

## A C1069G SNP of the *MC4R* gene and its association with economic traits in Korean native cattle (brown, brindle, and black)

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### Abstract

**Background:** The melanocortin-4 receptor gene (*MC4R*) plays an important role in regulating food intake and body weight in mammals. In the present study, we identified the *MC4R* gene in native Korean brown, brindle, and black cattle by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), and investigated its association with economic traits. A total of 413 cattle from the three breeds were tested for backfat thickness, carcass weight, longissimus muscle area, and marbling score, and statistical data were analyzed using the SAS program.

**Results:** The C allele had the highest frequency in brown and brindle cattle, and the G allele frequency was highest in black cattle. The C1069G SNP was significantly ( $p < 0.05$ ) associated with only marbling scores in brown and brindle cattle but no significant association was detected between the marbling scores and polymorphism in black cattle.

**Conclusions:** These results suggest that a C1069G SNP of the *MC4R* gene may be useful as a genetic marker for marbling scores in Korean brown and brindle cattle.

**Keywords:** economic trait, marbling scores, *MC4R* gene, PCR-RFLP, SNP.

### INTRODUCTION

The native breeds of Korean cattle are the brown, black, and brindle. Brown (also called Hanwoo) is the main breed with the other two breeds having endangered status. Various conservation and research initiatives have been initiated to ensure a stable population of these two endangered breeds with attention now focused on the Korean cattle industry. All three breeds are beef cattle and thus carcass traits, including marbling score, are considered critical economic traits. To satisfy consumer demand, genetic and molecular techniques have been applied to native cattle for the production of better-quality beef (Chung and Kim, 2005).

Genetic improvement has long been considered an important factor for the competitiveness of beef cattle production. Gene or marker-assisted selection (MAS) is a promising strategy for the genetic improvement of economically important quantitative traits, such as growth and carcass traits in beef cattle (Dekkers, 2004).

The identification of genes or polymorphisms highlighting quantitative traits, which are involved in different phenotypes, and an understanding of how these genes/polymorphisms interact with the environment or with other genes affecting economic traits, might be the key to successful application of MAS to the commercial animal population.

As one of those economic traits, marbling in inter-muscular fat gives meat flavour and tenderness. Therefore, an increase in the degree of marbling raises the meat quality (Cheong et al. 2007). In some countries, research is ongoing to determine the association of candidate genes with growth and economic traits in different species. Also the development of DNA markers for genetic breeding is currently a research topic, and attempts to identify the relationship between meat quality traits (intramuscular fat, tenderness, meat and fat colour) and quantitative trait loci (QTL) are proceeding in various countries (Van Eenennaam et al. 2007; Solberg et al. 2008).

Phenotypic expressions of economic traits are controlled by many genes and the melanocortin-4 receptor gene (*MC4R*) is believed to be one of them. The gene is a type of G-protein-coupled membrane receptor. It regulates both food intake and energy expenditure (Huszar et al. 1997), and it is also known to be a representative gene for the obesity response to the leptin hormone in vertebrates, including humans (Farooqi et al. 2003)

In this study, we performed using the SNP C1069G which is a missense mutation that replaced Leu with Val at the position identical to amino acid 286 of bovine *MC4R* protein. Many scientists have reported relationships between the *MC4R* gene and phenotypic traits in cattle breeds. However, the results obtained have contradicted each other in many cases (Haegeman et al. 2001; Thue et al. 2001; Óvilo et al. 2006; Meng et al. 2010; McLean and Schmutz, 2011). Polymorphism of the *MC4R* gene is associated with economic (carcass weight, backfat thickness, and marbling) and growth traits (birth weight, average daily gain) in Chinese Qinchuan and Nanyang cattle (Zhang et al. 2009; Liu et al. 2010). An association between backfat thickness and the *MC4R* gene has been reported in Korean brown cattle (Seong et al. 2012). However, this gene has not been reported to be associated with other economic traits in black and brindle cattle. Therefore, this is the first study describing the relationship between the *MC4R* gene and economic traits in Korean black and brindle cattle.

## MATERIALS AND METHODS

### Animals

Three different Korean native cattle breeds, including brown (n = 281), brindle (n = 111), and black (n = 21), were slaughtered at ages between 24 and 36 months. Databases for four carcass traits (carcass weight [CW], longissimus muscle area [LMA], backfat thickness [BF], and marbling score [MS]) were obtained from the Korea Institute for Animal Products Quality Evaluation (KAPE) and used for the comparative analysis of economic traits.

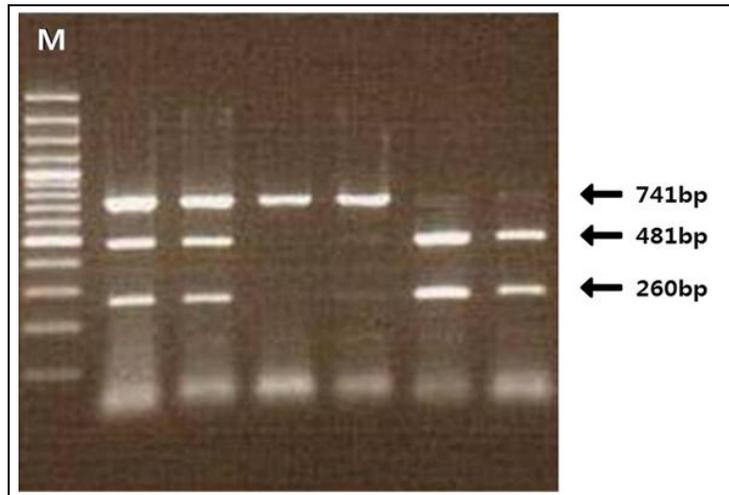
### DNA extraction

Genomic DNA was extracted from 25 mg Hanwoo beef samples using i-genomic CTB DNA Extraction Mini Kit (Intron Biotechnology, Inc., Sungnam, Korea). DNA concentration and purity (A260/A280 ratio) for each sample was assessed using a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA). The measured DNA samples were stored at -80°C until further analysis.

### PCR-RFLP genotyping

Genotyping for the *MC4R* polymorphism (SNP C1069G) was performed using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method, with primers (5'-TGACTCGGTGATCTGTAGC-3', 5'-TTCCTCCATGCCCTACA-3'), as described by Liu et al. (2010). The PCR was carried out in a 20 µL reaction volume, containing 0.25 µL each primer (10 pmol/mL), 20 ng ovine genomic DNA, and 1.5 µL 10X PCR buffer containing 15 mM MgCl<sub>2</sub>/mL and 5U Taq DNA polymerase (Applied Bio systems, USA).

The PCR conditions were carried out at 94°C for 5 min and 35 cycles of 30 sec at 94°C, 30 sec at 55°C, 30 sec at 72°C, and a final step of 10 min at 72°C using the GeneAmp® PCR system 9700 (Applied Bio systems, Foster city, CA, USA). To genotype the *MC4R* gene, PCR products were digested directly with *Tai*I (Fermentas, Burlington, Canada) in accordance with the manual and then subjected to electrophoresis on a 2% agarose gel stained with ethidium bromide. The banding patterns could be divided into three genotypes CC, CG, and GG (Figure 1).



**Fig. 1** PCR-RFLP genotyping of C1069G in the *MC4R* gene with restriction enzyme *Tai*I in Korean native cattle breeds. Three SNP genotypes, C/C (481, 260 bp), C/G (741, 481, 260 bp) and GG (741 bp) were detected. M: 100 bp DNA ladder.

### Statistical analysis

Allele and genotype frequencies were calculated by the simple allele counting method. The correlation analysis was tested by comparing expected and observed genotype frequencies using Cervus v2.0 (Marshall et al. 1998). The association between the genotypes of *MC4R* candidate genes and economic traits was evaluated using the least squares method (GLM procedure of the SAS v9.2 package; SAS Institute, USA). The statistical model was as follows:

$$Y_{ij} = \mu + G_i + Ma_j + \varepsilon_{ij}$$

[Equation 1]

where  $Y_{ij}$  is the carcass trait,  $\mu$  is the whole average for each trait,  $G_i$  is the effect of genotype,  $Ma_j$  is the regression variable for measured age, and  $\varepsilon_{ij}$  is a random error effect for each observation.

### RESULTS AND DISCUSSION

The observed genotype and allele frequencies for the SNP C1069G of the *MC4R* gene are shown in Table 1. In the brown cattle breed, the PCR-RFLP analysis for the SNP indicated that the frequency of alleles C and G were 0.532 and 0.468, and those of genotypes CC, CG, and GG were 0.271, 0.523, and 0.206, respectively. Similarly in brindle cattle, the frequencies were 0.513, 0.487, 0.216, 0.595, and 0.189 for the C and G alleles and the CC, CG, and GG genotypes, respectively. However, the CC genotype did not appear in black cattle, in which only the CG (0.714) and GG (0.286) genotypes were obtained (Table 1).

In a previous study, Qinchuan cattle exhibited a higher CG genotype frequency (0.54) than the CC (0.28) and GG (0.18) genotypes (Liu et al. 2010). In a previous study of Korean cattle (brown), the genotype frequencies had almost the same pattern (0.42, 0.19, and 0.39 for the CG, CC, and GG genotypes, respectively) (Seong et al. 2012).

Table 2 presents the effects of the SNP C1069G on carcass weight, longissimus muscle area, back fat thickness, and marbling score. The SNP in brown ( $p < 0.05$ ) and brindle ( $p < 0.01$ ) cattle were significantly associated with marbling score. For brown cattle in particular, the mean value of the marbling score was higher in the CC genotype (6.24) than the CG (5.57) and GG (4.93) genotypes. In brindle cattle, the CC genotype also had a better marbling score (5.67) than the CG and GG genotypes (4.05, 3.48 respectively). However, there were no significant associations with any economic traits in black cattle. These results differed from a study by Seong et al. (2012) which demonstrated that the SNP had a significant influence on backfat thickness in 57 head of Korean brown cattle ( $p < 0.01$ ) but was not significantly related to marbling score. However, number of samples in the previous experiment was much lower than the sample number in the present experiment; using of large sample number increases the accuracy of the result (Seong et al. 2012). Therefore, the present result has more reliability than the observation of Seong et al. (2012).

So far, to the best of our knowledge, no experiment has been carried to find out the association between *MC4R* gene and economic traits in the Brindle cattle. These findings might help to setup the selection criteria for Brindle cattle as well as other two types of Korean cattle to improve economic their traits. In conclusion, the SNP C1069G of the *MC4R* gene is significantly associated with marbling score in Korean brown and brindle cattle. Our results provide evidence that the *MC4R* gene may affect the economic traits of bovine breeds. Further work is necessary to use the SNP for MAS in a large population.

**Table 1. Genotype and allele frequencies of the *MC4R* gene in Korean native cattle.**

Breeds	Genotype frequencies (n)			Allele frequencies	
	C/C	C/G	G/G	C	G
Brown	0.271 (76)	0.523 (147)	0.206 (58)	0.532	0.468
Brindle	0.216 (24)	0.595 (66)	0.189 (21)	0.513	0.487
Black	-	0.714 (15)	0.286 (6)	0.357	0.643

**Table 2. Associations of 1069C > G SNP genotypes with economic traits at Korean native cattle.**

Breeds	Traits	SNP Genotype			P-value
		CC (mean $\pm$ SE)	CG (mean $\pm$ SE)	GG (mean $\pm$ SE)	
Brown (281)	CW	410.50 $\pm$ 6.93	398.95 $\pm$ 5.80	399.81 $\pm$ 8.11	0.442
	LMA	91.95 $\pm$ 1.53	88.47 $\pm$ 1.27	87.41 $\pm$ 1.72	0.135
	BF	10.72 $\pm$ 0.50	11.87 $\pm$ 0.46	12.19 $\pm$ 0.70	0.208
	MS	6.24 $\pm$ 0.31 <sup>a</sup>	5.57 $\pm$ 0.23 <sup>ab</sup>	4.93 $\pm$ 0.34 <sup>b</sup>	<0.05
Brindle (111)	CW	358.17 $\pm$ 11.24	349.47 $\pm$ 8.90	362.52 $\pm$ 12.53	0.688
	LMA	82.21 $\pm$ 2.40	77.91 $\pm$ 1.99	78.62 $\pm$ 2.21	0.452
	BF	10.75 $\pm$ 1.15	9.77 $\pm$ 0.56	8.57 $\pm$ 0.77	0.296
	MS	5.67 $\pm$ 0.29 <sup>a</sup>	4.05 $\pm$ 0.31 <sup>b</sup>	3.48 $\pm$ 0.46 <sup>b</sup>	<0.01
Black (21)	CW	-	384.20 $\pm$ 13.01	397.33 $\pm$ 17.71	0.863
	LMA	-	79.87 $\pm$ 2.53	85.17 $\pm$ 3.47	0.541
	BF	-	14.13 $\pm$ 1.20	15.17 $\pm$ 1.92	0.905
	MS	-	4.87 $\pm$ 0.53	5.33 $\pm$ 1.09	0.915

CW: carcass weight; LMA: longissimus muscle area; BF: backfat thickness; MS: marbling scores.

<sup>a, b</sup> Different superscripts within columns are significantly different ( $p < 0.05$ ).

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