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SNP Detection of Carboxypeptidase E Gene and Its Association with Meat Quality and Carcass Traits in Korean Cattle

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ABSTRACT : Carboxypeptidase E (CPE) plays an important role in the regulation of the body fat content. Therefore, it has been suggested as candidate gene for traits related to meat quality in beef cattle. This study was conducted to identify single nucleotide polymorphisms (SNPs) in the CPE gene and to investigate association of SNP marker with carcass and meat quality traits in Korean cattle. Three SNPs were identified in the intron 4 (A309G SNP and C445T SNP) and exon 5 (C601T SNP) of the CPE gene by sequence analyses of CPE cDNA and genomic DNA samples. The sequences have been deposited in GenBank database with accession numbers AY970664 and AY970663. Genotyping of the gene-specific SNP marker was carried out using the PCR-RFLP with restriction enzymes *Dde*I for C445T SNP and *Nla*III for C601T SNP. The frequencies of C and T alleles were 0.43 and 0.57 for C445T SNP and 0.42 and 0.58 for C601T SNP, respectively. Statistical analysis indicated that the C445T SNP showed a significant effect ($p<0.05$) on marbling score (MS) and breeding value of backfat thickness (BF-EBV), respectively. Animals with the CT genotype showed higher marbling score and backfat thickness than those with the TT genotype. This marker also showed a significant dominance effect for the MS and BF-EBV ($p<0.05$). However, no significant associations were observed between C601T SNP genotypes and all traits examined. The results suggest that the CPE gene may be used as a marker for carcass traits in Korean cattle. (**Key Words** : Carboxypeptidase E Gene, SNP Marker, Carcass Traits and Meat Quality, Korean Cattle)

INTRODUCTION

Meat quality and carcass composition are the most economically important traits in beef cattle production. Carcass and meat quality traits are typical quantitative characteristics controlled by a number of genes. Mutations in their sequences may alter animal performance as well as their breeding values. However, genes affecting polygenic traits such as meat traits are difficult to identify. Recently, advances in molecular genetic techniques focused on genome analysis open new possibilities for genetic evaluation of economically important traits in farm animals. These molecular technologies allow the isolation and mapping of specific regions of the genome that influence quantitative traits. Marker or gene-assisted selection is a promising strategy for genetic improvement of such traits (Meuwissen and Goddard, 1996). It has been proposed that candidate gene analysis can be used to identify major genes responsible for traits of economic importance (Rothschild and Soller, 1997). The identification of individual genes or anonymous genetic markers associated with such traits could be contributed to an increase rate of genetic gain in beef cattle populations (Ge et al., 2001). The DNA markers could be particularly useful for genetic evaluation of economic traits for which phenotypic measurements or data are difficult or expensive to obtain. A large number of potential candidate genes in livestock have been recognized to date, but relatively little is known about which markers could be useful in evaluation of specific traits. The candidate genes may be selected on the basis of a known relationship between physiological or biochemical processes and production traits (Shin and Chung a, b, 2007).

Carboxypetidase E (CPE) is a prohormone-processing exopeptidase found in secretory granules of endocrine and neuroendocrine cells (Fricker et al., 1986). The CPE plays an important role in the regulation of the body fat content (Konfortov and Miller, 1998). A single point mutation in the CPE gene was identified as the cause of obesity in the *fat/fat* mouse (Naggert et al., 1995). The fat gene encodes CPE, a candidate gene as markers in genetic studies of human obesity (Winick and Friedman, 1998). Therefore,

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Table 1. Overall means and standard deviations (SD), minimum (Min) and maximum (Max) of traits analyzed in this study (n = 309)

Carcass and meat quality traits	Mean	SD	Min	Max
Live weight (kg)	538.22	51.56	390	690
Carcass weight (kg)	307.29	32.56	212	401
Dressing percentage (%)	57.1	1.6	53.1	61.5
Backfat thickness (cm)	0.7	0.29	0.2	1.6
M. *longissimus dorsi* area (cm)	75.47	8.19	57	92
Marbling score (1~7)	2.35	1.43	1	7
Carcass weight-EBV (kg)	1.57	10.21	-22.804	33.076
Backfat thickness-EBV (cm)	0.5	3.42	-8.245	7.48
M. *longissimus dorsi* area-EBV (cm^2)	0.03	0.412	-0.706	0.197
Marbling score-EBV (1~7)	0.06	0.6	-0.281	0.384

Table 2. Primer sequences, amplified region and fragment size for PCR amplification in SNP genotyping of CPE gene

Primer	Primer sequence (5′ - 3′)	Nucleotide substitution	Amplified region	Product size (bp)	Restriction enzyme	GenBank accession no.
CPE 1-F	TCTCCTTACTGTCTTCCCAA	C445T	Intron 4	355	*Dde*I	AY970663
CPE 1-R	TAAGCTTTGGAAGATGTCGT					
CPE 2-F	GTTCACAGTGCCTATTGGAT	C601T	Exon 5	511	*Nla*III	AY970663
CPE 2-R	CCTTCTACAAAGCTGCTGTC					

based on the role of CPE in obesity, the gene encoding CPE was chosen as a candidate gene for association tests with meat traits and quality in beef cattle. The objective of this study was to identify single nucleotide polymorphisms (SNP) in the CPE gene and to investigate the association of these polymorphisms with meat quality and carcass 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). The mean and standard deviations for traits analyzed in this study are presented in Table 1.

SNP discovery

To detect SNP in CPE gene, primer pairs were designed based on the cDNA sequence of the bovine CPE gene from nucleotides 598-817 of GenBank accession no. X04411 that correspond to exons 4 and 5. The CPE gene was amplified by PCR using the following primers: forward primer: 5′-TGCTCTCTGCCAATCTTCA-3′ and reverse primer: 5′-TGGTCGTTCCTTCATCTACAAA -3′. The PCR reaction was performed in a 20 μl reaction mixture containing 10 pmol of each primer, 1.5 mM $MgCl_2$, 200 μM of each dNTP and 1 unit of *Taq* DNA polymerase, 10×reaction buffer and 30 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, 57°C for 30 sec and 72°C for 30 sec. with a final extension at 72°C for 5 min. After completion of the PCR reaction, amplified fragment was subjected to sequence analysis.

Sequence analysis

For sequencing of the CPE 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

We developed a PCR-RFLP procedure for SNP genotyping in the CPE gene. Two different primer pairs were designed to amplify SNP regions that were detected in the cDNA sequence analysis (Table 2). The first primer pair (CPE1) was used to amplify a 355 bp fragment of intron 4 and the second primer pair (CPE2) was used to amplify a 511 bp fragment of exon 5. The PCR reaction contained 30-50 ng of genomic DNA, 10×PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 10 pmol of each primer and 1 unit of *Taq* DNA polymerase in a total volume of 20 μl. The PCR conditions were 5 min at 94°C, followed by 35 cycles of 94°C for 30 sec, 52-57°C for 30 sec, 72°C 45 sec and a final extension 72°C for 5 min. The PCR products were digested with *Dde*I for a fragment of 355 bp and *Nla*III for a fragment of 511 bp. The digested fragments were separated by electrophoresis on 8% non-denaturation polyacrylamide gels stained with silver for a 355 bp and 3% agarose gels stained with ethidium bromide for a 511 bp.

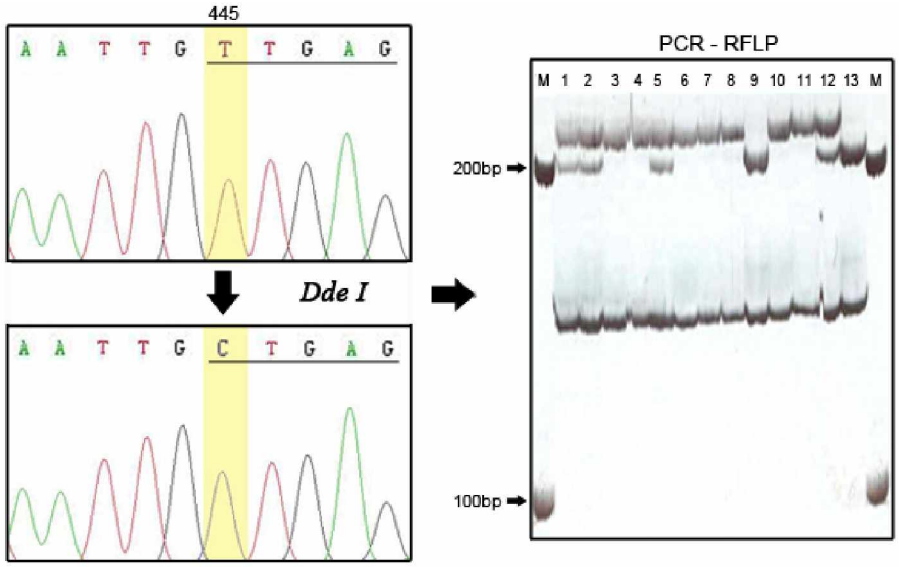

Figure 1. Sequence chromatograms and PCR-RFLP for the detection of the gene specific SNP of the CPE gene in Korean cattle. SNP with the C/T transition was detected at positions 445 (C445T) within intron 4 region of the CPE gene. Lanes 9 and 13: CC genotype; lanes 3, 4, 6, 7, 8, 10 and 11: TT genotype; lanes 1, 2, 5 and 12: CT genotype. M: molecular size marker (100 bp DNA ladder).

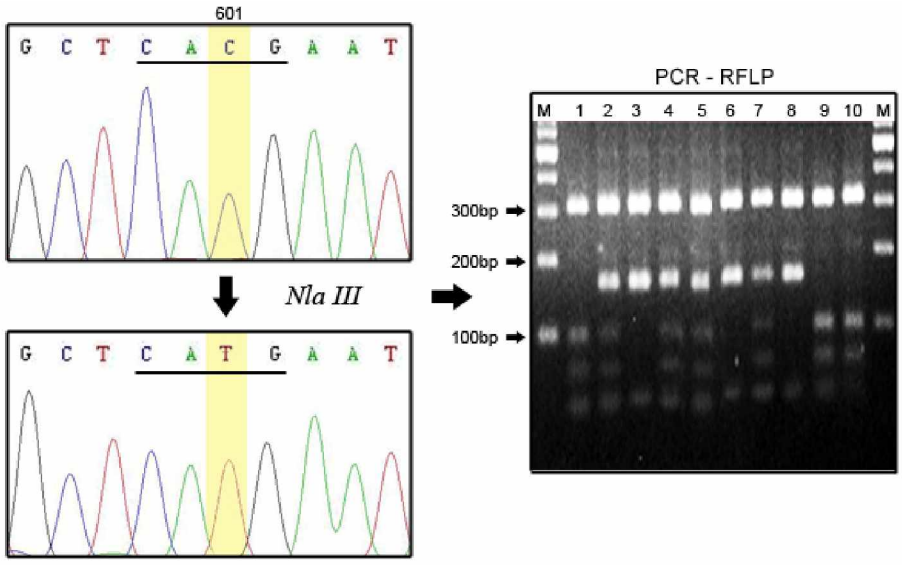

Figure 2. Sequence chromatograms and PCR-RFLP for the detection of the gene specific SNP of the CPE gene in Korean cattle. SNP with the C/T transition was detected at positions 601 (C601T) within exon 5 region of the CPE gene. Lanes 3, 6 and 8: CC genotype; lanes 1, 9 and 10: TT genotype; lanes 2, 4, 5 and 7: CT genotype. M: molecular size marker (100 bp DNA ladder).

Statistical analysis

The association between genotypes of CPE 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 genetic effects were estimated by pair-wise comparison of the two homozygous genotypes and the dominance effects were calculated as the deviation of the heterozygote effect from the average of the two homozygous genotypes. The estimated effects were tested for significance using the t-test.

RESULTS

SNP identification and genotyping

DNA samples from sixty unrelated Korean cattle were amplified and sequenced. We identified three polymorphic sites (SNPs) by sequencing analysis of the bovine CPE cDNA. The two primer pairs were designed for the PCR-RFLP genotyping of these SNPs on genomic DNA samples. The primers amplified a 355 bp fragment in intron 4 and a 511 bp fragment in exon 5, respectively. These SNPs were confirmed by sequencing analysis of the PCR products corresponding to positions 309 (A/G SNP) and 445 (C/T SNP) in intron 4 and 601 (C/T SNP) in exon 5 of the bovine CPE gene. The sequences have been deposited in GenBank database with accession numbers AY970664 and AY970663. These transitions resulted in no changes to the amino acid substitution. Two of these SNPs can be detected by PCR-RFLP using digestion of the amplified fragment with *Dde*I(C$^{\blacktriangledown}$TNAG) and *Nla*III(CATG$^{\blacktriangledown}$) for SNPs at sites 445 in intron 4 and 601 in exon 5, respectively. Two alleles, C and T, showing three different genotypes CC, CT and TT were observed for each RFLP (Figures 1 and 2). In the C445T SNP, the C allele showed three fragments of 13, 144 and 198 bp, while the T allele showed two fragments of 144 and 211 bp. For the C601T SNP, the C allele showed three fragments of 36, 162 and 313 bp, while the T allele showed four fragments of 36, 65, 97 and 313 bp.

The allele and genotype frequencies of the CPE gene estimated for the Korean cattle population are shown in Table 3. In both C445T and C601T SNP, the frequencies of allele T (0.571 and 0.582) were higher than those of allele C (0.429 and 0.418), respectively. The genotypic frequencies

Table 3. Allele and genotype frequencies of CPE gene in Korean cattle

SNP	Genotype frequencies (%)			Allele frequencies		Heterozygosity
	C/C	C/T	T/T	C	T	
C445T	21.1	49.7	29.2	0.429	0.571	0.489
C601T	19.0	45.6	35.4	0.418	0.582	0.486

Table 4. Least squares means and standard errors for carcass traits and meat quality of different CPE (T445C) genotype in Korean cattle

Traits	SNP genotype			p-value	Additive effect	Dominance effect
	C/C	C/T	T/T			
LW (kg)	544.647±9.181	540.867±6.401	550.156±7.090	0.6242	-5.545±11.637	-14.598±17.315
CW (kg)	313.074±5.904	309.720±4.116	313.569±4.559	0.7982	-0.517±7.480	-8.155±11.129
DP (%)	57.426±0.278	57.244±0.194	56.914±0.215	0.3041	0.511±0.351	0.128±0.522
BF (cm)	0.627±0.048	0.636±0.033	0.670±0.037	0.7150	-0.044±0.062	-0.039±0.092
LDA (cm^2)	75.279±1.483	75.610±1.034	74.467±1.145	0.7561	0.809±1.869	1.385±2.781
MS (1~7)	2.053±0.212^{ab}	2.344±0.147^{a}	1.756±0.163^{b}	0.0311*	0.295±0.278	0.791±0.414*
CW-EBV (kg)	5.528±1.834	2.767±1.278	3.705±1.416	0.4686	1.818±2.314	-3.875±3.443
LDA-EBV (cm^2)	1.089±0.625	0.666±0.436	0.056±0.483	0.3980	1.032±0.787	0.206±1.171
BF-EBV (cm)	0.062±0.098^{ab}	0.215±0.068^{a}	-0.105±0.075^{b}	0.0114*	0.166±0.128	0.411±0.191*
MS-EBV (1~7)	-0.057±0.022	-0.030±0.016	-0.009±0.017	0.2566	-0.048±0.029	0.0004±0.043

LWT = Live weight; CWT = Carcass weight; DP = Dressing percentage; BF = Backfat thickness.
LDA = M. *Longissimus dori* area; MS = Marbling score; EBV = Estimated breeding value.
* Effect was significant at $p<0.05$.
^{a,b} Within a row, means without a common superscript letter differ ($p<0.05$).

Table 5. Least squares means and standard errors for carcass traits and meat quality of different CPE (T601C) genotype in Korean cattle

Traits	SNP genotype			p-value	Additive effect	Dominance effect
	C/C	C/T	T/T			
LW (kg)	542.142±9.684	544.155±6.263	547.500±7.106	0.8919	-5.357±12.057	-1.881±17.4170
CW (kg)	311.428±6.221	311.722±4.023	312.076±4.565	0.9962	-0.648±7.742	-0.401±11.18
DP (%)	57.414±0.293	57.246±0.189	56.928±0.215	0.3515	0.485±0.363	0.143±0.524
BF (cm)	0.653±0.050	0.620±0.032	0.676±0.037	0.5204	-0.023±0.064	-0.094±0.092
LDA (cm^2)	75.928±1.555	75.701±1.005	73.980±1.141	0.4512	1.947±1.923	1.463±2.778
MS (1~7)	2.071±0.226	2.223±0.146	1.884±0.166	0.3140	0.186±0.291	0.461±0.420
CW-EBV (kg)	5.388±1.932	3.477±1.250	3.027±1.418	0.6017	2.361±2.395	-1.525±3.460
LDA-EBV (cm^2)	1.441±0.651	0.745±0.421	-0.209±0.478	0.1036	1.651±0.806	0.265±1.164
BF-EBV (cm)	0.041±0.105	0.155±0.068	-0.038±0.077	0.1667	0.0797±0.135	0.294±0.195
MS-EBV (1~7)	-0.0537±0.024	-0.038±0.015	-0.003±0.017	0.1817	-0.050±0.030	-0.021±0.043

LWT = Live weight; CWT = Carcass weight; DP = Dressing percentage; BF = Backfat thickness.
LDA = M. *Longissimus dori* area; MS = Marbling score; EBV = Estimated breeding value.

were as follows: 21.1% CC, 49.7% CT and 29.2% TT for the C445T SNP; 19.0% CC, 45.6% CT and 35.4% TT for the C601T SNP.

Gene-specific SNP marker association analysis

Results of the gene-specific SNP marker association analysis for the CPE gene are presented Table 4 and 5. At the SNP marker of C445T in intron 4, there was a significant effect on the MS and BF-EBV. Animals with the genotype CT had higher MS than animals with TT genotype ($p<0.05$) and higher BF-EBV compared with TT genotype ($p<0.05$). This marker also showed a significant dominance effect for the MS and BF-EBV ($p<0.05$). No significant associations were observed between C445T SNP genotypes and other traits. The association analysis between the C601T SNP in exon 5 and all traits examined in this study showed no significant genotype effects.

Discussion

There is considerable interest in the application of molecular technologies in the form of specific DNA markers that are associated with various QTL to promote more efficient and relatively easy selection and breeding of beef cattle with an advantage for quantitative traits of growth and carcass traits (Ge et al., 2003; Haegeman et al., 2003; Li et al., 2004; Zhao et al., 2004; Buchanan et al., 2005; Nkrumah et al., 2005). Several QTL for performance and meat production in cattle have been identified, and a number of potential candidate genes have been identified and selected for analyses based on a known relationship with physiological or biochemical processes and production traits (Moore et al., 2003; Thaller et al., 2003; Casas et al., 2005; Curi et al., 2005; Schenkel et al., 2005; Maj et al., 2006) Gene encoding the carboxypeptidase is considered candidate gene for traits related to meat quality due to an important role in the regulation of the body fat content. In mice, the pathological obesity condition (fat) gas been proved to be caused by a mutation within this gene (Naggert et al., 1995). The bovine CPE cDNA and genomic DNA sequences reported in this study allowed the identification of three SNPs (A309G and C445T in intron 4 and C601T in exon 5). Two (C445T and C602T SNPs) of these SNPs creat a polymorphic *Dde*I and *Nla*III restriction sites, respectively. In the case of exon 5, although the mutation is located in the coding region, it does not alter the amino acid sequence of the CEP gene. The C602T SNP may not be a causative or close to the causative mutation that affects the carcass and meat quality traits in the populations of Korean cattle examined in this study. With respect to C445T SNP in intron 4, the results presented showed that MS score was 33% higher for CT animals than for TT animals. BF-EBV was 51% higher in CT animals than TT animals. The dominance effect was also significant for MS and BF-EBV. Thus, heterozygous animals were shown to have more desirable marbling score. This may explain the phenomenon of heterosis. Although this mutation is not located in the CPE coding sequence, it is possible that they affect mRNA stability or translation efficiency, resulting in specific biological effects (Kennes et al., 2001). The SNP reported in this study may also act as molecular markers linked to a specific locus which controls carcass and meat quality traits. The CPE gene in bovine is located on chromosome 17 and this gene plays in the inheritance of some beef quality traits (Konfortov and Miller, 1998). Cargill et al. (1998) detected a PCR- RFLP of the porcine CPE gene using the restriction endonuclease *Msp*I. They reported that the observed role of CPE in obesity and fertility phenotypes makes it an interesting candidate gene in the study of body composition and fecundity in pigs and in other mammals. The exact molecular and physiological mechanisms underlying the association of the SNP with the meat quality traits reported in the present study are unknown. This study is the first to investigate the association of CPE gene with carcass and meat quality traits in beef cattle. Although data sets for some of the individual SNP genotype were limited, these results indicate that CPE or a closely linked gene to CPE may be important in carcass fat and meat quality traits in Korean cattle. The results represent the initial association of the polymorphisms with these traits and further efforts are required to validate these findings in other populations before their application in marker-assisted selection. Consequently, this study suggest that the C455T SNP in intron 4 of CPE gene may be used as a genetic marker for selection of higher meat quality in Korean cattle.

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