5	1

Table 2. The primer sequence, PCR product sizes and corresponding annealing temperatures for ACOX1 and CSRP3 genes.

Gene	F/R	Primer sequence (5'→3')	PCR product size (bp)	Annealing temp (°C)
ACOX1	F	CCCGTTACCATGAATCCAGA	625	61
	R	ATGGCGAATTCAAGGTTTCAG		
	F	CGTGGAACCTAACGTCCATT	664	61
	R	CTTGGCCCATTCAAACAAGT		
CSRP3	F	ACGCTCAAGGACAACACTAC	560	53
	R	GGAACAGAATGACCTACC		

MT, MM, MQLG) were measured at the 11th~12th rib interface after an hour chill. MS was determined by assessing the degree of marbling in the cut surface of the rib eye on a scale from 1 to 5 according to the Korean Beef Marbling Standard (BMS) from Animal product Grading Service, Korea. The means and standard deviations for the traits analyzed in this study are given in Table 1.

2. Primer design and PCR amplification

Three primer pairs were designed using bovine sequence data from NCBI (accession number no: NC_007317, NC_07330). This primer used to amplify exon 3 of CSRP3 gene, and exon 1 and 9 of ACOX1 gene. The primer sequences, PCR product sizes and corresponding annealing temperatures are shown in Table 2. The PCR mixture contained approximately 50 ng of genomic DNA, 1X PCR gold buffer (50 mM KCl, 10 nM Tris-HCl pH 8.3), 1.5 mM MgCl₂, 200 μM dNTPs, 0.4 pmol of each primer and 1 U Taq polymerase (Ampli Tag GoldTM, applied Bio system, USA) in a final volume of 20 μl. The PCR amplification was performed in a GeneAmp 2700 (Applied Biosystems, USA) thermocycler with an initial denaturation temperature 94°C for 10 min followed by 32~35 cycles of 30 second at 94°C, 30 second at specific annealing temperature for each primer set (Table 2), and 30~40 second at 72°C, final extension temperature at 72°C for 10 minute. All the PCR products were run on agarose gel and the bands confirmed under UV light.

3. SNP detection and genotyping

DNA samples from eight Hanwoo individuals were randomly chosen for DNA polymorphism investigation. The PCR products were purified with AccuPrep[®] PCR purification kit (Bioneer, Korea) according to manufacture's

Table 3. Identified SNPs in ACOX1 and CSRP3 genes in Hanwoo.

Gene (Access. no.)	SNP ^a	Location	Amino acid change	Restriction enzyme
ACOX1 (NC_007317)	g.224G > A	Exon 1	Silent	<i>Mbo</i> II
	g.19491G > A	Exon 9	Silent	<i>Tsp</i> 509 I
CSRP3 (NC_007330)	g.14859C > T	Exon 3	Silent	<i>Nla</i> III

^a Nucleotide positions are numbered according to the first base of each gene as it appears in GenBank.

instructions. Sequencing reaction of purified PCR products were performed by a 3100 automated DNA sequencer (Applied Biosystems, USA) using Big Dye Terminator Cycle Sequencing Ready Reaction Kit (ver 3.0, Applied Biosystem, USA). The DNA sequences were verified using Chromas program ver. 2.01 (www.technelysium.com.au) and polymorphisms were detected by comparing the obtained sequence data with the sequences in the NCBI data base (<http://www.ncbi.nlm.nih.gov>). Alignment of multiple sequences was performed using ClustalW program (Thompson et al., 1994) and mutations were detected by MEGA software ver. 4.0 (Tamura et al., 2007). For investigating mutation type, translation of the nucleotide sequences into amino acids was performed using the Open Reading Frame Finder at NCBI (<http://www.ncbi.nlm.nih.gov/gorf/gorf.html>).

A total of 227 individuals from Hanwoo cattle were used to detect PCR-RFLP restriction patterns for estimating the allele frequency in a 20 μl reaction volume. The restriction enzymes were used for digestion of PCR products are listed in Table 3. The resulting RFLP fragments were separated on 3~5% agarose gel by electrophoresis with 1X TAE buffer and stained using ethidium bromide. The RFLP fragment bands were visualized under UV light.

4. Statistical analysis

Genotype and allele frequencies were calculated accor-

ding to Falconer and Mackay (1996). The genotype allele frequency of each single nucleotide polymorphism (SNP) were examined for deviations from Hardy-Weinberg equilibrium by chi-square (χ^2) tests (Falconer and Mackay, 1996). Single marker association analyses were performed between genotype of each SNP, and carcass and meat quality association traits to evaluate the relationships in Hanwoo. In this population, the PROC GLM (generalized linear model) procedure of SAS, was used.

The statistical model used as follows:

$$\text{Model} : Y_{ijk} = \mu + \text{Sex}_i + \text{Genotype}_j + bx_{ijk} + e_{ijk}$$

Y_{ijk} is the observation of each carcass and meat quality traits;

Sex_i is the effect of i th sex, i is sex = 1(♀), 2(♂).

Genotype_j is the effect of j th genotype.

b is the regression coefficient of carcass weight.

x_{ijk} is the set up covariate of carcass weight.

e_{ijk} is the random residual error $\text{NID} \sim (0, \sigma_e^2)$,

NID is normally independent distribution, 0 is average

and σ_e^2 is variance.

III. Results and Discussion

1. Identification of SNPs

Amplification and partial sequencing of ACOX1 and CSRP3 genes among the Hanwoo individuals revealed 3 polymorphisms. The obtained nucleotide sequences were compared with the sequences in the NCBI database. Detailed information on all SNPs, mutation type and corresponding restriction enzymes are shown in Table 3. Two SNPs (g.224G > A and g.19491G > A) of ACOX1 gene and one SNP g.14859C > T of CSRP3 gene were identified in Hanwoo cattle and these SNPs were located in the coding regions. The g.224G > A and g.19491G > A SNPs were found in exon 1 and exon 9 of ACOX1 gene, respectively and g.14859C > T SNP was found in exon 3 of CSRP3 gene. All three SNPs were found silent mutations. The SNPs of ACOX1 and CSRP3 genes were used for allele frequency analysis and association studies.

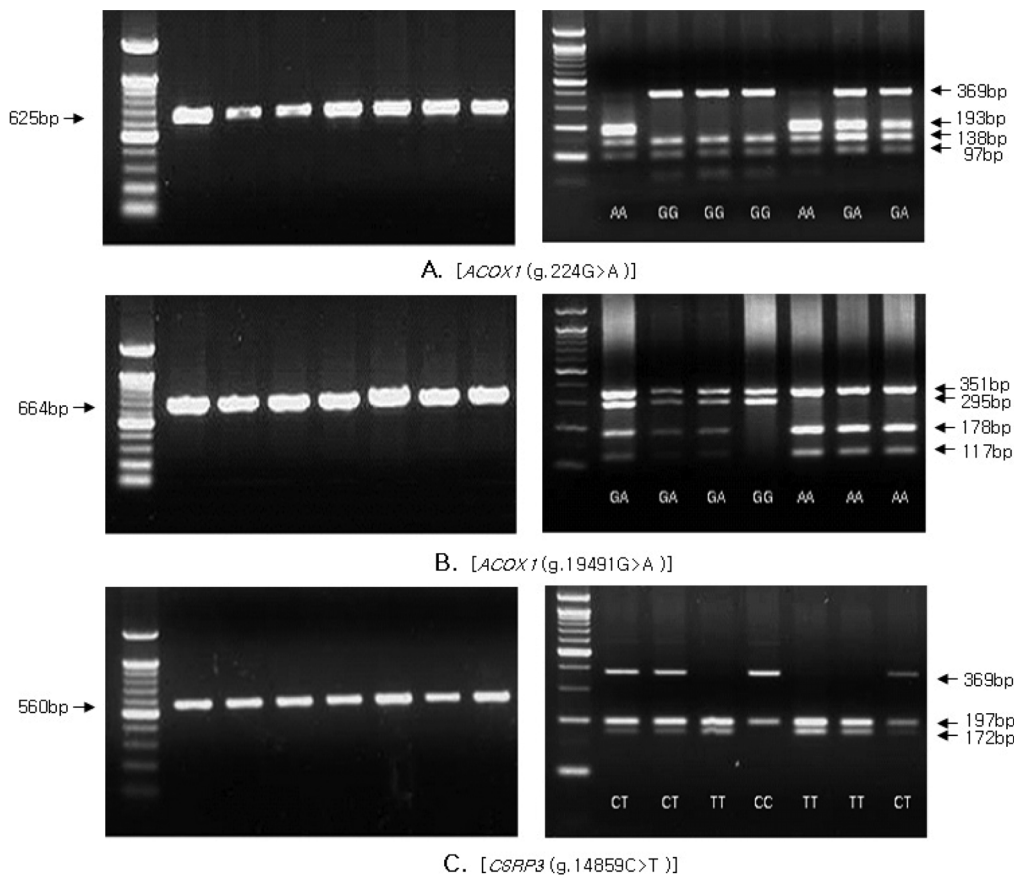


Fig. 1. PCR-RFLP patterns for the SNPs in ACOX1 and CSRP3 genes in Hanwoo.

Table 4. SNP genotype and allele frequencies of CSRP3 and ACOX1 genes in Hanwoo.

Gene	SNP	Genotype frequency			Allele frequency		N	P-value
		GG	GA	AA	G	A		
ACOX1	g.224G > A	0.282(61)	0.569(123)	0.148(32)	0.57	0.43	216	p < 0.05
		0.277(59)	0.563(120)	0.160(34)	0.56	0.44		
CSRP3	g.14859C > T	0.434(85)	0.413(81)	0.153(30)	0.64	0.36	196	p > 0.05

Hardy-weinberg equilibrium test for each locus according to Falconer and Mackay(1996). N denotes total number of animals investigated.

2. Genotyping and allele frequencies

Genotyping was performed by PCR-RFLP method and different SNP loci of restriction patterns are shown in Fig. 1. The SNP genotypes and allele frequencies of ACOX1 and CSRP3 genes in Hanwoo population are shown in Table 4. The genotype frequencies were in agreement with Hardy-Weinberg equilibrium (Falconer and Mackay, 1996) for CSRP3 gene but deviated results were found for the two SNPs of ACOX1 gene. This deviation might be due to non random sampling from Hanwoo population.

The g.224G > A polymorphism in ACOX1 gene, PCR products with *Mbo*II restriction enzyme resulted in fragment of 97, 138 and 369 bp for G allele and 97, 138, and 193 bp for A allele (Fig. 1. A). The GG, GA and AA genotype frequencies were 0.282, 0.569 and 0.148, respectively, and the corresponding G and A allele frequencies were 0.57 and 0.43, respectively. The g.19491G > A polymorphism showed 295, 351 bp fragments for G allele and 117, 178 and 351 bp for A allele with *Tsp*509 I restriction enzyme (Fig. 1. B). The genotype and allele frequencies were 0.277, 0.563 and 0.160, and 0.56 and 0.44, respectively.

In CSRP3 gene, the g.14859C > T polymorphism digested with *Nla*III restriction enzyme created 197 and 369 bp for C allele and 172, 197 bp for T allele (Fig. 1. C). For this SNP, the frequencies of CC, CT and TT genotypes in Hanwoo population were 0.434, 0.413 and 0.153, respectively, and the C and T allele frequencies were 0.64 and 0.36, respectively.

3. Association of polymorphism with carcass and meat quality traits

Using phenotype data of 227 Hanwoo individuals,

Table 5. Association of g.224G > A SNP in ACOX1 gene with carcass and meat quality traits in Hanwoo¹.

Trait ²	SNP genotype			P-value
	GG (n = 61)	GA (n = 123)	AA (n = 32)	
CW (kg)	328.85±7.67	325.84±6.30	328.58±9.17	0.89
BT (mm)	8.16±0.69	6.94±0.56	8.48±0.82	0.06
LMA (cm ²)	80.79±1.69	80.30±1.39	79.96±2.02	0.92
MQNI	69.28±0.56	69.97±0.46	68.97±0.66	0.20
MQNG (1-3)	1.35±0.09 ^{ab}	1.20±0.08 ^b	1.48±0.11 ^a	0.03*
MS (1-5)	2.84±0.32	2.89±0.26	3.04±0.38	0.89
MG1 (1-5)	3.91±0.16	3.90±0.13	3.78±0.19	0.80
MC (1-7)	5.23±0.08	5.24±0.06	5.12±0.09	0.46
FC (1-7)	3.26±0.05	3.20±0.04	3.16±0.06	0.29
MT (1-3)	1.73±0.07	1.67±0.06	1.59±0.09	0.39
MM (1-9)	3.43±0.24	3.41±0.19	3.33±0.28	0.94
MQAG (1-5)	4.01±0.16	3.91±0.13	3.83±0.19	0.67

¹ Value are expressed as LSM±SE.

^{a, b} values in the same row with different superscript differ at p < 0.05.

² CW = carcass weight, BT = backfat thickness, LMA = longissimus muscle area, MQNI = meat quantity index, MQNG = meat quantity grade, MS = marbling score, MG1 = meat grade 1st, MC = meat color, FC = fat color, MT = meat texture, MM = meat maturity, MQAG = meat quality grade.

association studies between each SNP genotypes and carcass and meat quality traits are shown in Table 5, 6 and 7. In ACOX1 gene, significant association was found between the g.224G > A SNP genotypes and MQNG (Table 5). Hanwoo with AA genotype had 8.8% and 19.0% higher MQNG than those from GG and GA genotypes, respectively (P < 0.05). Moreover, BT tended to be greater (P = 0.06) with AA genotypes than other genotypes. However, no significant association was found between g.19491G > A SNP in the ACOX1 and g.14859C > T SNP genotypes of CSRP3 gene and considered carcass and meat quality traits (Table 6 and 7).

CSRP3 gene encodes a member of CSRP family of

Table 6. Association of g.19491G>A SNP ACOX1 gene with carcass and meat quality traits in Hanwoo¹.

Trait ²	SNP genotype			P-value
	GG (n = 59)	GA (n = 120)	AA (n = 34)	
CW (kg)	326.33±7.55	329.89±5.97	315.88±9.29	0.27
BT (mm)	7.61±0.71	7.35±0.56	7.97±0.86	0.74
LMA (cm ²)	80.02±1.70	80.54±1.35	81.04±2.08	0.89
MQNI	69.50±0.57	69.73±0.45	69.41±0.70	0.85
MQNG (1-3)	1.31±0.10	1.27±0.08	1.26±0.12	0.91
MS (1-5)	2.98±0.31	2.80±0.25	2.91±0.38	0.81
MG1 (1-5)	3.85±0.15	3.91±0.12	3.95±0.19	0.84
MC (1-7)	5.16±0.08	5.26±0.06	5.14±0.10	0.24
FC (1-7)	3.22±0.05	3.20±0.04	3.29±0.06	0.33
MT (1-3)	1.67±0.07	1.67±0.06	1.69±0.09	0.96
MM (1-9)	3.66±0.15	3.30±0.19	3.52±0.29	0.28
MQAG (1-5)	3.93±0.15	3.94±0.12	3.99±0.19	0.95

¹ Value are expressed as LSM±SE.

^{a, b} values in the same row with different superscript differ at $p < 0.05$.

² CW = carcass weight, BT = backfat thickness, LMA = longissimus muscle area, MQNI = meat quantity index, MQNG = meat quantity grade, MS = marbling score, MG1 = meat grade 1st, MC = meat color, FC = fat color, MT = meat texture, MM = meat maturity, MQAG = meat quality grade.

Table 7. Association of g.14859C>T CSRP3 gene with carcass and meat quality traits in Hanwoo¹.

Trait ²	SNP genotype			P-value
	CC (n = 85)	CT (n = 81)	TT (n = 30)	
CW (kg)	328.81±6.58	331.64±7.11	337.64±10.17	0.66
BT (mm)	7.18±0.59	7.32±0.64	8.21±0.92	0.49
LMA (cm ²)	80.44±1.43	80.96±1.55	77.82±2.21	0.32
MQNI	69.84±0.47	69.82±0.51	68.85±0.73	0.32
MQNG (1-3)	1.28±0.08	1.25±0.09	1.37±0.13	0.66
MS (1-5)	2.90±0.28	2.80±0.31	3.38±0.44	0.39
MG1 (1-5)	3.89±0.14	3.93±0.15	3.57±0.22	0.22
MC (1-7)	5.26±0.07	5.15±0.07	5.12±0.11	0.29
FC (1-7)	3.21±0.05	3.25±0.05	3.28±0.07	0.61
MT (1-3)	1.67±0.06	1.67±0.07	1.69±0.10	0.97
MM (1-9)	3.45±0.20	3.46±0.22	3.21±0.32	0.70
MQAG (1-5)	3.93±0.14	3.95±0.15	3.79±0.22	0.76

¹ Value are expressed as LSM±SE.

^{a, b} values in the same row with different superscript differ at $p < 0.05$.

² CW = carcass weight, BT = backfat thickness, LMA = longissimus muscle area, MQNI = meat quantity index, MQNG = meat quantity grade, MS = marbling score, MG1 = meat grade 1st, MC = meat color, FC = fat color, MT = meat texture, MM = meat maturity, MQAG = meat quality grade.

CSRP3 gene encodes a member of CSRP family of

LIM domain proteins which is involved in the regularity process important for development and cellular differentiation in mammals. The CSRP3 gene has also been implicated in the regulation of myogenesis (Kong et al., 1997). Besides, ACOX1 gene encodes the first enzyme of the fatty acid beta-oxidation pathway, which catalyzes the desaturation of acyl-CoAs to 2-trans-enol-CoAs (Munoz, 2008). The candidate gene study allows the identification of SNPs in genes likely to cause variation in a trait based on the functions of gene (Te Pas and Soumilion, 2001). The candidate gene association study is necessary to develop marker assisted selection strategy of animals for the development of growth and meat quality traits in cattle. Until now, there are no studies on ACOX1 gene in cattle. And this is the first result for identification of SNPs in this gene. Several studies have been carried out with ACOX1 genes in pigs. Association of the polymorphism in GYS1 and ACOX1 genes with meat quality traits in pigs (Zuo et al., 2007). The previously identified g.14859C>T SNP was used in this study and the obtained genotype and allele frequencies are almost similar with the previous results reported by Bhuiyan et al. (2007). However, using this SNP, the association studies showed no significant results among the carcass and meat quality traits investigated. Table 5 shows the g.224G>A SNP genotypes of ACOX1 gene, which was significantly associated with meat quantity grade at slaughter ($P < 0.03$) and backfat thickness tended to be greater ($P = 0.06$) in Hanwoo. This results agree with previous investigation by Zuo et al. (2007), where they found ACOX1 gene polymorphism affect significantly the meat quality traits in pigs. In addition, the significant QTL which harbor ACOX1 gene affecting meat quality traits in pigs was also reported by De Koning et al. (1999) and Clop et al. (2003). These results also support our findings. Very recently, Munoz (2008) identified 7 SNPs in different pig breeds and two of them were missense mutations. The association studies between the identified SNPs and fatty acid composition traits were not statistically significant. In this study, partial sequencing (exon 1 and 9) has been carried out for ACOX1 gene. This gene has mapped on BTA19 where several QTLs affecting beef fatty acid composition (Zhang et al., 2008), adipose fat and milk fat content (Roy et al., 2006; Morris et al., 2007) were detected. Therefore, it would be better to investigate SNPs in the entire sequence of this gene and association

study between the identified SNPs with carcass and meat quality traits in Hanwoo. In conclusion, the information on the identified SNPs in CSRP3 and ACOX1 genes could be useful for further association study and haplotype analysis for the development of carcass and meat quality traits in Hanwoo.

IV. Summary and Conclusion

한국의 고유 소 품종인 한우는 최근 육량과 육질을 개량하기 위하여 많은 노력을 기울이고 있다. 그러나 현재까지 한우에서 분자유전학적인 방법을 이용하여 육질관련 형질들에 대한 연구는 많이 부족한 상태이다. ACOX1과 CSRP3 유전자들은 도체와 육질에 중요한 역할을 하는 것으로 보고되고 있으나 한우에서 이들 유전자의 연구는 전무한 실정이다. 따라서 본 실험은 한우의 도체와 육질관련 후보유전자인 ACOX1과 CSRP3 유전자의 단일염기 다형성을 확인하고 형질과의 연관성을 알아보기 위하여 실시하였다. 본 연구에 사용된 227두의 한우에서 혈액샘플을 채취해 DNA를 추출하였으며 ACOX1과 CSRP3 유전자들의 단일염기다형(SNP)을 찾기 위하여 NCBI database에서 유전자들의 정보를 얻어 6개의 primer들을 만들었다. 그 후 PCR을 실시하여 sequencing한 결과 ACOX1 유전자에서 2개의 단일염기 다형성(SNP)을, CSRP3 유전자에서 1개의 SNP를 확인하였다. 또한 PCR-RFLP 방법을 이용하여 이 SNP들의 유전자 빈도와 유전자형 빈도를 한우에서 구할 수 있었으며 도체와 육질관련 형질들과의 관계를 SAS program을 이용하여 유의성 검정을 실시하였다. 그 결과, ACOX1 유전자 g.224G > A SNP 유전자형에서 도체 육량등급과 유의적 상관관계가 있음이 밝혀졌다($P < 0.03$). 그리고 통계적인 유의성은 없었으나 등지방두께 역시 영향을 받는 것으로 드러났다($P = 0.06$). 그러나 본 연구에서 CSRP3 유전자의 SNP와 형질과의 연관성을 확인할 수 없었다. 본 논문의 결과는 분자유전학적인 정보를 이용하여 소의 도체와 육질을 개량하기 위한 기초자료로 이용될 수 있을 것으로 사료된다.

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