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Polymorphisms in the *SIRT5* gene and their association with body measurement and ultrasound traits in Qinchuan cattle

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ABSTRACT. Silent information regulator 5 (SIRT5), a member of the Sirtuin family class III nicotinamide adenine dinucleotide-dependent protein deacetylases, plays an important role in metabolic and aging processes in mammals. We identified 4 single-nucleotide polymorphisms (SNPs) (G22010A, G22052A, G22119T, and G22245C) in the 3' untranslated regions of the *SIRT5* gene from 572 Qinchuan cattle by sequencing and investigating their association with growth and ultrasound traits. The frequencies of genotype GG and allele G were high at the 4 SNPs. Based on the χ^2 test, the genotypic distributions of the 4 SNPs were not in Hardy-Weinberg equilibrium ($P < 0.05$ or $P < 0.01$). Association analysis of individual SNPs and haplotype combinations revealed that the 4 loci were significantly associated with some body measurement and ultrasound traits in Qinchuan cattle,

and the H_1H_5 (AG-GA-GG-GG) diplotypes had better performance than other combinations in Qinchuan cattle. Our results demonstrate that *SIRT5* may be a candidate for marker-assisted selection in future breeding programs for Qinchuan cattle.

Key words: Genetic variability; Body measurement; Ultrasound; Silent information regulator 5; Single-nucleotide polymorphism

INTRODUCTION

Sirtuins are protein deacetylases that hydrolyze 1 nicotinamide adenine dinucleotide (NAD^+) cosubstrate for each lysine side chain they deacetylate (Blander and Guarente, 2004). Sirtuins play a regulatory role in many biological processes, including genetic transcription, DNA repair, genome stability, and energy metabolism (Marmorstein, 2004), which has been generally attributed to their NAD-dependent deacetylase activity (Haigis and Guarente, 2006). Mammals have 7 isoforms, SIRT1-SIRT7, which have been described to have different target proteins and cellular localizations, including the cytoplasm (SIRT1 and 2), nucleus (SIRT1, 2, 3, 6, and 7), and mitochondria (SIRT3, 4, and 5). SIRT5 is the only mammalian Sirtuin grouped in subfamily III, which includes mainly prokaryotic sirtuins (Gertz and Steegborn, 2010).

SIRT5 is an endogenous protein localized in the mitochondrial matrix (Hirschey, 2011) and is primarily expressed in the brain, muscle, heart, liver, and kidney tissues (Michishita et al., 2005). A number of research studies have suggested that the *SIRT1* and *SIRT2* genes enhance proliferation of preadipocytes and inhibit preadipocyte apoptosis through their acetylation/deacetylation functions (Bai et al., 2007; Wang and Tong, 2009). Similarly, recent studies have suggested that SIRT5 has a similar function in humans (Nakagawa, 2009), but its mechanism of action remains unclear. In addition, SIRT5 interacts with carbamoyl phosphate synthetase 1, which catalyzes the first step of ammonia detoxification and disposal via the urea cycle. It is possible that SIRT5 stimulates the deacetylation function of carbamoyl phosphate synthetase 1with NAD^+ *in vitro* (Schuetz et al., 2007). Lysine succinylation has been consistently shown to be a new posttranslational modification and SIRT5 serves as a key enzyme for removing succinyl groups from lysine residues of multiple cellular proteins (Nakagawa et al., 2009). Based on its localization and biological functions, these findings indicate that SIRT5 links metabolic and aging processes in mammals.

However, studies of polymorphisms in the *SIRT5* gene have not been reported. Therefore, we estimated genetic variations in the *SIRT5* gene in 572 Qinchuan cattle using DNA sequencing methods and determined the association between its quantitative traits loci and body measurement and ultrasound traits. Our results are useful for further studies of the *SIRT5* gene.

MATERIAL AND METHODS

Animal handling procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals, and the animal study protocol was approved by the Institutional Animal Care and Use Committee of Northwest A&F University.

Genomic DNA preparation and phenotypic data collection

A total of 572 adult animals were randomly selected from Qinchuan cattle breeding populations (without genetic relationships), and stratified into age categories of 18-24 months. DNA samples were extracted from blood samples and then stored at -80°C (Sambrook et al., 1989). DNA content was estimated spectrophotometrically, and then the genomic DNA was diluted to 50 ng/L. All DNA samples were stored at -20°C until subsequent analysis.

For further association studies, body measurement traits, including body length (BL), withers height (WH), hip height (HH), rump length (RL), hip width (HW), chest depth (CD), chest circumference (CC), and pin bone width (PBW), were measured as described previously (Gilbert et al., 1993). Ultrasound traits, including backfat thickness (BT), ultrasound loin muscle area (ULA), and intramuscular fat (IF), were measured using the Rincon method (Rincon et al., 2009). To reduce systematic error, a single person measured each of the 11 traits in all animals.

Primer design and polymerase chain reaction (PCR) conditions

Primers to amplify of the bovine *SIRT5* gene were designed based on sequences in the NCBI database (GenBank accession No. NP_001029467.1) using the Primer v5.0 software (PREMIER Biosoft, Palo Alto, CA, USA). Forward primer 3'-Region-F (5'-TGCTGTGTTCG TCCTTCTGT-3') and reverse primer 3'-Region-R (5'-CTATCCCCACCCCGAACTT-3') were used to amplify a fragment of 582 base pair in the 3' untranslated region (UTR) of the *SIRT5* gene. PCR was conducted in 20-μL reactions 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, Shiga, Japan). The following PCR reaction conditions were used: 5 min at 95°C; 35 cycles of 30 s at 94°C, 35 s at 58.7°C, 40 s at 72°C, and final extension at 72°C for 10 min. Digested products were detected by electrophoresis on 1.5% agarose gels.

Genotyping of *SIRT5* alleles by sequencing

Using single-strand conformation polymorphism to detect the 4 mutations would be time-consuming and very complicated. Additionally, there are no suitable restriction endonucleases for restriction fragment length polymorphism, which is commonly used to detect mutation genotypes. Thus, DNA from the 572 Qinchuan cattle were sequenced to distinguish the genotypes of the 4 mutations in *SIRT5*.

Statistical analysis

Gene frequencies, allelic frequencies, and Hardy-Weinberg equilibrium were evaluated by direct counting. Population genetic indices including gene heterozygosity (H_E), gene homozygosity (H_O), effective allele numbers (N_E), and polymorphism information content (PIC) were calculated according to Nei's methods (Nei and Roychoudhury, 1974). Linkage disequilibrium and haplotype distribution of the SNPs were analyzed using the expectation maximization algorithm with Haploview software (Barrett et al., 2005).

SPSS v. 13.0 (SPSS, Inc., Chicago, IL, USA) was used to analyze the relationship between different genotypes of the *SIRT5* gene, body measurement traits (BL, WH, HH, RL,

HW, CD, CC, and PBW), and ultrasound traits (BT, ULA, and IF) in Qinchuan cattle. The following statistical linear model was used: $Yij = \mu + Gi + Ai + Eijk$, where *Yij* was the traits measured on each of the individual cattle, μ was the overall population mean for the traits, *Gi* was the fixed effect associated with the genotype, A_i was the fixed effect due to age, and E_{ijk} was the standard error.

RESULTS

Polymorphisms and genetic diversity

Four polymorphic sites in the *SIRT5* gene (Figures 1-4), including G22010A, G22052A, G22119T, and G22245C, located in 3'UTR of *SIRT5* were identified by sequencing. Data showed that both G22119T and G22245C had 2 genotypes and the TT and CC genotypes were not observed in the animals sampled. Genotype and allele frequencies for the 4 loci are shown in Table 1. The G allele was predominant at the 4 SNPs. According to the χ^2 test, the genotypic distributions of 4 SNPs were not in Hardy-Weinberg equilibrium ($P < 0.05$ or $P < 0.01$).

According to Nei's methods, population genetic indices, including H_E, N_E, and PIC, were calculated and the results are shown in Table 1. The PIC value is an effective indicator of genetic diversity from different loci of the candidate gene. Our results showed that both G22010A and G22052A had medium polymorphism levels ($0.250 < PIC < 0.500$), whereas G22119T and G22245C had low polymorphism levels ($0.250 > PIC$).

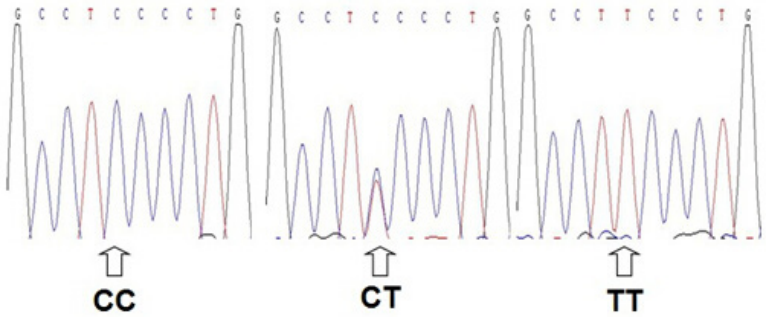

Figure 1. Sequencing map of the SNP22010 locus (reverse direction).

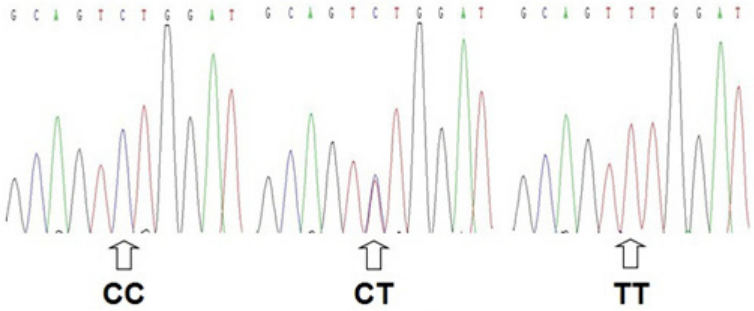

Figure 2. Sequencing map of the SNP22052 locus (reverse direction).

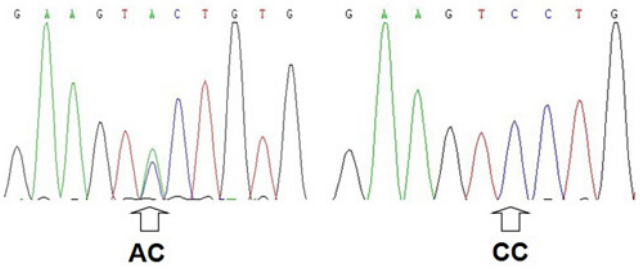

Figure 3. Sequencing map of the SNP22119 locus (reverse direction).

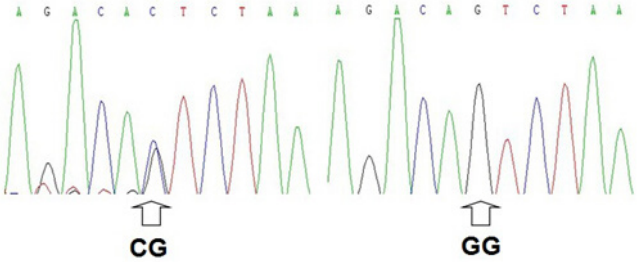

Figure 4. Sequencing map of the SNP22245 locus (reverse direction).

Table 1. Genotype frequencies (%) of the *SIRT5* gene for SNPs in *Qinchuan* cattle populations.

Site	Sample	Genotypic frequency			Allele frequency		H_E	N_E	PIC	χ^2 (HWE*)
G22010A	572	GG	GA	AA	G	A				
		0.5769	0.3357	0.0874	0.7448	0.2552	0.3802	1.6134	0.3079	7.8453
G22052A	572	GG	AG	AA	G	A				
		0.5664	0.3322	0.1014	0.7325	0.2675	0.3919	1.6444	0.3151	13.2772
G22119T	572	GG	GT		G	T				
		0.7762	0.2238	0	0.8881	0.119	0.1987	1.2480	0.1790	9.0788
G22245C	572	GG	GC		G	C				
		0.7972	0.2028	0	0.8986	0.1014	0.1822	1.1228	0.1656	7.2833

HWE = Hardy-Weinberg equilibrium; $\chi 0.05^2 = 5.991$, $\chi 0.01^2 = 9.210$.

Linkage disequilibrium and haplotype analysis

Linkage disequilibrium between polymorphism pairs and haplotype structure analyses of the *SIRT5* gene are shown in Table 2 and 3. To determine linkage relationships among the 4 SNPs, the linkage disequilibrium between the 4 sites was estimated. The results indicated that the r^2 values ranged from 0.000-1.000. According to a previous study, if the value of r^2 is over 0.33, linkage disequilibrium is considered to be strong (Ardlie et al., 2002). Our results revealed strong linkage between G22052A and G22245C, while others linkages with pair-wise $r^2 < 0.33$ were weak.

Haplotype analyses for the 4 SNPs showed that 5 different haplotypes were present in the populations studied, with frequencies greater than 0.05 (haplotypes with frequency < 0.05 were ignored). Hap2 (-GGGG-) showed the highest haplotype frequencies (41.30%), followed by Hap5 (-GAGG-), and Hap1 (-AGGG-). The high-frequency haplotypes were likely been

present in the population for a long time, which may be directly or indirectly regulated by different rearing environments (Gui et al., 2015).

Table 2. Estimated values of linkage disequilibrium for SNPs in bovine *SIRT5* of *Qinchuan* cattle.

SNP	G22010A	G22052A	G22119T	G22245C
G22010A	-	D = 0.220	D = 0.042	D = 0.033
G22052A	r^2 = 0.006	-	D = 0.455	D = 0.961
G22119T	r^2 = 0.000	r^2 = 0.009	-	D = 0.102
G22245C	r^2 = 0.000	r^2 = 0.038	r^2 = 0.009	-

Table 3. Haplotypes of *SIRT5* gene and their frequencies in *Qinchuan* cattle.

Haplotype	G22010A	G22052A	G22119T	G22245C	Frequency
Hap1	A	G	G	G	0.167
Hap2	G	G	G	G	0.413
Hap3	G	G	G	C	0.059
Hap4	G	G	T	G	0.060
Hap5	G	A	G	G	0.187

Effects of single markers/haplotype combinations on growth traits and ultrasound traits in Qinchuan cattle

Table 4 shows the effects of the SNPs on growth performance and ultrasound in Qinchuan cattle. The 4 polymorphisms mainly affected cattle body measurement traits and ultrasound trait. At the SNP22010 locus, individuals with genotype GG had higher values than those with GA for WH, HH, RL, and CC ($P < 0.05$). Additionally, the HW and PBW of individuals with genotype AA were higher than those with genotype AG ($P < 0.01$). At the SNP22052 locus, individuals with genotype AG had higher values than those with AA on BL, WH, RL CD, and CC ($P < 0.05$), while genotype AG had higher mean values for WH, HH, CC, and ULA than those with genotype GG ($P < 0.05$). Significant differences in HW and PBW were observed between the AG and GG genotypes ($P < 0.01$). Compared with AA, individuals with the AG genotype showed better performance for HH and PBW ($P < 0.01$). At the SNP22119 locus, individuals with GG genotype had very significantly ($P < 0.05$) greater values of BL, RL, HW, CC, and IF than those with the GT genotype. At the SNP22245 locus, significant differences in BL, HH, RL, CD, PBW, and IF were observed between the GG and GC genotypes ($P < 0.05$). However, no significant correlations were observed in the remaining indices for the 4 SNPs.

Multiple effects of the 4 SNPs were evaluated and a total of 18 haplotype combinations were identified. Combinations with frequencies lower than 5.0% (data not shown) were not included and the remaining combinations were further analyzed. In Table 5, individuals with H_1H_5 and H_2H_2 showed significantly higher HW than those with H_1H_2 ($P < 0.05$). Individuals with diplotypes H_1H_5 had higher values than those with H_1H_2 and H_4H_5 for CC ($P < 0.05$), and similar results were observed between H_1H_2 and H_5H_5 ($P < 0.01$). In addition, individuals with H_1H_5 exhibited significantly larger PBW than those with H_1H_2 ($P < 0.01$). For ultrasound traits, individuals with the combined genotype H_1H_5 showed a significantly difference than the others ($P < 0.01$) for ULA. H_4H_5 had the lowest IF value compared with the other diplotypes ($P < 0.01$).

Table 4. Association between different SNPs in SIRT5 and growth traits and ultrasound traits in Qinchuan cattle.

		Body measurement (Means ± SD)								Ultrasound trait (Means ± SD)		
		BL (cm)	WH (cm)	HH (cm)	RL (cm)	HW (cm)	CD (cm)	CC (cm)	PBW (cm)	BT (cm)	ULA (cm^2)	IF (%)
G22010A	GG	135.723 ± 11.698	122.282 ± 10.589^{a}	124.502 ± 8.262^{a}	42.861 ± 4.259^{a}	39.639 ± 2.978^{A}	59.921 ± 4.363	165.629 ± 12.456^{a}	19.442 ± 0.778	0.903 ± 0.063	46.348 ± 4.387^{b}	7.323 ± 0.525
	GA	134.354 ± 10.756	120.126 ± 9.659^{b}	123.044 ± 8.691^{b}	41.760 ± 4.564^{b}	37.839 ± 4.210^{B}	58.602 ± 4.106	162.026 ± 15.297^{b}	18.281 ± 0.820	0.892 ± 0.079	46.164 ± 3.789^{B}	7.410 ± 0.623
	AA	136.080 ± 12.058	122.120 ± 9.585	123.740 ± 7.632	42.680 ± 3.223	38.420 ± 4.066	59.110 ± 3.266	164.340 ± 13.641	18.400 ± 0.699	0.945 ± 0.052	49.975 ± 3.577^{Aa}	7.427 ± 0.417
	P	0.295	0.020	0.035	0.013	0.010	0.056	0.042	0.330	0.094	0.008	0.399
G22052A	GG	134.823 ± 11.858	120.733 ± 9.695^{b}	123.637 ± 9.564^{b}	42.293 ± 4.542	38.299 ± 3.681^{B}	59.085 ± 4.782	163.238 ± 13.055^{B}	18.586 ± 0.996	0897 ± 0.062	45.673 ± 3.542	7.282 ± 0.609
	GA	136.934 ± 10.660^{a}	122.903 ± 10.396^{a}	125.124 ± 6.423^{Aa}	43.116 ± 3.211^{a}	40.163 ± 2.071^{A}	60.466 ± 4.003^{a}	166.966 ± 15.005^{A}	19.802 ± 0.985	0.921 ± 0.080^{a}	48.358 ± 2.089	7.483 ± 0.550
	AA	132.560 ± 11.252^{b}	119.267 ± 10.698^{b}	121.810 ± 8.952^{B}	41.396 ± 3.221^{b}	38.392 ± 3.586	57.741 ± 5.021^{b}	161.569 ± 14.200^{B}	18.310 ± 1.314	0.878 ± 0.072^{b}	46.049 ± 2.420	7.407 ± 0.442
	P	0.042	0.032	0.004	0.034	0.007	0.036	0.006	0.405	0.040	0.107	0.053
G22119T	GG	135.940 ± 11.552^{a}	121.489 ± 10.542	124.197 ± 9.695	42.728 ± 4.003^{a}	39.243 ± 2.157^{a}	59.677 ± 3.775	165.303 ± 13.825^{a}	19.110 ± 1.035	0.915 ± 0.059	46.822 ± 3.584	7.436 ± 0.589^{a}
	GT	133.055 ± 10.452^{b}	120.670 ± 11.222	123.074 ± 10.025	41.602 ± 2.687^{b}	37.836 ± 3.051^{b}	58.473 ± 4.051	160.852 ± 14.054^{b}	18.445 ± 0.680	0.860 ± 0.071	45.843 ± 3.226	7.102 ± 0.668^{b}
	P	0.045	0.472	0.142	0.021	0.024	0.115	0.023	0.067	0.100	0.310	0.037
G22245C	GG	136.043 ± 9.695^{a}	121.537 ± 8.259	124.315 ± 10.231^{a}	42.686 ± 3.778^{a}	39.160 ± 4.541	59.731 ± 3.234^{a}	165.109 ± 14.651	18.328 ± 0.758^{a}	0.902 ± 0.058	46.700 ± 3.324	7.413 ± 0.501^{a}
	GC	132.354 ± 10.445^{b}	120.394 ± 10.761	122.504 ± 8.330^{b}	41.647 ± 2.355^{b}	38.017 ± 3.772	58.133 ± 4.599^{b}	161.155 ± 13.065	18.328 ± 1.556^{b}	0.908 ± 0.067	46.236 ± 4.039	7.160 ± 0.516^{b}
	P	0.013	0.333	0.022	0.040	0.077	0.043	0.051	0.035	0.849	0.127	0.032

^{a,b}Means with different superscripts were significantly different ($P < 0.05$), ^{A,B}Means with different superscripts were significantly different ($P < 0.01$).

Table 5. Associations between haplotypes and growth and ultrasound traits in Qinchuan cattle.

		Body measurement (means ± SD)								Ultrasound trait (means ± SD)		
Hap	Frequency (%)	BL (cm)	WH (cm)	HH (cm)	RL (cm)	HW (cm)	CD (cm)	CC (cm)	PBW (cm)	BT (cm)	ULA (cm^2)	IF (%)
Hap5/5	15.03	133.231 ± 8.335	120.230 ± 8.779	122.587 ± 11.642	41.514 ± 2.001	38.029 ± 2.569	58.614 ± 3.779	160.586 ± 12.692^{B}	18.829 ± 1.535	0.897 ± 0.045	46.287 ± 4.252^{A}	7.933 ± 0.489^{A}
Hap1/5	7.34	135.272 ± 10.362	122.022 ± 9.658	124.544 ± 9.698	42.944 ± 3.457	39.900 ± 2.993^{a}	60.072 ± 5.482	166.067 ± 10.776^{Aa}	19.856 ± 0.957^{A}	0.899 ± 0.074	46.152 ± 3.568^{A}	7.364 ± 0.533^{B}
Hap2/2	6.12	136.195 ± 9.956	121.006 ± 7.986	124.316 ± 9.775	42.943 ± 3.452	39.448 ± 2.480^{a}	60.218 ± 4.631	165.874 ± 10.594	19.414 ± 0.8223^{a}	0.887 ± 0.058	45.427 ± 3.893^{B}	7.462 ± 0.5127^{AB}
Hap4/5	17.31	133.929 ± 12.365	122.179 ± 9.652	125.286 ± 8.230	42.405 ± 4.124	38.524 ± 2.119	58.524 ± 4.522	162.762 ± 12.612^{b}	18.786 ± 1.067	0.854 ± 0.064	47.917 ± 4.201^{A}	6.850 ± 0.485^{C}
Hap1/2	15.21	134.895 ± 9.568	119.511 ± 10.036	123.326 ± 9.781	42.023 ± 3.993	37.849 ± 3.028^{b}	58.924 ± 4.778	162.756 ± 11.793^{b}	18.221 ± 1.695^{Bb}	0.908 ± 0.062	46.472 ± 3.842^{A}	7.467 ± 0.572^{AB}
P		0.326	0.441	0.289	0.075	0.039	0.227	0.003	0.004	0.524	0.005	0.001

^{a,b}Means with different superscripts were significantly different ($P < 0.05$), ^{A,B}Means with different superscripts were significantly different ($P < 0.01$).

DISCUSSION

Mitochondria are essential organelles in cellular energy metabolism because they supply the cell with metabolic energy in the form of ATP generated by oxidative phosphorylation (Martinou and Youle, 2011). They are also important in processes such as apoptosis, cellular senescence, and lifespan regulation (Balaban et al., 2005). Among the mammalian sirtuins gene, SIRT5 is located in the mitochondrial matrix and is the only protein shown to possess efficient demalonylase and desuccinylase activity (Du et al., 2011). Schlicker et al. (2008) reported that the *SIRT5* gene could deacetylate cytochrome c, a protein in the mitochondrial intermembrane space with a central function in oxidative metabolism and apoptosis. A previous proteomic survey verified that lysine succinylation is a new posttranslational modification in proteins using *Escherichia coli* extracts (Zhang et al., 2011), and Peng et al. (2011) reported that SIRT5 served as a key enzyme that removed succinyl groups from the lysine residues of multiple cellular proteins. Because of its location and its function in the mitochondria, we hypothesized that the *SIRT5* gene is associated with cattle body measurement and ultrasound traits.

To evaluate the effects of the 4 SNPs in the *SIRT5* gene on growth traits and ultrasound traits in Qinchuan cattle, the association between *SIRT5* polymorphisms and 11 traits, including 8 body measurement traits and 3 ultrasound traits, were examined. Our results showed that the AA genotypes (G22010A), GG genotypes (G22052A), AG genotypes (G22119T), and GG genotypes (G22245C) were highly associated growth traits and ultrasound traits compared to other genotypes in single analysis. Furthermore, we examined the association between haplotype combinations and the above traits. The diplotypes H_2H_2 (GG-GG-GG-GG), H_1H_5 (GA-GA-GG-CG), and H_1H_2 (GA-GG-GG-GG) showed higher associations than the other haplotype combinations. Compared with the combination results, individuals with the H_2H_2 haplotype showed better performance for growth and ultrasound.

The 4 SNPs identified in this study were located in the 3'UTR region, and did not change the structure of the encoded proteins; however, the 3'UTRs of eukaryotic genes were reported to regulate mRNA stability, localization, and translation, and the molecular genetic variability of the 3' region sequence or its binding and splicing factors may be responsible for these modifications (Rigo et al., 2008). Previous reports showed that mutations in the 3'UTR could affect gene expression, phenotype, and consequently respective physiological functions. For instance, Yie et al. (2008) identified a single base pair mutation in the human leukocyte antigen-G 3'UTR, which was thought to be a fundamental mechanism for lowering the levels of placental human leukocyte antigen-G protein expression in patients with preeclampsia. Zhou et al. (2011) identified an SNP in the malic enzyme 1 gene 3'UTR, and showed that it was correlated with ultrasound in Chinese red cattle.

CONCLUSIONS

In summary, we reported polymorphisms in the *SIRT5* gene in Qinchuan cattle and demonstrated an association between the *SIRT5* gene and body measurement and ultrasound traits. Our data strongly suggest that the combined genotype H_2H_2 can be used as a genetic marker for the selection and breeding of Qinchuan. Our results suggest that the *SIRT5* gene can be used as a candidate gene for breeding. Further studies should be conducted in a large population before using this gene for molecular marker-assisted selection.

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