Association between polymorphisms of the *CRH* and *POMC* genes with economic traits in Korean cattle (Hanwoo)

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ABSTRACT. The *corticotrophin-releasing hormone* (*CRH*) and *proopiomelanocortin* (*POMC*) genes are considered to play an important role in the growth and development of mammals. In this study, the bovine *CRH* and *POMC* genes were characterized to detect genetic variation at these loci in relation to economic traits in Korean cattle (Hanwoo). Nine single nucleotide polymorphisms (SNPs; C148T, A186G, A234C, G269A, G1030A, G1084A, A1136C, G1179C, and A1439G) were detected in the *CRH* gene, and six SNPs (C7017T, A7027T, C7050T, G7063T, C7160T, and C7221T) were detected in the *POMC* gene. Three SNPs in the *CRH* gene (G1030A, G1084A, and G1179C) were missense mutations, and three SNPs in the *POMC* gene (C7017T, A7027T, and C7160T) were missense mutations. Statistical analysis indicated that one *CRH* polymorphism (G1084A) was significantly (P = 0.05) associated with the longissimus dorsi muscle area (LMA), and a *POMC* polymorphism (C7221T) significantly influenced LMA and marbling scores. A significant interaction was detected between *CRH* and *POMC* in relation to carcass weight and LMA. These

results indicate that *CRH* and *POMC* may be candidate genes for carcass traits, and suggest that the interaction between *CRH* and *POMC* strongly affects carcass traits in cattle.

Key words: Korean cattle; Hanwoo; *CRH*; *POMC*; SNP; Economic traits

INTRODUCTION

The native cattle in Korea is considered to belong to European cattle breeds (Mannen et al., 1998, 2004; Seong et al., 2011). Hanwoo (Korean native cattle) refers to a breed of cattle raised in Korea (*Bos taurus coreanae*) and Hanwoo is regarded as a premium beef because of its high palatability and desired chewiness. For breeding and selection of founders with a high potential for meat production and quality, molecular approaches have been used to develop useful selection markers (Han et al., 2009; Seong et al., 2011, 2012).

Corticotrophin releasing hormone (CRH), a major regulator of neuroendocrine response to stress, is purported to be a growth inhibitor (Sharpe et al., 1986; Buchanan et al., 2005; Muráni et al., 2006). CRH indirectly causes the release of glucocorticoids, which reduce appetite by increasing proopiomelanocortin (POMC) production (Buchanan et al., 2005). In bovines, the *CRH* gene is located on chromosome 14, is 1617-bp long, and has two exons (GenBank Accession No. NC_007312). In a previous study, the C22G single nucleotide polymorphism (SNP) in the *CRH* gene was associated with the longissimus dorsi muscle area (LMA) and carcass weight (CW), while the C240G SNP was associated with CW (Buchanan et al., 2005).

POMC is a prohormone that codes for many different peptides, including adrenocorticotrophin, melanocyte stimulating hormone, and β-endorphin (Pritchard et al., 2002; Deobald and Buchanan 2011). In bovines, the *POMC* gene is located on chromosome 11, is 7479-bp long, and has three exons (GenBank Accession No. NC_007309). A *POMC* SNP has previously been associated with CW and shipping weight in cattle (Buchanan et al., 2005).

In a previous study, the interaction effect between *CRH* (C22G) and *POMC* polymorphisms significantly affected LMA in Charolais-cross steers (Buchanan et al., 2005). However, the interaction effect between *CRH* and *POMC* polymorphisms in Hanwoo has not been reported. Therefore, the present study investigated the interaction effect between the *CRH* and *POMC* genes and the association of SNPs with economic traits in Hanwoo.

MATERIAL AND METHODS

Animals and data collection

Two hundred Hanwoo (steers) with progeny from 2 stations: Korean Cattle Improvement Center of Agriculture Cooperative Federation and National Livestock Research Institute were used in the study. Abdominal muscle was collected and genomic DNA was isolated. The weight at slaughter was recorded as the CW at slaughter age. Backfat thickness and LMA were measured at the 12 and 13th rib interface. Marbling score (MS) was evaluated using a cross section at the 12 and 13th rib interface and scored on a scale from 1 to 7, where 7 was associated with the most marbling. The overall means ± standard deviations of the analyzed traits are shown in Table 1.

Table 1. Overall means ± standard deviation (SD), minimum (Min) and maximum (Max) of traits analyzed in Korean Cattle (Hanwoo).

Economic traits	Means ± SD	Min	Max
CW(kg)	415.67 ± 63.92	260.00	546.00
LMA (cm²)	89.99 ± 11.97	67.00	135.00
BF (cm)	12.40 ± 6.12	3.00	34.00
MS (1-7)	5.25 ± 2.71	1.00	9.00

CW, carcass weight; LMA, longissimus dorsi muscle area; BF, backfat thickness; MS, marbling score.

SNP identification and genotyping

Six pairs of primers were designed based upon the bovine *CRH* and *POMC* sequences using the Primer 3 software (http://bioinfo.ut.ee/primer3/; Table 2). Polymerase chain reaction (PCR) conditions were 95°C for 5 min and 35 cycles of 95°C for 30 s, 55°C for 30 s, 72°C for 30 s, and a final step of 10 min at 72°C, using a Peltier Thermal Cycler 200 (MJ Research, USA). DNA sequencing was performed using an ABI 3130 Genetic Analyzer (Applied Bio-systems, USA). Identification and visualization of sequence mutations were performed using the SeqMAN II software (DNA Star, USA).

Table 2. Primers used to amplify the *CRH* and *POMC* genes in Korean cattle (Hanwoo).

Primer	Primer sequence (5'-3')	Location
CRH_1	F: 5'-AGAAGGGAAGAGAACCGCTG-3'	Exon 1, Intron1
	R: 5'-GCTCGTTTACACAGCCACCA-3'	
CRH_2	F: 5'-CGTGCCACACGATTCCAATAG-3'	Intron 1
	R: 5'-CCTGTCTAACGATTCCC-3'	
CRH_3	F: 5'-GAGCCCTTGACACCACGAAT-3'	Intron 1
	R: 5'-GCGCTAATTGCGGTACAGAG-3'	
CRH_4	F: 5'-TTTTCTCTCTCATTCCGCCC-3'	Exon 2
	R: 5'-TTTTCTCTCTCATTCCGCCC-3'	
CRH_5	F: 5'-AGGAATACTTCCTCCGCCTG-3'	Exon 2
	R: 5'-TCCCAGCAATGTCCAACAGT-3'	
POMC	F: 5'-CCTCGAATTCAAGAGGGAGC-3'	Exon 3
	R: 5'-AGAGGCCTTCAGGGTCAACT-3'	

Statistical analysis

Allele and genotype frequencies were calculated by simple counting. Hardy-Weinberg equilibrium was tested by comparing expected and observed genotypes of frequencies using a chi-square test. The association between the genotypes of the *CRH* and *POMC* candidate genes and economic traits were evaluated using the least square method (GLM undertaken in the SAS software package: SAS Institute, USA).

$$Y_{ijkl} = \mu + YS_i + P_j + M_k + e_{ijkl} \qquad \text{(Equation 1)}$$

where, Y_{ijkl}: observation of the carcass traits; μ: overall mean for each trait; YS_i: effect of ith year and season of calving; P_j: fixed effect of jth parity; M_k: fixed effect of kth SNP (*CRH* or *POMC*) genotype; e_{ijkl}: random residual effect.

$$Y_{ijklm} = \mu + YS_i + P_j + M1_k + M2_l + e_{ijklm} \qquad \text{(Equation 2)}$$

where, Y_{ijklm}: observation of the carcass traits; μ: overall mean for each trait; YS_i: effect of ith year and season of calving; P_j: fixed effect of jth parity; $M1_k$: fixed effect of kth SNP (*CRH*) genotype; $M2_l$: fixed effect of lth SNP (*POMC*) genotype; e_{ijklm}: random residual effect.

RESULTS

DNA samples were amplified and sequenced for the *CRH* and *POMC* genes. Following sequencing analysis, ten SNPs were identified in the *CRH* gene. One SNP (C148T) was detected in the exon 1 region, three SNPs (A186G, A234C, and G269A) were detected in the intron 1 region, and five SNPs (G1030A, G1084A, A1136C, G1179C, and A1439G) were detected in the exon 2 region (Figure 1). G1030A, G1084A, and G1179C were missense mutations. G1030A displayed a change in amino acid from Ser to Asn, G1084A resulted in a change in amino acid from Ser to Asn, and G1179C resulted in a change in amino acid from Asp to His (Table 3). Six SNPs were identified following sequencing analysis of the *POMC* gene. Six SNPs (C7017T, A7027T, C7050T, G7063T, C7160T, and C7221T) were detected in the exon 3 region (Figure 1). SNPs C7017T, A7027T, and C7160T were missense mutations. C7017T resulted in a change in amino acid from Ala to Val, A7027T resulted in a change in amino acid from Arg to Trp, and C7160T resulted in a change in amino acid from Ser to Leu (Table 3).

Estimated CRH allele and genotype frequencies for the Hanwoo are shown in Table 3. The G1084A frequency for allele G was higher than that for allele A, with 29.7 and 70.3% displaying the GG and GA genotype, respectively. To investigate the effects of SNPs on variation in the economic traits of Hanwoo, the association of the CRH genotype was analyzed. The SNP marker of G1084A was found to significantly affect LMA ($P < 0.05$; Table 4).

The results of the SNP markers association analysis for the *POMC* gene are presented in Table 5. The C7221T genotypes were significantly associated with LMA and MS ($P < 0.05$).

The interaction effect between polymorphisms of *CRH* and *POMC* in Hanwoo was investigated and the results are presented in Table 6. Polymorphisms were observed to significantly affect LMA and CW.
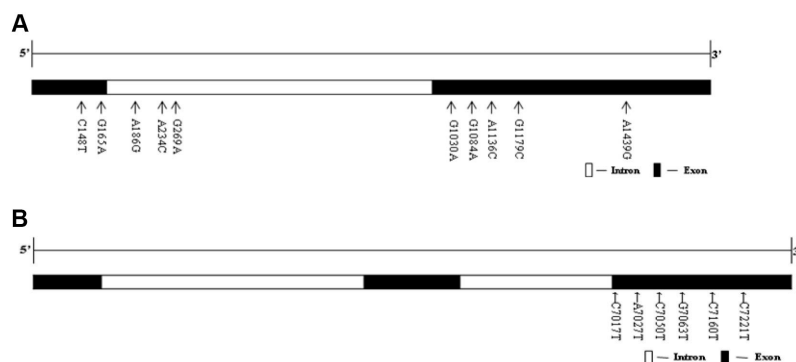
Figure 1. Structure of the *CRH* and *POMC* genes in cattle. **A.** *CRH* gene length of 1617 bp with two exons and one intron (GenBank accession No. NC_007312); **B.** *POMC* gene length of 7479 bp with three exons and two introns (GenBank accession No. NC_007309).

Table 3. Genotype frequencies and allele frequencies of the *CRH* and *POMC* genes in Korean cattle (Hanwoo).

Gene	SNPs	Amino acid change	Genotype frequency (%)			Allele frequency (%)	
CRH	C148T		CC	CT	TT	C	T
			95.8	4.2	0.0	97.9	2.1
	A186G		AA	AG	GG	A	G
			58.3	41.7	0.0	79.2	20.8
	A234C		AA	AC	CC	A	C
			58.3	41.7	0.0	79.2	20.8
	G269A		GG	GA	AA	G	A
			58.3	41.7	0.0	79.2	20.8
	G1030A	Ser27Asn	GG	GA	AA	G	A
			71.8	18.2	0.0	90.9	9.1
	G1084A	Ser45Asn	GG	GA	AA	G	A
			29.7	70.3	0.0	64.8	35.2
	A1136C		AA	AC	CC	A	C
			72.3	24.9	2.8	84.7	15.3
	G1179C	Asp77His	GG	GC	CC	G	C
			83.2	13.8	3.0	90.1	9.9
	A1439G		AA	AG	GG	A	G
			0.0	25.0	75.0	12.5	87.5
POMC	C7017T	Ala121Val	CC	CT	TT	C	T
			1.6	98.4	0.0	50.8	49.2
	A7027T	Arg128Trp	AA	AT	TT	A	T
			7.9	92.1	0.0	54.0	46.0
	C7050T		CC	CT	TT	C	T
			10.8	89.2	0.0	55.4	44.6
	G7063T		GG	GT	TT	G	T
			12.3	87.7	0.0	56.2	43.8
	C7160T	Ser172Leu	CC	CT	TT	C	T
			2.9	97.1	0.0	51.5	48.5
	C7221T		CC	CT	TT	C	T
			43.5	50	6.4	68.5	31.5

Table 4. Least square mean ± standard error (SE) for economic traits of the CRH (G1084A) genotype in Korean cattle (Hanwoo).

Traits	SNP genotype		P value
	GG (Mean ± SE)	GA (Mean ± SE)	
CW (kg)	430.500 ± 17.71	408.89 ± 5.69	
LMA (cm²)	97.17 ± 3.30	88.44 ± 1.06	0.013
BF (cm)	13.58 ± 1.78	12.57 ± 0.57	
MS (1-7)	6.00 ± 0.75	5.02 ± 0.24	

CW = carcass weight; LMA = longissimus dorsi muscle area; BF = backfat thickness; MS = marbling score.

Table 5. Least square mean ± standard error (SE) for economic traits of the POMC (C7221T) genotype in Korean cattle (Hanwoo).

Traits	SNP genotype			P value
	CC (Mean ± SE)	CT (Mean ± SE)	TT (Mean ± SE)	
CW (kg)	397.38 ± 8.95	402.37 ± 8.35	390.71 ± 23.20	
LMA (cm²)	85.26 ± 1.51	90.7 ± 1.41	88.9 ± 3.91	0.009
BF (cm)	11.38 ± 0.80	11.06 ± 0.75	10.43 ± 2.08	
MS (1-7)	4.93 ± 0.35	5.41 ± 0.33	3.4 ± 0.90	0.042

CW = carcass weight; LMA = longissimus dorsi muscle area; BF = backfat thickness; MS = marbling score.

Table 6. Genotype frequencies between the *CRH* and *POMC* genes in Korean cattle (Hanwoo).

SNPs		Traits	SNPs		Traits	
G1084A (*CRH*)	C7221T (*POMC*)	LMA (cm²)	A1136C (*CRH*)	C7221T (*POMC*)	CW (kg)	LMA (cm²)
GG	CC	95.6 ± 6.8	AA	CC	418.5 ± 11.8[a]	86.3 ± 2.2[b]
	CT	96.5 ± 5.9		CT	417.0 ± 9.20[c]	92.3 ± 1.7[a,c]
	TT	89.0 ± 11.8		TT	438.7 ± 36.0	94.0 ± 6.6
GA	CC	84.2 ± 0.3[a]	AC	CC	347.0 ± 31.2[b,d]	76.8 ± 5.7[d]
	CT	91.6 ± 1.8[b]		CT	435.88 ± 22.1	95.4 ± 4.0
	TT	94.3 ± 6.8		TT	345.0 ± 62.4	95.4 ± 11.4
			CC	CT	358.0 ± 62.4	90.0 ± 11.4
				TT	336.0 ± 62.4	89.0 ± 11.4

CW = carcass weight; LMA = longissimus dorsi muscle area; BF = backfat thickness; MS = marbling score; [a,b]Different superscripts within columns differ significantly (P < 0.05).

DISCUSSION

In this study, we detected nine SNPs (C148T, A186G, A234C, G269A, G1030A, G1084A, A1136C, G1179C, and A1439G) in the *CRH* gene. G1030A resulted in a change in amino acid from Ser to Asn, G1084A resulted in a change in amino acid from Ser to Asn, G1179C resulted in a change in amino acid from Asp to His (Table 3), and SNP G1084A significantly affected LMA (Table 4). Previous studies have detected several SNPs (C22G, A145G, and C240G) in the *CRH* gene of cattle, and have linked these SNPs to growth traits (Buchanan et al., 2005; Pugh et al., 2011). The relationship between the A145G polymorphism and milk production traits has been assessed in Jersey cows (Kulig et al., 2011), and SNP G10936C was found to be significantly associated with MS in a Wagyu x Limousin F2 population (Wibowo et al., 2007).

SNP C228T has been identified in the *POMC* intron region in cattle and has been linked to growth traits (Buchanan et al., 2005; Deobald and Buchanan 2011). Thus, we identified SNPs in the coding region in Hanwoo. Following sequencing, six SNPs (C7017T, A7027T, C7050T, G7063T, C7160T, and C7221T) were detected in the exon 3 region (Figure 1). C7017T resulted in a change in amino acid from Ala to Val, A7027T resulted in a change in amino acid from Arg to Trp, C7160T resulted in a change in amino acid from Ser to Leu (Table 3), and SNP C7221T was observed to significantly affect LMA and MS (Table 5). Thus, we detected novel SNP regions in the *CRH* and *POMC* genes that may be candidate genes for carcass traits in Hanwoo.

The present study investigated the interaction effect between polymorphisms of *CRH* and *POMC* in Hanwoo, and polymorphisms were observed to significantly affect LMA and CW. The results suggest that *CRH* and *POMC* may be candidate genes for carcass traits, and the interaction between *CRH* and *POMC* may strongly affect carcass traits in cattle. These results provide evidence that interaction between *CRH* and *POMC* polymorphisms have potential effects for carcass traits.

ACKNOWLEDGMENTS

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