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New polymorphisms in the novel LYRM1 gene are associated with body measurement and meat quality traits in Qinchuan cattle

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ABSTRACT. Body measurement and meat quality traits play important roles in the evaluation of productivity in cattle; they are influenced by genetic and environmental factors. Recent studies have shown that LYRM1 is a novel gene related to obesity and may influence fat deposition. We screened for new polymorphisms in the bovine LYRM1 gene and analyzed their association with body measurement and meat quality traits in cattle. DNA samples were obtained from 572 Qinchuan cattle aged from 18 to 24 months. DNA sequencing was used to find the LYRM1 single nucleotide polymorphisms (SNPs). Sequence analysis of LYRM1 revealed four novel SNPs in exon 3: G50A in coding region, C126A, A127T, and T128A in a 3'-untranslated region. G50A, A127T and T128A showed two genotypes: AG and GG, AA and AT, AT and TT, respectively; while C126A showed three genotypes: AA, AC and CC. Analysis showed that these four polymorphisms were significantly associated with body measurement and meat quality traits in the

Qinchuan cattle population. We suggest that the LYRM1 gene can be used for marker-assisted selection to improve body measurement and meat quality traits in the Qinchuan cattle population.

Key words: Body measurement; Meat quality traits; LYRM1; Qinchuan cattle; Single nucleotide polymorphism

INTRODUCTION

In recent decades, obesity is rapidly developing in most parts of the world, and it has become a major global public health problem (Jeffery and Sherwood, 2008), which is associated with serious metabolic and cardiovascular complications (Kopelman, 2000; Spiegelman and Flier, 2001). Common obesity is a complex and multifactorial disorder with high heritability, and it results from interactions between genetics, environment and social psychology (Lee, 2009; Walley et al., 2009). Obesity is also related to many severe diseases, including type II diabetes, work disability and some kinds of cancer (Mokdad et al., 2003). LYR containing 1 (LYRM1) is a novel gene related to obesity, and is highly expressed in adipose tissue of obese patients (Cao et al., 2010).

LYRM1 plays a key role in human obesity, and can promote the proliferation of preadipocytes and inhibit preadipocyte apoptosis; LYRM1 protein is localized in the nucleus, and it has an LYR domain associated with mitochondrial function and metabolism (Qiu et al., 2007, 2009). One study showed that obesity/diabetes is accompanied with impaired mitochondria in adipose tissue (Choo et al., 2006). The association between mitochondrial dysfunction in adipose tissue and obesity/diabetes has also been suggested (Hammarstedt et al., 2003; Dahlman et al., 2006). In addition, overexpression of LYRM1 not only can cause lower insulin-stimulated glucose uptake and a decline in intracellular ATP synthesis in 3T3-L1 adipocytes, but can also inhibit glucose transport in skeletal muscle cells through attenuated PI3K (phosphatidylinositol-3-kinase) and Akt phosphorylation (Cao et al., 2010; Kou et al., 2011). Studies have also shown that the development of insulin resistance can cause obesity (Ferrannini et al., 2007).

Fat deposition is an important factor to improve the quality of beef, and the identification of the genes influencing individual growth, via marker-assisted selection, has the potential to alter the genetic improvement rate (Nkrumah et al., 2003). Therefore, exploring the function of the novel gene LYRM1 related to obesity is necessary.

So far, there has been little information about the polymorphism of LYRM1. Thus, it would be interesting to conduct preliminary study to determine and discuss the genetic variations in the LYRM1 gene in 572 Qinchuan cattle. DNA sequencing was used to identify the genetic variations in the LYRM1 gene, which will possibly contribute to constructing genetic markers for the analysis of the association between genotype and body measurement and meat quality traits and evaluating them. Perhaps the information obtained in this study will provide some useful information for further research on the LYRM1 gene.

MATERIAL AND METHODS

DNA sample preparation and data collection

DNA samples were obtained from blood of the jugular vein of 572 adult animals (18-

24 months), which were randomly selected from Qinchuan cattle breeding populations and used to analyze the LYRM1 allelic frequencies. DNA extraction was performed by a standard phenol-chloroform method (Mullenbach et al., 1989).

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). Two ultrasound traits, ultrasound backfat thickness (UBT) and ultrasound loin muscle area (ULMA), were also measured for evaluating meat quality treats (Brethour, 1994; Hamlin et al., 1995). To minimize systematic error, a single person was assigned to measure each of the 10 traits in all animals.

Polymerase chain reaction (PCR) amplification and sequencing

According to the sequence of the bovine LYRM1 gene (GenBank accession No. NP_001192566.1), primer A (sense: 5'-CTTAGTTGCTCCCAGACCAG-3', anti-sense: 5'-ATCCTCATTCCGACAGTACC-3') was designed to amplify a 380-bp fragment of LYRM1 in exon 3 by the Primer 5 software. PCR amplifications were performed in a 20-μL reaction mixture containing 50 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 followed by 35 cycles at 94°C for 30 s, 52.9°C annealing for 30 s and 72°C extension for 30 s, and then followed by a final extension at 72°C for 10 min. The products for sequencing were first electrophoresed on 1.5% agarose gels, then purified by Axygen kits (BMI Fermentas, Glen Burnie, MD, USA), and finally sequenced in both directions in an ABI PRIZM 377 DNA sequencer (Perkin-Elmer, Dalian, China). The sequence maps were analyzed with the SeqMan software. DNA sequencing revealed four novel mutations (G50A, C126A, A127T, and T128A) in exon 3.

Genotyping of the LYRM1 allele by sequencing

The use of single-strand conformation polymorphism to detect the 4 mutations was time-consuming and the process very complicated. Also, there were no suitable restriction endonucleases for restriction fragment length polymorphism, which is another common method to determine the genotypes of the gene with mutation. Therefore, the products obtained from the DNA samples of the 572 Qinchuan cattle were all sequenced for distinguishing the genotypes of the 4 mutations in LYRM1.

Statistical analysis

We tested the genotypic and allelic frequencies for Hardy-Weinberg equilibriums (HWE) by use of the χ^2 test (Nielsen et al., 1998). The genotype and allele frequencies for H_E (expected heterozygosity), N_E (effective number of alleles) and PIC (polymorphism information content) were evaluated according to the previous approaches of Nei and Roychoudhury (1974) and Nei and Li (1979). The trait means were calculated by the general linear model. The association between SNP marker genotype of the LYRM1 gene and records of body measurement and meat quality traits (BL, WH, HH, RL, HW, CD, HG, PBW, UBT, ULMA) was analyzed by use of the SPSS (version 17.0) software according to the following statistical linear model:

$$Y_{ijk} = \mu + G_i + A_j + \varepsilon_{ijk}$$

where Y_{ijk} represents the observation for the traits, μ represents the overall mean for each trait, G_i represents the genotype effect, A_j represents the fixed effect of age, and ε_{ijk} represents the random error.

RESULTS

Genetic polymorphism of the Qinchuan cattle LYRM1 gene and χ^2 test

Sequence analysis of the LYRM1 gene revealed 4 novel mutations in exon 3: G>A mutation at 50 bp (Figure 1A and B), C>A mutation at 126 bp (Figure 2A-C), A>T mutation at 127 bp (Figure 3A and B) and T>A mutation at 128 bp (Figure 4A and B), named G50A, C126A, A127T and T128A, respectively. G50A, a missense mutation leading to the change of the 100th amino acid arginine to glutamine, was in the coding region, whereas the other 3 mutations were all in the 3'-UTR of the Qinchuan cattle LYRM1 gene. C126A showed 3 genotypes, namely AA, AC and CC (Figure 2A-C); while G50A, A127T and T128A all showed 2 genotypes, namely AG and GG (Figure 1A and B), AA and AT (Figure 3A and B), AT and TT (Figure 4A and B), respectively.

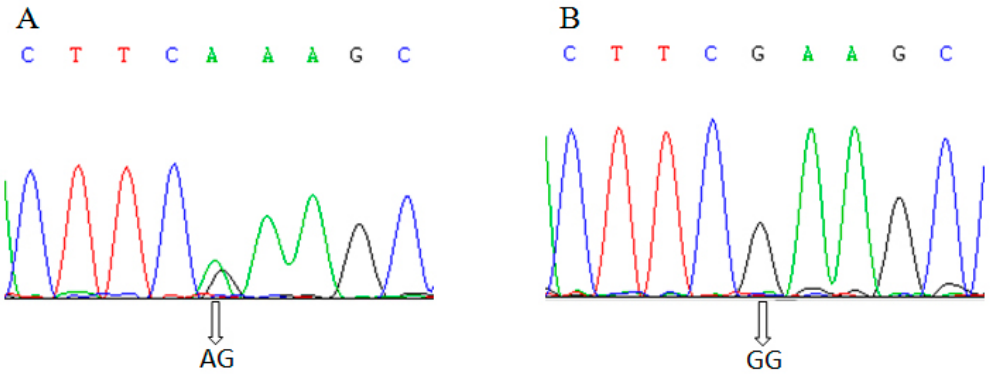

Figure 1. A. B. Sequencing map of the novel SNP of the Qinchuan cattle LYRM1 gene in exon 3 (50-bp locus).

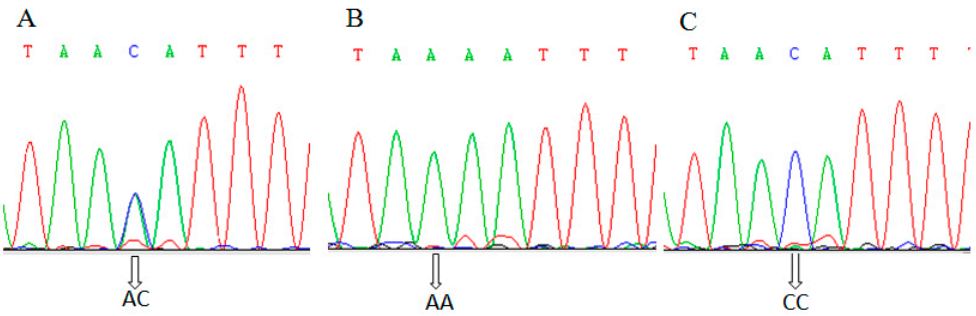

Figure 2. A. B. C. Sequencing map of the novel SNP of the Qinchuan cattle LYRM1 gene in exon 3 (126-bp locus).

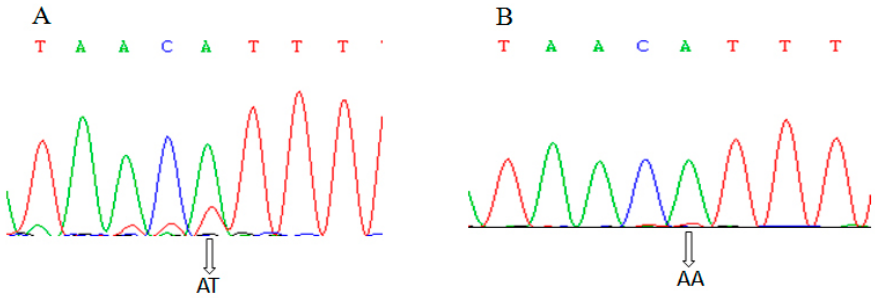

Figure 3. A. B. Sequencing map of the novel SNP of the Qinchuan cattle LYRM1 gene in exon 3 (127-bp locus).

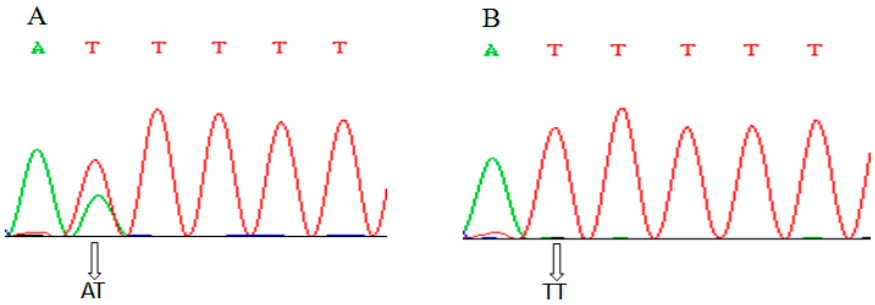

Figure 4. A. B. Sequencing map of the novel SNP of the Qinchuan cattle LYRM1 gene in exon 3 (128-bp locus).

For the 4 mutations, the genotype and allele frequencies were analyzed, and the results are shown in Tables 1 to 4, respectively. The χ^2 test indicated that the genotype distributions of the 4 mutations were all in Hardy-Weinberg equilibrium ($\chi^2 < \chi_{0.05}^2$). For the G50A mutation, GG was the main genotype with a very high frequency of 0.9580 in all populations studied; as to the C126A mutation, CC was the main genotype with the frequency 0.5594; at the A127T mutation, AA was the main genotype with the frequency 0.8811; the frequency of the TT genotype was the highest (0.9545) as to the T128A mutation. According to the allele frequencies of the 4 mutations, PIC, H_E and N_E for G50A, C126A, A127T, and T128A were, respectively: 0.0402, 0.3100, 0.1056, and 0.0434; 0.0411, 0.3836, 0.1118, and 0.0444; and, 1.0428, 1.6223, 1.1259, and 0.1547. On the basis of the classification of PIC (high polymorphism if PIC value was greater than 0.5, medium polymorphism if PIC value was between 0.25 and 0.5, and low polymorphism if PIC value was less than 0.25), the population studied for G50A, A127T and T128A was at the low polymorphism level; whereas C126A was at the medium polymorphism level.

Table 1. Genotype frequencies (%) of the G50A locus of the LYRM1 gene in Qinchuan cattle populations.

	Genotypic frequencies		Total	Allelic frequencies		χ^2 (HW)
	AG	GG		A	G	
Population	0.0420 (24)	0.9580 (548)	572	0.0210	0.9790	0.2627

HW = Hardy-Weinberg equilibrium; $\chi_{0.05}^2 = 5.991$, $\chi_{0.01}^2 = 9.21$.

Table 2. Genotype frequencies (%) of the C126A locus of the LYRM1 gene in Qinchuan cattle populations.

	Genotypic frequencies			Total	Allelic frequencies		χ^2 (HW)
	AA	AC	CC		A	C	
Population	0.0769 (44)	0.3636 (208)	0.5594 (320)	572	0.2587	0.7413	1.5475

HW = Hardy-Weinberg equilibrium; $\chi_{0.05}^2 = 5.991$, $\chi_{0.01}^2 = 9.21$.

Table 3. Genotype frequencies (%) of the A127T locus of the LYRM1 gene in Qinchuan cattle populations.

	Genotypic frequencies		Total	Allelic frequencies		χ^2 (HW)
	AA	AT		A	T	
Population	0.8811 (504)	0.1189 (68)	572	0.9406	0.0594	2.2845

HW = Hardy-Weinberg equilibrium; $\chi_{0.05}^2 = 5.991$, $\chi_{0.01}^2 = 9.21$.

Table 4. Genotype frequencies (%) of the T128A locus of the LYRM1 gene in Qinchuan cattle populations.

	Genotypic frequencies		Total	Allelic frequencies		χ^2 (HW)
	AT	TT		A	T	
Population	0.0455 (26)	0.9545 (546)	572	0.0227	0.9773	0.3094

HW = Hardy-Weinberg equilibrium; $\chi_{0.05}^2 = 5.991$, $\chi_{0.01}^2 = 9.21$.

Association of polymorphism with body measurement and meat quality traits at the G50A locus

The association of the LYRM1 polymorphism with 8 BMTs and 2 ultrasound traits for the G50A was analyzed, and the results are shown in Table 5. There was very significant difference between AG and GG genotypes with BL, CD and UBT ($P < 0.01$). Meanwhile, the mean value of animals with the AG genotype was significantly different from the GG genotype for the traits WH, HH, HW, and ULMA ($P < 0.05$). No difference was found between the genotypes AG and GG for the other BMTs and ultrasound traits studied ($P > 0.05$). Although the frequency of genotype AG was much less than that of genotype GG, the mean values for all BMTs and ultrasound traits of genotype AG were all higher compared to genotype GG. Therefore, genotype AG may be used for marker-assisted selection.

Table 5. Association of G50A SNP genotypes (AG and GG) of the LYRM1 gene with body measurement and ultrasound traits in Qinchuan cattle.

Traits (means ± SE)	AG	GG
BL (cm)	139.875 ± 1.974^{A}	131.971 ± 0.413^{B}
WH (cm)	123.708 ± 1.295^{a}	119.513 ± 0.271^{b}
HH (cm)	124.750 ± 1.152^{a}	122.374 ± 0.241^{b}
RL (cm)	42.667 ± 0.797	41.460 ± 0.167
HW (cm)	40.833 ± 1.031^{a}	37.810 ± 0.216^{b}
CD (cm)	64.708 ± 1.266^{A}	58.686 ± 0.265^{B}
HG (cm)	167.167 ± 2.998	162.604 ± 0.627
PBW (cm)	18.583 ± 0.610	18.785 ± 0.128
UBT (cm)	1.128 ± 0.059^{A}	0.898 ± 0.012^{B}
ULMA (cm^2)	56.640 ± 2.820^{a}	47.121 ± 0.590^{b}

^{a,b}Means with different superscripts are significantly different ($P < 0.05$). ^{A,B}Means with different superscripts are significantly different ($P < 0.01$). 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; UBT = ultrasound backfat thickness; ULMA = ultrasound loin muscle area.

Association of polymorphism with body measurement and meat quality traits at the C126A locus

Analysis of the association of polymorphism with BMTs and ultrasound traits at the C126A locus showed that mean values of genotype AA for the 8 BMTs and 2 ultrasound traits were higher than genotype AC, and mean values of AC for the traits studied were all higher than CC. There was a very significant difference between genotypes AA and CC with regard to BL, WH, HH, RL, and HG ($P < 0.01$). The difference between AA and AC was also very significant for RL ($P < 0.01$). Mean values of AC were significantly higher than CC for WH, HH, RL, HW, CD, HG, and PBW ($P < 0.05$). Also, mean values of AA were significantly higher than CC for HW, CD, PBW, and ULMA ($P < 0.05$). However, there was no association between genotypes for UBT ($P > 0.05$) (Table 6).

Table 6. Association of C126A SNP genotypes of the LYRM1 gene with body measurement and ultrsound traits in Qinchuan cattle.

Traits (means ± SE)	Genotypes		
	AA	AC	CC
BL (cm)	136.977 ± 1.448^{Aa}	133.764 ± 0.666^{Ab}	130.709 ± 0.537^{B}
WH (cm)	123.182 ± 0.946^{Aa}	120.428 ± 0.435^{b}	118.728 ± 0.351^{Ba}
HH (cm)	125.477 ± 0.836^{Aa}	123.250 ± 0.385^{b}	121.556 ± 0.310^{Ba}
RL (cm)	44.182 ± 0.575^{A}	41.865 ± 0.264^{Ba}	40.912 ± 0.213^{Bb}
HW (cm)	39.273 ± 0.758^{a}	38.721 ± 0.349^{a}	37.244 ± 0.281^{b}
CD (cm)	61.023 ± 0.942^{a}	59.726 ± 0.433^{a}	58.141 ± 0.349^{b}
HG (cm)	169.727 ± 2.182^{Aa}	164.606 ± 1.003^{b}	160.666 ± 0.809^{Ba}
PBW (cm)	19.733 ± 0.447^{a}	19.029 ± 0.206^{a}	18.475 ± 0.166^{b}
UBT (cm)	0.945 ± 0.044	0.911 ± 0.020	0.900 ± 0.016
ULMA (cm^2)	53.125 ± 2.086^{a}	48.207 ± 0.959^{b}	46.304 ± 0.773^{b}

^{a,b}Means with different superscripts are different ($P < 0.05$). ^{A,B}Means with different superscripts are significantly different ($P < 0.01$). For abbreviations, see legend to Table 5.

In most cases, the AA genotype had the highest mean value for the 10 traits studied and could be the most beneficial genotype.

Association of polymorphism with body measurement and meat quality traits at the A127T locus

Analysis of the association of polymorphism with body measurement and meat quality traits at A127T locus is shown in Table 7. The results showed that there was a very significant difference between AA and AT genotypes for the parameters WH, HH, CD, HG, and PBW ($P < 0.01$). Between the 2 genotypes, the difference was significant for BL, RL and HW ($P < 0.05$). No difference was found between AA and AT for the 2 ultrasound traits: UBT and ULMA ($P > 0.05$).

For all traits, AT had a higher mean value than AA, which could be the beneficial genotype in the population studied.

Table 7. Association of A127T SNP genotypes of the LYRM1 gene with body measurement and ultrasound traits in Qinchuan cattle.

Traits (means ± SE)	Genotypes	
	AA	AT
BL (cm)	131.819 ± 0.433^{a}	135.882 ± 1.178^{b}
WH (cm)	119.075 ± 0.275^{A}	124.235 ± 0.749^{B}
HH (cm)	121.992 ± 0.245^{A}	126.044 ± 0.668^{B}
RL (cm)	41.361 ± 0.173^{a}	42.618 ± 0.472^{b}
HW (cm)	37.698 ± 0.225^{a}	39.706 ± 0.612^{b}
CD (cm)	58.554 ± 0.278^{A}	61.794 ± 0.756^{B}
HG (cm)	161.958 ± 0.648^{A}	169.000 ± 1.763^{B}
PBW (cm)	18.603 ± 0.131^{A}	20.059 ± 0.758^{B}
UBT (cm)	0.904 ± 0.013	0.929 ± 0.036
ULMA (cm^2)	47.118 ± 0.619	50.503 ± 1.686

^{a,b}Means with different superscripts are significantly different ($P < 0.05$). ^{A,B}Means with different superscripts are significantly different ($P < 0.01$). For abbreviations, see legend to Table 5.

Association of polymorphism with body measurement and meat quality traits at the T128A locus

The results in Table 8 show that mean values of genotype AT were very significantly higher than TT for HH, RL, HW, and HG ($P < 0.01$). There was a significant difference between AT and TT genotypes for BL, WH, CD, PBW, and UBT ($P < 0.05$), while no difference was found between the 2 genotypes for the other traits ($P > 0.05$). For the 10 traits studied, the mean values of genotype AT were all higher than with TT in the research population.

Table 8. Association of T128A SNP genotypes of the LYRM1 gene with body measurement and ultrasound traits in Qinchuan cattle.

Traits (means ± SE)	Genotypes	
	AT	TT
BL (cm)	136.692 ± 1.913^{a}	132.093 ± 0.417^{b}
WH (cm)	123.846 ± 1.242^{a}	119.491 ± 0.271^{b}
HH (cm)	126.538 ± 1.097^{A}	122.280 ± 0.239^{B}
RL (cm)	44.538 ± 0.756^{A}	41.366 ± 0.165^{B}
HW (cm)	42.308 ± 0.980^{A}	37.729 ± 0.214^{B}
CD (cm)	62.269 ± 1.231^{a}	58.780 ± 0.269^{b}
HG (cm)	173.769 ± 2.847^{A}	162.273 ± 0.621^{B}
PBW (cm)	20.462 ± 0.582^{a}	18.696 ± 0.127^{b}
UBT (cm)	1.031 ± 0.058^{a}	0.901 ± 0.013^{b}
ULMA (cm^2)	50.454 ± 2.732	47.381 ± 0.596

^{a,b}Means with different superscripts are significantly different ($P < 0.05$). ^{A,B}Means with different superscripts are significantly different ($P < 0.01$). For abbreviations, see legend to Table 5.

DISCUSSION

The prevalence of individuals with obesity and overweight continues to rise around the world. Furthermore, obesity and overweight could result in many negative health problems such as diabetes, cardiovascular disease, osteoarthritis, and some forms of cancer, which will increase healthcare requirements and economic burden (Anonymous, 2002). Obesity itself has

a high heritability and is significantly influenced by susceptibility genes and environmental factors (Walley, 2009). LYRM1 is a novel gene related to obesity, and it has effects on insulin resistance and mitochondrial dysfunction in adipocytes (Cao et al., 2010; Zhang et al., 2012), which is important to fat deposition.

As LYRM1 is related to obesity, we considered a candidate gene to explore its effects on animals' growth. The candidate gene approach is a very effective method to analyze the association between a gene's polymorphisms and valuable economical traits in farm animals (Rothschild and Soller, 1997). Through the candidate gene approach, much research has been done on animal reproduction (Chu et al., 2010), growth (Li et al., 2010) and meat quality traits (Jiao et al., 2010), which will provide more valuable data for further research. To facilitate the Chinese indigenous cattle-breeding program, more molecular genetic information on quantitative trait locus should be collected (Adoligbe et al., 2012).

As a novel gene, LYRM1 is expressed at a high level in omental adipose tissue of obese patients, and studies have demonstrated that LYRM1 is associated with mitochondrial function and energy metabolism (Qiu et al., 2007, 2009). These findings suggested that LYRM1 may have effects on BMTs and meat quality traits on animals.

In this study, sequence analysis of LYRM1 revealed 4 novel SNPs in exon 3: G50A in the coding region with a missense mutation leading to the change of the 100th amino acid arginine to glutamine; and C126A, A127T and T128A in 3'-UTR. C126A showed 3 genotypes whereas the other 3 mutations all showed 2 genotypes. The association of the 4 novel SNPs in LYRM1 with 8 BMTs and 2 ultrasound traits was analyzed using DNA samples from 572 Qinchuan cattle population. It seems that G50A is associated with BL, WH, HH, HW, CD, UBT, and ULMA, and AG appears to be the beneficial genotype; C126A is associated with BL, WH, HH, RL, HW, CD, HG, PBW, and ULMA, and AA seems to be the beneficial genotype; A127T is associated with BL, WH, HH, HW, CD, RL, HG, and PBW, and AT genotype seems to be the beneficial one; T128A is associated with BL, WH, HH, RL, HW, CD, HG, PBW, and UBT, and AT seems to be the beneficial genotype. The change in amino acid by G50A mutation may have an impact in terms of function of the protein produced by the LYRM1 gene. C126A, A127T and T128A, in 3'-UTR, may have effects on energy mechanism via mitochondria and may also have an impact on glucose transport through PI3K and Akt phosphorylation by the LYRM1 gene (Qin et al., 2012). The absence of the AA genotype at the 50-bp locus, the TT genotype at 127-bp locus and the AA genotype at 128-bp locus can be explained in 2 ways: first, these genotypes do not exist in the population studied; second, the experimental population is small. On the basis of the results obtained in this study, we suggest that the LYRM1 gene may have potential effect on BMTs and meat quality traits in Qinchuan cattle population.

Studies have reported that LYRM1 is associated with obesity. Not only can it influence glucose uptake and transport, but it can also have an effect on mitochondrial function (Cao et al., 2010; Kou et al., 2011). On the basis of the results in this study, it can be inferred that the mutations of LYRM1 have effects on BMTs and meat quality traits in Chinese native indigenous cattle. Therefore, we suggest that further research should be done in a larger population size with the goal of using SNPs for marker-assisted selection.

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