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Polymorphisms of the bovine *MC3R* gene and their associations with body measurement traits and meat quality traits in Qinchuan cattle

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ABSTRACT. The melanocortin 3 receptor (*MC3R*) gene, which belongs to the rhodopsin-like family A of the G protein-coupled receptor family, plays a crucial role in feed efficiency and energy homeostasis. The aim of this study was to examine associations between bovine *MC3R* gene polymorphisms and body measurement traits (BMTs) and meat quality traits (MQTs). We identified three synonymous mutations (T429C, T537C, and T663C) in exon 1 of the *MC3R* gene in Chinese Qinchuan beef cattle (N = 271) by sequencing. *D*' and r^2 values revealed that these three SNPs were in strong linkage disequilibrium (LD) ($r^2 >$ 0.33); the T429C and T537C SNPs were in complete LD (*D*' = 1 and $r^2 = 1$). Association analyses revealed that the SNPs were significantly associated with BMTs and MQTs in Qinchuan cattle. Individuals with the wild homozygotic genotypes g.TTTT and g.TT had significantly higher values of chest depth, heart girth, back fat thickness, intramuscular fat content, and loin muscle area than the mutant heterozygotic genotypes g.TCTC and g.TC. These results suggest that the *MC3R* gene affects

MQTs in Qinchuan cattle, and that it may be a good candidate gene for marker-assisted selection.

Key words: *MC3R*; Qinchuan cattle; Body measurement trait; Meat quality trait; Single-nucleotide polymorphism

INTRODUCTION

Qinchuan cattle are the best-known native breed for beef production in China, and are mainly reared in Shaanxi province. Body measurement traits (BMTs) and meat quality traits (MQTs) play an important role in the evaluation of beef cattle productivity, and have received increasing attention in cattle selection and breeding. The identification of quantitative trait loci and major-effect genes that are associated with these traits is a powerful and efficient strategy for the molecular breeding of beef cattle, and has been the main objective of several genetic studies (Pedersen et al., 2009; Ribeca et al., 2014).

The melanocortin 3 receptor (*MC3R*) gene, which belongs to the rhodopsin-like family A of the G protein-coupled receptor family, plays a crucial role in feed efficiency and energy homeostasis (Begriche et al., 2011; Irani et al., 2011), and its polymorphisms have been studied in terms of their effects on growth traits, fat deposition, and obesity (Jiang et al., 2002; Sharma et al., 2008; Tao, 2010; Santos et al., 2011; Müller et al., 2012). Previous studies have shown that *MC3R* knock-out (-/-) mice exhibit reduced lean mass, increased fat mass, and metabolic adaptation to restricted feeding (Chen et al., 2000). In humans, over 20 mutations in *MC3R* have been identified (Cieslak et al., 2013), and 6Thr>Val, 81Val>Ile, and 335Ile>Ser are strongly predisposing to obesity (Tao, 2007). In the porcine *MC3R*, two silent single-nucleotide polymorphisms (SNPs) have been detected, and are significantly related to daily weight gain (Weisz et al., 2011). Furthermore, extensive studies on the *MC3R* gene in the chicken, dog, red fox, and Chinese raccoon dog have revealed variable levels of polymorphism (Sharma et al., 2008; Skorczyk et al., 2011), and association studies conducted on chickens and red foxes have revealed significant relationships with body weight, carcass weight, feed efficiency, and abdominal fat mass. However, few studies have been conducted on *MC3R* and bovine BMTs and MQTs.

Therefore, we investigated the effects of *MC3R* on bovine BMTs and MQTs. Sequencing was used to detect variants of the *MC3R* gene in cattle, and relationships with BMTs and MQTs were evaluated in order to detect potential markers that could be used for selection by breeders.

MATERIAL AND METHODS

DNA samples and data collection

In total, 271 blood samples were collected from pure-bred Qinchuan cattle, which were fed corn and corn silage after weaning at 6 months of age. At 24 months of age the following BMTs were measured, as previously described (Ozkaya and Bozkurt, 2009): 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). The following MQTs were mea-

sured using an ultrasound scanner (Brethour, 1994; Hamlin et al., 1995), also at 24 months of age: back fat thickness (BFT), intramuscular fat content (IFC), and loin muscle area (LMA). Genomic DNA samples were obtained from the blood samples using standard methods (Sambrook and Russell, 2001).

SNP detection and genotyping

A pair of primers (1F, 5'-AACAGTCCCAGACAGCCTACA-3' and 5'-CCTTCT TTCACTCCCATTTCC-3') was designed based on the DNA sequence of bovine *MC3R* (GenBank accession No. ID281798) using the oligonucleotide design tool Primer 5.0 software, in order to amplify a 421-bp product to find SNPs. Mutations were detected using DNA pools as templates from 20 individual genomic samples that were randomly selected from the cattle. SNPs and novel mutations were identified by direct sequencing, using an ABI PRIZM 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

Statistical analysis

Gene frequencies were determined by direct counting, and the Hardy-Weinberg equilibrium was analyzed by a chi-square test using SAS 8.1 (SAS Institute Inc., Cary, NC, USA). Pairwise linkage disequilibrium (LD) was measured using the SHEsis platform (Shi and He, 2005). Association analyses between the SNP-marker genotypes of the *MC3R* gene and the BMTs and MQTs (BL, WH, HH, RL, HW, CD, HG, PBW, BFT, LMA, and IFC) were performed using the SAS 8.1 software. All of the analyses were conducted in two steps: firstly using a full animal model and then using a reduced animal model. The full animal model included marker genotype, season of birth (spring or fall), birth year, sire, farm, and sex as fixed effects, and individual animal as a random effect. Sire, farm, sex, birth year, and season of birth were not significant in the full model; hence, the following reduced model was used for the final analysis:

$$y_{ik} = u + G_i + e_{ik}$$

where y_{ik} is the value of the trait, u is the population mean, G_i is the fixed effect of genotype, and e_{ik} is the random error.

RESULTS

Genotypic and allelic frequencies

We amplified and sequenced pooled DNA data (Pool-Seq) from an exon of the *MC3R* gene in 20 Qinchuan cattle. The sequences obtained were compared with a previously reported sequence (GenBank accession No. 505405), and three synonymous mutations (T429C, T537C, and T663C) in exon 1 of *MC3R* were identified (Figure 1). The genotyping of these SNPs, which was conducted using DNA sequencing, revealed three genotypes at each locus (Figure 1). The chi-square test indicated that the three mutations were in Hardy-Weinberg equilibrium ($P > 0.05$) (Table 1).

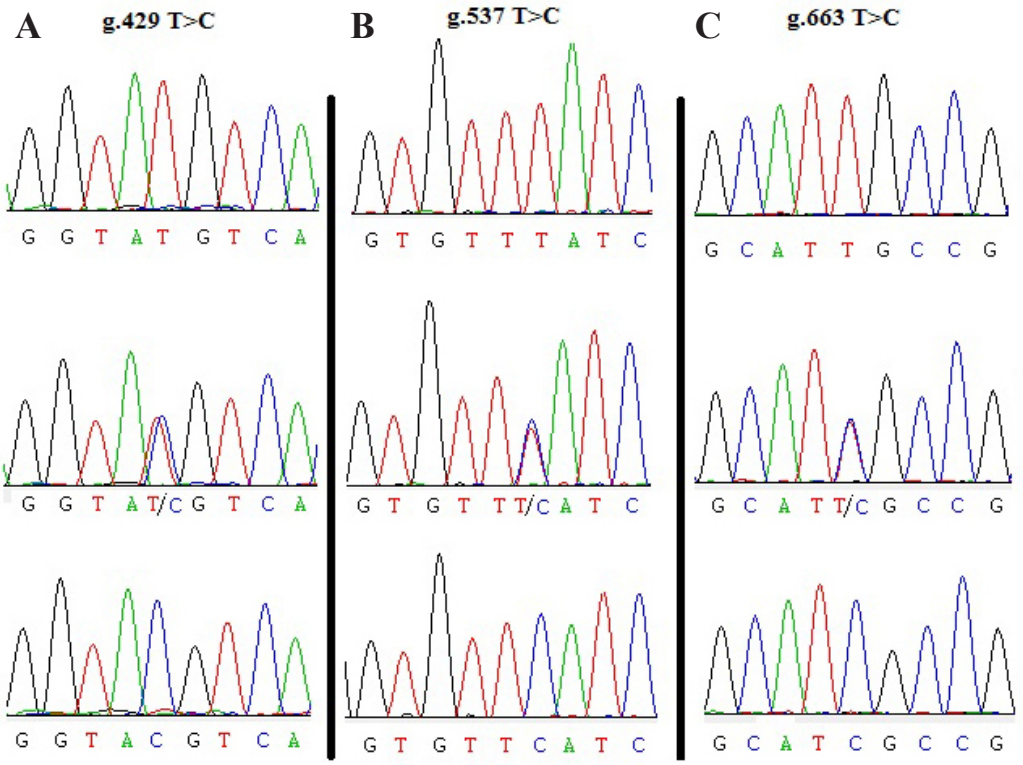

Figure 1. Sequencing maps of three mutations in the bovine *MC3R* gene. **A. B. C.** Sequencing map of T429C, T537C and T663C, respectively.

Table 1. Genotypic and allelic frequencies (%) at the *MC3R* gene for the SNP in Qinchuan cattle populations.

Loci	Observed genotypes (N)	Frequencies		χ^2 (P value)
		Genotypes	Alleles	
T429C	TT (216)	0.797	T 0.886	4.312 (P = 0.116)
	TC (48)	0.177	C 0.114	
	CC (7)	0.026		
T537C	TT (216)	0.797	T 0.886	4.312 (P = 0.116)
	TC (48)	0.177	C 0.114	
	CC (7)	0.026		
T663C	TT (190)	0.701	T 0.828	2.756 (P = 0.252)
	TC (69)	0.255	C 0.172	
	CC (12)	0.044		

LD and haplotype analyses

In order to determine the linkage relationships between the three variants, two popular measures of LD, commonly denoted by r^2 and D', were used between pairs of biallelic markers (Ardlie et al., 2002); if $r^2 > 0.33$, the LD was considered to be strong (Ardlie et al., 2002). The

D' and r^2 values we obtained suggested that the three SNPs were in strong LD (Table 2), and the T429C and T537C SNPs were in complete LD (*D*' = 1 and r^2 = 1) (Table 2). We found three genotypes: a wild homozygotic g.TTTT genotype, a heterozygotic g.TCTC genotype, and a mutant homozygotic g.CCCC genotype. Therefore, T429C and T537C were analyzed together and marked as a single locus for the association analysis.

Table 2. Estimated values of linkage disequilibrium for SNPs bovine *MC3R* Qinchuan cattle.

SNPs	T429C-T537C	T429C-T663C	T537C-T663C
r^2	1.000	0.661	0.661
D'	1.000	1.000	1.000

Association between *MC3R* genotypes and BMTs and MQTs

Associations between the *MC3R* genotypes and the BMTs and MQTs were analyzed, and the results are presented in Tables 3 and 4 (genotypes with frequencies lower than 0.05 were ignored). Animals with the g.TTTT genotype had significantly higher values of BL, CD, HG, BFT, LMA, and IFC than those with the g.TCTC genotype ($P < 0.05$). At the polymorphic locus 663, individuals with the g.TT genotype had significantly higher values of WH, CD, HG, BFT, LMA, and IFC than those with the g.TC genotype ($P < 0.05$).

Table 3. Associations of combined genotypes of the *MC3R* gene with meat quality traits in Qinchuan cattle.

Loci	Genotypes	Meat quality traits		
		BFT	LMA	IFC
T429C-T537C	g.TTTT (216)	0.917 ± 0.030^a	47.496 ± 1.347^a	7.346 ± 0.114^a
	g.TCTC (48)	0.824 ± 0.048^b	38.460 ± 2.765^b	6.912 ± 0.271^b
T663C	g.TT (190)	0.923 ± 0.033^a	48.293 ± 1.440^a	7.405 ± 0.113^a
	g.TC (69)	0.835 ± 0.038^b	39.500 ± 2.252^b	6.949 ± 0.234^b

Values with different superscript letters (a, b) within the same line differ significantly at $P < 0.05$.

DISCUSSION

The bovine *MC3R* gene, mapped to chromosome 13 and containing a single 972-bp exon (GenBank accession No. JN210913), is very similar to the human (86.7%) and mouse (87.6%) *MC3R* gene. We amplified and sequenced the exon within the bovine *MC3R* gene. A comparison with a previously reported sequence identified three SNPs within the bovine *MC3R* gene by Pool-Seq (T429C, T537C, and T663C), and a chi-square test revealed that they were in Hardy-Weinberg equilibrium and exhibited strong LD. Interestingly, a previous study also found three SNPs of the *MC3R* gene in Xiangxi cattle, and these were in strong LD (Luoreng et al., 2014). These results indicate that *MC3R* could be used as a molecular marker in marker-assisted selection.

Our statistical analyses revealed that the three synonymous SNPs affected BMTs and MQTs. Individuals with the wild homozygotic genotypes g.TTTT and g.TT had significantly higher values of CD, HG, BFT, LMA, and IFC than did the mutant heterozygotic genotypes g.TCTC and g.TC. Although these three mutations do not change amino acid sequences, synonymous SNPs can affect *in vivo* protein folding, mRNA stability (Duan et al., 2003), and

Table 4. Associations of combined genotypes of the *MC3R* gene with body measurement traits in Qinchuan cattle.

Loci	Genotypes	Body measurement traits							
		BL	WH	HH	RL	HW	CD	HG	PBW
T429C	g.TTTT (216)	134.66 ± 1.130^{a}	120.101 ± 1.014	123.010 ± 0.578	42.520 ± 0.390	38.931 ± 0.543	60.202 ± 0.636^{a}	165.468 ± 1.589^{a}	18.597 ± 0.267
T537C	g.TCTC (48)	127.28 ± 1.836^{b}	117.25 ± 1.382	120.515 ± 1.247	41.563 ± 0.851	37.437 ± 0.998	56.631 ± 1.228^{b}	156.000 ± 3.077^{b}	17.218 ± 0.499
T663C	g.TT (190)	134.881 ± 1.203	121.031 ± 0.755^{a}	123.250 ± 0.6331	42.535 ± 0.417	38.969 ± 0.582	60.424 ± 0.674^{a}	166.023 ± 1.689^{a}	18.677 ± 0.287
	g.TC (69)	129.565 ± 1.752	115.663 ± 2.492^{b}	120.750 ± 0.928	41.848 ± 0.706	37.696 ± 0.876	57.174 ± 1.093^{b}	157.761 ± 2.672^{b}	17.522 ± 0.433

Values with different superscript letters (a, b) within the same line differ significantly at $P < 0.05$.

splicing (Pagani et al., 2005), and consequently affect function as well as gene expression and phenotype (Kimchi et al., 2007; Sauna et al., 2007). Synonymous mutations in *MC3R* are closely associated with daily weight gain, body weight, and feed efficiency in pigs and red foxes (Skorczyk et al., 2011; Weisz et al., 2011); further studies are required in order to investigate how SNPs affect variations in these traits. BMTs and MQTs are quantitative traits controlled by multiple genes (Boukha et al., 2011). So, due to the negative effects of other genes, it is not sure that a candidate gene may have effect in another breed (Ma et al., 2011; Liu et al., 2014). Therefore, our results need to be confirmed with much larger sample sizes and in other populations.

In conclusion, we found that T429C, T537C, and T663C polymorphisms in the bovine *MC3R* gene were associated with BMTs and MQTs. These data strongly suggest that *MC3R* polymorphisms may be used as genetic markers for the breeding of beef cattle; however, further studies with larger sample sizes and in other populations are required to confirm these findings.

Conflicts of interest

The authors declare no conflict of interest.

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