SINGLE NUCLEOTIDE POLYMORPHISM (SNP) ANALYSIS OF BOVINE CANDIDATE GENES FOR CARCASS AND MEAT QUALITY TRAITS

M. S. A. Bhuiyan[1], S. L. Yu[1], D. H. Yoon[2] and J. H. Lee[1]

[1]Division of Animal Science & Resources, College of Agriculture and Life Sciences, Chungnam National University, Daejeon 305-764, Korea; [2]Division of Animal Genomics and Bioinformatics, National Livestock Research Institute, Suwon 441-350, Korea

Key Words: Candidate gene, SNP, meat quality, beef cattle

Introduction

Carcass and meat quality traits such as marbling, muscle tenderness, meat color and palatability are important to consumers and these factors ultimately determine the market value of beef. Quantitative trait is controlled by a single pair to many pairs of genes and DNA polymorphisms exert a quite variation in animal performance among the different breeds of cattle. Polymorphisms in different candidate genes which are associated with variation in meat quality traits have been described previously (Haegeman *et al.*, 2003; Nkrumah *et al.*, 2005 and Schenkel *et al.*, 2005). In this study, we investigated 5 candidate genes whose effects in cattle or other species on carcass traits have been reported previously. These genes are caveolin (Cav)-1, Cav-2 and Cav-3, cysteine and glycine rich protein 3 (CSRP3) and high mobility group AT-hook 1 (HMGA1).

Caveolins serve as markers and structural proteins for caveolae and their functions include intracellular trafficking of cellular components, lipid homeostasis and signal transduction. Cav-1 and 2 are co-expressed in most cell types, but are enriched in adipocytes, endothelial cells. In contrast, the expression of Cav-3 is restricted in muscles (Williams and Lisanti, 2004). Cav-1 is a major fatty acid binding protein and Cav-1 deficient mice are lean and resistant to diet induced obesity (Razani *et al.*, 2002). Zhu *et al*. (2006) reported Cav-3 gene might be a candidate gene of meat production traits in pig. In another study, Lehnert *et al*. (2006) found CSRP3 gene has putative roles in skeletal muscle of cattle for regulation of myogenic differentiation. Studies showed that HMGA1 gene polymorphisms are associated with growth, fat deposition traits and lean meat content in pig (Kim *et al.*, 2004 and 2006). The objective of the present study was to identify SNPs in 5 different genes and this in turn will help to examine associations of such polymorphisms with carcass and meat quality traits in cattle.

Materials and Methods

Twenty-six animals from six different *Bos taurus* genotypes (Jersey, Hereford, Limousin, Angus, Cross breed and Hanwoo) were used in this investigation. Blood samples were collected in a sampling tube containing heparin anticoagulant and placed on ice for subsequent DNA extraction. Genomic DNA was extracted using QIAprep® Spin Miniprep Kit (Qiagen, USA) according to the manufacturer's instructions. Twenty three primer pairs (five pairs from Cav-1, two pairs each from Cav-2 and 3, six pairs from CSRP3 and eight pairs from HMGA1 gene) were designed from bovine sequence data (GenBank accession no. NC_007302, NC_007320, NC_007324 and NC_007330). The primers used to amplify coding regions of candidate genes also included some parts of intron. The polymerase chain reaction (PCR) amplification was carried out in a 25 µl reaction volume containing ~50 ng of genomic DNA, 1X PCR gold buffer (50 mM KCL, 10 mM Tris - HCl, p^H 8.3), 1.5mM $MgCl_2$, 200µM dNTPs, 0.4 pM of each primer and 1U *Taq* polymerase (Ampli Tag Gold ™, Applied Biosystems, USA). Amplification was performed in a GenAmp 2700 (Applied Biosystems, USA) thermocycler with an initial denaturation at 94^0C for10 min followed by 30-35 cycles of 30 sec at 94^0C, 30 sec at 52-60^0C, 30 sec.-1 min. at 72^0C and a final extension at 72^0C for 10 min.

The PCR products were purified with Accuprep® PCR purification kit (Bioneer, Korea) according to the manufacturer's instructions. The sequencing reactions for some samples in mixed condition were found problematic repeatedly. Therefore, PCR products of some samples were chosen for cloning in order to sequence separately and cloning was performed using TOPO TA cloning kit (Invitrogen, UK) Sequencing reaction was performed by a 3100 automated DNA sequencer (Applied Biosystems, USA). The sequence data were verified using Chromas program (ver. 1.41, McCarthy, 1997). Alignment of multiple sequences was performed with ClustalW program (Thompson *et al.*, 1994) and mutations were scored using MEGA software (ver.3.1, Kumar *et al*., 2004). A set of restriction enzymes were used to digest PCR products of five candidate genes. The resulting fragments were subjected to electrophoresis in 3% agarose gels and bands were visualized under UV light.

Results and Discussion

The sequence information obtained by sequencing of the PCR fragments was used as an initial step to detect polymorphism in meat quality related genes among the six *Bos taurus* genotypes. The obtained sequences were compared with the sequences in the NCBI database (www.ncbi.nlm.nih.gov). In caveolin gene family, four A ↔ G transition mutations were detected at position 33503, 33782, 34233 and 34365 bp in the exon-3 region of Cav-1 gene and the resulting three restriction sites were *Rsa*I, *Hinf*I and *Ale*I or *Sfc*I, respectively. However, no

restriction enzyme was found to detect SNP at 34365 bp position. All of these polymorphisms were silent mutation and found only in Hanwoo. The enzymatic digestion created the following patterns: 21+124+189+522 (allele A) and 21+292+522 (allele B) for *Rsa*I; 341+494 (allele A) and 146+341+348+494 (allele B) for *Hinf*I (Fig. 1B), and 99+295+386 (allele A) and 99+141+154+386 (allele B) for *Ale*I restriction enzyme. Hanwoo and Crossbreed cattle specific mutations were found in both coding and intronic region of Cav-2 gene and revealed two SNPs at position 560 (A ↔ C) and 623 (G ↔ T) in the exon-2. But these were silent mutation and had *Fav*I restriction site only for G to T substitution. In addition, 3 mutations were identified at position of 259 (C ↔ T), 397 (A ↔ G) and 409 bp (C ↔ T) in intron-1 of Cav-2 gene and the following digestion pattern with *Rsa*I enzyme (Fig. 1A) was observed for C to T change at 409 bp: 397 + 284 (allele A) and 397 + 305 + 284 (allele B).

Two silent transition mutations were identified at position 86 (C ↔ T, *Msp*I or *Hpa*II site) and 238 bp (A ↔ G, *Nla*III site) in the exon-1 of Cav-3 gene of all genotypes except Hereford. On the other hand, one SNP (C ↔ T) was detected at 30122 bp in the exon-2 region of the same gene. This silent mutation created no restriction site and was also specific for Hanwoo and Crossbreed cattle. This finding agrees with the previous results reported by Haegeman *et al.* (2003). They found no mutation in the coding region of Cav-3 gene among several European cattle breeds. Furthermore, two other SNPs (C ↔ T, *BsrB*I and *Aci*I site and A ↔ G, *Bcc*I site) were also found in the adjacent intronic 1 region of Cav-3 gene.

For CSRP3 gene, a C to T transition mutation was detected at position 14859 bp in the exon-3 of all studied cattle breeds except Jersey. This was a silent mutation and 560 bp PCR product creates the following digestion pattern (Fig.1C) with *Nla*III restriction endonuclease: 363+197 (allele A) and 172+191+197+363 (allele B). In addition, one A to G mutation was found at 15000 bp in intron-3 (*Aci*I cutting site) and the three others were observed in the intron-4 which as follows: G to A change (18555bp, *Nla*IV site), C to T substitution (18582 bp, no restriction site) and A to G substitution (18952 bp, no cutting site). On the contrary, there was no mutation identified in the coding region of HMGA1 gene. Six SNPs were identified only from the intron-5 region. These mutations included a C to T transition (at position 310 bp and creation of *Hinf*I site); two A to G substitution (719 and 810 bp, *BstN*I and *Hpy*CH4IV restriction site); two T to C change (952 and 1301 bp, *Hpy*CH4IV and *Bbs*I cutting site) and a C to T mutation at position 1432 bp facilitated *Nla*IV cutting site. The PCR-RFLP restriction patterns with *Hpy*CH4IV endonuclease is shown in Fig.1D.

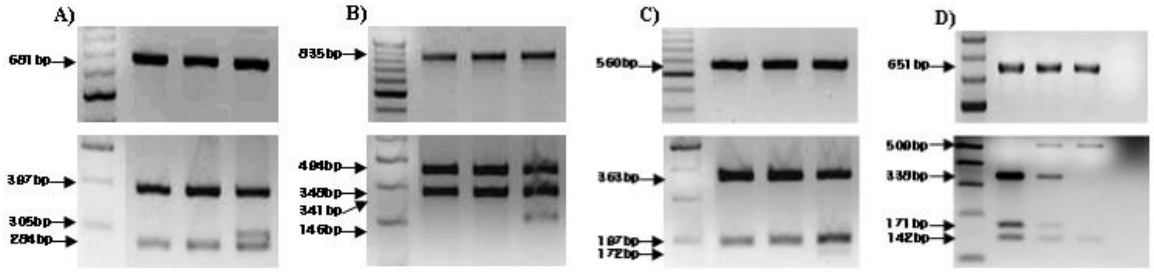

Figure 1. PCR-RFLP patterns of four SNPs in Cav-2 (A), Cav-1 (B), CSRP3 (C) and HMGA1 (D) gene.

Conclusions

Mutations in the gene's coding region are particularly important in studies of the effects of SNPs on gene expressions and protein functions. Our findings identified some silent mutations in the coding regions of caveolin genes family and CSRP3 gene. Moreover, we found Hanwoo specific SNPs in Cav-1, 2 and 3 genes. Thus, the results of our study will be useful in a further search for association between polymorphisms in candidate genes studied and meat quality traits.

References

1. Haegeman, A., Williums, J. L., Law, A., Van Zeveren, A. and Peelman, L. J. (2003). Mapping and SNP analysis of bovine candidate genes for meat and carcass quality. *Animal Genetics, 34*, 349-353.
2. Kim, K. S., Lee, J. J., Shin, H. Y., Choi, B. H., Lee, C. K., Kim, J. J., Cho, B. W. and Kim T.H. (2006). Association of melanocortin 4 receptor (MC4R) and high mobility group AT-hook 1(HMGA1) polymorphisms with pig growth and fat deposition traits. *Animal Genetics, 37*, 419-421.
3. Schenkel, F. S., Miller, S. P.,Ye, X., Moore, S. S., Nkrumah, J. D., Li, C., Yu, J., Mandell, I. B., Wilton, J. W.and Williams, J. L. (2005). Association of single nucleotide polymorphisms in the leptin gene with carcass and meat quality traits of beef cattle. *Journal of Animal Science, 83*, 2009-2020.
4. S. A. Lehnert, S. A., Byrne, K. A., Reverter, A., Natrass, G. S., Greenwood, P. L., Wang, Y. H., Hudson, N. J. and Harper, G. S. (2006). Gene expression profiling of bovine skeletal muscle in response to and during recovery from chronic and severe undernutrition. *Journal of Animal Science, 84*, 3239-3250.
5. Williams, T. M. and Lisanti, M. P. (2004). The caveolin genes: from cell biology to medicine. *Annals of Medicine, 36*, 584-595.
6. Zhu, Z., Li, Y., Mo, D., Li, K., Zhao, S. (2006). Molecular characterization and expression analysis of the porcine caveolin-3 gene. *Biochemical and Biophysical Research Communications, 346*, 7-13.