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A Single Nucleotide Polymorphism in LOC534614 as an Unknown Gene Associated with Body Weight and Cold Carcass Weight in Hanwoo (Korean Cattle)*

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ABSTRACT : A major aim of cattle genome research is to identify candidate genes associated with meat quantity and quality through QTL analysis for application in the livestock industry. Therefore, this study focused on discovery of useful SNPs within the LOC534614 gene, containing 12273_165 SNP which is located on the same site as the QTL on chromosome 6, and evaluation of the association between SNP and body weight and cold carcass weight in Hanwoo (Korean cattle) As a result of a BLAST search of the NCBI web site, we discovered that the mRNA sequence of the LOC534614 gene was similar to that of the coiled-coil domain containing 158 (CCDC158) for dog and human. According to the direct DNA sequence from the CCDC158 gene, we identified 19 polymorphic SNPs within exons and their flanking regions. Among them, 17 polymorphic SNPs were selected for genotyping in Hanwoo (n = 476) and seventeen marker haplotypes containing 12273_165 SNP (frequency >0.1) were identified. As a result of the association between 17 polymorphic SNPs and Hanwoo (n = 476), g.8778G>A SNP in exon 6 was found to be a non-synonymous SNP, and was significantly associated with body weight and cold carcass weight ($p<0.05$). We discovered 19 polymorphic SNPs in the CCDC158 gene on the QTL region of BTA 6 in Hanwoo and identified that the g.8778G>A SNP was significantly associated with body weight and cold carcass weight ($p<0.05$), which causes an amino acid variation from valine to methionine. Furthermore, statistical analysis demonstrated that the CCDC158 gene is strongly associated with body weight and cold carcass weight in Hanwoo. In this regard, the g.8778G>A SNP in the CCDC158 gene can be useful as a positional candidate for body weight and cold carcass weight for marker-assisted selection in Hanwoo. (**Key Words :** SNP, Hanwoo, QTL, CCDC158 Gene, Weight)

INTRODUCTION

Identification of QTL-related economic traits is a major aim of cattle genome research. In order to verify QTL, we conducted a high-density map and then collected candidate genes associated with economic traits from the QTL region. Two main approaches were used to obtain the gene: positional cloning and the candidate gene approach. In recent studies, researchers have used the candidate gene approach for identification of causal SNPs that affect gene function for use as DNA-based markers.

This approach has also been proven to be extremely powerful for study of the genetic component of complex traits, which is a far more effective and economical method for direct gene discovery. The thyroglobulin (TG) gene was found from a QTL which was associated with marbling score and located near the CSSM66 microsatelltie in the QTL region of BTA14 (Barendse et al., 2004). This study suggested an association between a single nucleotide polymorphism (SNP) in the 5 leader sequence of the thyroglobulin gene and marbling score in cattle fed for a period lasting longer than 250 days. Fitzsimmons et al. (1998; 1999) reported that the leptin gene, near the BM1500 microsatellite located within the QTL region of BTA4, was significantly associated with a mis-sense SNP in eight beef bulls. Single-nucleotide polymorphisms within the micromolar calcium-activated neutral protease

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(CAPN1) gene, encoding the protease μ-calpain, were associated with meat tenderness on bovine chromosome 29 (Page et al., 2002). The CAPN1 gene has been mapped to the QTL interval known to influence meat tenderness on chromosome 29 (Casas et al., 2000). Moreover, SNPs within TG, leptin, and CAPN1 genes have been used in the commercial cattle industry as useful tools for marker-assisted selection.

Previous studies have suggested that the 12273_165 SNP was related to body weight and cold carcass weight in a Hanwoo half-sib population and that it was located in the same position as that of the ILSTS035 microsatellite (Lee et al., 2008). Therefore, the LOC534614 gene containing the 12273_165 SNP could be a potential candidate gene for weight in Hanwoo. Therefore, the objective of this study was to develop SNPs in the LOC534614 gene and to evaluate the association between SNP and body weight and cold carcass weight in Hanwoo.

MATERIALS AND METHODS

Animals and phenotypes

The Hanwoo population (n = 476) was reared under the progeny-testing program of the National Livestock Research Institute (NLRI) of Korea. The pedigree record of 476 steers was produced from 50 sires collected by the Korea Animal Improvement Association (Seoul, Korea). All steers were fed under the tightly controlled conditions of the feeding program in the Daekwanryeong and Namwon branches. The animals were born between the spring of 1998 and autumn of 2002. After two years, all steers were slaughtered in the spring of 2002 to autumn of 2004. They were castrated at 6 months of age and were raised 4 animals per pen (4 m×8 m). After 6 months of age, they were fed with concentrates consisting of 15% crude protein (CP)/ 71% totally digestible nutrients (TDN) for a period of 60 to 90 days; 15% CP/71% TDN for a period of 180 days; and 13% CP/72% TDN for a period of 90 to 120 days of self-feeding. Roughage was offered *ad libitum*, and steers had free access to fresh water throughout the entire period. Live weights were determined before slaughter using electronic scales. Following a 24-h chill, cold carcass weight was measured. The mean and standard deviation of live weight and cold carcass weight was 569.016±57.301 kg and 316.510±33.985 kg, respectively. Genomic DNA from white blood cells was extracted using the phenol-chloroform method (Sambrook et al., 2001).

BLAST and Sequencing of the LOC534614 gene

Using the LOC534614 mRNA (GenBank:XM_614439) sequence for comparison of homology among species, we performed a search with NCBI's BLASTX tool. We sequenced 25 exons and their flanking regions for discovery of variants of the SNP in 50 unrelated Korean cattle (Hanwoo) using the BigDye Terminator (Ver. 3.1) cycle sequencing kit (Applied Biosystems, Foster City, CA) on an ABI 3730XL DNA analyzer (Applied Biosystems). Twenty-five primer sets for amplification and sequencing analysis were designed on the basis of the GenBank sequence (Accession no. NC_007304) using Primer3 software. Primer information is provided in the supplementary data. Sequence editing was generated by visual confirmation using the Sequencher 4.6 program (Gene Codes Corp., Ann Arbor, MI).

SNP genotyping

For genotyping of polymorphic sites, primers for amplification and extension were designed for single-base extension (SBE) (Vreeland et al., 2002). Primer extension reactions were conducted using the SNaPshot ddNTP Primer Extension Kit (Applied Biosystems, Foster City, CA). In order to clean up the primer extension reaction, one unit of SAP (shrimp alkaline phosphatase) was added to the reaction mixture which was incubated for 1 h at 37°C, followed by 15 min at 72°C for enzyme inactivation. DNA samples containing extension products and Genescan 120 LIZ size standard solution were added to HiDi formamide (Applied Biosystems, Foster City, CA) in accordance with the manufacturer's recommendations. The mixture was incubated for 5 min at 95°C, followed by 5 min on ice, after which electrophoresis was conducted using the ABI PRISM 3130XL Genetic Analyzer. Results were analyzed using GeneMapper v4.0 software (Applied Biosystems, Foster City, CA).

Statistical analysis

x^2 tests were used to determine whether or not the individual variant was in equilibrium at each locus in the population (Hardy-Weinberg equilibrium). We examined a widely used measure of linkage disequilibrium between all pairs of bi-allelic loci, D' (the correlation coefficient [Delta, |D'|]), LOD (logarithm of odds), and r^2. Strength of LD between pairs of SNPs was measured as D' using Haploview. Regions of strongly associated markers (LD blocks) were inferred by Gabriel's method, as implemented in Haploview (Gabriel et al., 2002; Barrett et al., 2005) Using Gabriel's method, pairs of SNPs are considered to be in strong LD if the one-sided 95% D' confidence boundary is between 0.7 and 0.98. The method defines a block if 95% of pair-wise SNP comparisons are in strong LD. r^2 was also used to determine whether or not the pairs of sites were in absolute LD. Haplotypes and their frequencies were inferred using the algorithm developed by Stephens et al. (2001) Phase probabilities for each site were calculated for each individual using this software (PHASE) (input option: ignoring families). Using this software, phase probabilities

of all polymorphic sites for haplotypes were calculated for each individual. Because 95% of samples had phase probabilities greater than 97%, 97% was chosen as the threshold for phase probability. Associations between individual SNPs and body weight and cold carcass weight were determined by the mixed effect model, treating "sire" as a random effect; "age" at slaughter was also included in the model as a covariate in the SPSS statistics v17.0 package. Other covariates were not available for this analysis. We used a single SNP model. Single SNP/haplotype effects were tested in the mixed effect model. For haplotype analyses, we fitted the model with the same covariates in a similar manner.

RESULTS

The LOC534614 gene included the 12273_165 SNP, which was associated with meat quantity (Figure 1B) (Lee et al., 2008). However, the function of the LOC534614 gene is not yet known. Therefore, results of a BLASTX search of the NCBI web site using the mRNA sequence of the LOC534614 gene found that the mRNA sequence of the LOC534614 gene was very similar to that of the coiled coil domain containing the 158 (CCDC158) gene, which has been found in dogs and humans (Table 1).

By direct sequencing for discovery of SNPs within the CCDC158 gene, 19 polymorphic SNPs within exons and their flanking regions of CCDC158 were identified: 3 in coding exons, and 16 in introns. Among 3 polymorphic SNPs in coding exons, the g.8778G>A SNP was a non-synonymous SNP characterized by an amino acid change from valine to methionine. Locations and allele frequencies of the polymorphisms are shown in Table 2 and in Figure 1C.

Eighteen polymorphic SNPs, including the 12273_165 SNP reported by Lee et al. (2008), were selected for pair-wise linkage disequilibrium analysis based on location (polymorphisms in exons were preferred) and a minor allele frequency exceeding 0.05 and LD (a polymorphism was chosen if it was in absolute LD[$r^2 = 1$] with one or more other polymorphisms) (Gabriel et al., 2002). Pair-wise linkage disequilibrium analysis with the 18 polymorphic SNPs showed that the CCDC158 gene can be conducted in LD blocks (*Block1*), the 66 kb region spanning from exon3 to intron24 (*Block1*). We have conducted haplotype analyses from 3 SNPs in exon regions associated with an influence on gene function. There were four common haplotypes in *Block1* (Figure 2); frequencies are shown in Table 3.

The 17 polymorphic SNPs (g.-8606+137C>T, g.-74-34G>T, g.70+20C>T, g.3885-18C>G, g.4102+36T>G, g.8420-137T>C, g.8529+19G>A, g.8643-21T>C, g.8778G>A, g.11500-125A>G, g.11500-117C>G, g.11521T>C, g.11614+19G>T, g.18765G>A, g.32330-48A>G, g.34425+102A>T, g.66995-169insdelC) and 2 haplotypes (Exon_ht1, Exon_ht2) were selected for genotyping from the large-scale Hanwoo population.

The g.-74-34G>T, g.8420-137T>C, g.8529+19G>A, g.8778G>A, g.11500-125A>G SNPs and Exon_ht1 haplotype were significantly associated with body weight ($p<0.05$, Table 4). As for cold carcass weight, only the g.8778G>A SNP showed a significant difference ($p>0.05$, Table 5).

This g.8778G>A was a non-synonymous SNP, in which valine is changed to methionine. With regard to body weight, the least square mean of the group with the GG genotype (576.096 kg) of g.8778G>A was higher than in the AG or AA genotypes (560.261 kg, 558.423 kg, respectively). The difference between the GG and AA genotypes was 17.673 kg, which was the largest among significant differences between SNPs and haplotypes. Also, the least square mean and frequency of Exon_ht1 type were quite similar to those of the g.8778G>A genotype. In cold carcass weight only, g.8778G>A showed a significant difference. In conclusion, we would predict that the g.8778G>A SNP was the causal mutation that directly affects CCDC158 gene function.

DISCUSSION

Traits associated with weight are economically relevant in the Hanwoo industry. A previous study reported on identification of a significant QTL for growth traits on BTA6 from the Belgian Blue×MARC III and Piedmontese× Angus population and reported detection of a suggestive QTL on the same chromosome for the *longissimus dorsi* muscle area (Casas et al., 2003) and hot carcass weight in a *Bos indicus*×*Bos taurus* population (Casas et al., 2000). Also, a significant QTL was identified for birth weight and pre-weaning average daily gain in *Bos taurus* (Kneeland et al., 2004). Recent studies have reported on association of the non-SMC condensing I complex, a subunit of the G (NCAPG) gene, with body weight and carcass weight in the QTL region of chromosome 6 in the Japanese Black half-sib family (Takasuga et al., 2007; Eberlein et al., 2009; Setoguchi et al., 2009). In another study, a significant QTL was detected for ADG on chromosome 6 and the 12273_165 SNP, located at a position similar to that of ILSTS035 QTL, was identified in Hanwoo (Kim et al., 2003; Lee et al., 2008). Therefore, we would predict CCDC158 as a candidate gene for association with final weight and cold carcass weight in Hanwoo.

Weight gain is associated with skeletal muscle mass. Principal determinants of skeletal muscle mass include

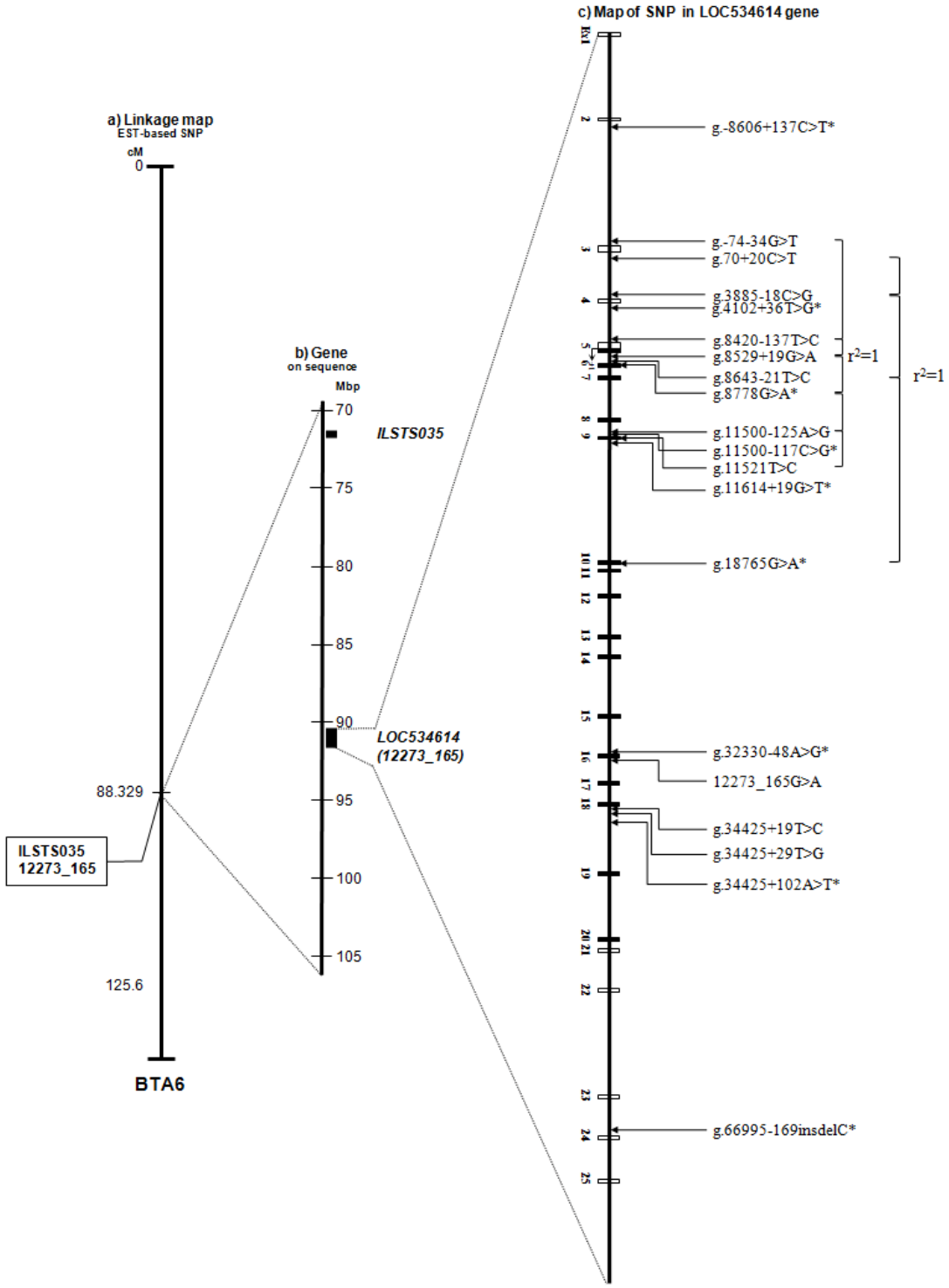

Figure 1. Map of 19 polymorphic SNPs within LOC534614 associated with QTL of BTA 6. a) The 12273_165 SNP and QTL associated with meat quantity is located in a position similar to that of 88.329 cM on the EST-based SNP linkage map of BTA 6 in Hanwoo [21]. b) The 12273_165 SNP for meat quantity is located within the LOC534614 gene on the Gene sequence map (NCBI Map viewer). c) The coding exon is marked by black blocks, and 5' UTRs and 3'UTRs by white blocks. The first base of the translational site is denoted as nucleotide +1.

Table 1. Similarity between LOC534614 and sequences from other species using the BLASTX tool

Species	Gene ID (Symbol)	Identities (%)	Description
Canis lupus familiaris (Dog)	478436 (CCDC158)	93	coiled-coil domain containing 158
Homo sapiens (Human)	339965 (CCDC158)	90	coiled-coil domain containing 158

Table 2. Genotype and allele frequencies of the 19 polymorphic SNPs within the LOC534614 gene

SNP	Region	NCBI assay ID	Genotype (Number of animals) Frequency			H[1]	MAF[2]	HWE[3]
g.-8606+137C>T	Intron	ss147452114	CC(10) 0.023	CT(159) 0.362	TT(270) 0.615	0.325	0.205	0.017
g.-74-34G>T	Intron	ss147452123	GG(214) 0.470	GT(193) 0.424	TT(48) 0.105	0.435	0.320	0.737
g.70+20C>T	Intron	ss147452131	CC(214) 0.491	CT(176) 0.404	TT(46) 0.106	0.426	0.308	0.306
g.3885-18C>G	Intron	ss147452136	CC(215) 0.489	CG(177) 0.402	GG(48) 0.109	0.428	0.311	0.262
g.4102+36T>G	Intron	ss147452144	TT(161) 0.363	GT(209) 0.471	GG(74) 0.167	0.481	0.403	0.737
g.8420-137T>C	Intron	ss147452151	TT(208) 0.464	CT(190) 0.424	CC(50) 0.112	0.438	0.324	0.561
g.8529+19G>A	Intron	ss147452160	TT(74) 0.158	CT(188) 0.401	CC(207) 0.441	0.443	0.331	0.262
g.8643-21T>C	Intron	ss147452168	TT(223) 0.500	CT(180) 0.404	CC(43) 0.096	0.419	0.298	0.503
g.8778G>A	Exon	ss147452173	GG(212) 0.471	GA(189) 0.420	AA(49) 0.109	0.435	0.320	0.564
g.11500-125A>G	Intron	ss147452178	AA(223) 0.474	AG(196) 0.417	GG(51) 0.109	0.437	0.323	0.514
g.11500-117C>G	Intron	ss147452185	CC(42) 0.091	CG(183) 0.396	GG(237) 0.513	0.407	0.284	0.378
g.11521T>C	Exon	ss147452191	TT(224) 0.489	CT(185) 0.404	CC(49) 0.107	0.436	0.320	0.452
g.11614+19G>T	Intron	ss147452199	GG(223) 0.484	GT(191) 0.414	TT(47) 0.102	0.432	0.316	0.567
g.18765G>A	Exon	ss147452205	GG(220) 0.487	GA(185) 0.409	AA(47) 0.104	0.427	0.308	0.521
g.32330-48A>G	Intron	ss147452209	AA(275) 0.611	AG(149) 0.331	GG(26) 0.058	0.348	0.224	0.406
g.34425+19T>C	Intron	ss147452221	TT(0) 0.000	CT(444) 0.982	CC(8) 0.018	0.500	0.497	0.000
g.34425+29T>G	Intron	ss147452226	TT(442) 0.993	TG(3) 0.007	GG(0) 0.000	0.007	0.003	1.000
g.34425+102A>T	Intron	rs43469994	AA(125) 0.286	AT(215) 0.492	TT(97) 0.222	0.498	0.468	0.859
g.66995-169insdelC	Intron	ss147452232	del(118) 0.267	Ins/del(227) 0.514	ins(97) 0.219	0.499	0.477	0.586

[1] Heterozygosity. [2] Minor allele frequency. [3] Hardy-Weinberg principle.

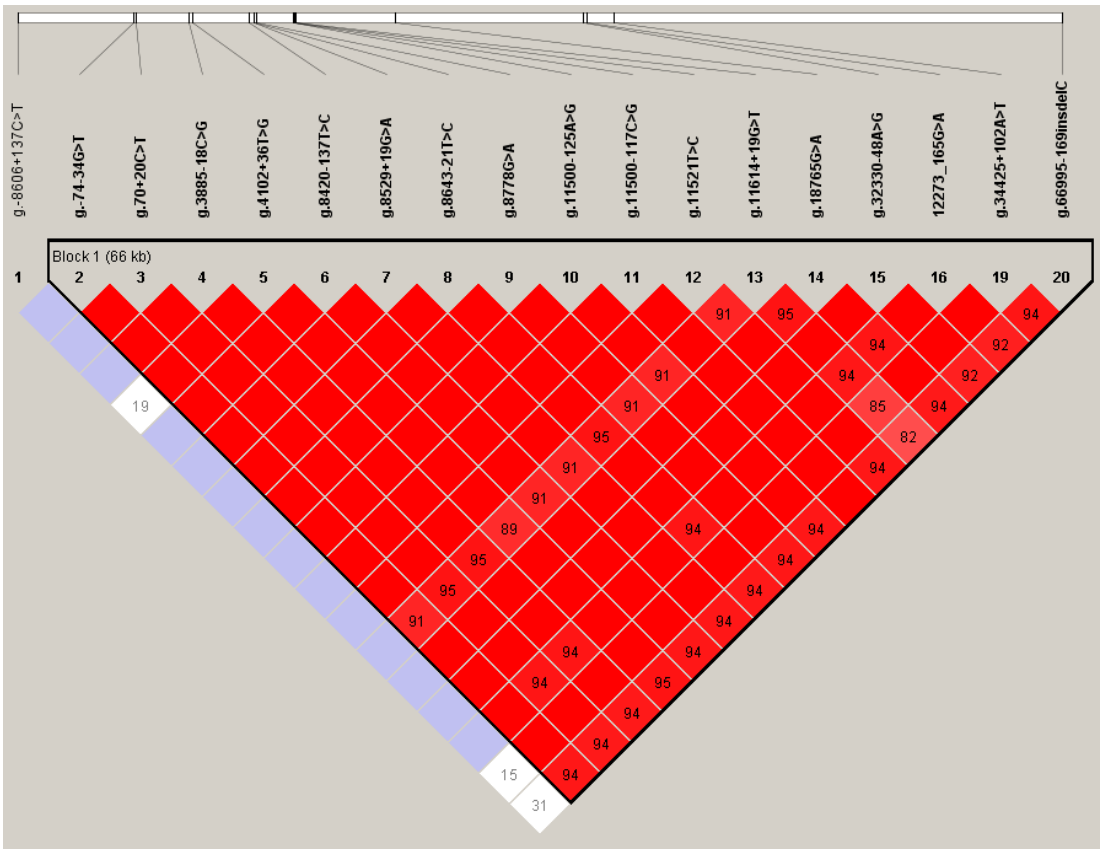

Figure 2. Linkage disequilibrium between SNPs within the LOC534614 gene. The color code on the Haploview plot follow the standard color scheme: white (|D□|<1, LOD<2); shades of pink/red (|D□|<1, LOD≥2); blue (|D□| = 1, LOD<2); bright red (|D□| = 1, LOD≥2). Numbers in cells are D□ value. However, the D□ value of 1.0 is not shown (empty).

Table 3. Haplotype blocks of the exon region within the LOC534614 gene and their frequencies

Haplotype	g.8778G>A	g.18765G>A	Frequency
Exon_ht1	G	G	0.670
Exon_ht2	A	A	0.299
Exon_ht3	A	G	0.024
Exon_ht4	G	A	0.007

Table 4. Least-square mean and standard error of SNP and haplotype for body weight within the LOC53614 gene in Korean cattle (Hanwoo)

Traits	Position	SNP	Amino acid change	Genotype (No. of animals) LSMEAN±SE			p-value
BW	Intron	g.-8606+137C>T	-	CC(9)	CT(156)	TT(260)	0.148
				599.888±18.377	572.603±5.222	564.996±3.585	
	Intron	g.-74-34G>T	-	GG(202)	GT(183)	TT(46)	0.044
				575.535±4.307[a]	560.251±4.112[b]	564.490±8.923[ab]	
	Intron	g.70+20C>T	-	CC(208)	CT(169)	TT(44)	0.196
				573.952±4.316	562.681±4.317	567.357±9.367	
	Intron	g.3885-18C>G	-	CC(209)	CG(171)	GG(47)	0.122
				573.588±4.307	560.847±4.280	567.071±9.018	
	Intron	g.4102+36T>G	-	GG(71)	GT(203)	TT(156)	0.077
				566.922±7.109	561.331±3.895	575.255±4.721	
	Intron	g.8420-137T>C	-	CC(48)	CT(181)	TT(201)	0.042
				562.577±8.764[ab]	560.624±4.128[a]	575.781±4.292[b]	
	Intron	g.8529+19G>A	-	AA(53)	AG(178)	GG(200)	0.046
				565.335±8.537[ab]	560.144±4.179[a]	575.340±4.309[b]	
	Intron	g.8643-21T>C	-	CC(41)	CT(172)	TT(215)	0.281
				561.666±9.441	563.332±4.282	572.749±4.230	
	Exon	g.8778G>A	V132M	AA(47)	AG(181)	GG(201)	0.025
				558.423±8.819[a]	560.261±4.118[ab]	576.096±4.283[b]	
	Intron	g.11500-125A>G	-	AA(202)	AG(181)	GG(48)	0.044
				575.644±4.288[a]	560.572±4.131[b]	562.487±8.756[ab]	
	Intron	g.11500-117C>G	-	CC(40)	CG(168)	GG(221)	0.895
				563.715±9.360	566.942±4.490	568.501±3.914	
	Exon	g.11521T>C	N22N	CC(48)	CT(178)	TT(200)	0.055
				562.732±8.783	560.530±4.188	575.308±4.336	
	Intron	g.11614+19G>T	-	GG(204)	GT(176)	TT(46)	0.068
				575.299±4.339	561.600±4.203	559.371±9.057	
	Exon	g.18765G>A	T45T	AA(44)	AG(176)	GG(210)	0.136
				561.704±9.218	562.024±4.205	574.059±4.272	
	Intron	g.32330-48A>G	-	AA(262)	AG(142)	GG(25)	0.202
				571.742±3.812	562.595±4.893	552.242±11.831	
	Intron	g.34425+102A>T	-	AA(120)	AT(211)	TT(94)	0.138
				566.925±5.414	563.228±3.946	577.632±6.100	
	Intron	g.66995-169insdelC	-	del(114)	insdel(221)	ins(92)	0.345
				573.017±5.411	563.423±3.783	567.484±6.166	
	-	Exon_ht1	-	ht1*ht1(201)	ht1*R(182)	R*R(48)	0.040
				575.847±4.310[a]	560.485±4.122[b]	562.501±8.753[ab]	
	-	Exon_ht2	-	ht2*ht2(44)	ht2*R(176)	R*R(211)	0.135
				561.644±9.206	561.965±4.199	573.998±4.262	

[a,b] Means with different superscripts within the same column are significantly different ($p<0.05$).

muscle fiber number and muscle fiber size. During development, these factors are controlled by a series of events, including myoblast proliferation, myotube formation, and myofiber maturation. Throughout physical development, the CCDC158 gene, which is associated with final weight and cold carcass weight, expresses the coiled-coil domain containing 158 proteins of the coiled coil type. The function of the CCDC158 gene is not yet known; however, the coiled coil type protein has been detected in transcription factors during cell growth and proliferation and in muscle protein (Glover et al., 1995; Mason et al., 2004) and the g.8778G>A SNP within the CCDC158 gene has also been associated with final weight and cold carcass weight.

Thus, we would suggest that the g.8778G>A SNP within the CCDC158 gene was influenced during transformation of the coiled-coil structure by the nonsynonymous SNP found in this study.

Table 5. Least-square mean and standard error of the SNP and haplotype for cold carcass weight traits within the LOC53614 gene in Korean cattle (Hanwoo)

Traits	Position	SNP	Amino acid change	Genotype (No. of animals) LSMEAN±SE			p-value
CWT	Intron	g.-8606+137C>T	-	CC(9)	CT(156)	TT(260)	0.102
				335.628±10.716	318.377±3.032	313.466±2.090	
	Intron	g.-74-34G>T	-	GG(202)	GT(183)	TT(46)	0.064
				319.499±2.510	311.154±2.394	313.127±5.202	
	Intron	g.70+20C>T	-	CC(208)	CT(169)	TT(44)	0.323
				318.075±2.526	312.633±2.528	313.202±5.492	
	Intron	g.3885-18C>G	-	CC(209)	CG(171)	GG(47)	0.260
				317.959±2.506	312.005±2.494	313.636±5.256	
	Intron	g.4102+36T>G	-	GG(71)	GT(203)	TT(156)	0.171
				314.993±4.143	311.980±2.264	318.692±2.751	
	Intron	g.8420-137T>C	-	CC(48)	CT(181)	TT(201)	0.062
				311.752±5.111	311.350±2.403	319.552±2.502	
	Intron	g.8529+19G>A	-	AA(53)	AG(178)	GG(200)	0.069
				312.287±4.978	311.386±2.432	319.497±2.511	
	Intron	g.8643-21T>C	-	CC(41)	CT(172)	TT(215)	0.360
				310.286±5.505	313.381±2.497	317.714±2.461	
	Exon	g.8778G>A	V132M	AA(47)	AG(181)	GG(201)	0.033
				309.278±5.134[a]	311.176±2.393[ab]	319.880±2.492[b]	
	Intron	g.11500-125A>G	-	AA(202)	AG(181)	GG(48)	0.062
				319.594±2.498	311.368±2.403	311.780±5.105	
	Intron	g.11500-117C>G	-	CC(40)	CG(168)	GG(221)	0.966
				314.892±5.451	314.552±2.615	315.482±2.270	
	Exon	g.11521T>C	N22N	CC(48)	CT(178)	TT(200)	0.066
				311.835±5.110	311.097±2.433	319.374±2.521	
	Intron	g.11614+19G>T	-	GG(204)	GT(176)	TT(46)	0.124
				318.949±2.524	312.211±2.445	309.799±5.273	
	Exon	g.18765G>A	T45T	AA(44)	AG(176)	GG(210)	0.257
				311.471±5.374	312.567±2.451	318.195±2.486	
	Intron	g.32330-48A>G	-	AA(262)	AG(142)	GG(25)	0.122
				317.450±2.206	313.164±2.846	302.484±6.886	
	Intron	g.34425+102A>T	-	AA(120)	AT(211)	TT(94)	0.101
				315.463±3.131	312.133±2.277	321.012±3.528	
	Intron	g.66995-169insdelC	-	del(114)	insdel(221)	ins(92)	0.263
				319.080±3.156	312.756±2.593	313.987±3.594	
	-	Exon_ht1	-	ht1*ht1(201)	ht1*R(182)	R*R(48)	0.056
				319.713±2.511	311.311±2.399	311.786±5.102	
	-	Exon_ht2	-	ht2*ht2(44)	ht2*R(176)	R*R(211)	0.249
				311.468±5.370	312.544±2.448	318.235±2.482	

[a,b] Means with different superscripts within the same column are significantly different ($p<0.05$).

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