RESEARCH ARTICLE

Association and expression analyses of the *Ucp2* and *Ucp3* gene polymorphisms with body measurement and meat quality traits in Qinchuan cattle

YANING WANG[1], WUCAI YANG[1,2], LINSHENG GUI[1], HONGBAO WANG[1,2] and LINSEN ZAN[1,2]*

[1]*College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, People's Republic of China*
[2]*National Beef Cattle Improvement Center of China, Yangling, Shaanxi 712100, People's Republic of China*

Abstract

The uncoupling proteins (UCPs) belong to the mitochondrial inner membrane anion carrier superfamily and play an important role in energy homeostasis. Genetic studies have demonstrated that *Ucp2* and *Ucp3* gene variants are involved in obesity and metabolic syndrome. The aim of this study was to identify associations between polymorphisms of *Ucp2* and *Ucp3* genes and economically-important traits in Qinchuan cattle. In the present study, one single-nucleotide polymorphism (SNP) in the 5′UTR region (SNP1: g.C-754G) of the *Ucp2* gene was identified by direct sequencing of 441 Qinchuan cattle. Two SNPs in exon 3 (SNP2: g.G4877A; SNP3: g.C4902T) of the *Ucp3* gene were identified by sequencing and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) among 441 Qinchuan cattle. Association analysis showed that SNP1 and SNP2 were associated with the meat quality traits (MQTs) including back fat thickness, loin muscle area and intramuscular fat content. SNP3 was found to be associated with part of the body measurement traits (BMTs) which referred to withers height and chest depth. In addition, QTL pyramiding analysis showed that individuals with diplotype *P3P3* (*GG–GG–CC*) exhibited the best performance in terms of back fat thickness, loin muscle area, intramuscular fat content, rump length, hip width, chest depth and chest circumference. With regard to the G4877A mutation, real time PCR analysis revealed that individuals with *AA* genotype of the *Ucp3* gene expressed higher mRNA levels than those with *GG* genotype. These results suggest that the diplotype *P3P3* (*GG–GG–CC*) could be used as a molecular marker of the combined genotypes for future selection of body measurement traits and meat quality traits in Qinchuan cattle.

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Introduction

Body measurement traits (BMTs) and meat quality traits (MQTs) are economically important traits that receive considerable attention in cattle breeding. It is widely accepted that BMTs and MQTs are controlled by multiple genes, which make it difficult to realize rapid genetic improvement by traditional phenotype-dependent breeding methods. During the past several decades, marker-assisted selection (MAS) had led to great achievements in livestock selection and breeding (Pedersen *et al.* 2009). Consequently, it would be reasonable to place considerable emphasis on sifting out numerous candidate genes and elaborating the significant associations between their genetic variations, and BMTs and MQTs (Hirwa *et al.* 2011).

Uncoupling proteins (UCPs) belong to the mitochondrial inner membrane anion carrier superfamily and are considered pivotal regulators of energy homeostasis (Stefan *et al.* 2005). A group of five different UCPs (UCP1–5) have been identified, with each protein exhibiting distinct tissue distributions and performing different functions (Ricquier and Bouillaud 2000). Among these genes, *Ucp2* is widely expressed in almost all mammalian tissues and regulates energy homeostasis at multiple levels, including gene expression, transcription and posttranslational regulation (Donadelli *et al.* 2014). *Ucp3* is mainly expressed in skeletal muscle, brown adipose tissue, white adipose tissue and heart (Azzu and Brand 2010). It is reported that UCP2 and UCP3 are highly active in various signalling pathways related to

*For correspondence. E-mail: zanlinsen@163.com.
Yaning Wang and Wucai Yang contributed equally to this work.

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energy metabolism (Brand and Esteves 2005), oxidative stress (Azzu and Brand 2010) and body mass index (Sherman *et al.* 2008). Meanwhile, relationships between *Ucp2* or *Ucp3* polymorphisms and phenotypes have been identified. For example, genetic variation studies of *Ucp2* gene showed that the −866G/A and Ala55Val C/T polymorphisms were associated with human obesity and insulin resistance (Srivastava *et al.* 2010; Andersen *et al.* 2013; Say *et al.* 2014). In addition, *Ucp3* gene polymorphisms were found to be related to human obesity and porcine meat quality (Cieslak *et al.* 2009; Han *et al.* 2012; de Almeida Brondani *et al.* 2014).

Meat quality is determined by various factors, such as intramuscular fat content and myofibre fineness, which are regulated by a set of genes concerning adipogenesis and myogenesis (Hideyuki 2011; Muhammad *et al.* 2015). Although many researches have been devoted to the regulation of the *Ucp2* and *Ucp3* genes on energy metabolism, little information is available on the associations with BMTs or MQTs in cattle. Therefore, in this study, we made an attempt to identify *Ucp2* and *Ucp3* gene polymorphisms and evaluate their effects on BMTs and MQTs in Qinchuan cattle. The findings obtained from this study may not only suggest a broader hypothesis for further research into the role of *Ucp2* and *Ucp3* genes, but may also set a better stage for MAS-based cattle breeding.

Materials and methods

DNA samples and data acquisition

A total of 441 female Qinchuan cattle that were unrelated for at least three generations were randomly selected from Qinchuan beef cattle breeding farm of National Beef Cattle Improvement Center. The cattle were all fed a diet of corn and corn silage after weaning at 6 months old under the same management conditions. Qinchuan cattle is the most common native breed used for beef production in China and is mainly reared in Shaanxi province. The eight BMTs (body length, BL; withers height, WH; hip height, HH; rump length, RL; hip width, HW; chest depth, CD; chest circumference, CC; ischium width, IW) and three MQTs (back fat thickness, BFT; loin muscle area, LMA; intramuscular fat content, IFC) were measured at 24 months of age according to the standard protocol proposed by Gilbert *et al.* (1993). All procedures involving the animals were approved by the Animal Care and Use Committee of Northwest A&F University. Genomic DNA were obtained from blood samples according to standard methods and were stored at −20°C for further study.

SNP discovery and genotyping

The primers used to amplify the bovine *Ucp2* and *Ucp3* genes were designed using Primer-BLAST based on the sequence of the *Ucp2* gene (accession number: ID:281562) and the *Ucp3* gene (accession number: ID:281563) (table 1). For the *Ucp2* gene, novel single-nucleotide polymorphisms (SNPs) were detected and genotyped by direct sequencing of 441 DNA samples using the ABIPRIZM 377 DNA Sequencer (Applied Biosystems, Foster City, USA). For the *Ucp3* gene, the genetic variations were obtained by sequencing 25 DNA samples, and two restriction enzymes (*Sca*I and *Nsb*I, Thermo) were employed to genotype the SNPs using PCR-RFLP among the 441 individuals. The digestion mixture contained 3 μL (∼1.5 μg) PCR products, 1× digestion buffer, 3.0 U of each enzyme, and was digested at 37°C overnight.

Total RNA isolation and real-time PCR

Back adipose tissue samples were collected at slaughter from 10 female Qinchuan cattle (24 months of age from the National Beef Cattle Improvement Center, fed and maintained under the same management conditions) and snap-frozen in liquid nitrogen. All samples were stored at −80°C. Total RNA was extracted from each tissue sample using the RNAprep pure Tissue kit (Tiangen, Beijing, China) according to the manufacturer's instructions. First strand cDNA was synthesized using a reverse transcription kit (Fermentas Life Science, Hanover, USA). Quantitative real-time PCR (ABI7500) was carried out using SYBR Green (TaKaRa, Dalian, China). For evaluation of relative *Ucp3* gene expression, β-actin was used as an internal control. Primers were designed using Primer 5.0 software (table 2). Each experiment was performed at least thrice in duplicate. The relative mRNA expression levels from real-time PCR were calculated using the $2^{-\Delta\Delta CT}$ method.

Qualitative trait loci (QTL) pyramiding analysis

QTL pyramid breeding combines two or more QTLs from two or more genes which are dominant for various beneficial phenotypic breeding traits (Motoyuki and Makoto 2006; An *et al.* 2013). This method requires a donor animal to be repeatedly backcrossed with another recurrent parent to obtain introgression lines (ILs) (Motoyuki and Makoto 2006). To combine the beneficial traits, the ILs will then be crossed to produce pyramiding lines carrying all the

Table 1. Summary of PCR condition for bovine *Ucp2* and *Ucp3* genes.

Item	Function	Primer sequences	T_m (°C)	Location	Production size
Primer 1	*Ucp2* PCR	F: 5-AACAGTCCCAGACAGCCTACA-3 R: 5-CCTTCTTTCACTCCCATTTCC-3	58.9	5′UTR region	767 (bp)
Primer 2	*Ucp3* PCR	F: 5-ACTATCCACGACCCTACCTCA-3 R: 5-GGCATCCATTGTCCCACT-3	56.2	Exon 3, intron 3, part of intron 2 and exon 4	981 (bp)

Table 2. Sequences of primer pairs and amplification conditions for RT-PCR.

Item	Function	Primer sequences	T_m (°C)	Production size
Ucp3	RT-PCR	F: 5-CTGCTTTGCTGACCTCCTC-3 R: 5-GGTAATGATGCTGGAGTGGTC-3	60.5	283 bp
β-actin	Internal control	F: 5-CACCAACTGGGACGACAT-3 R: 5-ATACAGGGACAGCACAGC-3	61	202 bp

beneficial QTLs (Motoyuki and Makoto 2006). In the present study, it was determined that the SNP1 *GG* genotype of the *Ucp2* gene was beneficial for MQTs and the SNP3 *CC* genotype of the *Ucp3* gene exhibited better performance in the aspect of BMTs. Consequently, to ensure whether these two genes could be used for QTL pyramid breeding in the future, a prepyramiding analysis that first analysed the haplotypes and then genotype for all the experimental animals was conducted to confirm whether the three beneficial genotypes would be still well-performed after they were combined.

Statistical analysis

The genotypic and allelic frequencies were calculated by direct counting and additional genetic information including gene heterozygosis (H_e), effective allele numbers (N_e), polymorphism information content (PIC) and Hardy–Weinberg equilibrium (HWE) were analysed using Genpop32 software (Raymond and Rousset 1995). The haplotype analysis was performed using haploview program (http://analysis.bio-x.cn/myAnalysis.php) (Wang *et al.* 2014). Association analyses between SNPs and BMTs or MQTs were performed using SPSS 18.0 software (Wang *et al.* 2015a). Both additive and dominant effects were estimated using models in the REG procedure of the SAS 8.1 based on the method introduced by Liu (1998). The phenotypic trait records for the 24-month-old Qinchuan cattle were used for the analysis. All analyses were done in two steps, first using a full animal model, and then a reduced animal model. The adjusted linear model included fixed effects for marker genotype, season of birth (spring versus fall), sex and random animal effects. The effects associated with sex and season of birth were not matched in the linear model, as the preliminary statistical analyses indicated that these effects did not have a significant influence on trait variability in the analysed population. Therefore, the linear model is shown below:

$$Y_{ij} = \mu + G_i + e_j.$$

In this model, Y_{ij} is the phenotypic value of the traits measured on each individual cattle, μ is the overall population mean of the traits, G_i is the fixed effect of genotype and e_j is the random residual error.

The mRNA expression levels of *Ucp3* gene with different genotypes was determined by analysis of variance (ANOVA) using SPSS 18.0 software, and Tukey's posthoc test was used for subsequent individual group comparisons.

Results

Genotypic and allelic frequencies

The 5′UTR region of *Ucp2* gene from 441 Qinchuan cattle and all exons of *Ucp3* gene from 25 animals were amplified and sequenced. The obtained sequences were compared with those previously reported. One novel *Ucp2* gene SNP (SNP1: g.C-754G) and two novel SNPs in exon 3 of the *Ucp3* gene (SNP2: g.G4877A; SNP3: g.C4902T) were identified (figure 1). The SNP3 was a missense mutation of Ala70Thr and SNP2 was a synonymous mutation. The SNP1 was genotyped through direct sequencing, while the SNP2 and SNP3 were genotyped by PCR-RFLP, and three genotypes were detected at each locus (figures 1 and 2). The genetic information of the three SNPs is shown in table 3.

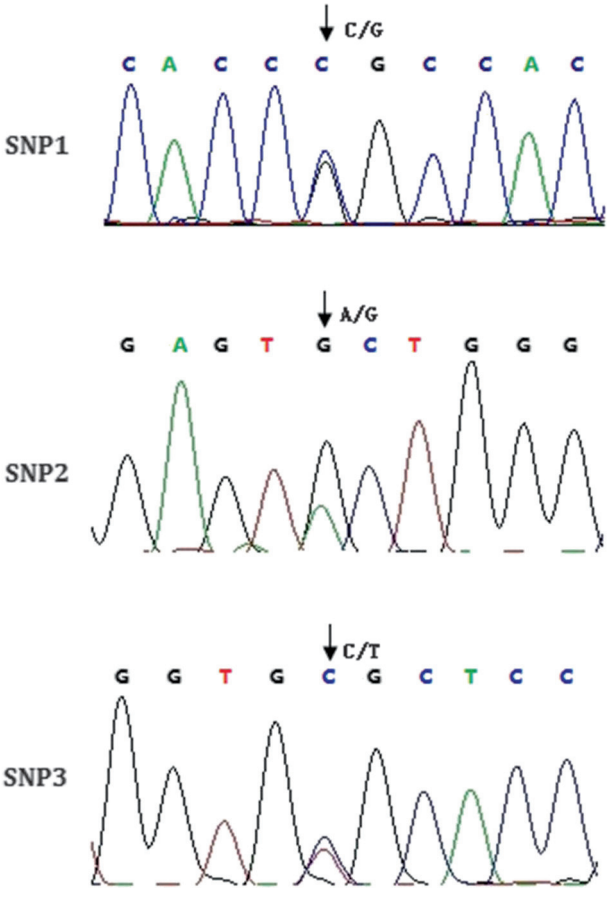

Figure 1. Sequence maps of the three SNPs of the *Ucp2* and *Ucp3* genes. The mutant nucleotides are marked with arrows.

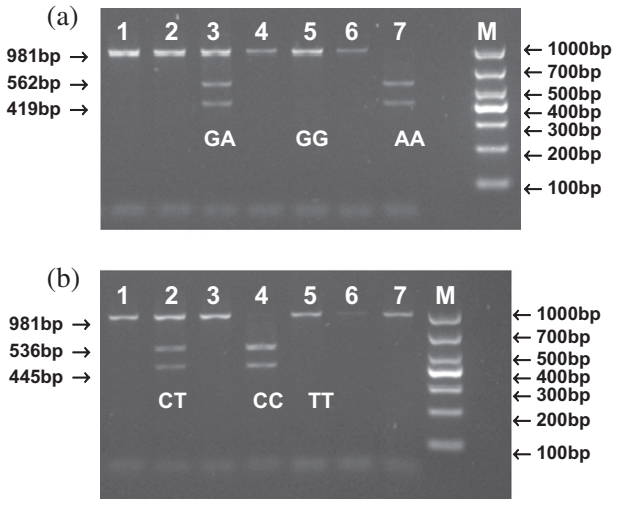

Figure 2. Electrophoresis patterns of PCR-RFLP analysis of (a) SNP2 and (b) SNP3 in bovine *Ucp3* gene.

The higher allelic frequencies were *C* (70.75%), *G* (86.96%) and *C G* (86.96%) and *C* (81.75%) for SNP1, SNP2 and SNP3, respectively. The χ^2 test indicated that SNP1 and SNP3 were in agreement with HWE ($\chi^2 < \chi^2_{0.05}$), while SNP2 was not ($\chi^2 > \chi^2_{0.01}$).

Linkage disequilibrium (LD) and haplotype analysis

To reveal the linkage relationships among SNP1, SNP2 and SNP3, LD between these three SNPs was estimated. Theoretically, values for D' and r^2 should range from 0.000 to 1.000 and $r^2 > 0.33$ is indicative of strong LD (Ardlie *et al.* 2002). However, in some cases, analysis based on haplotypes may be more advantageous than single markers due to the ancestral structure captured in the distribution of haplotypes, especially, when LD between single markers is weak (Akey *et al.* 2001; Morris and Kaplan 2002; Xue *et al.* 2013). In the present study, LD was weak among the three SNPs. The r^2 values ($r^2 < 0.33$) implied that SNP1, SNP2 and SNP3 were weakly linked (data not shown). Further, haplotype analysis of the three mutational loci among the 441 individuals was conducted, and the results are shown in table 4. All haplotypes with frequency < 0.05 were ignored as the study was interested only in common genetic polymorphisms (frequency >0.05) (Wang *et al.* 2014). Obviously, haplotype *C–G–C* showed higher frequency (50.4%) than all others. It is likely that the high frequency haplotypes have probably been present in the population for a long time and have been adapted to the environment.

Table 4. QTL haplotype analysis of *Ucp2* and *Ucp3* genes in Qinchuan cattle population.

Item	SNP1	SNP2	SNP3	Frequencies
P1	*C*	*A*	*C*	0.083
P2	*C*	*G*	*C*	0.504
P3	*G*	*G*	*C*	0.208
P4	*C*	*G*	*T*	0.124

All those frequency < 0.05 had been ignored in analysis.

Association analysis with BMTs and MQTs

Association analysis of these three SNPs with BMTs and MQTs in Qinchuan cattle are listed in tables 5–7. SNP1 was significantly associated with some of the MQTs. Individuals with genotype *GG* had significantly larger HWE ($P < 0.05$), BFT ($P < 0.01$), LMA ($P < 0.01$) and IFC ($P < 0.01$) than those with other genotypes. At this locus, there were additive effect ($P < 0.05$) on BFT, LMA and IFC. For SNP2, individuals with *GA* and *GG* genotypes had better performance in terms of IFC ($P < 0.01$), whereas no significant differences were found between each of the three genotypes and BMTs. At this locus, there were dominant effect ($P < 0.05$) on LMA and IFC. No additive effects were found on the 11 traits. For SNP3, statistical analysis revealed that this locus was associated with WH ($P < 0.01$) and CD ($P < 0.01$). However, there were no associations found between SNP3 and MQTs. At this locus, there were dominant effects ($P < 0.05$) on WH and RL, and no additive effects were found on the 11 traits.

To identify whether there were associations between the pyramided genotypes and BMTs or MQTs, further association analysis was conducted. As shown in table 8, asso-

Table 3. Genetic information of the three SNPs within *Ucp2* and *Ucp3* genes in Qinchuan cattle.

Loci	Genotypic frequencies		Allelic frequencies		H_e	N_e	PIC	χ^2 (HWE)
SNP1	*CC* (228)	0.5170	*C*	0.7075				
	CG (168)	0.3810	*G*	0.2925	0.4139	1.7062	0.3282	2.7947
	GG (45)	0.1020						
	GG (343)	0.7778	*G*	0.8696				
SNP2	*GA* (81)	0.1837	*A*	0.1304	0.2268	1.2933	0.2011	15.9278
	AA (17)	0.0385						
	CC (298)	0.6757	*C*	0.8175				
SNP3	*CT* (125)	0.2834	*T*	0.1825	0.2984	1.4254	0.2539	1.1128
	TT (18)	0.0408						

$\chi^2_{0.05} = 5.991$, $\chi^2_{0.01} = 9.21$; high polymorphism: PIC > 0.05; medium polymorphism: 0.25 < PIC ≤ 0.5; low polymorphism: PIC ≤ 0.25.

Table 5. Association analysis between SNP1 and economically important traits in Qinchuan cattle (data donate 'mean ± SE').

	Trait	SNP1			Additive effect	Dominant effect
		CC	*CG*	*GG*		
BMTs	BL (cm)	134.43 ± 0.93	134.54 ± 1.05	135.19 ± 2.00	0.38 ± 1.12	0.13 ± 0.77
	WH (cm)	119.58 ± 0.91	121.13 ± 0.65	121.04 ± 1.25	0.73 ± 0.93	−0.41 ± 0.64
	HH (cm)	121.96 ± 0.48	123.31 ± 0.52	122.92 ± 0.8	−0.02 ± 0.57	−0.18 ± 0.39
	RL (cm)	42.15 ± 0.33	42.21 ± 0.34	42.69 ± 0.63	0.27 ± 0.38	0.11 ± 0.26
	HW (cm)	38.17 ± 0.44^{b}	39.11 ± 0.46^{ab}	40.42 ± 0.87^{a}	1.13 ± 0.52*	0.09 ± 0.36
	CD (cm)	59.45 ± 0.51	60.00 ± 0.56	60.18 ± 0.95	0.36 ± 0.60	−0.09 ± 0.42
	CC (cm)	163.47 ± 1.44	165.43 ± 1.39	169.18 ± 2.65	2.85 ± 1.65	0.45 ± 1.13
	IW (cm)	19.29 ± 0.28	19.36 ± 0.30	19.40 ± 0.54	0.06 ± 0.33	−0.01 ± 0.23
MQTs	BFT (cm)	0.91 ± 0.02^{B}	0.92 ± 0.03^{B}	1.12 ± 0.75^{A}	0.11 ± 0.03**	0.04 ± 0.02
	LMA (cm^2)	46.93 ± 0.94^{B}	47.59 ± 1.09^{B}	55.23 ± 3.46^{A}	4.15 ± 1.38**	1.74 ± 0.90
	IFC (%)	7.08 ± 0.12^{b}	7.26 ± 0.10^{ab}	7.62 ± 0.16^{a}	0.27 ± 0.13*	0.04 ± 0.09

The significant level between different superscript letters of lowercase 'a' and 'b' in the same now is $P < 0.05$.The significant level between different uppercase letters 'A' and 'B' in the same row $P < 0.01$. ^{ab} Has no difference with other data in the same row * additive effect of the three genotypes of the same phenotype in the same row represent significant level at $P < 0.05$. '**' Additive effect of the three genotypes of the same phenotype in the same row represent significant level at $P < 0.01$.

Table 6. Association analysis between SNP2 and economically important traits in Qinchuan cattle (data donate 'mean ± SE').

	Trait	SNP2			Additive effect	Dominant effect
		GG	*GA*	*AA*		
BMTs	BL (cm)	134.23 ± 0.74	136.31 ± 1.5	133.19 ± 3.4	1.23 ± 1.66	1.47 ± 1.03
	WH (cm)	120.21 ± 0.62	120.98 ± 1.28	120.03 ± 2.86	−0.49 ± .143	−0.63 ± 0.96
	HH (cm)	123.31 ± 0.37	122.60 ± 0.77	121.69 ± 0.73	−1.12 ± 0.86	−0.21 ± 0.58
	RL (cm)	39.04 ± 0.34	37.69 ± 0.71	38.19 ± 0.60	−0.99 ± 0.58	−0.26 ± 0.39
	HW (cm)	59.72 ± 0.70	60.12 ± 0.82	58.50 ± 1.85	−0.49 ± 0.79	0.43 ± 0.53
	CD (cm)	42.39 ± 0.25	41.93 ± 0.52	40.81 ± 1.17	−0.77 ± 0.92	−0.59 ± 0.62
	CC (cm)	164.33 ± 1.08	166.37 ± 2.33	166.75 ± 5.02	1.21 ± 2.57	−0.42 ± 1.70
	IW (cm)	19.31 ± 0.22	19.44 ± 0.45	19.31 ± 1.01	−0.07 ± 0.50	−0.10 ± 0.34
MQTs	BFT (cm)	1.03 ± 0.11	0.95 ± 0.05	0.91 ± 0.02	0.04 ± 0.05	0.01 ± 0.03
	LMA (cm^2)	48.05 ± 3.03	53.93 ± 1.89	45.94 ± 0.89	−1.08 ± 1.82	−3.71 ± 1.22**
	IFC (%)	7.43 ± 0.08^{A}	7.65 ± 0.17^{A}	6.46 ± 0.38^{B}	−0.29 ± 0.19	−0.41 ± 0.13*

The significant level between different superscript letters of uppercase 'A' and 'B' in the same row is $P < 0.01$.
* Dominant effect of the three genotypes of the same phenotype in the same row represent significant level at $P < 0.05$.
** Dominant effect of the three genotypes of the same phenotype in the same row represent significant level at $P < 0.01$.

Table 7. Association analysis between SNP3 and economically important traits in Qinchuan cattle (data donate 'mean ± SE').

	Trait	SNP3			Additive effect	Dominant effect
		CC	*CT*	*TT*		
BMTs	BL (cm)	134.98 ± 0.80	133.23 ± 1.23	137.42 ± 3.23	1.24 ± 1.66	1.47 ± 1.03
	WH (cm)	120.69 ± 0.66^{AB}	118.76 ± 1.02^{B}	125.78 ± 2.68^{A}	2.57 ± 1.38	2.23 ± 0.86**
	HH (cm)	123.26 ± 0.42	122.44 ± 0.62	125.47 ± 1.63	1.13 ± 0.84	0.95 ± 0.52
	RL (cm)	39.08 ± 0.37	38.16 ± 0.57	37.78 ± 1.50	0.90 ± 0.57	0.91 ± 0.35*
	HW (cm)	59.76 ± 0.43	59.44 ± 0.66	61.78 ± 1.75	−0.64 ± 0.77	0.13 ± 0.48
	CD (cm)	42.44 ± 0.27^{ABa}	41.50 ± 0.42^{Bb}	44.22 ± 1.10^{Aa}	1.02 ± 0.90	0.66 ± 0.56
	CC (cm)	165.54 ± 1.16	162.58 ± 1.80	167.79 ± 4.73	1.12 ± 2.44	2.04 ± 1.51
	IW (cm)	19.57 ± 0.23	18.75 ± 0.36	19.44 ± 0.95	−0.06 ± 0.49	0.38 ± 0.30
MQTs	BFT (cm)	0.94 ± 0.21	0.91 ± 0.32	0.98 ± 0.16	0.01 ± 0.04	0.02 ± 0.03
	LMA (cm^2)	48.05 ± 0.87	47.27 ± 1.34	47.66 ± 3.54	−0.19 ± 0.82	0.29 ± 1.13
	IFC (%)	7.15 ± 0.90	7.36 ± 0.14	6.85 ± 0.55	−0.15 ± 0.19	−0.18 ± 0.12

The significant level between different uppercase letters 'A' and 'B' in the same row is $P<0.01$. ^{AB}Has no difference with the data in the same row. ^{ABa}CC genotypes has significant level of $P<0.05$ with CT genotype. ^{Aa}TT genotypes has significant level of $P<0.01$ with CT genotype. *Dominant effect of the three genotypes of the same phenotype in the same row represent significant level at $P<0.05$. **Dominant effect of the three genotypes of the same phenotype in the same row represent significant level at $P<0.01$.

Table 8. QTL pyramiding analysis of the *Ucp2* and *Ucp3* genes and economic traits in Qinchuan cattle (data donate 'mean ± SE').

Genotype	Body measurement traits							Meat quality traits		
	BL (cm)	WH (cm)	HH (cm)	RL (cm)	HW (cm)	CD (cm)	CC (cm)	BFT (cm)	LMA (cm^2)	IFC (%) [-1.1pc]
P1P1 (CC–AA–CC)	129.78 ± 5.20	113.50 ± 3.24^{B}	117.56 ± 2.00^{B}	38.89 ± 1.55^{B}	35.11 ± 1.84^{B}	54.67 ± 2.38^{B}	158.50 ± 8.49^{B}	0.90 ± 0.11^{B}	45.21 ± 1.33^{AB}	5.40 ± 0.91^{B}
P1P2 (CC–AG–CC)	136.02 ± 2.69	119.77 ± 1.70^{AB}	122.05 ± 0.80^{AB}	41.00 ± 0.76^{AB}	35.85 ± 1.16^{B}	59.11 ± 1.40^{AB}	162.85 ± 3.40^{AB}	0.90 ± 0.05^{B}	51.91 ± 2.44^{AB}	7.90 ± 0.11^{A}
P1P3 (CG–AG–CC)	135.85 ± 3.07	123.38 ± 1.55^{AB}	124.46 ± 1.02^{A}	43.15 ± 1.49^{AB}	41.85 ± 1.52^{A}	59.58 ± 1.49^{AB}	172.77 ± 3.54^{AB}	1.00 ± 0.12^{AB}	56.18 ± 4.44^{A}	7.60 ± 0.16^{A}
P1P4 (CC–AG–CT)	134.03 ± 4.87	120.07 ± 2.60^{AB}	120.70 ± 2.14^{AB}	42.53 ± 1.36^{AB}	38.27 ± 2.06^{B}	61.03 ± 2.54^{AB}	166.60 ± 7.40^{AB}	1.06 ± 0.15^{AB}	56.20 ± 4.88^{A}	7.45 ± 0.25^{A}
P2P2 (CC–GG–CC)	133.27 ± 1.14	119.57 ± 1.15^{AB}	123.25 ± 0.66^{AB}	42.44 ± 0.46^{AB}	38.81 ± 0.59^{AB}	59.22 ± 0.63^{AB}	163.46 ± 1.70^{AB}	0.89 ± 0.03^{B}	44.60 ± 1.24^{AB}	6.89 ± 0.17^{A}
P2P3 (CG–GG–CC)	135.71 ± 1.38	121.93 ± 0.85^{AB}	123.72 ± 0.65^{AB}	42.73 ± 0.43^{AB}	39.70 ± 0.62^{AB}	60.37 ± 0.75^{AB}	166.28 ± 1.85^{AB}	0.94 ± 0.03^{B}	48.36 ± 0.34^{AB}	7.19 ± 0.16^{A}
P2P4 (CC–GG–CT)	134.80 ± 2.03	117.27 ± 3.13^{AB}	123.63 ± 1.31^{AB}	41.58 ± 0.62^{AB}	37.95 ± 0.74^{B}	58.77 ± 1.21^{AB}	159.09 ± 4.05^{B}	0.89 ± 0.04^{B}	44.60 ± 1.24^{AB}	7.44 ± 0.13^{A}
P3P3 (GG–GG–CC)	142.03 ± 2.94	123.28 ± 1.65^{AB}	123.81 ± 1.40^{AB}	44.94 ± 0.80^{A}	43.89 ± 1.06^{A}	63.44 ± 1.22^{A}	178.22 ± 3.44^{A}	1.28 ± 0.12^{A}	57.65 ± 5.71^{A}	7.70 ± 0.34^{A}
P3P4 (GC–GG–CT)	129.51 ± 2.21	118.11 ± 1.31^{AB}	121.13 ± 1.20^{AB}	40.50 ± 0.73^{B}	36.88 ± 0.93^{B}	58.52 ± 1.28^{AB}	159.00 ± 3.01^{B}	0.87 ± 0.07^{B}	41.80 ± 2.16^{B}	7.10 ± 0.18^{A}
P4P4 (CC–GG–TT)	138.60 ± 4.27	125.70 ± 2.40^{A}	125.00 ± 1.23^{A}	45.20 ± 2.16^{A}	37.80 ± 3.01^{B}	64.50 ± 2.67^{A}	169.00 ± 7.39^{AB}	1.00 ± 0.14^{AB}	51.68 ± 5.83^{AB}	6.80 ± 0.91^{A}

^{a,b}Significantly different ($P < 0.05$); The significant level between different superscript letters of uppercase 'A' and 'B' in the same column is $P < 0.01$. ^{AB} Has no difference with other data in the same row.

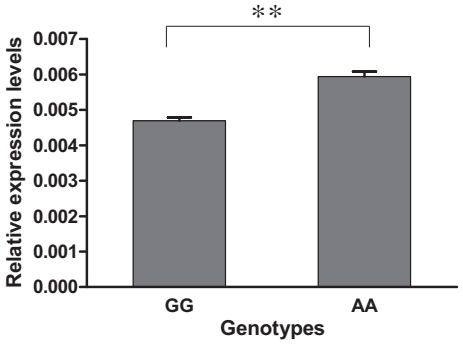

Figure 3. The *Ucp3* gene expression pattern of different genotypes in Qinchuan cattle (bars donate SE).

ciation analysis indicated that there were significant relationships between different pyramided genotypes and the 11 phenotypic traits. Among the 10 pyramided genotypes, *GG–GG–CC* proved to be the superior genotype with regard to both BMTs and MQTs, which was in agreement with single SNP association analyses.

Effect of various genotypes on Ucp3 mRNA expression

The mRNA expression levels of different genotypes in the *Ucp3* gene were analysed and the results are shown in figure 3. For SNP2, individuals with the mutant homozygotic genotype *AA* had significantly higher *Ucp3* mRNA expression levels than the wild homozygotic genotype *GG* ($P < 0.01$).

Discussion

Several studies have shown that UCP2 and UCP3 are involved in energy metabolism regulation (Brand and Esteves 2005), oxidative stress (Azzu and Brand 2010), body mass index (Sherman *et al.* 2008), obesity and hyperinsulinemia (Donadelli *et al.* 2014) through physiological and pathological processes. Further, previous studies found that genetic variation in the *Ucp2* and *Ucp3* genes were significantly associated with human obesity (Srivastava *et al.* 2010), insulin resistance (Andersen *et al.* 2013; Say *et al.* 2014) and porcine meat quality (Cieslak *et al.* 2009; Han *et al.* 2012; de Almeida Brondani *et al.* 2014). These findings suggest that the *Ucp2* and *Ucp3* genes may influence BMTs and MQTs. In this study, one SNP in the 5′UTR region (SNP1: g.C-754G) of bovine *Ucp2* gene and two SNPs in the exon 3 of bovine *Ucp3* gene (SNP2: g.G4877A; SNP3: g.C4902T) were identified. The G4877A mutation was a synonymous mutation, whereas the C4902T mutation was a missense mutation. The χ^2 test showed that the SNP2 was not in HWE. This may be due to several factors including artificial selective breeding from draft cattle to beef cattle and smaller sample sizes (Pan *et al.* 2013).

In this study, the results showed that the three SNPs were significantly associated with BMTs and some of the

MQTs. Through TFSEARCH predictions, the mutation of g.C-754G locus in the 5′UTR of bovine *Ucp2* gene was found to partly alter the binding sites of transcription factors. Previous studies reported that the function of transcriptional regulation may be defected by point mutations in the regulatory regions and altered regulatory region configurations (Mayo *et al.* 2006). Meanwhile, many mutants have been found through genetic screening for developmentally-important genes involved in transcriptional regulation (Gibson and Honeycutt 2002). Thus, the SNP1 may be associated with production traits through the promoter region or upstream *cis*-acting element of the *Ucp2* gene. For example, Wang *et al.* (2015a, b) revealed that genetic variants of the CIDEC gene promoter region could affect growth traits in Nanyang beef cattle. Meanwhile, in a recent genomewide RNA sequencing study, Tizioto *et al.* (2015) found that *Ucp2* mRNA expression level was higher in cattle populations with lower residual feed intake (high feed efficiency), suggesting that UCP2 might affect beef cattle feed efficiency. However, whether or not the *Ucp2* gene *GG* genotype identified in the present study which was associated with improved MQTs was also related to higher feed efficiency required further investigation. Although SNP2 g.G4877A mutation did not change the amino acid sequence, some reports confirmed that synonymous SNPs could affect *in vivo* protein folding and function, as well as gene expression and phenotype (Kimchi *et al.* 2007). In the present study, SNP3 was proved to be a missense mutation. It is postulated that this mutation may change the amino acid sequence of a protein, which, in turn, may affect the translation efficiency, thereby altering the function of UCP3. In addition, different genotypes may affect the mRNA stability and result in different mRNA expression levels. For example, it is reported that mutations in 3′UTR of porcine *Ucp3* gene resulted in three genotypes, with whose mRNA free energy is much lower, expresses higher mRNA level (Li *et al.* 2012). For the SNP2, g.G4877A mutation in the present study, quantitative realtime-polymerase chain reaction (qRT-PCR) analysis revealed that individuals with *AA* genotype expressed higher mRNA levels than those with *GG* genotype. But it requires further investigation to determine whether this difference is also associated with mRNA stability. It is widely accepted that UCP3 is a negative regulator of energy homeostasis and lipid metabolism, and increased *Ucp3* mRNA expression levels could cause lower fat accumulation (Azzu and Brand 2010). From these results, we can postulate that the *Ucp3* gene is negatively associated with bovine adipogenesis, or that the *Ucp3* gene may affect fatty acid (FA) metabolism and inhibit fat accumulation by preventing storage of cytosolic triglyceride (Musa *et al.* 2012). However, further verification is needed. Incontrovertibly, there exists data biases in the present study. The deviation of lower frequencies but close relationships between some genotypes (*AA* for SNP2 and *TT* for SNP3) and production traits might have been influenced by the small study population. As a consequence, the experimental population size should be increased for future studies to avoid such biases and obtain more precise data.

QTL pyramiding technology is an effective tool to allow rapid improvement in animal breeding, as it allows the integration of MAS for multiple traits of interest from several functionally-related genes (Ashikari and Matsuoka 2006). This technology has been used to produce new rice varieties with great achievements (Ruengphayak *et al.* 2015). Multigene pyramid breeding has also been used to select litter size-related genes in sheep with high efficiency (Wang *et al.* 2015a, b). The results in the present study indicated that these three SNPs were not in LD. Notably, in the present study, it was found that SNP1 of the *Ucp2* gene was more related to MQTs and SNP3 of the *Ucp3* gene to BMTs, suggesting that the two genes may potentially be used for QTL pyramid breeding to obtain individuals with better performance both in BMTs and MQTs in the future. By pyramiding three QTLs from the *Ucp2* and *Ucp3* genes, we found that one genotype *GG–GG–CC* was associated with both of the bovine BMTs and MQTs. It is likely that this genotype may have potential effects on bovine bone development, myogenesis and adipogenesis. However, further much work is required to investigate the regulatory mechanisms.

Conclusions

Our results showed for the first time that G4877A and C4902T polymorphisms in bovine *Ucp3* gene, and C-754G polymorphism in bovine *Ucp2* gene were associated with some of the BMTs and MQTs, and affected the *Ucp3* mRNA expression. *GG–GG–CC* genotype had the best performance on both of the BMTs. These results suggest that the genotype *GG–GG–CC* could be used as a molecular marker of the combined genotype for future selection for BMTs and MQTs in Qinchuan cattle.

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