GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Novel polymorphisms of the *PRKAG2* gene and their association with body measurement and meat quality traits in Qinchuan cattle

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Genet. Mol. Res. 14 (2): 3669-3679 (2015)
Received May 22, 2014
Accepted August 22, 2014
Published April 17, 2015
DOI http://dx.doi.org/10.4238/2015.April.17.16

ABSTRACT. Body measurement and meat quality traits play important roles in the evaluation of productivity and economy in cattle, which are influenced by genes and environmental factors. *PRKAG2*, which encodes the γ2 regulatory subunit of AMPK, is associated with key metabolic pathways in muscle. We detected bovine *PRKAG2* gene polymorphisms and analyzed their associations with body measurement and meat quality traits of cattle. DNA samples were taken from 578 Qinchuan cattle aged 18-24 months. DNA sequencing, polymerase chain reaction-restriction fragment length polymorphism, and time-of-flight mass spectrometry were used to detect *PRKAG2* single nucleotide polymorphisms (SNPs). Sequence analysis revealed three SNPs in exon 3 (g.95925G>A, g.95973G>C, and g.95992A>G) and

one g.96058T>C mutation in intron 3. g.95973G>C, g.95992A>G, and g.96058T>C each showed 3 genotypes: GG, GC, and CC; AA, AG, and GG; and TT, TC, and CC, respectively. In contrast, g.95925G>A only showed 2 genotypes, GG and GA. Analysis showed that g.95925G>A had no effects on body measurement and meat quality traits, whereas the other 3 polymorphisms were significantly associated with some of the body measurement and meat quality traits in the Qinchuan cattle population. It is inferred that the *PRKAG2* gene can be used for marker-assisted selection to improve the body measurement and meat quality traits in the Qinchuan cattle population.

Key words: Body measurement traits; Meat quality traits; *PRKAG2*; Qinchuan cattle; Single nucleotide polymorphism

INTRODUCTION

Some genetic markers are associated with important economical traits of livestock species; via marker-assisted selection, the identification of the marker has the potential to alter the genetic improvement rate (Nkrumah et al., 2003). The genes associated with adult height have been identified and provided important insights to analyze the genetics of polygenic quantitative traits (Weedon et al., 2007).

AMP-activated protein kinase (AMPK) is a metabolic regulator that is responsible for adjusting the energy supply to match demands (Oliveira et al., 2012). AMPK, consisting of an α catalytic subunit and β and γ regulatory subunits, is a heterotrimer complex found in all eukaryotic cells. During hypoxia and ischemia, it plays an essential role in activating glucose transport (Ofir et al., 2008). Dominant mutations in the γ2 regulatory subunit of AMPK, which is encoded by the gene *PRKAG2*, could cause cardiac hypertrophy and increase the risk of sudden cardiac death. Mutations in *PRKAG2* produce a distinctive cardiac histopathology characterized by enlarged myocytes with vacuoles containing glycogen derivatives (Blair et al., 2001; Gollob et al., 2001; Arad et al., 2002). In transgenic mouse models, it has been confirmed that *PRKAG2* mutations were associated with glycogen cardiomyopathy (Arad et al., 2003; Sidhu et al., 2005; Davies et al., 2006). Human mutations in *PRKAG2* could cause unique cardiomyopathy characterized by ventricular pre-excitation, myocardial hypertrophy, and progressive conduction system disease (Arad et al., 2002). In transgenic mice overexpressing the human *PRKAG2* mutation in their hearts, glycogen storage and other features of this disease were completely recapitulated (Arad et al., 2003). In contrast, transgenic mice overexpressing the wild-type *PRKAG2* gene showed a milder increase in glycogen, which could cause mild hypertrophy without cardiomyopathy (Ofir et al., 2008).

So far, no polymorphisms of the *PRKAG2* gene in Qinchuan cattle have been reported. Thus, discovering and discussing the genetic variations in the *PRKAG2* gene in Qinchuan cattle is preliminary and interesting work. The genetic variations in the *PRKAG2* gene were first identified by DNA sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technology, which will possibly contribute to constructing genetic markers to analyze the association between the genotype and the body measurement and meat quality traits. The information about the *PRKAG2* gene obtained in this study will provide some useful assistance for further research.

MATERIAL AND METHODS

DNA samples and data collection

A total of 578 adult animals aged 18-24 months were randomly selected to be unrelated for at least 3 generations from 1 Qinchuan cattle-breeding population and used to analyze the *PRKAG2* allelic frequencies. The body measurement traits (BMTs), including body length (BL), withers height (WH), hip height (HH), rump length (RL), hip width (HW), chest depth (CD), heart girth (HG), and pin bone width (PBW), were measured as previously described (Gilbert et al., 1993). Ultrasound measurements were available for meat quality traits (MQTs) (Brethour, 1994; Hamlin et al., 1995), including backfat thickness (BT), loin muscle area (LMA), and intramuscular fat content (IFC). A single person was assigned to measure 1 of the 11 traits in all animals to minimize systematic error. DNA samples were extracted from the blood samples derived from the jugular vein of the 578 Qinchuan cattle using a standard phenol-chloroform protocol (Müllenbach et al., 1989).

PCR amplification and DNA sequencing

According to the sequence of the bovine *PRKAG2* gene (GenBank accession No. NC_007302.5), 1 pair of primer sequences (F: 5'-TCCGAGGGACCTCACCTGT-3'; R: 5'-CACCCACCTGGAACAAAAGC-3') were designed by the Primer Premier 5.0 software to amplify PCR products including exon 3 and partial intron 3. PCR amplifications were performed in a 20-μL reaction mixture containing 20 ng DNA, 10 pM of each primer, 0.20 mM dNTPs, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The PCR protocol was as follows: 95°C for 5 min; 35 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s; and a final extension at 72°C for 10 min. The products for sequencing were first electrophoresed on 1.5% agarose gels, purified by Gel Extraction Kit (Omega, USA), and sequenced in both directions in an ABI PRIZM 377 DNA sequencer (ABI, USA). The sequence maps were analyzed by the SeqMan software. Through the DNA sequencing, 4 novel mutations (g.95925G>A, g.95973G>C, g.95992A>G, and g.96058T>C) were found. Furthermore, exon 1, exon 2, and exon 4 were carefully analyzed, and no variations were found.

Genotyping of *PRKAG2* alleles by PCR-RFLP and time-of-flight (TOF) technology

PCR-RFLP was used to identify the g.95992A>G mutation. A new primer pair (S: 5'-CGTGTTCCCGTTCTCCTAC-3'; A: 5'-CTACAGCCACCTGCCAGC-3') was designed to obtain 251-bp products from all cattle. The cycling protocol was 95°C for 5 min; 35 cycles of denaturing at 94°C for 30 s, 61.3°C annealing for 30 s and extension at 72°C for 30 s; and a final extension at 72°C for 10 min. Aliquots of 10 μL of the PCR products were digested with 5 U *Msp*I (TaKaRa) following the supplier manual. The digested products were detected by electrophoresis on a 2.5% agarose gel stained with ethidium bromide. To verify the results of the PCR-RFLP technique, the products with different electrophoresis patterns were sequenced.

As for the mutations g.95925G>A, g.95973G>C, and g.96058T>C, the products from the DNA samples were all sequenced to distinguish the genotypes of the 3 mutations by TOF mass spectrometry, which was completed at Sangon Biotech (Shanghai, China).

Statistical analysis

Trait means were calculated by a generalized linear model. Genotypic and allelic frequencies, gene homozygosity, gene heterozygosity, Hardy-Weinberg equilibrium, effective allele numbers, and polymorphism information content (PIC) were statistically analyzed according to previous approaches (Nei and Roychoudhury, 1974; Nei and Li, 1979). The association between SNP marker genotypes of the *PRKAG2* gene and BMTs and MQTs (BL, WH, HH, RL, HW, CD, HG, PBW, BT, LMA, and IFC) was analyzed by the SPSS software (version 17.0) according to the following statistical linear model:

$$Y_{ijl}=\mu+G_i+S_j+A_l+\varepsilon_{ijl}$$

where Y_{ijl} is the observation of the BMTs and ultrasound measurement traits, μ is the mean value for each trait, G_i is the genotype effect, S_j is the fixed effect of sex, A_l is the fixed effect of age, and ε_{ijl} is the random error.

RESULTS

SNP identification and the *PRKAG2* genotypes

The novel SNPs identified in this study were synonymous mutations g.95925G>A (Figure 1a) and g.95973G>C (Figure 1b) and the missense mutation g.95992A>G (Figure 1c), resulting in a Ser to Gly substitution (75th amino acid), in exon 3 and a g.96058T>C mutation (Figure 1d) in intron 3. By TOF technology, all three 96058T>C genotypes were identified: TT, TC, and CC; only 2 g.95925G>A genotypes were identified: GG and GA. The 3 g.95973G>C genotypes (GG, GC, and CC) were also identified. For the g.95992A>G polymorphism with 3 genotypes (AA, AG, and GG), the products digested with *Msp*I showed 3 fragments (251, 154, and 97 bp) for genotype AG, 1 fragment (251 bp) for genotype AA, and 2 fragments (154 and 97 bp) for genotype GG (Figure 2).

Genetic polymorphism of the Qinchuan cattle *PRKAG2* gene and χ^2 test

For the mutations g.95925G>A, g.95973G>C, g.95992A>G, and g.96058T>C, the genotype and allele frequencies were analyzed, and the results are shown in Table 1. The allele frequencies of g.95925G>A were 0.9542(G)/0.0458(A), those of g.95973G>C were 0.7439(G)/0.2561(C), those of g.95992A>G were 0.7050(A)/0.2950(G), and those of g.96058T>C were 0.7301(T)/0.2699(C). The χ^2 test indicated that the genotype distributions of the 4 mutations were all in good accordance with Hardy-Weinberg equilibrium (χ^2 < 0.052). The genetic indexes of each locus including homozygosity, heterozygosity, effective number of alleles, and PIC were calculated (Table 2). The PIC value of the mutations g.95925G>A, g.95973G>C, g.95992A>G, and g.96058T>C were 0.0837, 0.3084, 0.3294, and 0.3164, respectively. According to the classification of PIC (high polymorphism if the PIC value is greater than 0.5, medium polymorphism if the PIC value is between 0.25 and 0.5, and low polymorphism if the PIC value is less than 0.25), the experimental Qinchuan cattle were in the medium polymorphism level at g.95973G>C, g.95992A>G, and g.96058T>C; at g.95925G>A, the population studied was in the low polymorphism level.

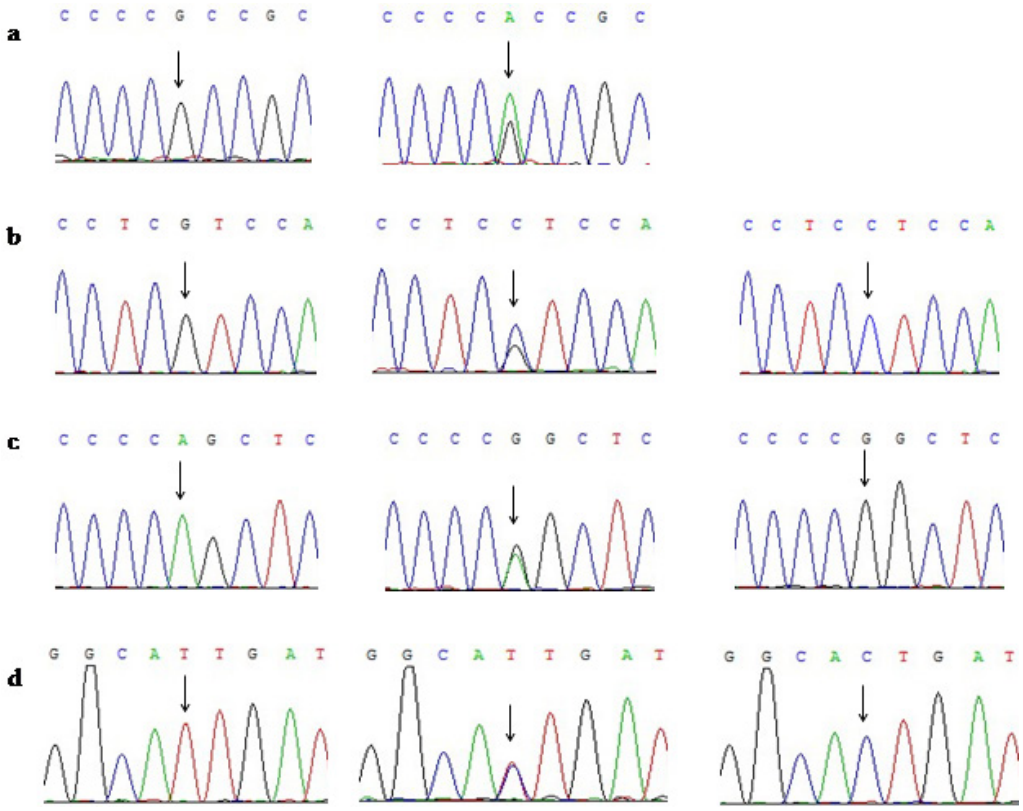

Figure 1. Sequencing map of the bovine *PRKAG2* gene. Single nucleotide polymorphisms are indicated using arrows. **a.** g.95925G>A, **b.** g.95973G>C, **c.** g.95992A>G, and **d.** g.96058T>C.

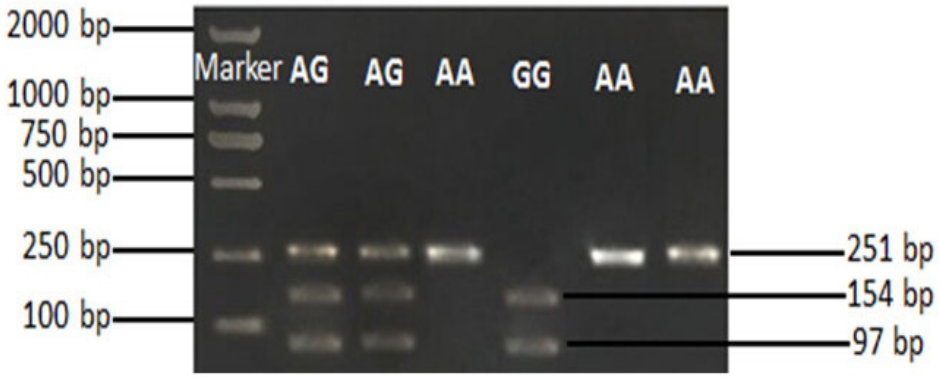

Figure 2. Restriction fragment length polymorphism electrophoresis pattern of the g.95992A>G mutation in exon 3 of the Qinchuan cattle *PRKAG2* gene. Marker: DL2000 (TaKaRa, Dalian, China).

Table 1. Genotypic and allelic frequencies of *PRKAG2* gene single nucleotide polymorphisms (SNPs) in the Qinchuan cattle population.

SNP	Genotypic frequencies (number)			Total	Allelic frequencies		χ^2 (HWE)
g.95925G/A	GG	GA	AA	578	G	A	1.3345
	0.9083	0.0917	0		0.9542	0.0458	
	(526)	(51)	(0)				
g.95973G/C	GG	GC	CC	578	G	C	5.8785
	0.5727	0.3426	0.0848		0.7439	0.2561	
	(320)	(220)	(38)				
g.95992A/G	AA	AG	GG	578	A	G	4.5845
	0.5156	0.3789	0.1055		0.7050	0.2950	
	(287)	(240)	(50)				
g.96058T/C	TT	CT	CC	578	T	C	1.0681
	0.5415	0.3772	0.0813		0.7301	0.2699	
	(308)	(228)	(42)				

HWE = Hardy-Weinberg equilibrium; $\chi 0.05^2 = 5.991$, $\chi 0.01^2 = 9.21$.

Table 2. Genetic indexes of *PRKAG2* gene single nucleotide polymorphisms (SNPs) in the Qinchuan cattle population.

SNP	Gene homozygosity	Gene heterozygosity	Effective allele number	Polymorphic information content
g.95925G/A	0.9125	0.0875	1.0959	0.0837
g.95973G/C	0.6190	0.3810	1.6155	0.3084
g.95992A/G	0.5841	0.4159	1.7121	0.3294
g.96058T/C	0.6059	0.3941	1.6504	0.3164

PIC > 0.5 means high polymorphism; 0.25 < PIC < 0.5 means moderate polymorphism; PIC < 0.25 means low polymorphism.

Effects of the polymorphism locus on BMTs

The association between *PRKAG2* variants and 8 BMTs was analyzed, and the results are shown in Table 3. For the g.95925G>A mutation, no difference was found between the 2 genotypes (GG and GA) for the 8 BMTs studied ($P > 0.05$). At the g.95973G>C locus, the mean value of BMTs of animals with genotype GG was significantly different from that with genotype CC for parameters HH and CD ($P < 0.05$); meanwhile, differences in RL and HW were observed between genotypes GG and GC with RL and HW. HH was different between genotypes GC and CC ($P < 0.05$). No difference was observed between genotypes for BL, WH, HG, and PBW ($P > 0.05$). For g.95992A>G, there was a significant difference in WH and HW between genotypes AA and GG, and there was also a significant difference in WH and CD between genotypes AG and GG ($P < 0.05$). Individuals with genotype AA had higher HH and CD than individuals with genotype GG ($P < 0.01$). Individuals with genotype AG also had higher HH than individuals with genotype GG ($P < 0.01$). No difference was observed between genotypes AA, AG, and GG for BL, RL, HG, and PBW ($P > 0.05$). At the g.96058T>C locus, there was a significant difference between genotypes TT and CC and also between TC and CC for WH and CD ($P < 0.05$). A very significant difference was observed between genotypes TT and CC for HH ($P < 0.01$); individuals with the TC genotype also had a significantly higher HH than individuals with the CC genotype ($P < 0.01$). No difference was observed between any genotype for BL, RL, HW, HG, and PBW ($P > 0.05$). These results suggested that g.95973G>C, g.95992A>G, and g.96058T>C could be used as valuable markers to select excellent Qinchuan cattle with desired BMTs.

Table 3. Association of *PRKAG2* gene single nucleotide polymorphisms (SNPs) with body measurement traits in the Qinchuan cattle population.

SNP	Genotypes	Body measurement traits (cm)							
		BL	WH	HH	RL	HW	CD	HG	PBW
g.95925G>A	GG	126.983 ± 1.021	117.382 ± 0.587	120.873 ± 0.557	39.214 ± 0.379	36.443 ± 0.471	55.992 ± 0.533	155.479 ± 1.875	18.224 ± 0.238
	GA	123.637 ± 3.335	115.175 ± 2.239	119.743 ± 1.859	37.885 ± 1.297	35.515 ± 1.558	54.817 ± 1.886	153.495 ± 3.558	17.427 ± 0.975
g.95973G>C	GG	126.713 ± 1.035	116.182 ± 0.775	120.113 ± 0.638^{b}	38.765 ± 0.467^{b}	35.279 ± 0.575^{b}	55.327 ± 0.625^{b}	153.457 ± 2.163	17.933 ± 0.312
	GC	126.988 ± 1.433	117.537 ± 1.216	120.798 ± 0.847^{b}	40.513 ± 0.631^{a}	37.854 ± 0.742^{a}	56.972 ± 0.793	158.832 ± 2.579	18.018 ± 0.352
	CC	131.481 ± 4.469	121.438 ± 3.458	126.620 ± 2.857^{a}	40.079 ± 1.839	37.453 ± 2.158	60.477 ± 2.342^{a}	162.735 ± 4.270	18.316 ± 1.269
g.95992A>G	AA	126.527 ± 1.034	116.427 ± 0.874^{b}	120.415 ± 0.721^{B}	39.143 ± 0.641	35.487 ± 0.538^{b}	55.347 ± 0.758^{B}	152.733 ± 2.358	17.758 ± 0.311
	AG	126.635 ± 1.258	116.574 ± 0.947^{b}	120.256 ± 0.815^{B}	39.369 ± 0.637	37.611 ± 0.658	56.440 ± 0.784^{b}	158.173 ± 2.741	18.329 ± 0.379
	GG	131.149 ± 3.377	122.958 ± 2.844^{a}	126.722 ± 2.115^{A}	41.173 ± 1.395	39.835 ± 1.693^{a}	61.659 ± 1.987^{Aa}	166.866 ± 7.858	19.032 ± 0.922
g.96058T>C	TT	126.748 ± 1.184	116.472 ± 0.883^{b}	120.437 ± 0.685^{B}	39.258 ± 0.524	35.976 ± 0.635	56.284 ± 0.598^{b}	153.255 ± 2.174	17.835 ± 0.377
	TC	126.274 ± 1.318	116.683 ± 0.978^{b}	120.836 ± 0.753^{B}	39.536 ± 0.584	36.479 ± 0.684	56.036 ± 0.875^{b}	157.026 ± 2.974	18.142 ± 0.414
	CC	129.679 ± 3.742	122.735 ± 2.468^{a}	125.949 ± 2.014^{A}	41.195 ± 1.842	38.883 ± 1.879	59.749 ± 2.147^{a}	162.579 ± 7.894	18.985 ± 1.057

BL = body length; WH = withers height; HH = hip height; RL = rump length; HW = hip width; CD = chest depth; HG = heart girth; PBW = pin bone width. ^{a,b}Means with different superscripts are significantly different ($P < 0.05$). ^{A,B}Means with different superscripts are significantly different ($P < 0.01$).

Effects of polymorphisms on MQTs

The associations between the 4 mutations and the 3 MQTs were analyzed, and the results are shown in Table 4. g.95925G>A had no significant effects on the 3 MQTs: BT, LMA, and IFC ($P > 0.05$). For g.95973G>C, the mean value of MQTs of individuals with genotype GG was very significantly higher than that of genotype GC for BT ($P < 0.01$); LMA was significantly different between genotypes GG and GC ($P < 0.05$). At the g.95992A>G locus, there was a significant difference between genotypes AA and AG for BT and LMA ($P < 0.05$). For the g.96058T>C mutation, a difference was only found between genotypes TT and TC for LMA ($P < 0.05$). For the 4 mutations, there was no difference between any genotype for IFC ($P > 0.05$).

Table 4. Association of *PRKAG2* gene single nucleotide polymorphisms (SNPs) with meat quality traits in the Qinchuan cattle population.

SNP	Genotypes	Meat quality traits		
		BT (cm)	LMA (cm^2)	IFC (%)
g.95925G>A				
	GG	0.847 ± 0.023	47.874 ± 1.477	7.585 ± 0.131
	GA	0.797 ± 0.078	43.538 ± 4.968	7.046 ± 0.355
g.95973G>C				
	GG	0.764 ± 0.027^{B}	44.827 ± 1.473^{b}	7.483 ± 0.137
	GC	0.938 ± 0.036^{A}	50.018 ± 2.287^{a}	7.579 ± 0.147
	CC	0.836 ± 0.131	46.573 ± 5.625	7.405 ± 0.544
g.95992A>G				
	AA	0.793 ± 0.029^{b}	45.487 ± 1.858^{b}	7.385 ± 0.121
	AG	0.907 ± 0.033^{a}	50.794 ± 2.173^{a}	7.636 ± 0.143
	GG	0.845 ± 0.079	48.752 ± 3.798	7.697 ± 0.349
g.96058T>C				
	TT	0.817 ± 0.025	45.795 ± 1.864^{b}	7.517 ± 0.114
	TC	0.884 ± 0.035	51.335 ± 1.989^{a}	7.603 ± 0.146
	CC	0.849 ± 0.089	47.887 ± 4.753	7.627 ± 0.375

BT = backfat thickness; LMA = loin muscle area; IFC = intramuscular fat content. ^{a,b}Means with different superscripts are significantly different ($P < 0.05$). ^{A,B}Means with different superscripts are significantly different ($P < 0.01$).

DISCUSSION

AMPK, a heterotrimer composed of α-, β-, and γ-subunits, is a serine/threonine kinase; it works in the regulation of the glucose metabolic pathway in muscle (Gollob et al., 2002). The α-subunit has effects on catalytic activity, whereas the γ-subunit is involved in regulating enzyme activation. *PRKAG2*, which encodes the γ2 regulatory subunit of AMPK, is highly expressed in skeletal and cardiac muscle (Cheung et al., 2000). An essential role of AMPK is to function as a metabolic sensor in muscle, responding to cellular energy demands by depleting ATP stores (Hardie and Hawley, 2001; Winder, 2001). This function of AMPK suggested that *PRKAG2* might be associated with maintaining an adequate ATP resource (Blair et al., 2001).

PRKAG2 plays a role in regulating key metabolic pathways in muscle, particularly glucose metabolism (Gollob et al., 2002). Therefore, we considered it as a candidate gene to explore its effects on animal growth. Identifying quantitative trait loci will facilitate the Chinese indigenous cattle breeding program, and molecular genetic information can also bring about significant positive developments for animals (Adoligbe et al., 2012). The candidate gene approach is a very effective method to analyze the association between gene polymorphisms and

valuable economical traits in farm animals (Rothschild and Soller, 1997). Through the candidate gene approach, studies have been performed on animal reproduction (Chu et al., 2010), growth (Li et al., 2010), and MQTs (Jiao et al., 2010), which will provide more valuable data for further research. To facilitate the Chinese indigenous cattle-breeding program, further molecular genetic information on quantitative trait loci should be collected (Adoligbe et al., 2012).

PRKAG2, which is related to conduction system disease and cardiac hypertrophy (Gollob et al., 2002), is expressed at a high level in skeletal and cardiac muscle. Studies have demonstrated that *PRKAG2* is associated with regulating key metabolic pathways in muscle, particularly in glucose metabolism (Gollob et al., 2002). These findings suggest that *PRKAG2* may have effects on BMTs and MQTs in animals.

In this study, sequence analysis of *PRKAG2* revealed 4 novel SNPs: 2 synonymous mutations, g.95925G>A and g.95973G>C, in exon 3; 1 missense mutation, g.95992A>G, in exon 3 leading to the Ser to Gly change of the 75th amino acid; and 1 g.96058T>C mutation in intron 3. g.95973G>C, g.95992A>G, and g.96058T>C showed 3 genotypes, whereas g.95925G>A showed 2 genotypes. The associations of *PRKAG2* polymorphisms with 8 BMTs (BL, WH, HH, RL, HW, CD, HG, and PBW) were analyzed using blood samples from 578 Qinchuan cattle. The possible relationships between the *PRKAG2* polymorphisms and the 3 MQTs (BT, LMA, and IFC) were also evaluated. The results of the association analysis show that g.95925G>A has no significant effects on the BMTs and MQTs studied. g.95973G>C is associated with HH, RL, HW, CD, HG, BT, and LMA. CC seems to be the beneficial genotype for BMTs; however, for MQTs, the GC genotype is better. G.95992A>G is associated with WH, HH, HW, CD, BT, and LMA. The GG genotype is better for BMTs; AG seems to be the beneficial genotype for MQTs. g.96058T>C is associated with WH, HH, CD, and LMA. CC is the best genotype for BMTs, and TC is the best genotype for MQTs. The change of amino acid by the g.95992A>G mutation may have effects on the function of the protein produced by the *PRKAG2* gene, which may play a key role in glucose metabolism through AMPK. Studies suggested that introns and their removal by the spliceosome might have effects on gene expression at many different levels, including polyadenylation, transcription, translational efficiency, mRNA export, and the rate of mRNA decay (Nott et al., 2002). The absence of genotype AA at the g.95925G>A locus can be explained in 2 ways: this genotype does not exist at all, or it is due to the artificial selection. According to the results of this study, it is suggested that the *PRKAG2* gene may have potential effects on BMTs and MQTs in the Qinchuan cattle population.

CONCLUSIONS

In this study, 4 novel SNPs of the *PRKAG2* gene were revealed. g.95925G>A, g.95973G>C, and g.95992A>G were located in exon 3. g.96058T>C was located in intron 3. Association analysis results showed that g.95925G>A did not influence the traits that were studied, while the other 3 SNPs had significant effects on some of the BMTs and MQTs in the Qinchuan cattle population. Therefore, the *PRKAG2* gene could be considered for marker-assisted selection to improve growth and MQTs of Qinchuan cattle.

ACKNOWLEDGMENTS

We thank all the research assistants and laboratory technicians who contributed to

this study. We extend special thanks to Professor Zan for his assistance. Research supported by the National 'Twelfth Five-Year' ''863'' Plan (#2013AA102505), the National 'Twelfth Five-Year' ''863'' Plan (#2011AA100307-02), the National Natural Science Foundation of China (#31272411), the National Major Project of 'Twelfth Five-Year' Transgenic Breeding (#2013ZX08007-002), and the Project of Constructing National Modern Agricultural and Industrial Technology System (#CARS-38).

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