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Full Length Research Paper

Association analysis of *PRKAG3* gene variants with carcass and meat quality traits in beef cattle

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The bovine *PRKAG3* gene encodes a muscle-specific isoform of the regulatory gamma-subunit of adenosine monophosphate activated protein kinase (AMPK), which plays a key role in regulating energy homeostasis in eukaryotes. It is well known that mutations in the *PRKAG3* gene affect high glycogen content in the porcine skeletal muscle and, consequently, meat quality. Therefore, this gene has been proposed as a positional and functional candidate gene for a quantitative trait locus (QTL) with an effect on meat quality traits. In this study, we detected four single nucleotide polymorphisms (SNPs) at the *PRKAG*3 gene (DQ082736) in 267 beef cattle. The SNP marker association analysis indicated that the SNP markers T2885C was significantly associated with tenderness trait. Animals with the TT genotype had lower Warner-Bratzler shear force (WBS) than those with the other genotypes. Results of this study suggest that the *PRKAG3*-gene-specific SNP may be a useful marker for meat quality traits in future marker-assisted selection programmes in beef cattle.

Key words: Association analysis, beef cattle, single nucleotide polymorphism (SNP) polymorphism, *PRKAG3* gene.

INTRODUCTION

In cattle, tenderness is the primary quality attribute for consumer acceptance of meat, followed by juiciness and flavor. Extensive efforts are being made to control and improve these qualities but are still impaired by the fact that these characters are subjected to many genetic and environmental factors and the elucidation of the mechanisms involved requires extensive studies (Roux et al., 2006).

The adenosine monophosphate (AMP)-activated protein kinase (AMPK) has been pointed out as one of the main actors in the regulation of intracellular energy metabolism (Carling, 2004). AMPK is activated by phosphorylation of the α-subunit at threonine 172. The phosphorylation is catalysed by an upstream kinase, the AMP-activated protein kinase kinase (AMPKK), which in turn is directly activated by AMP (Hawley et al., 1996) or by the binding of AMP to AMPK and AMPKK (Hardie et al., 1998). AMPK is activated by the catabolic pathway responsible for the switch to aerobic metabolism, which is necessary to sustain exercise for a long period. Once AMPK is activated, it stimulates both an increase in fatty acid oxidation and an increase in glucose uptake to meet the energy demands of the working muscle (Winder, 2001).

Numerous mechanisms of AMPK action on lipid and carbohydrate metabolism have been proposed (Ferre et al., 2003; Hardie et al., 2003). AMPK is a heterotrimeric enzyme complex comprising a catalytic α subunit and regulatory β and γ subunits. Seven different isoforms (α1, α2, β1, β2, γ1, γ2 and γ3), each encoded by a different gene, have been characterized so far and all the combinations (12 in total) are possible. The different combinations depend on the tissue type and they have different levels of activity.

The γ3-peptide, encoded by the PRKAG3 gene, is one

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Table 1. Number of records, means and standard deviations for traits analyzed in this study (N = 267).

Trait	Mean	SD
Live weight (kg)	548.15	64.05
Carcass weight (kg)	301.13	39.50
Dressing percentage (%)	55.00	0.03
Backfat thickness (cm)	1.03	0.45
Loin muscle area (cm^2)	68.81	12.82
Meat color (1-6)	4.45	0.74
Fat color (1-7)	1.32	0.41
Marbling score (1-5)	2.14	1.00
Tenderness (kg)	3.95	1.40

of the three γ-isoforms for the γ-regulatory subunit of AMPK and shows muscle specific expression (Stapleton et al., 1996; Thornton et al., 1998; Kemp et al., 1999; Cheung et al., 2000). Previous work has indicated that the PRKAG3 gene affects the glycogen content in muscle and hence, meat quality traits in pigs, including ultimate pH, meat color, water-holding capacity, drip loss, tenderness and cooking loss (Milan et al., 2000; Ciobanu et al., 2001).

Knowledge of muscle biochemical characteristics related to meat quality in cattle is still limited. However, it is now well established that there is an important variability in muscle biology which is directly associated with downstream parameters like postmortem meat maturation, drip loss, cooking loss, tenderness, juiciness and taste. This is because of the large effect of AMPK γ3 subunit mutations on muscle glycogen content and pH observed in pig and the close association between glycogen metabolism, pH, and meat quality in cattle (Immonen et al., 2000).

The objectives of this study were to detect polymorphisms in the *PRKAG3* gene, and to analyze associations between these polymorphisms and carcass and meat traits in several cattle breeds. The results of this study could add new evidence that *PRKAG3* is an important candidate gene to be used for selection of meat traits in the beef cattle industry.

MATERIALS AND METHODS

Animals, carcass and meat quality data

A total of 267 animals, including Simmental (N =107), Angus (N = 44), Hereford (N = 30), Charolais (N = 28), Limousin (N = 19), Luxi (N = 20) and Jinnan (N = 19), were randomly selected from commercial populations and used in the association analysis. The animals (405 ± 50.5 kg; 30 ± 2 months of age at slaughter) were reared in the provinces of Inner Mongolia and Hebei. Carcass and meat quality traits were measured according to the criterion GB/T 17238-1998 Cutting Standard of Fresh and Chilled Beef in China (China Standard Publishing House). Nine traits were measured or calculated (Table 1): live weight (LW), carcass weight (CW), dressing percentage (DP), backfat thickness (BF), marbling score (MS) loin muscle area (LMA), meat color (MC), fat color (FC) and tenderness (TD). BF and LMA were measured between the 12 and 13th rib. MC, FC and MS for quality grade were evaluated on a cross section of the loin muscle between the 12 and 13th rib, which are scored on a scale of 1 to 7, 1 to 7 and 1 to 5, respectively. TD was also measured on a cross section of the loin muscle between the 12 and 13th rib, the measurement type is called measurement of Warner-Bratzler shear force (WBS), which was recorded in kg. All experimental procedures were performed according to authorization granted by the Chinese Ministry of Agriculture.

Polymerase chain reaction (PCR) amplification and sequencing

DNA samples were extracted from blood samples according to Mullenbach et al. (1989), which was diluted to 50 ng μl^{-1} for PCR. According to the bovine *PRKAG3* gene sequence cloned in the 8048 bp DNA sequence (published data: DQ082736), four pairs of primers were designed to amplify four fragments (checked by DNA sequencing) within it (Table 2). PCR amplifications were performed in a 30 µl volume containing 50 ng of DNA template, 10 pM each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 U of DNA Taq polymerase (TaKaRa, Dalian, China). The PCR protocol was 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, annealing for 30 s and 72°C for 30 to 60 s, and a final extension at 72°C for 10 min. The products were purified by using a Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology, P. R. China) and sequenced (Beijing Aolaibo Biotechnology, P. R. China; Applied Biosystems 3730xl DNA sequencer, Foster city, CA, USA).

Polymorphism discovery and PCR-restriction fragment length polymorphism (RFLP) analysis

Four polymorphisms were identified by sequencing (Figure 1) and confirmed by PCR-RFLP using *Bfm* I (primer sets 1), *Bcn* I (primer sets 2), *Rsa* I (primer sets 3) and *Tai* I (primer sets 4) restriction enzymes (New England BioLabs, Beverley, MA, USA). A1428G site has three genotypes, which are GG (221bp), AG (221/119/102bp) and AA (119/102bp); T2643C site has three genotypes, which are TT (230/96bp), TC (230/187/96/43bp) and CC (187/96/43 bp); T2885C site has three genotypes, which are TT (203 bp), TC (203/104/99 bp) and CC (104/99 bp); G3869C site has three genotypes, which are CC (669/174bp), GC (669/559/174/70bp) and

Table 2. Primer sequences used for amplification and polymorphism in the bovine *PRKAG3* gene.

Fragment[a] (primer sets)	Sequence (5'-3')	PCR product size(bp)	Genomic position	SNP location	Nucleotide polymorphism
P1	F: CTCTCGCCTCCTTCCTCTTT R: TCCAAAGGTCTTTTCCTCCA	221	Intron2	1428	A/G
P2	F: CAAGCTGGTCATCTTCGACA R: AAATGCTCCCAGTCATCCTG	326	Intron4	2643	T/C
P3	F: CAGGATGACTGGGAGCATTT R: CCATACGTGCTGGTTGTAGC	203	Intron4	2885	T/C
P4	F: GGTAAACCCCACTCCTCTCTCT R: CAAAGATGTGCAGGAACTTGAG	843	Exon7	3869	G/C

[a]The position of fragments are shown in Figure 1.

GG (599/174/70 bp) (Figure 2).

Statistical analysis

The associations between SNP marker genotypes of the *PRKAG3* gene and carcass and meat quality traits were analyzed by the least-squares method as applied in the GLM procedure of SAS (SAS Institute Inc., Cary, NC, USA) according to the following linear model:

$$Y_{ijkl} = \mu + BF_i + Month_j + G_k + e_{ijkl},$$

Where, Y_{ijkl} = observed value; μ = overall mean for each trait; BF_i = fixed effect of i^{th} breed and farm; $Month_j$ = fixed effect of j^{th} month of slaughtering; G_k = fixed effect of k^{th} single SNP marker genotype; e_{ijkl} = random error.

RESULTS AND DISCUSSION

We amplified and sequenced four fragments at the *PRKAG3* gene (GenBank accession number DQ082736) in 267 animals. The comparisons among these sequences revealed four mutations: A1428G, T2643C, T2885C and G3869C (Figure 1). Sequence analysis showed that these alleles were caused by A to G, T to C, T to C and G to C mutations at positions 1428, 2643, 2885 and 3869, respectively. The allele and genotype frequencies of the four SNPs are shown in Table 3.

The genotypes of 267 individuals were tested for correlation with phenotypic data for nine traits. The gene-specific SNP marker correlation analysis indicated that the SNP marker T2885C was significantly correlated with tenderness trait (P<0.05). Animals with the TT genotype had lower tenderness WBS value than those with TC and CC genotypes (P<0.05) (Table 4). No significant correlations were observed between any of the marker genotypes at T2885C and other traits. The other SNPs showed no significant association with traits examined in this study.

Rothschild et al. (2005) gained the US Patent for they found PRKAG3 alleles and use the same as genetic markers for reproductive and meat quality traits in pigs. Roux et al. (2006) reported the bovine PRKAG3 gene and a polymorphism analysis in three cattle breed; 32 SNPs were identified among which 13 are in the coding region, one is in the 3' UTR and 18 are in the introns; five of them change an amino acid in the PRKAG3 protein sequence; allelic frequencies were determined in the three breeds considered, and mutant alleles affecting the coding sequence were found at a very low frequency. Similarly, Yu et al. (2005) also reported that the genomic structure and sequence of the bovine PRKAG3 were analyzed from a Korean cattle BAC clone; the bovine PRKAG3 gene comprises 13 exons and spans approximately 6.8 kb on BTA2; from 5' and 3'-rapid amplification of cDNA ends experiments, the full-length cDNA of bovine PRKAG3 had been identified, encoding a deduced protein of 465 amino acids; seven single nucleotide polymorphisms were detected in four *Bos taurus* cattle breeds; the bovine PRKAG3 gene described in that study might be involved in muscle-related genetic diseases or meat quality traits in cattle.

However, in the study of these two groups, they did not measure any associated traits, and also did not test association analysis.

Therefore, in our study, by measuring carcass and meat quality traits and association analysis of gene-specific SNP markers in 267 animals, we found a significant correlation between the T2885C SNP in the *PRKAG3* gene and tenderness trait. Since the power of detection of associations for such a relatively small sample size as ours is low, we cannot exclude the possible association with other traits considered.

Conclusions

Our results provide evidence that the *PRKAG3* gene has

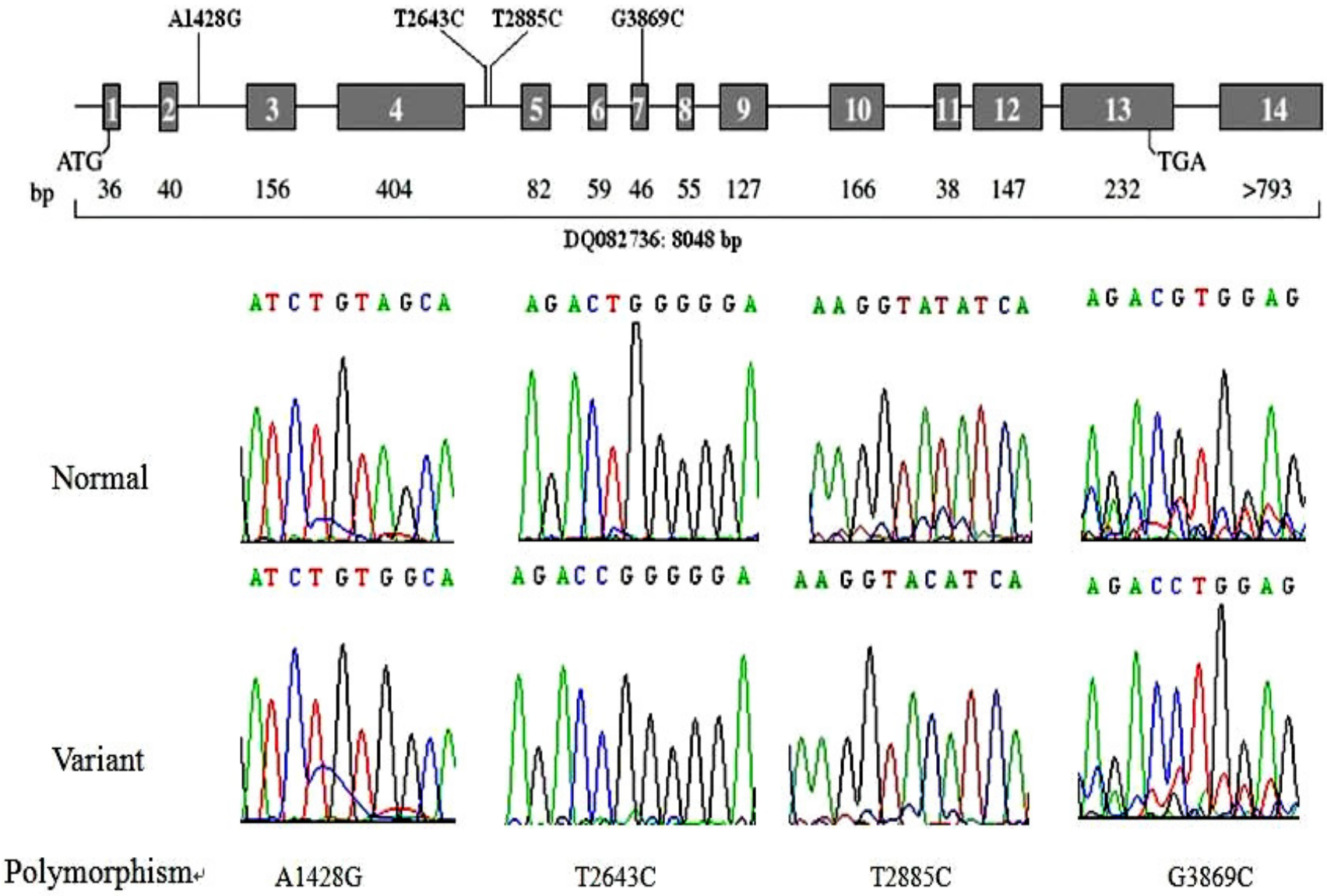

Figure 1. Chromatograms showing sequence variation at positions 1428 (A1428G), 2643 (T2643C), 2885 (T2885C) and 3869(G3869C) within the 8048 bp fragment of the *PRKAG3*

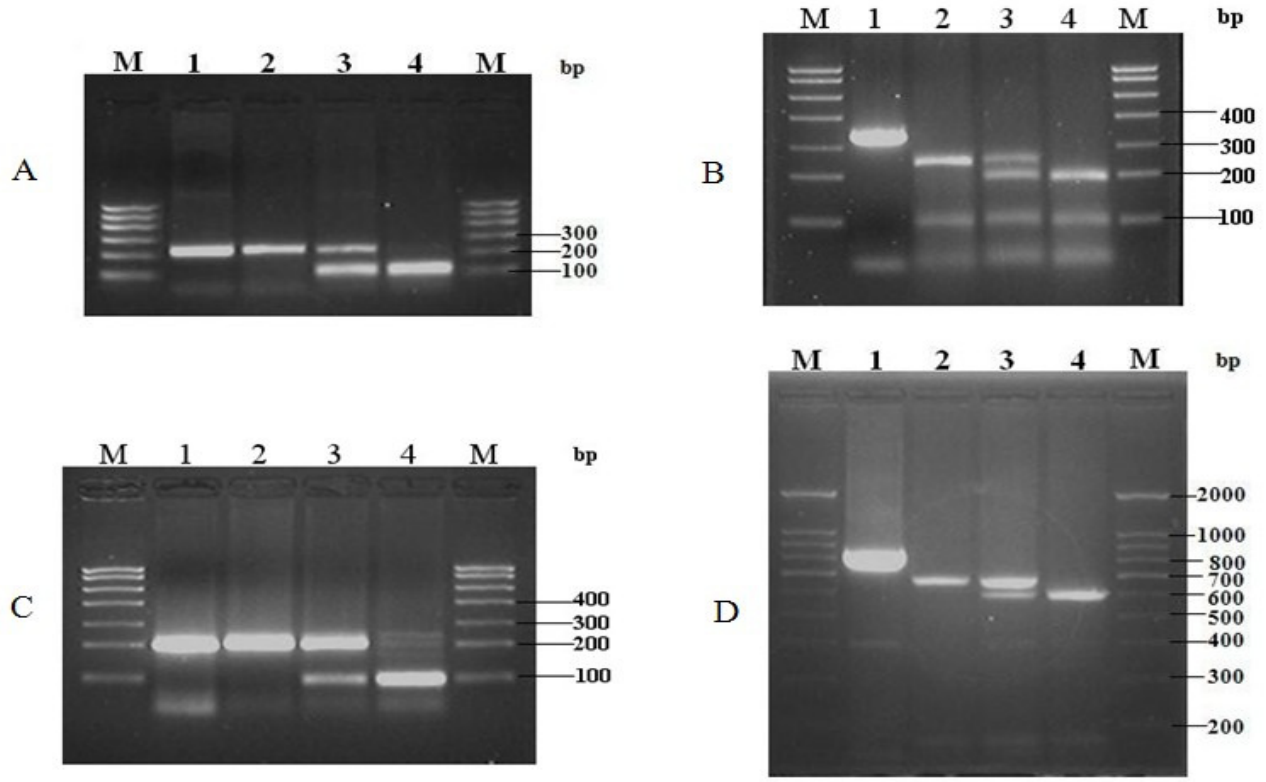

Figure 2. Sequence variation at positions 1428 (A1428G), 2643 (T2643C), 2885 (T2885C) and 3869 (G3869C) within the 8048 bp fragment of the *PRKAG3* gene (DQ082736) that is cut by respective endonuclease in 3% agarose gel. A, *Bfm* I-RFLP patterns of PCR products of A1428G site: 1, 221 bp PCR product of P1 primer; 2, GG; 3, AG; 4, AA; B, *Bcn* I-RFLP patterns of PCR product of T2643C site: 1, 326 bp PCR product of P2 primer; 2, TT; 3, TC; 4, CC; C, *Rsa* I-RFLP patterns of PCR products of T2885C site: 1 203 bp PCR product of P3 primer; 2, TT; 3, TC; 4, CC; D, *Tai* I-RFLP patterns of PCR products of G3869C site: 1, 843 bp PCR product of P4 primer; 2, CC; 3, GC; 4, GG; M, DNA marker, respectively.

Table 3. Genotype frequencies within different breeds for the four SNPs in the PRKAG3 gene.

SNP	Genotype	Breed (number)							Total (N = 267)
		Simmental (N = 107)	Angus (N = 44)	Hereford (N = 30)	Charolais (N = 28)	Limousin (N = 19)	Luxi (N = 20)	Jinnan (N = 19)	
	AA	39.25 (42)	20.45 (9)	10.00 (3)	39.29 (11)	26.32 (5)	5.00 (1)	5.26 (1)	26.97 (72)
A1428G	AG	49.53 (53)	56.82 (25)	36.67 (11)	46.43 (13)	57.89 (11)	40.00 (8)	63.16 (12)	49.81 (133)
	GG	11.21 (12)	22.73 (10)	53.33 (16)	14.29 (4)	15.79 (3)	55.00 (11)	31.58 (6)	23.22 (62)
	TT	42.99 (46)	20.45 (9)	3.3 3(1)	35.71 (10)	26.32(5)	5.00 (1)	5.26 (1)	27.34 (73)
T2643C	TC	42.99 (46)	54.55 (24)	36.67 (11)	53.57 (15)	63.16 (12)	50.00 (10)	78.95 (15)	49.81 (133)
	CC	14.02 (15)	25.00 (11)	60.00 (18)	10.71 (3)	10.53 (2)	45.00 (9)	15.79 (3)	22.85 (61)
	TT	33.64 (36)	38.64 (17)	86.67(26)	28.57 (8)	42.11 (8)	70.00 (14)	42.11 (8)	43.82 (117)
T2885C	TC	42.06 (45)	54.55 (24)	13.33 (4)	53.57 (15)	47.37 (9)	25.00 (5)	52.63 (10)	41.95 (112)
	CC	24.30 (26)	6.82 (3)	0.00 (0)	17.86 (5)	10.52 (2)	5.00 (1)	5.26 (1)	14.23 (38)
	GG	76.64 (82)	47.73 (21)	70.00 (21)	78.57 (22)	68.42 (13)	35.00 (7)	26.32 (5)	64.04 (171)
G3869C	GC	20.56 (22)	43.18 (19)	30.00 (9)	17.86 (5)	15.79 (3)	45.00 (9)	68.42 (13)	29.96 (80)
	CC	2.80 (3)	9.09 (4)	0.00 (0)	3.57 (1)	15.79 (3)	20.00 (4)	5.26(1)	6.00 (16)

The location of the SNP in the sequence DQ082736.

Table 4. Effects of four different SNPs genotypes on phenotypic traits in beef cattle.

SNP	Genotype	Number	Traits* (mean ± SE)								
			LW (kg)	CW (kg)	DP (%)	BF (cm)	LMA (cm^2)	MC(1-6)	FC(1-7)	MS (1-5)	TD (kg)
A1428G	AA	72	553.84±9.71	311.69±6.44	55.76±0.53	1.21±0.08	70.22±1.90	4.58±0.13	1.38±0.07	2.29±0.16	4.50±0.23
	AG	133	567.29±8.94	313.94±5.93	55.09±0.49	1.27±0.07	71.46±1.75	4.56±0.12	1.32±0.07	2.12±0.15	4.16±0.21
	GG	62	576.48±10.58	321.88±7.01	55.61±0.58	1.20±0.08	72.79±2.07	4.51±0.14	1.23±0.08	2.33±0.19	4.09±0.25
	P		0.0947	0.3028	0.2677	0.5499	0.4653	0.8945	0.1635	0.2519	0.1639
T2643C	TT	73	556.30±10.01	313.03±6.62	55.87±0.54	1.25±0.08	69.91±1.95	4.64±0.13	1.41±0.07	2.22±0.17	4.43±0.24
	TC	113	565.78±8.90	313.18±5.88	54.99±0.48	1.23±0.07	71.24±1.73	4.55±0.12	1.31±0.06	2.23±0.15	4.23±0.21
	CC	61	573.94±10.68	321.16±7.06	55.79±0.58	1.22±0.08	73.46±2.08	4.45±0.14	1.21±0.08	2.24±0.18	4.10±0.25
	P		0.2707	0.3895	0.0832	0.9434	0.2429	0.4384	0.0557	0.9910	0.4152
T2885C	TT	117	565.23±9.43	314.07±6.23	55.28±0.01	1.23±0.07	72.56±1.82	4.46 ±0.13	1.25±0.07	2.25±0.16	3.98^{a}±0.22
	TC	112	568.15±9.20	316.79±6.08	55.49±0.01	1.28±0.07	71.68±1.78	4.54±0.12	1.37±0.07	2.26±0.15	4.38^{b}±0.21
	CC	38	557.28±11.58	312.88±7.65	55.60±0.01	1.17±0.09	68.42±2.24	4.77±0.15	1.36±0.09	2.11±0.19	4.55^{b}±0.27
	P		0.5986	0.7967	0.8441	0.3583	0.1766	0.1303	0.1384	0.7052	0.0388
G3869C	GG	171	564.37±8.50	314.97±5.62	55.50±0.46	1.22±0.07	70.55±1.63	4.58±0.11	1.32±0.06	2.23±0.14	4.32±0.20
	GC	80	565.15±10.47	312.54±6.92	55.04±0.57	1.23±0.08	73.77±2.01	4.62±0.14	1.34±0.08	2.26±0.18	4.10±0.24
	CC	16	545.83±16.43	306.11±10.7	55.77±0.89	1.24±0.13	71.64±3.16	4.31±0.22	1.36±0.12	2.03±0.28	4.08±0.38
	P		0.4295	0.6505	0.5099	0.9661	0.1309	0.3063	0.8982	0.6605	0.4795

* LW, Live weight; CW, carcass weight; DP, dressing percentage; BF, backfat thickness; LMA, loin muscle area; MC, meat color; FC, fat color; MS, marbling score; TD, tenderness. ^{a,b}Means of traits with different superscripts were significantly different ($P<0.05$).

potential effects on tenderness trait. Therefore, further work will be necessary to use these SNPs for marker assisted selection (MAS) in a larger population and to investigate whether the *PRKAG3* gene plays a role in intramuscular fat deposition or is in linkage disequilibrium with other causative mutations.

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