

## Association between SNP Marker of Uncoupling Protein 3 Gene and Meat Yield and Marbling Score Traits in Korean Cattle

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### Abstract

It is well established that uncoupling protein 3 (UCP3) is expressed largely in skeletal muscle, white adipose tissue and brown adipose tissue and has been suggested to play important roles in regulating energy expenditure, body weight, thermoregulation as well as fatty acid metabolism and obesity. Therefore, the UCP3 gene was selected as a candidate gene for carcass and meat quality traits in Korean cattle. The objective of this study was to identify single nucleotide polymorphisms (SNPs) in the UCP3 gene and to evaluate the association of UCP3 SNP markers with carcass and meat quality traits in Korean cattle. The five exons in the UCP3 gene were sequenced, and ten SNPs were identified. The PCR-SSCP method was then developed to genotype the individuals examined. The g.3076A>G genotype was significantly associated with marbling score (MS) of Korean cattle. Animals with the AA genotype had a higher MS than those with the AG and GG genotypes. No significant associations of the SNP g.3076A>G were observed for any traits. In conclusion, although SNP g.3076A>G, which showed an association with MS, does not cause amino acid changes, this SNP may be used as a DNA marker to select animals that have higher intramuscular fat content.

**Key words:** uncoupling protein 3 gene, single nucleotide polymorphisms marker, meat quality traits, marbling score, Korean cattle

### Introduction

Meat quality is of great importance to the beef industry where the consumer is willing to pay more for high quality products (Gill *et al.*, 2010). Intramuscular fat, so called marbling, is an important factor for evaluation of beef quality, and increasing intramuscular fat is the major target in beef industries in Korea. Deposition of intramuscular fat in beef cattle contributes to meat quality variables, including taste, juiciness, flavor, and tenderness. Therefore, it would be beneficial to producers if a method for ranking animals for economic traits such as marbling and tenderness were developed. However, meat quality traits can usually only be measured post-slaughter, so that making progress using direct measurements is difficult (Gill *et al.*, 2010). Also, the degree of marbling is hard to predict because it is not strongly correlated with subcutaneous fat, so fattening cattle to improve marbling and then

choosing to turn off animals on the basis of the fatness under the skin is not an accurate way to get high marbling scores (Barendse *et al.*, 2009). The accumulation of intramuscular fat is largely influenced by the genetic background of cattle, as well as their age and nutrition (Wang *et al.*, 2009). Recent research has suggested that DNA markers are related to marbling deposition. Thus, it is important to identify candidate genes that can be used as early biomarkers for the prediction of marbling development in cattle. Biomarkers found in various candidate genes linked to economically important traits have been identified and incorporated into commercially available DNA marker tests for meat quality in beef cattle (Gill *et al.*, 2010; Van Eenennaam *et al.*, 2007). Candidate genes are selected based on their potential effects on carcass and meat traits. The possible effects of these genes on carcass and meat traits can be judged based on the known involvement of the gene product in cellular or metabolic processes such as energy homeostasis (Haegeman *et al.*, 2003).

Uncoupling protein 3 (UCP3) is a member of the mitochondrial anion carrier family primarily expressed in skeletal muscle and brown adipose tissue (Boss *et al.*,

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1997; Matsuda *et al.*, 1997). Due to its high homology to UCP1, which plays an important role in adaptive thermogenesis, a primary function of UCP3 was thought to be control of thermogenesis (Katsumata *et al.*, 2004). The genes that codes for novel uncoupling protein play an important role in energy metabolism, and it has been reported that UCP gene was partially involved in the obesity (Fleury *et al.*, 1997; Zhao *et al.*, 2002). In addition, UCP gene also can be regulated by leptin, the production of the obesity gene, which plays an important role in the energy intake and expenditure (Zhao *et al.*, 2005). Therefore, based on the physiological and biochemical roles of UCP3 in fat and energy metabolisms, the gene encoding UCP3 was chosen as a candidate gene for association tests with carcass and meat quality traits in beef cattle. The objective of this study was to identify single nucleotide polymorphisms (SNPs) of the UCP3 gene and to evaluate associations between SNP marker of the UCP3 gene and carcass and meat quality traits in Korean cattle.

## Materials and Methods

### Animals and carcass data

A total of 309 Hanwoo steers produced from 49 sires used in progeny testing program of National Livestock Research Institute (NLRI) of Korea were used in this study. All steers of the national progeny-testing population were fed under the tightly controlled conditions of the feeding program. Meat samples were collected from 13<sup>th</sup> thoracic rib to the first lumbar vertebrae of the steers within 24 h of slaughter and evaluated by mechanical and physical methods according to the Animal Product Grading System of Korea. The carcass data analyzed were live weight (LW), carcass weight (CW), dressing percentage (DP), backfat thickness (BF), M. *Longissimus dorsi* area (LDA) and marbling score (MS). BF and LDA were measured at the 12<sup>th</sup>- and 13<sup>th</sup>- rib interface. MS for quality grade was evaluated on a cross section of the longissimus muscle at the 12<sup>th</sup>- to 13<sup>th</sup>-rib interface by official graders

**Table 1. Mean, standard deviation (SD) and extreme values of phenotypic values measured on each trait in Korean cattle**

Traits	No.	Mean	SD	Minimum	Maximum
LW/kg	309	538.22	51.564	390	690
CW/kg	309	307.29	32.558	212	401
DP/%	309	57.1	1.6	52.6	62.4
BF/cm	309	0.7	0.3	0.2	1.8
EMA/cm <sup>2</sup>	309	75.466	8.195	54	97
MS1/1-7	309	2.35	1.43	1	7
MS2/1-3	309	2.25	0.78	1	3
CW-EBV	309	1.568	10.214	-24.119	33.076
EMA-EBV	309	0.503	3.416	-8.548	8.889
MS-EBV	309	0.062	0.598	-1.062	2.917
BF-EBV	309	0.026	0.412	-0.706	2.226

LW, live weight; CW, carcass weight; DP, dressing percentage; BF, backfat thickness; EMA, M. *Longissimus dorsi* area; MS, marbling score ; EBV, estimated breeding value

according to the Korean Beef Marbling Standard. MS is scored on a scale from 1 to 7 with 7 being associated with the best marbling. The mean and standard deviations for traits analyzed in this study are presented in Table 1. For each animal, genomic DNA was extracted from whole blood by using a NaCl precipitation protocol (Miller *et al.*, 1988). The DNA sample was suspended in TE buffer (10 mM Tris-HCl, pH 7.4; 1 mM EDTA) and stored at -20°C until analysis.

### Sequence analysis and SNP discovery

Three pairs of primers for amplification and sequencing analysis were designed based on the genomic DNA sequence of the bovine UCP3 gene from nucleotides 820-3463 of GenBank accession no. AF127030 that included five exon regions (exons 2-6) to amplify 461-1293 bp fragments using Primer 3 program (<http://frodo.wi.mit.edu/>). The sequences of primers are shown in Table 2. To determine SNP identification, pooled DNA samples from the sixty unrelated animals were amplified by PCR using the each primer pair. The PCR amplification was performed in a DNA thermal cycler (Perkin Elmer Cetus,

**Table 2. Primer pairs used for sequencing and SNP discovery of the UCP3 gene in Korean cattle**

Primer sequences (5'-3')	Location	Fragment size (bp)	Annealing temp. (°C)	GenBank accession no.
F-GCCCGGAGTGCCAGTA R-CGCCAAAATCCGGGTAATG	820-1281	461	54	
F-ACGGGGGCCATGGCAGTGAC R-TCCTCGTGATGTTGGGCAGAAT	1291-2583	1293	60	AF127030
F-TCTGCCCAACATCACGAGGAAT R-CCTGGGGGCGAGTTCATA	2564-3463	900	58	

USA). The PCR reaction was performed in a 20  $\mu$ L reaction mixture containing 10 pmol of each primer, 1.5 mM  $MgCl_2$ , 200  $\mu$ M of each dNTP and 1 unit of *Taq* DNA polymerase, 10 $\times$  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 1 min, 54-60°C and for 1 min and 72°C for 1 min, with a final extension at 72°C for 5 min. The product of the PCR was verified by 2.5% agarose gel electrophoresis and purified with Wizard Prep PCR purification kit (SolGent, Korea). The purified DNA fragments were directly sequenced in both directions using BigDye<sup>TM</sup> Terminator V3.1 Cycle Sequencing kit in an ABI 3130 genetic analyzer (Applied Biosystems, USA). Sequencer Software V5.1 (Applied Biosystems) was used to assemble the sequences and to identify polymorphisms.

#### SNP genotyping using PCR-SSCP

Genotyping of the SNP located at position g.3076A>G (numbering according to GenBank database accession no. AF127030) within intron V region of the UCP3 gene was performed by a PCR-single strand conformation polymorphism (SSCP) method, because no restriction site exists for this SNP. The PCR amplification was carried out using forward (5'-ACTGCAAGAGATTTGGAAAA-3') and reverse (5'-TCCGTCAAATCTGACTCTTT-3') primers designed for amplification of 230 bp fragment including SNP g.3076A>G site. The 20  $\mu$ L reaction mixture contained 50 ng of genomic DNA, 0.1  $\mu$ M of each primer, 2  $\mu$ L 10 $\times$  PCR buffer, 1.5 mM  $MgCl_2$ , 250  $\mu$ M of each dNTP and 1 unit *Taq* polymerase. Amplification conditions were 94°C for 5 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 30 s, with a final extension at 72°C for 5 min in a DNA thermal cycler (Perkin Elmer Cetus). After PCR amplification, 1  $\mu$ L of PCR product was mixed with 4  $\mu$ L of gel loading solution containing 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol. The mixture was then denatured at 95°C for 5 min, cooled on ice for 10 min and loaded on non-denaturing 12% polyacrylamide gels (49:1 acrylamide to bis-acrylamide). Electrophoresis was performed in 1 $\times$  TBE buffer at 250 V for 4-6 h at room temperature. After electrophoresis, the DNA fragments in the gel were detected by silver staining. To define each genotype according to band patterns, the PCR products of different SSCP patterns corresponding to each genotype were sequenced and analyzed for nucleotide changes.

#### Statistical analysis

Allele and genotype frequencies were calculated by simple allele counting method. Hardy-Weinberg equilibrium (HWE) in examined population was tested by comparing expected and observed genotype frequencies using a Chi-square test. The PROC GLM procedure of SAS (Release 9.1, SAS, Inst. Inc., Cary NC, 2008) was used to test the association between SNP marker genotypes of the UCP3 gene and carcass and meat quality traits. The linear model used was as follows:

$$Y_{ijklm} = \mu + S_i + YS_j + SP_k + A_l + G_m + e_{ijklm}$$

Where  $Y_{ijklm}$  is the observation of the carcass traits,  $\mu$  is the overall mean for each trait,  $S_i$  is the effect of sire,  $YS_j$  is the effect of  $i_{th}$  year and season of calving,  $SP_k$  is the effect of slaughter place,  $A_l$  is the effect of age at slaughter (covariate),  $G_m$  is the fixed effect of SNP genotype and  $e_{ijklm}$  is the random residual effect. Significance differences among mean values of different genotypes were calculated using Duncan's multiple range test, and determined to be statistically significant at  $p < 0.05$ .

The additive and dominance effects were also estimated using REG procedure of SAS version 9.2, where the additive effect was denoted as -1, 0 and 1 for AA, AG and GG, respectively, and the dominance effect was denoted as 1, -2 and 1 for AA, AG and GG, respectively.

## Results

#### SNP identification in the Hanwoo UCP3 gene

For SNP detection of the UCP3 gene in Hanwoo, we sequenced five regions from exon II to exon VI of the UCP3 gene. Ten nucleotide substitutions were identified, which were one (g.1099G>A) in intron III, six (g.1594A>C, g.1638G>A, g.1794Del(A), g.1796A>G, g.1801Del(T) and g.2457T>C) in intron IV, and three (g.2689C>T, g.2966A>C and g.3076A>G) in intron V (Fig. 1).

#### SNP genotyping by using PCR-SSCP

Genotyping of the SNP located at position g.3076A>G within the intron V region of the UCP3 gene was performed by a PCR-SSCP method (Fig. 2). The allele and genotype frequencies of the UCP3 gene estimated for the Hanwoo population are shown in Table 3. In g.3076A>G SNP, the frequency of allele A (53.1%) was higher than that of allele G (46.9%). In the genotype frequencies, the heterozygous genotype AG showed higher frequency (53.1%) compared to either homozygous genotype AA or GG, whereas the two homozygous genotypes AA and GG

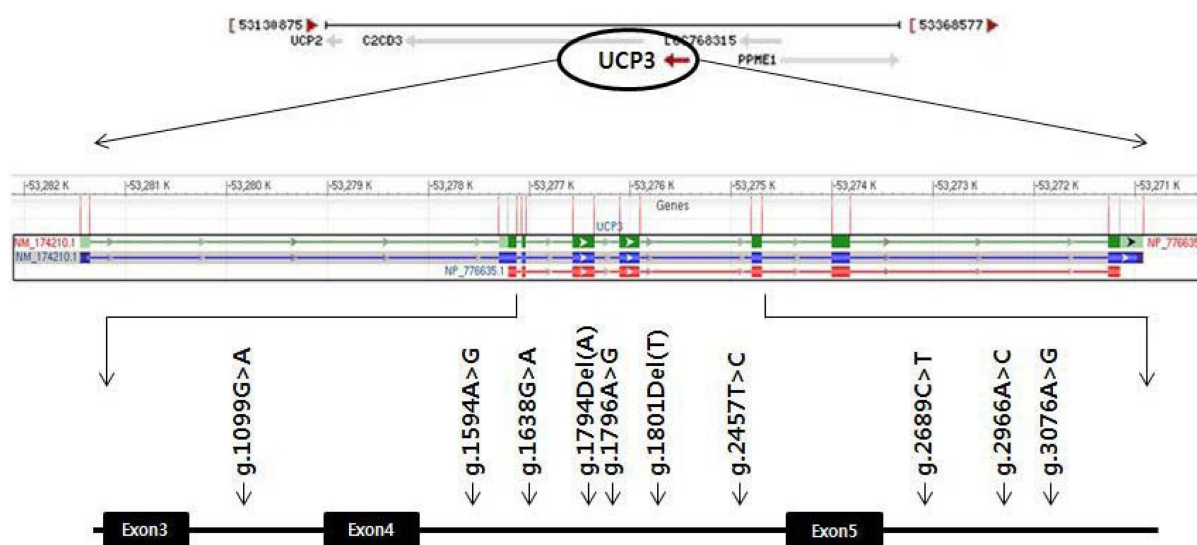


Fig. 1. Map position of SNPs identified in the UCP3 gene of Korean cattle. The SNPs were detected in the introns 3, 4, and 5, respectively.

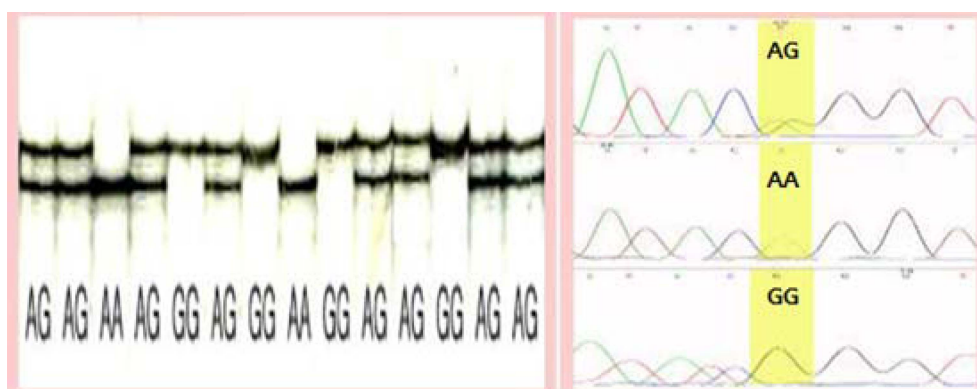


Fig. 2. PCR-SSCP analysis and sequence chromatograms for the detection of the gene specific SNP of the UCP3 gene in Korean cattle. SNP with the A>G substitution were detected at position 3076 (g.3076A>G) within intron 5 region of the UCP3 gene.

Table 3. Genotype and allele frequencies for g.3076A>G marker of the UCP3 gene in Korean cattle

SNP marker	No. of animals	Frequencies (%)					He <sup>1)</sup>	PIC <sup>2)</sup>	HWE <sup>3)</sup>	
		Genotype			Allele				ccc <sup>2</sup>	p-value
UCP3 g.3076A>G	309	AA	AG	GG	A	G	0.606	0.537	1.329	0.514
		26.5	53.1	20.4	53.1	46.9				

<sup>1)</sup>heterozygosity

<sup>2)</sup>polymorphic information content

<sup>3)</sup>Hardy-Weinberg equilibrium

had similar frequencies (26.5 and 20.4%, respectively). The observed genotype distributions were in good agreement with those expected according to the Hardy-Weinberg equilibrium in this population.

**Association of gene-specific SNP marker with carcass traits**

Results of the SNP markers association analysis are pre-

sented in Table 4. The SNP marker of g.3076A>G was significantly associated ( $p < 0.0209$ ) with the MS. Animals with the AA ( $2.538 \pm 0.194$ ) genotype had higher MS than those with the AG ( $1.910 \pm 0.137$ ) and GG ( $1.866 \pm 0.222$ ) genotypes. This SNP genotype also showed a significant additive genetic effect for the MS ( $p < 0.05$ ). No significant associations, however, was detected between any of the SNP genotype and other carcass traits measured in this study.

**Table 4. Associations of UCP3 SNP g.3076A>G variation with carcass and meat quality traits in Korea cattle**

Traits	SNP genotype (mean±SE)			p-value	Genetic Effects (mean±SE)	
	AA (n=82)	AG (n=164)	GG (n=63)		Additive	Dominance
LW/kg	539.487±8.221	548.205±5.813	543.000±9.373	0.6719	-3.512±12.467	-13.923±17.047
CW/kg	309.461±5.278	313.615±3.732	309.700±6.018	0.7587	-0.238± 8.005	-8.069±10.946
DP/%	57.346±0.248	57.153±0.176	56.956±0.283	0.5862	0.389± 0.377	-0.004± 0.516
BF/cm	0.633±0.043	0.665±0.031	0.610±0.050	0.6113	0.023± 0.066	-0.087± 0.091
EMA/cm <sup>2</sup>	76.743±1.312	74.512±0.927	74.633±1.495	0.3587	2.110± 1.989	2.351± 2.720
MS/1-7	2.538±0.194 <sup>a</sup>	1.910±0.137 <sup>b</sup>	1.866±0.222 <sup>b</sup>	0.0209*	0.671± 0.295*	0.584± 0.404

LW, live weight; CW, carcass weight; DP, dressing percentage; BF, backfat thickness; EMA, M. *Longissimus dorsi* area; MS, marbling score

\*Effect was significant at  $p < 0.05$ .

<sup>a,b</sup> Within a row, means with different superscript letter differ ( $p < 0.05$ ).

## Discussion

Intramuscular fat content, commonly called marbling, refers to the visually discernible deposits of fat within muscle. The intramuscular fat is generally considered to increase juiciness and tenderness of meat, and therefore to have a positive association with eating quality (Gutierrez-Gil *et al.*, 2008; Wood *et al.*, 1995). Increased marbling improves the palatability and acceptability of the meat and is an economically important trait of beef cattle in Korea. A better knowledge of the molecular mechanism of marbling is important as it may generate new opportunities for more effective marker assisted selection, leading to economic benefits to the beef industry (Yamada *et al.*, 2009). Recently, the development of genomics has stimulated interest in improving meat quality. DNA markers have been reported in several genes responsible for carcass and meat quality traits in cattle and pigs (Gill *et al.*, 2010; Maj *et al.*, 2006; Otto *et al.*, 2007; Plastow *et al.*, 2005). Genes that regulate metabolism and energy partitioning have the potential to influence economically important traits in farm animals, as do polymorphisms within these genes (Sherman *et al.*, 2008).

The UCP3 is a member of the mitochondrial anion carrier superfamily with high homology (57%) to UCP1, a well characterized uncoupling protein. Uncoupling protein genes family has five members that named UCP1, UCP2, UCP3, BMCP1, and UCP4 (Vidal-Puig *et al.*, 2000). The tissue distribution of UCP1, UCP2, and UCP3 is markedly different. UCP1 is expressed only in mitochondria of brown adipose tissue where it plays a central role in heat production and thermoregulation by uncoupling respiration from ATP synthesis (Stone *et al.*, 1999). UCP2 is expressed in a variety of tissues, whereas UCP3 is expressed largely in skeletal muscle, white adipose tissue and brown adipose tissue (Fleury *et al.*, 1997; Vidal-

Puig *et al.*, 1997). Thus, UCP2 and UCP3 may have an important roles in determining feed conversion and /or maintenance energy requirements in cattle (Stone *et al.*, 1999). This energy expenditure mechanism can be associated with the metabolism of fat, regulation of body weight and obesity (Fleury *et al.*, 1997; Vidal-Puig *et al.*, 2000; Zhao *et al.*, 2005). Therefore, UCP3 gene has been suggested to play important roles in regulating energy expenditure, body weight and thermoregulation including regulation of fatty acid metabolism. It has also been proposed that UCP3 could prevent the formation of oxygen-free radicals in skeletal muscle (Vidal-Puig *et al.*, 2000).

In this study, we identified ten novel SNPs within the UCP3 gene and the g.3076A>G SNP markers were evaluated with respect to their effects on several carcass and meat quality traits in Korean cattle. A significant difference ( $p < 0.021$ ) was obtained for marbling score between SNP genotypes of g.3076A>G marker but not for other measured traits. The animals with homozygous genotype AA showed a significantly higher marbling score compared with those of heterozygous genotype AG or homozygous genotype GG. The results of this study give strong evidence for the potential for marker assisted selection for meat quality, especially intramuscular fat content. Therefore, the g.3076A>G SNP marker of UCP3 gene can be used for substantial improvement of meat quality traits in Korean cattle. However, this UCP3 SNP marker did not contributed to other traits such as live weight, carcass weight, dressing percentage, backfat thickness, and M. *Longissimus dorsi* area.

As a first step in assessing the potential role of UCP3 in economically important traits of livestock such as feed conversion and maintenance energy requirements as well as fat metabolism. Stone *et al.* (1999) first identified five SNPs within the UCP3 gene of cattle: 679, T/C, intron 2; 820, G/A, exon 3; 1099, G/A, intron 3; 2967, A/C, intron

5; 3076, A/G, intron 5 (GenBank accession no. AF127030). Among the ten SNPs identified in our study, 3 SNPs (g.1099G>A, g.2966A>C and g.3076A>G) are identical to a previously reported SNPs on this UCP3 gene. However, seven SNPs (UCP3g.2689C>T, UCP3g.2457T>C, UCP3 g.1801Del(T), UCP3g.1796A>G, UCP31794Del (A), UCP3g.1638G>A and UCP3g.1594A>C) detected in this study were recognized as new SNPs identified in Korean cattle. It was found that UCP3 gene was assigned to BTA15, a chromosome previously shown to harbor a locus influencing meat tenderness (Stone *et al.*, 1999). Sherman *et al.* (2008) reported that g.1099G>A SNP marker, which was previously reported (Stone *et al.*, 1999), showed associations with average daily gain ( $p=0.025$ ), feed conversion ratio and partial efficiency of growth ( $p<0.01$ ) in beef cattle, and three SNPs in UCP2 gene showed associations with lean meat yield, yield grade and dry matter intake ( $p<0.05$ ). These results are consistent with the possible functions of these genes. However, there was no significant association between the g.1099G>A SNP marker and meat quality traits such as carcass marbling score, M. *Longissimus dorsi* area, backfat thickness and quality grade. Haegeman *et al.* (2003) selected the UCP2 gene as a bovine candidate gene based on potential effects on carcass and meat traits. On the other hand, Zhao *et al.* (2002) reported that SNPs in the 3'-UTR region of chicken UCP gene was associated with variation of weight as well as percentage of abdominal fat. In addition, Oh *et al.* (2006) identified ten SNPs within the UCP gene of the Korean native chicken and showed association of the SNP markers with daily percent lay and mean body weight. Also, SNP markers encompassing the location of UCP2/UCP3 in humans have been associated with resting metabolic rate, body mass, body fatness and fat mass (Sherman *et al.*, 2008). Even though these SNPs in the intron regions of UCP3 gene do not cause amino acid changes, the functions show that UCP gene could be affecting fatness and may be an important candidate gene controlling variation on energy metabolism, growth and body composition traits, especially the fatness traits in domestic animals. In conclusion, SNPs in UCP3 gene were shown to be useful as genetic markers in the selection of Korean cattle with superior meat quality traits. Further studies are necessary to determine the effect of several other SNP markers of UCP3 gene on carcass and meat quality traits.

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