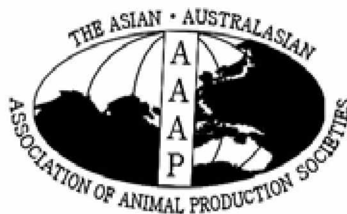

Asian-Aust. J. Anim. Sci.
Vol. 20, No. 4 : 466 - 470
April 2007

www.ajas.info

Identification of a Novel SNP Associated with Meat Quality in C/EBPα Gene of Korean Cattle

S. C. Shin, M. J. Kang[1] and E. R. Chung*

Division of Animal Science and Resources, College of Life Science and Natural Resources
Sangji University, Wonju 220-702, Korea

ABSTRACT : CCAAT/enhancer binding protein α (C/EBPα) plays an important role in lipid deposition and adipocyte differentiation. In order to find genetic markers to improve the meat quality of Korean cattle, the bovine C/EBPα gene was chosen as a candidate gene to investigate its association with carcass and meat quality traits in Korean cattle. A single nucleotide polymorphism (SNP) was identified at position 271 (A/C substitution) of coding region in the C/EBPα gene. A PCR-RFLP procedure with restriction enzyme *Sma*I was developed for determining the marker genotypes. The frequencies of alleles C and A and were 0.374 and 0.626, respectively. The genotype frequencies for CC, AC and AA were 12.9, 49.0 and 38.1%, respectively, in Korean cattle population. The frequencies of genotype were in agreement with Hardy-Weinberg equilibrium. Association analysis indicated that the gene-specific SNP marker of C/EBPα showed a significant association with marbling score ($p<0.05$). The animals with AA genotype had higher marbling score than those with the AC or CC genotype. Although further studies are needed to validate our results, the C/EBPα gene could be useful as a genetic marker for carcass and meat quality traits in Korean cattle. (**Key Words :** C/EBPα Gene, SNP Marker, Meat Quality, Korean Cattle)

INTRODUCTION

Intramuscular fat deposition (marbling) is the most important meat quality trait in Korean beef cattle industry because carcass value is primarily determined by the degree of marbling (Shin and Chung, 2007a, b). The deposition of intramuscular fat is positively related to beef flavor and palatability (Wheeler et al., 1994). In particular, eating quality traits are influenced by the amount of intramuscular fat (Hovenier et al., 1993). However, improving meat quality by selective breeding is difficult because these traits are measured on the carcass.

The development of molecular genetic markers in bovine has made possible the identification of genomic regions that contain quantitative trait loci (QTL) that control economically important traits (Casas et al., 2001). The application of marker-assisted selection would be most beneficial for genetic improvement of such carcass composition and meat quality traits (Meuwissen and Goddard, 1996). The two main approaches that have been used to locate genes that affect carcass and meat quality traits in farm animals are the candidate gene approach and the genome scan approach (Rothschild and Plastow, 1999). The candidate gene approach utilizes knowledge from species that are in genome information (e.g., human, mouse), effects of mutations in other species, and/or knowledge of the physiological basis of traits to identify genes that are thought to play a role in the physiology of the trait (Dekkers et al., 2001).

Recently, the development of the bovine genome map and the extensive physiological analysis of adipocyte differentiation and lipid metabolism regulation resulted in the identification of genes which play a key role in the determination of carcass and meat quality traits. CCAAT/enhencer binding protein (C/EBP) family is a group of transcription factors expressed in the preadipocyte differentiation process. C/EBPα is a transcription factor that contains a conserved carboxyterminal domain (the bZIP), consisting of a region rich in basic amino acids and a flanking leucine zipper domain, that is necessary for DNA binding and dimmer formation (Taniguchi and Sasaki,

* Corresponding Author: E. R. Chung. Tel: +82-33-730-051, Fax: +82-33-730-0503, E-mail: erchung@sangji.ac.kr
[1] Department of Animal Science, College of Agriculture and Life Science, Chonnam National University, Gwangju 500-757, Korea.
Received June 20, 2006; Accepted August 22, 2006

1996). It has been recognized that C/EBPα plays an important role in lipid deposition and adipocyte differentiation (Taniguchi and Sasaki, 1996; Fajas et al., 1998; Morrison and Farmer, 1999; Rosen et al., 2000; Yamamoto et al., 2002). Therefore, the C/EBPα gene is an important candidate gene for the identification of genetic markers for carcass and meat quality traits in beef cattle. In a previous study, nucleotide and amino acid sequences of C/EBPα gene and expression of C/EBPα mRNA in adipose tissue were reported in Korean cattle (Hanwoo) (Jeoung et al., 2004). The objective of this study was to identify single nucleotide polymorphism (SNP) in the C/EBPα gene and examine association of SNP identified in this gene with carcass composition and meat quality traits in Korean cattle.

MATERIALS AND METHODS

Animals and carcass data

Three hundred-nine Korean native steers, which were animals of the 32rd and 33rd progeny test, were used from Hanwoo Experiment Station of the National Livestock Research Institute (NLRI). Genomic DNA was extracted from whole blood by using a NaCl precipitation protocol (Miller et al., 1988). The carcass data included were carcass weight (CW), carcass percentage (CP), M. *longissimus dorsi* area (LDA), backfat thickness (BF) and marbling score (MS).

SNP identification

To detect SNP in C/EBPα gene, primer pairs were designed based on the genomic DNA sequence of the bovine C/EBPα gene from nucleotides 4-1342(1,339 bp) of GenBank accession no. D82984. The C/EBPα gene was amplified by PCR using the following primers: forward primer: 5′-ACAAACCGGTATAAATGCTG-3′ and reverse primer: 5′-AATCTCCTGGTCCTGCTTAC-3′. The PCR reaction was performed in a 20 μl reaction mixture containing 10 pmol of each primer, 1.5 mM $MgCl_2$, 250 μM of each dNTP and 1 unit of *Taq* DNA polymerase, 10× reaction buffer and 50 ng of genomic DNA as template. The PCR conditions were 94°C for 5 min, followed by 35 cycles of 94°C for 30 sec, 50°C for 30 sec and 72°C for 30 sec, with a final extension at 72℃ for 5min. After completion of the PCR reaction, amplified fragment was subjected to sequence analysis.

Cloning and sequencing

The PCR-amplified DNA fragments were eluted from agarose gels using Power Gel Extraction Kit (TaKaRa Co., Japan) and purified with the Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA). For sequencing of the C/EBPα gene, the PCR products were cloned into PCR 2.1 TOPO (Invitrogen B.V., Groningen, The Netherlands) following the manufacturer's protocol. Positive clones were sequenced using an automated DNA sequencer (ABI 377, Perkin-Elmer, Foster City, CA, USA) with BigDye 3.1 reagents.

PCR-RFLP genotyping

For SNP genotyping of C/EBPα gene, we developed a PCR-RFLP procedure for detection of the C/A polymorphism at position 271 of the C/EBPα gene (GenBank accession no. DQ068270). A pair of primers was designed on the basis of the sequence information to amplify a 421 bp fragment from nucleotides 3 to 423, including the SNP under analysis. The PCR amplification was performed using primers sense (5′-GACAAACCGGTATAAATGCT-3′) and anti-sense (5′-GCTGTGTTGGAACAGGTC-3′). The 20 μl reaction mixture contained 50 ng of genomic DNA, 10 pmol of each primer, 10×PCR buffer, 1.5 mM $MgCl_2$, 250 μM of each dNTP and 1.0 unit *Taq* polymerase. Amplification conditions were 94°C for 4 min followed by 35 cycles of 94°C for 30 sec, 55°C for 30 sec and 72°C for 45 sec, with a final extension at 72°C for 5 min in a DNA thermal cycler (Perkin Elmer Cetus, Norwalk, CT). For the RFLP analysis, amplified fragments were digested with restriction enzyme *Sma*I at 25°C for at least 2 h. The digested DNA fragments were separated on 2% agarose gel by electrophoresis with 1×TBE buffer.

Statistical analysis

Allele and genotype frequencies were calculated by simple allele counting method (Falconer and Mackay, 1996). Hardy-Weinberg equilibrium in examined population was tested by comparing expected and observed genotype frequencies using a chi-square test. The association between genotypes of C/EBPα candidate gene and carcass and meat quality traits was evaluated with the least square method (GLM procedure of SAS software package, SAS Institute Inc., 2002) using the following statistical linear model:

$$Y_{ijkl} = \mu + YS_i + P_j + G_k + e_{ijkl}$$

Where Y_{ijkl} is the observation of the carcass traits, μ is the overall mean for each trait, YS_i is the effect of i_{th} year and season of calving, P_j is the effect of j_{th} parity, G_k is the fixed effect of K_{th} SNP genotype and e_{ijkl} is the random residual effect.

Additive effects were estimated by the difference between solutions for the two homozygous genotypes. Dominance effects were estimated by the differences between the solution for the heterozygous genotype and the

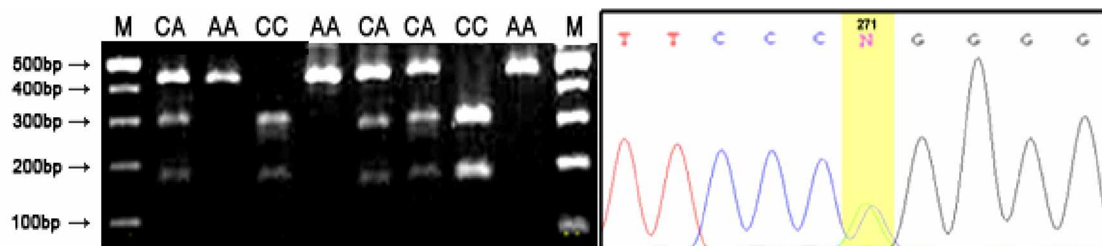

Figure 1. PCR-RFLP and sequence chromatogram for the detection of a SNP of the C/EBPα gene in Korean cattle. SNP with the C/A transition was detected at position 271 (C271A) within coding region (exon) of the C/EBPα gene. M: 100 bp DNA ladder.

Table 1. The observed and expected numbers and percentage of C/EBPα genotypes detected by *Sma*I RFLP analysis and allele frequencies in the Korean cattle population

Number	SNP genotype			Allele frequency	
	C/C	C/A	A/A	C	A
Observed	40	151	118	0.374	0.626
	(12.9)	(49.0)	(38.1)		
Expected	43.2	144.7	121.1		

χ^2 = 0. 590, 0.50<p<0.25.

average of the solutions for the two homozygous genotypes.

RESULTS

SNP identification and marker genotyping

Sequence analysis of PCR products revealed that a point mutation (C/A substitution) was identified at position 271 of the coding region in C/EBPα gene. The sequence data were deposited in the GenBank database with accession number DQ068270. A 421 bp fragment containing the C271A SNP was amplified with a primer pair designed in this study. The C271A SNP could be distinguished by digestion with the restriction enzyme *Sma*I using the PCR-RFLP method. The A allele remained uncut at 421 bp because of the absence of a *Sma*I recognition site, while the C allele, characterized by a *Sma*I restriction site, was cleaved into two fragments of 151 and 270 bp. All three possible SNP genotypes, CC, CA and AA were observed in Korean cattle (Figure 1). Allelic and genotypic frequencies for the C271A SNP detected in this study are shown in Table 1. The frequencies of alleles C and A and were 0.374 and 0.626, respectively. The genotype frequencies for CC, AC and AA were 12.9, 49.0 and 38.1%, respectively, in Korean cattle population. The frequencies of genotype were in agreement with Hardy-Weinberg equilibrium (p>0.05).

Gene-specific SNP marker association analysis

Levels of significance, least squares means and standard errors are presented in Table 2 for the effects of SNP marker in C/EBPα gene on live weight, carcass weight, dressing percentage, backfat thickness, M. *Longissimus dori* area and marbling score. The gene-specific SNP marker association analysis indicated that the C271A SNP marker was significantly associated (p<0.05) with marbling score. Animals with the AA genotype had higher marbling score than those with the AC and CC genotypes. No significant association, however, was detected between any of the marker genotypes and other traits measured in this study.

DISCUSSION

The C/EBP family of transcriptional regulators are critical for the activation of adipogenic genes during differentiation. The C/EBP β and δ isoforms are rapidly induced upon adipocyte differentiation and are responsible for activating the adipogenic regulators C/EBPα and peroxisome proliferator activated receptor γ (PPAR γ), which together activate the majority of genes expressed in differentiating adipocytes (Salma et al., 2006). Thus,

Table 2. Least squares means and standard errors for carcass traits and meat quality of different C/EBPα (C271A) genotype in Korean cattle population

Traits	SNP genotype			p-value	Additive effect	Dominance effect
	CC	CA	AA			
LW (kg)	537.368±11.684	539.444±6.002	554.285±6.805	0.2113	16.917±13.521	12.765±18.081
CW (kg)	309.631±7.485	307.277±3.845	318.125±4.630	0.1715	8.493±8.662	13.201±11.583
DP (%)	57.526±0.353	56.915±0.181	57.362±0.205	0.1489	-0.163±0.408	1.058±0.546
BF (cm)	0.647±0.062	0.615±0.032	0.683±0.036	0.3732	0.036±0.072	0.100±0.097
LDA (cm^2)	74.894±1.886	74.513±0.969	76.000±1.098	0.5936	1.105±2.183	1.866±2.919
MS (1-7)	$1.947±0.285^b$	$1.986±0.146^b$	$2.214±0.166^a$	0.0335*	0.266±0.330	0.189±0.442

LW = Live weight; CW = Carcass weight; DP = Dressing percentage; BF = Backfat thickness; LDA = M. *Longissimus dori* area; MS = Marbling score.
* Effect was significant at p<0.05.
a, b Within a row, means without a common superscript letter differ (p<0.05).

C/EBPα may influence body fat composition and distribution, which are economically important traits in beef cattle (Ihara et al., 2003). Bovine C/EBPα gene, a key regulator of adipogenesis and fat cell function, consists of only one exon (Taniguchi and Sasaki, 1996) and has been mapped on chromosome BTA18q24 (Ihara et al., 1998).

In this study, we identified a SNP (C271A) in the coding region (exon) of the C/EBPα by sequence analysis in Korean cattle. It may be the first time to report the polymorphism of bovine C/EBPα gene. Although the point mutation is located in the coding region, it dose not change the amino acid sequence of the C/EBPα gene. Our sequence (GenBank: DQ068270) was highly homologous to that reported by Taniguchi and Sasaki (1996) in Japanese Black cattle (GenBank: D82984). However, sequence comparison between the two breeds revealed three nucleotide variations: A↔C at position 733, T↔C at position 926 and T↔C at position 1253. The nucleotide substitution at positions 733 and 926 of the coding region in the C/EBPα gene of Korean cattle resulted in the changes from Asparagine (AAC) to histidine (CAC) and from valine (GTC) to alanine (GCC) in the amino acid sequence compared with Japanese Black cattle, respectively. The C to A substitution at the position 271 of C/EBPα gene discovered in this study creates a *Sma*I restriction site (CCC▼GGG). This SNP marker was genotyped by PCR-RFLP technique and its potential effect on carcass and meat quality traits was evaluated in Korean cattle population.

Association analysis revealed that C271A SNP of the C/EBPα gene had significant effects on marbling score related to meat quality in Korean cattle. The AA genotype was associated with higher marbling score compared with the CC genotype. Thus, increasing the frequency of the favorable allele A might be beneficial for the genetic improvement of the meat quality traits such as intramuscular fat deposition in Korean cattle population. The favorable allele A occurred at a high frequency of 63% compared with unfavorable allele C (37%) in the population examined. Because many other traits related to beef quality have not yet been examined, associations between the C271A SNP of the C/EBPα gene and other meat quality traits are to be expected through linked or pleiotropic effects or through nonrandom sampling of animals. In conclusion, although further analysis of other populations should be performed to confirm our results, the association of SNP marker with better marbling score is a very interesting finding and could be used in marker assisted selection to improve meat quality in Korean cattle.

The deposition of intramuscular fat, known as marbling, is an important factor for high beef quality in Korean cattle because it is associated with meat quality and thus makes animals more valuable. Therefore, understanding of the mechanisms of adipogenesis and lipid metabolism will be necessary in order to improve meat quality in beef cattle. However, the physiological and molecular mechanisms involved in marbling are not yet understood at the molecular level, although mapping of appropriate quantitative trait loci (QTL) is currently underway by linkage analysis (Casas et al., 1998; MacNeil and Grosz, 2002). Recently, genomic studies on mammalian species revealed several candidate genes, which may be play a key role in the control of fatness traits. Members of the C/EBP family (C/EBP-α, -β and -δ) as the first transcription factors play a major role in activation of adipocyte genes and adipocyte differentiation (Gregoire et al., 1998). The C/EBPα has been mapped at centromeric region of bovine chromosome 18. In the bovine QTL mapping data (Polineni et al., 2006), a QTL with an effect on carcass quality such as marbling has also mapped at the middle region of the chromosome 18. Therefore, the C/EBPα gene can also be considered as candidate QTL for meat quality, since it is involved in lipid deposition and adipogenesis and located at the same chromosome as the QTL for carcass quality. Fine mapping QTL and identification of causative genes that affect carcass quality traits will greatly enhance the progress in beef cattle breeding programs. The highest expression of C/EBPα mRNA was detected in mature adipocyte and adipose tissue of Korean cattle with 12 and 26 months (Jeoung et al., 2004). Moreover, expression level and polymorphism of adipocyte specific transcription factor such as C/EBPα may also cause phenotypic variation of the fatness traits. Further research will be useful for better understanding information on the effect of the C/EBPα gene polymorphism that regulates intramuscular fat deposition in beef cattle.

ACKNOWLEDGMENTS

This study was supported by a grant (Code# 20050301-034-442-016-01-00) from BioGreen 21 program, Rural Development Administration, Republic of Korea.

REFERENCES

Casas, E., J. W. Keele, S. D. Shackelford, M. Koohmaraie, T. S. Sonstegard, T. P. Smith, S. M. Kappes and R. T. Stone. 1998. Association of the muscle hypertrophy locus with carcass traits in beef cattle. J. Anim. Sci. 76:468-473.

Casas, E., R. T. Stone, J. W. Keele, S. D. Shackelford, S. M. Kappes and M. Koohmaraie. 2001. A comprehensive search for quantitative traits loci affecting growth and carcass composition of cattle segregating alternative forms of myostatin. J. Anim. Sci. 79:854-860.

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.

Dekkers, J. C. M., M. F. Rothschild and M. M. Malek. 2001. Potential and application of marker assisted selection for meat quality. Second International Virtual Conference on Pork Quality. 240-263.

Fajas, L., J. C. Fruchart and J. Auwerx. 1998. Transcriptional control of adipogenesis. Curr. Opin. Cell Biol. 10:165-173.

Falconer, D. S. and T. F. C. Mackay. 1996. Introduction to Quantitative Genetics. 4th ed. Addison Wesley Limited, Edinburg Gate, Harlow Essex, UK.

Gregoire, F. M., C. M. Smas and H. S. Sul. 1998. Understanding adipocyte differentiation. Physiol. Rev. 78:783-809.

Hovenier, R., E. Kanis, T. Van Asseldink and N. G. Westerink. 1993. Breeding for pig meat quality in halothane negative populations-a review. Pig News Info. 14:17N-35N.

Ihara, N., H. Yamakuchi, T. Hirano, H. Takeda, Y. Taniguchi, Y. Sasaki, S. K. Davis, J. F. Taylor, W. Barendse, Y. Sugimoto. 1998. Physical and genetic mapping of bovine CEBPα and PPARγ genes. Anim. Genet. 29:398-400.

Ihara, N., H. Yamakuchi, Y. Taniguchi, Y. Sasaki, G. L. Bennett, S. Kappes and Y. Sugimoto. 2003. Mapping of bovine CEBPD to BTA14q15-17. Anim. Genet. 34:465-476.

Jeoung, Y. H., S. M. Lee, H. Y. Park, D. H. Yoon, S. J. Moon, E. R. Chung and M. J. Kang. 2004. Molecular cloning and mRNA expression of the Hanwoo CCAAT/enhancing-binding Protein α(C/EBPα) gene. J. Anim. Sci. Technol. (Kor.). 46(6):909-916.

Macneil, M. D. and M. D. Grosz. 2002. Genome-wide scans for QTR affecting carcass traits in Hereford x composite double backcross populations. J. Anim. Sci. 80:2316-2324.

Meuwissen, T. H. E. and M. E. Goddard. 1996. The use of marker haplotypes in animal breeding schemes. Genet. Sel. Evol. 28(2):161-176.

Miller, S. A., D. D. Kykes and H. F. Polesky. 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16:1215.

Morrison, R. F. and S. R. Farmer. 1999. Insight into the transcriptional control of adipocyte differentiation. J. Cell Biochem. 32-33 (Suppl.). 59-67.

Polineni, P., P. Aragonda, S. R. Xavier, R. Furuta and D. L. Adelson. 2006. The bovine QTL viewer: a web accessible database of bovine quantitative trait loci. BMC Bioinfomatics 7:283.

Rosen, E. D., C. J. Walkey, P. Puiserver and B. M. Spiegelman. 2000. Transcriptional regulation of adipogenesis. Genes Dev. 14:1293-1307.

Rothschild, M. F. and G. S. Plastow. 1999. Advances in pig genomes and industry applications. AgBiotechNet 1, 1-8.

Salma, N., H. Xiao and A. N. Imbalzano. 2006. Temporal recruitment of CCAAT/enhancer-binding proteins to early and late adipogenic promoters *in vivo*. J. Mol. Endocrinol. 36:139-161.

Taniguchi, Y. and Y. Sasaki. 1996. Rapid communication: Nucleotide sequence of bovine C/EBPα gene. J. Anim. Sci. 74:2554.

Wheeler, T. L., L. V. Cundiff and R. M. Koch. 1994. Effect of marbling degree on beef palatability in *Bos indicus* cattle. J. Anim. Sci. 72:3145-3151.

Yamamoto, H., S. Kurebayashi, T. Hirose, H. Kouhara and S. Kasayama. 2002. Reduced IRS-2 and GLUT4 expression in PPARγ2-induced adipocytes derived from C/EBPβ and C/EBPγ-deficient mouse embryonic fibroblasts. J. Cell Sci. 115:3601-3607.