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# Identification and analysis of *MC4R* polymorphisms and their association with economic traits of Korean cattle (Hanwoo)

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**Abstract** Signaling by the melanocortin-4 receptor (MC4R) is important for mediation the effect of leptin on food intake and energy homeostasis, and is associated with obesity, energy homeostasis and control of feeding behavior. Presently, the bovine *MC4R* gene was characterized to detect genetic variation at this locus and to relate it to economic traits in Korean cattle (Hanwoo). Five single nucleotide polymorphisms (SNPs) were identified in the coding region (G709A, C927T, C1069G, C1343A, and C1786T). G709A changed amino acid 166 of the *MC4R* protein from valine to methionine and C1069G changed amino acid 286 of the *MC4R* protein from leucine to valine. A SNP at C927T significantly influenced the Marbling score, SNP markers C1069G and C1343A significantly affected the Backfat thickness, and the SNP marker C1786T significantly influenced backfat and Marbling score. The *MC4R* gene may thus be a candidate gene for carcass traits with *MC4R* SNPs being potentially valuable as genetic markers for economic traits in Hanwoo.

**Keywords** Korean cattle · Hanwoo · *MC4R* · Single nucleotide polymorphism · SNP · Economic trait

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## Introduction

The Hanwoo (*Bos taurus coreanae*), is a cattle breed that is native to the Korean peninsula and the Japanese islands, which is considered to belong to the European cattle (*Bos Taurus*) breeds [1–3]. In Korea, consumer demands are driving efforts to increase meat production and produce higher quality meat [4]. Breeding and selection of founders with high potential for meat production/quality is incorporating molecular approaches, in particular the identification of selection markers [5]. Knowledge of genetic polymorphisms that are involved in different quantitative trait phenotypes of quantitative traits, and increased understanding of how these polymorphisms interact with the environment or with other genes affecting economic traits is essential [6]. In particular, the identification of genetic markers associated with such traits could contribute to an increased rate of genetic gain in farmed animals. The application of marker-assisted selection in the cattle is a promising strategy for genetic improvement of economic traits [7].

Melanocortin-4 receptor (MC4R) is a G-protein-coupled receptor with seven transmembrane domains that is highly expressed in the hypothalamus, a region of the brain intimately involved in appetite regulation [8]. MC4R signaling is important for mediating the effect of leptin on food intake and energy homeostasis [9]. It is associated with obesity, energy homeostasis and control of feeding behavior. In bovines, *MC4R* is located on chromosome 24; the gene has a length of 1,808 bp and one exon (GenBank accession No. NC_007325).

Many of the identified mutations in the *MC4R* gene are associated with obesity, energy expenditure, and serum triglyceride levels in human and animals. Several frameshift and nonsense mutations are associated with dominantly

**Table 1** Primer sequences, amplified region and fragment size for PCR amplification in SNP genotyping of *MC4R* gene

| Primer | Primer sequences (5–3′) | SNPs | Location |
|---|---|---|---|
| MC4R_3_F | GCTCTTTGTCTCTCCCGAGG | G709A | Exon1 |
| MC4R_3_R | GCCAGCATGGTGAAGAACAC | | |
| MC4R_4_F | GTCGGGCGTCTTGTTCATC | C927T/C1069G | Exon1 |
| MC4R_4_R | GCTTGTGTTTAGCATCGCGT | | |
| MC4R_5_F | TGCAATTCCATCATTGACCC | C1343A | Exon1 |
| MC4R_5_R | AGCAAGCAAAGTGTCACATCC | | |
| MC4R_6_F | TCCACATCACAGGTTATAGGCAC | C1786T | Exon1 |
| MC4R_6_R | TCCCAAATTGCCTGTGAGAA | | |

inherited obesity in humans [8, 10, 11]. In pigs, a functional mutation (Asp298Asn) in a highly conserved region has been detected in the MC4R peptide [11]. Single nucleotide polymorphism (SNP) markers of the bovine *MC4R* gene have been detected [9, 12–14]. A SNP of C1069G was shown to be significantly associated with live weight (LW), carcass weight (CW), backfat thickness (BF), and Marbling score (MS) in Qinchuan cattle [15]. SNPs in the coding region of *MC4R* in Hanwoo have not been reported and were presently studied.

## Materials and Methods

### Animals and data collection

Ninety-four cattle consisted of 57 head of Korean cattle and 37 head of Angus cattle. The animals were collected from progeny test at two Korean stations (Korean Cattle Improvement Center, Agricultural Co-operative Federation; National Livestock Research Institute, Rural Development Administration). Genomic DNA was isolated from sperm and white blood cells that was used to genotype the *MC4R* genes.

The weight at slaughter was recorded as the carcass weight (CW) at slaughter age. Backfat thickness (BF) and longissimus muscle dorsi area (LMA) were measured at the 12th- and 13th- rib interface. Marbling score (MS) was evaluated on a cross section of the longissimus muscle at the 12th- to 13th-rib interface. MS is scored on a scale from 1 to 7 with 7 being associated with the most marbling. The estimated breeding value (EBV) based on measurements of performance, using phenotypic values on a number of its relatives. The overall means ± standard deviations of the analyzed traits are shown in Table 3.

### SNP identification and genotyping

Four pairs of primers were designed based upon bovine *MC4R* gene sequences using Primer 3 software (http://www-genome.wi.mit.edu/cgi-bin/primer3-www.results.cgiv) (Table 1). Polymerase chain reaction (PCR) was performed in 20 μl volumes, each containing 50 ng of genomic DNA, 10× PCR buffer (100 mM Tris pH 8.9, 50 mM KCl, 15 mM MgCl, 0.01% gelatin, 0.1% Triton X-100, 10 mg/ml bovine serum albumin), 10 pmol of each primer, 40 $\mu$M of dNTPs and 0.5 unit Taq DNA polymerase (GeNet Bio, Korea). PCR conditions were 94°C for 4 min and 35 cycles of 30 s at 94°C, 30 s at 56.4°C, 30 s at 72°C, and a final step of 10 min at 72°C using a Peltier Thermal Cycler 200 (MJ Research, USA). DNA sequencing was performed on an ABI 3130 Genetic Analyzer (Applied Biosystems, USA). Searching sequence mutation was using the SeqMAN II software (DNA Star, USA).

### Statistical analyses

Allele and genotype frequencies were calculated by a simple allele counting method. Hardy–Weinberg equilibrium was tested by comparing expected and observed genotype frequencies using a Chi-square test. The association between the genotypes of *MC4R* candidate gene and economic traits was evaluated with the least square method (GLM procedure of the SAS software package; SAS Institute, USA) using the following statistical linear model:

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

where $Y_{ijkl}$ is the observation of the carcass traits, $\mu$ is the overall mean for each trait, $YS_i$ is the effect of ith year and season of calving, $P_j$ is the fixed effect of jth parity, $M_k$ is the fixed effect of Kth SNP genotype, and $e_{ijkl}$ is the random residual effect [16].

## Results and Discussion

The DNA samples from 94 unrelated cattle were amplified and sequenced for the bovine *MC4R* gene. Five polymorphic sites (SNPs) were identified by sequencing analysis of the bovine DNA. The four primer pairs of the *MC4R* gene were designed for the SNP genotyping of these SNPs on

genomic DNA samples. Five SNPs (G709A, C927T, C1069G, C1343A, and C1786T) were detected in the exon 1 region (Fig. 1). C927T, C1343A, and C1786T were synonymous mutations, whereas G709A and C1069G were missense mutations. G709A displayed a change in amino acid 166 of the MC4R protein from valine (Val) to methionine (Met). C1069G exhibited a change of amino acid 286 of MC4R from leucine (Leu) to Val. Previous studies have detected several SNPs (C-293G, A-193T, T-192G, A-129G, T-84C, C927T, C1069G, Val145Ala, Ala172Thr) in the *MC4R* gene of cattle, and have linked the SNPs to growth traits [12–15, 17].

Estimated *MCR4* allele and genotype frequencies for the Hanwoo and Angus populations are shown in Table 2. The G709A frequency for allele G was higher than that for allele A. Among Hanwoo and Angus, 45.5 and 82.4% displayed the GG phenotype, respectively, and 54.5 and 17.6%, respectively, displayed the GA genotype. The


**Fig. 1** Structure of the cattle *MC4R* gene. The *MC4R* gene has a length of 1,808 bp (GenBank accession No. NC_007325)

C1069G frequency for allele G was higher than that for the C allele in Hanwoo. Moreover, 19.3, 42.1, and 38.6% displayed the CC, CG, and GG genotype, respectively. Among Angus, 57.1, 40.5, and 2.4% displayed the CC, CG, and GG genotype, respectively.

The genotypes of the 94 individuals were compared with their EBV. EBV is an estimate of the ability of an individual to produce superior offspring. Therefore, EBV is a useful tool to quicken the progress of breeding in animal [18]. The overall means ± standard deviations of the analyzed traits are summarized in Table 3.

To investigate the effects of SNPs, the association of the *MC4R* genotypes was analyzed to determine the effects on variation in the economic traits in Hanwoo. The SNP marker of C927T significantly affected the MS. Hanwoo cattle with a CT genotype displayed a significantly higher MS than genotype CC ($P < 0.05$) (Table 4). In both SNP markers of C1069G and C1343A, there was a significant effect on the BF. Hanwoo with the CC genotype had a higher BF than genotype GG in SNP markers of C1069G ($P < 0.05$) (Table 5). Hanwoo with the CA genotype had a higher BF than genotype CC in the SNP markers of C1343A ($P < 0.05$) (Table 6). The SNP marker of C1786T

**Table 3** Overall means ± standard deviations (SDs), minimum (Min) and maximum (Max) of traits analyzed in this study

| Economic traits | Mean± | Min | Max |
|---|---|---|---|
| CW (kg) | 3.35 ± 4.92 | −5.73 | 14.34 |
| LMA ($cm^2$) | 1.40 ± 1.29 | −1.29 | 3.51 |
| BF (cm) | −0.13 ± 0.58 | −1.29 | 0.73 |
| MS (1–7) | 0.15 ± 0.37 | −0.60 | 0.86 |

**Table 2** Genotype frequencies and allele frequencies of *MC4R* gene

| SNPs | Breeds | Amino acid change | Genotype frequencies (%) | | | Allele frequencies (%) | |
|---|---|---|---|---|---|---|---|
| G709A | | | GG | GA | AA | G | A |
| | Hanwoo | Val166Met | 45.5 | 54.5 | 0.0 | 72.7 | 27.3 |
| | Angus | | 82.4 | 17.6 | 0.0 | 91.2 | 8.8 |
| C927T | | | CC | CT | TT | C | T |
| | Hanwoo | | 64.9 | 31.6 | 3.5 | 80.7 | 19.3 |
| | Angus | | 100.0 | 0.0 | 0.0 | 100.0 | 0.0 |
| C1069G | | | CC | CG | GG | C | G |
| | Hanwoo | Leu286Val | 19.3 | 42.1 | 38.6 | 40.4 | 59.6 |
| | Angus | | 57.1 | 40.5 | 2.4 | 77.4 | 22.6 |
| C1343A | | | CC | CA | AA | C | A |
| | Hanwoo | | 88.5 | 11.5 | 0.0 | 94.2 | 5.8 |
| | Angus | | 100 | 0.0 | 0.0 | 100 | 0.0 |
| C1786T | | | CC | CT | TT | C | T |
| | Hanwoo | | 57.6 | 39.3 | 3.1 | 77.3 | 22.7 |
| | Angus | | 92.9 | 0.0 | 7.1 | 92.9 | 7.1 |

**Table 4** Least squares means and standard errors for economic traits of *MC4R* (C927T) genotype in Hanwoo

| Traits | SNP genotype | | | |
|---|---|---|---|---|
| | CC (mean ± SE) | CT (mean ± SE) | TT (mean ± SE) | *P* value |
| CW (kg) | 5.400 ± 1.317 | 2.242 ± 2.040 | −4.070 ± 4.562 | |
| LMA ($cm^2$) | 1.495 ± 0.427 | 1.538 ± 0.661 | 0.280 ± 1.478 | |
| BF (cm) | 0.028 ± 0.155 | −0.478 ± 0.240 | −0.540 ± 0.537 | |
| MS | $0.002^{a}$ ± 0.090 | $0.438^{b}$ ± 0.139 | $0.600^{ab}$ ± 0.312 | 0.0189 |

[a,b] Different superscripts within columns differ significantly ($P < 0.05$)

**Table 5** Least squares means and standard errors for economic traits of *MC4R* (C1069G) genotype in Hanwoo

| Traits | SNP genotype | | | |
|---|---|---|---|---|
| | CC (mean ± SE) | CG (mean ± SE) | GG (mean ± SE) | *P* value |
| CW (kg) | 4.330 ± 2.544 | 6.108 ± 2.544 | 3.016 ± 1.610 | |
| LMA ($cm^2$) | 1.485 ± 0.751 | 1.685 ± 0.751 | 1.323 ± 0.475 | |
| BF (cm) | $0.418^{a}$ ± 0.237 | $-0.033^{ab}$ ± 0.237 | $-0.413^{b}$ ± 0.150 | 0.010 |
| MS | −0.005 ± 0.191 | 0.143 ± 0.191 | 0.226 ± 0.121 | |

[a,b] Different superscripts within columns differ significantly ($P < 0.05$)

**Table 6** Least squares means and standard errors for economic traits of *MC4R* (C1343A) genotype in Hanwoo

| Traits | SNP genotype | | |
|---|---|---|---|
| | CA (mean ± SE) | CC (mean ± SE) | *P* value |
| CW (kg) | 2.760 ± 3.592 | 4.149 ± 1.270 | |
| LMA ($cm^2$) | 1.725 ± 1.031 | 1.404 ± 0.365 | |
| BF (cm) | $0.700^{a}$ ± 0.344 | $-0.249^{b}$ ± 0.122 | 0.019 |
| MS | 0.095 ± 0.270 | 0.164 ± 0.095 | |

[a,b] Different superscripts within columns differ significantly ($P < 0.05$)

**Table 7** Least squares means and standard errors for economic traits of *MC4R* (C1786T) genotype in Hanwoo

| Traits | SNP genotype | | | |
|---|---|---|---|---|
| | CC (mean ± SE) | CT (mean ± SE) | TT (mean ± SE) | *P* value |
| CW (kg) | 5.510 ± 1.660 | 3.544 ± 1.566 | −4.070 ± 4.698 | |
| LMA ($cm^2$) | 1.440 ± 0.522 | 1.568 ± 0.492 | 0.280 ± 1.477 | |
| BF (cm) | $0.213^{a}$ ± 0.172 | $-0.417^{b}$ ± 0.162 | $-0.540^{ab}$ ± 0.486 | 0.018 |
| MS | $-0.061^{a}$ ± 0.115 | $0.300^{b}$ ± 0.108 | $0.600^{ab}$ ± 0.324 | 0.037 |

[a,b] Different superscripts within columns differ significantly ($P < 0.05$)

significantly affected BF and MS. Hanwoo with the genotype CC had a higher BF than genotype CT, and genotype CT had a higher MS than genotype CC ($P < 0.05$) (Table 7).

SNP C1069G was a missense mutation that replaced Leu with Val at the position identical to amino acid 286 of bovine *MC4R* protein. It was significantly associated with BF. This result was similar to the previous report of a significant association of a SNP with LW, CW, BF and MS in Chinese (Qinchuan) cattle [15].

The present study identified SNPs in the coding region of the *MC4R* in Hanwoo. C927T, C1343A and C1786T were found to be synonymous mutations, whereas G709A (Val166Met) and C1069G (Leu286Val) were identified as missense mutations. Statistical analysis indicated that the polymorphisms in C927T, C1069G, and C1343A significantly affected MS. The results provide evidence that the *MC4R* gene is a candidate gene for carcass traits. Further studies with other populations are required.

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