

Association of *SIRT2* gene polymorphisms with body measurement and growth traits of Qinchuan cattle

L.S. Gui¹, W.C. Yang^{1,2}, C.P. Zhao^{1,2}, S.J. Wei¹, Z.D. Zhao¹ and L.S. Zan^{1,2}

¹College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi China ²National Beef Cattle Improvement Center of Northwest A&F University, Yangling, Shaanxi China

Corresponding author: L.S. Zan E-mail: zanlinsen@163.com

Genet. Mol. Res. 13 (4): 8834-8844 (2014) Received October 16, 2013 Accepted March 25, 2014 Published October 27, 2014 DOI http://dx.doi.org/10.4238/2014.October.27.24

ABSTRACT. Silent information regulator 2 (SIRT2), a member of the Sirtuin family of class III nicotinamide adenine dinucleotidedependent protein deacetylases, plays an important role in senescence, metabolism, and apoptosis. This study was conducted to detect potential polymorphisms of the bovine SIRT2 gene and explore their relationships with meat quality and body measurement traits (BMTs) in Qinchuan cattle. Four single nucleotide polymorphisms (A7445G, C7711T, G17937A, and G20937A) in the fourth intron, fourth exon, ninth exon, and twelfth exon of the SIRT2 gene, respectively, were identified according to the sequencing results of 520 individuals of a Qinchuan cattle population. The genotypic distributions of both A7445G and G20937A were in agreement with the Hardy-Weinberg equilibrium (P < 0.05), whereas the other two mutations were not (0.05 < P < 0.01), based on the χ^2 test. Association analysis indicated that the four loci were significantly correlated with several BMTs and meat quality traits. When in combination, the H₁H₁ (AA-CC-GG-CC) diplotypes showed better BMT and meat quality traits than those by other combinations. Collectively, the results show that SIRT2 is involved in the regulation of the growth and meat quality of cattle, suggesting that the *SIRT2* gene may be a candidate gene for marker-assisted selection in the development of future breeding programs for Qinchuan cattle.

Key words: Genetic variability; SIRT2; Body measurement; Meat quality; Single nucleotide polymorphism

INTRODUCTION

Chinese indigenous yellow cattle are known to have a strong trunk, high stress resistance, and good environmental adaptability. However, drawbacks of this breed remain, including their underdeveloped hind hips and slow growth rates. Therefore, it is necessary to select important functional genes of beef cattle through marker-assisted selection in order to increase their productivity, and thereby economic benefits, and promote the development of the Chinese cattle industry toward high quality and efficiency.

In mammals, homologs of silent information regulator 2 (SIRT2) are named sirtuins, and belong to the class III nicotinamide adenine dinucleotide (NAD)-dependent deacetylase family (Guarente, 2007). Seven sirtuin members, designated as SIRT1-SIRT7, have been identified to date. Among them, SIRT2, a tubulin deacetylase, is localized mainly in the cytoplasm and plays either detrimental or beneficial roles in cell survival under different conditions (Zhou et al., 2012). The *SIRT2* gene was found to be ubiquitously expressed as NAD⁺-dependent protein deacetylases (Peck et al., 2010), and plays critical roles in a variety of different biological processes, such as longevity and metabolism, through the deacetylation of histones (Blander and Guarente, 2004; Marmorstein, 2004).

Recent studies have suggested that the *SIRT2* gene could enhance the proliferation of preadipocytes and inhibit preadipocyte apoptosis. *SIRT2* could induce *FOXO1* gene deacetylation in rats (Wang and Tong, 2009). Overexpression of *SIRT2* caused FoxO1 acetylation/ deacetylation and reduced the expression of PPAR γ and C/EBP α in 3T3-L1 cells, and these changes led to abnormal mitochondrial morphology and inhibited adipogenesis (Jing et al., 2007). Moreover, transcriptional repression of *SIRT2* resulted in inhibition of fatty acid oxidation and energetic uncoupling via hypoxia-inducible factor 1 α accumulation in diabetic humans and mice (Krishnan et al., 2012). Based on the deduced biological function of *SIRT2* in humans and mice, we hypothesized that the *SIRT2* gene might be associated with cattle body measurement and meat quality traits.

However, polymorphisms of the *SIRT2* gene have not yet been reported for cattle. Therefore, this study aimed to determine the *SIRT2* gene function using bioinformatics information, and its tissue expression pattern was analyzed using real-time polymerase chain reaction (PCR). In addition, we sought to identify quantitative trait loci relevant to growth and meat quality traits in Qinchuan cattle.

MATERIAL AND METHODS

Genomic DNA preparation and phenotypic data collection

A total of 520 unrelated adult animals were randomly selected from Qinchuan cattle

Genetics and Molecular Research 13 (4): 8834-8844 (2014)

L.S.	Gui	et	al.	

breeding populations, which ranged in age from 18 to 24 months. DNA samples were extracted from blood samples collected from the jugular vein and stored at -80°C according to the standard phenol chloroform protocol (Sambrook and Russell, 2001). The DNA content was estimated spectrophotometrically, and then the genomic DNA was diluted to 50 ng/L. All DNA samples were stored at -20°C for subsequent analysis.

Body measurement traits (BMTs), 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). The meat quality traits, including backfat thickness (BT), ultrasound loin muscle area (ULA), and intramuscular fat (IF), were measured using the Rincon method (Rincon et al., 2009). In order to reduce systematic error, a single investigator was assigned to measure 1 of the 11 traits in all animals.

Primer design and PCR conditions

Based on the bovine *SIRT2* gene sequence (GenBank accession No. NM_001113531.1), three pairs of PCR primers were designed to amplify different fragments of the *SIRT2* gene. Primers, annealing temperature, and fragment sizes are given in Table 1. The PCR amplification product was amplified from a 20-µL mixture comprising 50 ng DNA, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM MgCl₂, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). Amplification was programmed for an initial 5 min at 95°C, followed by 35 cycles of 94°C for 30 s, annealing temperature of 63.3°, 63.3°, 58.5° and 65.5°C (for A7445G, C7711T, G17937A, and G20937A, respectively) for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The digested products were detected by electrophoresis on a 1.0% agarose gel stained with ethidium bromide, purified using Axygen kits (BMI Fermentas, Glen Burnie, MD, USA), and finally sequenced in both directions in an ABI PRISM 377 DNA sequencer (Perkin-Elmer). The sequence maps were analyzed with the SeqMan software (version 10.3).

Table 1. I	Primers used for polymerase chain reaction amplific	cation of the SIRT2 gene in Qinchuan	n cattle.
Primer	Primer sequence (5' to 3')	Length, location	Tm (°C)
P1	1F: 5'-TGTCCTAGAGCCCACACGC-3' 1R: 5'-GATACTCACTCTCTGCTTGTCC-3'	724 bp/intron 4 and exon 4	66.3
Р2	2F: 5'-GGTTCACTCCTGACCCTC-3' 2R: 5'-CATGGCCCAACTAAAGAC-3'	408 bp/exon 9	58.5
Р3	3F: 5'-CTGTCCCCGTGTCTGTCTGT-3' 3R: 5'-CCTGGAATCTGACCCCTGAG-3'	623 bp/exon 12	65.5

Genotyping of SIRT2 alleles by sequencing

The use of single-strand conformation polymorphism to detect four mutations is a time-consuming and complicated process. In addition, there are no suitable restriction endonucleases for restriction fragment length polymorphism, which is another common method for detecting genotype mutant forms of a gene. Therefore, all of the products obtained from the DNA samples of the 520 Qinchuan cattle were directly sequenced for distinguishing the genotypes of the four mutations in *SIRT2*.

Genetics and Molecular Research 13 (4): 8834-8844 (2014)

Statistical analysis

Gene frequencies, allelic frequencies, and deviations from Hardy-Weinberg equilibrium were determined by direct counting. Population genetic indices including heterozygosity (H_E) , 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 (LD) and haplotype distributions of the single nucleotide polymorphisms (SNPs) were analyzed using the expectation maximization algorithm, as obtained through the Haploview software (Barrett et al., 2005).

The SPSS software (version 13.0) was used to analyze the relationship between different genotypes of the *SIRT2* gene and the BMTs (BL, WH, HH, RL, HW, CD, CC, and PBW) and meat quality traits (BT, ULA, and IF) of Qinchuan cattle. The following statistical linear model was used: $Y_{ij} = \mu + G_i + A_i + E_{ijk}$, where Y_{ij} is the trait value for each individual, μ is the overall population mean for the traits, G_i is the fixed effect associated with genotype, A_i is the fixed effect of age, and E_{ijk} is the standard error.

RESULTS

Polymorphisms and genetic diversity

Four polymorphism sites in the *SIRT2* gene (Table 1), A7445G (Figure 1), C7711T (Figure 2), G17937A (Figure 3), and G20937A (Figure 4), were identified by sequencing, which were located in intron 4, exon 4, exon 9, and exon 12, respectively. Both G17937A and G20937A are missense mutations (Val221IIe and Val299IIe), whereas C7711T is a nonsense mutation.

The DNA restriction fragments for each SNP are shown in Figures 1-4. G17937A had only two genotypes, as the AA genotype was not detected in the sampled animals. Genotype and allele frequencies for the four loci are shown in Table 2. Allele G was predominant for G17937A and G20937A, whereas alleles A and C were predominant for A7445G and C7711T, respectively. Results of the χ^2 test illustrated that the genotypic distributions of both the A7445G and G20937A mutations were in agreement with Hardy-Weinberg equilibrium (P < 0.05), while the other two mutations were not (0.05 < P < 0.01).



Figure 1. Sequencing map of the snp7445 locus.

Genetics and Molecular Research 13 (4): 8834-8844 (2014)

[©]FUNPEC-RP www.funpecrp.com.br



Figure 2. Sequencing map of the snp7711 locus.



Figure 3. Sequencing map of the snp17937 locus.





Based on Nei and Roychoudhury (1974), the population genetic indices $H_{\rm E}$, $N_{\rm E}$, and PIC were calculated, and the results are shown in Table 2. The SNPs A7445G, C7711T, and G20937A showed a medium polymorphism level (0.25 < PIC < 0.50), whereas G17937A showed a low polymorphism level (PIC < 0.25).

Genetics and Molecular Research 13 (4): 8834-8844 (2014)

 Table 2. Genotype frequencies (%) of the SIRT2 gene for the single nucleotide polymorphisms in the Qinchuan cattle populations.

	Sample	Ger	notypic frequ	ency	Allele fr	requency	$H_{\rm E}$	$N_{\rm E}$	PIC	χ^2 (HWE)
A7445G	520	AA 0.4615	AG 0.4039	GG 0.1346	A 0.6635	G 0.3365	0.4466	1.8069	0.3469	4.7577
C7711T	520	CC 0.5731	CT 0 3365	TT 0.0904	C 0 7413	T 0.2587	0 3835	1 6221	0.3100	7 7987
G17937A	520	GG 0.8058	GA 0 1942	0.0901	G	A 0.0971	0.1754	1 2127	0.1600	6.0161
G20937A	520	GG 0.5346	GA 0.3654	AA 0.1000	G 0.7173	A 0.2827	0.4056	1.6822	0.3233	5.101

HWE = Hardy-Weinberg equilibrium; $\chi 0.05^2 = 5.991$, $\chi 0.01^2 = 9.21$. Genotype distributions of the mutation were in agreement with HWE if χ^2 (P value) was less than 0.05^2 (0.05), genotype distributions of the mutation were not in HWE if χ^2 (P value) was greater than $\chi 0.05^2$ (0.05). H_E = heterozygosity; N_E = effective number of alleles; PIC = polymorphism information content.

LD and haplotype analysis

LD between polymorphism pairs and haplotype structure analyses of the *SIRT2* gene in Qinchuan cattle are shown in Tables 3 and 4. The r^2 values for LD between the four sites ranged from 0.000 to 1.000. Ardlie et al. (2002) suggested that if the r^2 value is >0.33, LD is considered to be strong. Our results revealed strong linkage between A7445G and G17937A, as well as between C7711T and G20937A.

The four SNPs identified showed seven different haplotypes in the populations studied (frequency >0.05). Hap1 (-ACGC-) had the highest haplotype frequency (27.70%), followed by Hap2 (-ACGG-) and Hap7 (-GTGC-), 21.9 and 13.3%, respectively.

Table 3. Estimated values of linkage disequilibrium for single nucleotide polymorphisms of the bovine SIRT2 gene in Qinchuan cattle.

z	A7445G-G17937A	A7445G-C7711T	A7445G-G20937A	G17937A-C7711T	G17937A-G20937A	C7711T-G20937A
r ²	0.056	0.001	0.028	0.000	0.103	0.041

Table 4. Hap	olotypes of the SIRT	2 gene and their freq	uencies in Qinchuan	cattle.	
Haplotype	A7445G	C7711T	G17937A	G20937A	Frequency
Hap1	А	С	G	С	0.277
Hap2	А	С	G	G	0.219
Hap3	А	С	А	С	0.049
Hap4	А	Т	G	С	0.092
Hap5	G	С	G	С	0.121
Hap6	G	С	G	G	0.054
Hap7	G	Т	G	С	0.133

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

Table 5 summarizes the effects of the SNPs on growth performance and meat quality traits in Qinchuan cattle. At the 7445 SNP locus, individuals with genotype AA had higher values of BL, HH, HW, and CD compared to individuals with genotype GG (P < 0.01). In

Genetics and Molecular Research 13 (4): 8834-8844 (2014)

പ	
Ē	
Ē	
ü	
Ē	
ai	
n	
-R	
2	
·=.	
0	
g	
·=	
ts	
Ĩ	
1	
t2	
1	
Ia	
Ę	
a	
e	
μ	
Ч	
n	
а	
Ļ.	
1	
2	
Ĕ	
ad	
Ч	
it	
3	
-	
Je	
er	
ã	
0	
E.	
à	
\sim	
e	
- P	
Ξ	
-H	
ŝ	
В	
S	
1.	
d	
E	
2	
H	
\geq	
0	
ā	
e	
p	
÷.	
2	
le	
2	
JU	
-	
le	
ad	
II.	
ŝ	
f	
0	
SS	
ă	
5	
ot	
ŭ	
e	
on	
It	
G	
CLC	
fe	
if	
q	
Ŧ	
0	
n	
0	
Ē	
13	
S	
30	
S	
\triangleleft	
5	
e	
9	
3	
E	

					Body measureme	nt					Meat quality traits	
		BL (cm)	WH (cm)	(cm)	RL (cm)	HW (cm)	CD (cm)	CC (cm)	PBW (cm)	BT (cm)	$ULA (cm^2)$	IF (%)
A7445G	AG AG	137.194 ± 13.291^{A} 134.069 ± 10.852^{B}	121.564 ± 9.964 120.938 ± 9.133	124.473 ± 7.928^{a} 122.931 ± 9.134^{b}	42.838 ± 5.069^{a} 42.119 ± 4.745	$39.467 \pm 3.974^{\Lambda}$ 38.405 ± 4.275 37.366 ± 4.275	61.023 ± 6.317^{Aa} 59.201 ± 5.176^{b}	167.442 ± 13.956^{a} 163.455 ± 15.667^{b}	19.175 ± 0.895^{a} 18.914 ± 1.237	0.953 ± 0.057^{a} 0.892 ± 0.069	49.520 ± 4.334 48.406 ± 5.002	7.299 ± 0.568 7.373 ± 0.646
C7711T	385	136.571 ± 12.551^{A} 132.757 ± 10.453^{B}	121.604 ± 11.941 121.604 ± 11.941 119.912 ± 7.722	121.623 ± 5.970 124.182 ± 8.627^{A} 122.301 ± 5.867^{B}	42.694 ± 3.228 42.694 ± 3.228 41.875 ± 4.101	37.200 ± 4.700 38.810 ± 5.765 38.502 ± 4.001	60.335 ± 5.687 60.335 ± 5.687 59.269 ± 6.227	160.360 ± 14.060 166.360 ± 14.944^{a} 162.559 ± 15.921^{b}	18.900 ± 1.714 18.900 ± 1.714 18.820 ± 0.905	0.934 ± 0.065 0.934 ± 0.065 0.895 ± 0.076	49.842 ± 3.345 46.733 ± 5.017	$7.262 \pm 0.630^{\circ}$
G17937A	日名	133.805 ± 10.013 132.495 ± 12.629 ^B	120.573 ± 6.354 119 233 + 8 042	123.534 ± 4.185 122.609 ± 6.833	42.045 ± 3.387 42.139 ± 4.416	39.255 ± 3.880 37.842 ± 3.057	59.715 ± 4.094 58 649 + 5 416	164.952 ± 12.655 167.852 ± 12.655	19.570 ± 1.014 18.614 ± 2.045	0.882 ± 0.072 0.895 ± 0.049	47.897 ± 3.668 46.509 ± 2.784^{b}	7.722 ± 0.615^{a} 7.311 ± 0.613
A10011D	es es	$135.652 \pm 11.230^{\text{A}}$	121.347 ± 10.714	123.708 ± 6.718 123.708 ± 6.718	42.411 ± 4.254 $47.760 \pm 3.770^{\circ}$	38.962 ± 3.552	60.226 ± 5.410	$165.452 \pm 13.221^{\circ}$ $166.335 \pm 12.221^{\circ}$	19.012 ± 0.886 19.172 ± 1.203	0.918 ± 0.051	$49.130 \pm 3.440^{\circ}$	7.328 ± 0.509
	AA GA	133.472 ± 11.004 ^b 129.477 ± 9.92 ^{bc}	120.628 ± 9.745 119.780 ± 9.114	122.883 ± 8.540 121.961 ± 10.04	42.122 ± 2.352 41.275 ± 3.661^{b}	38.788 ± 2.442 38.199 ± 3.853	59.017 ± 5.065	162.815 ± 12.790	18.877 ± 1.272 18.235 ± 0.963	0.873 ± 0.077 0.954 ± 0.051^{b}	46.670 ± 5.245^{B} 44.025 ± 3.317^{B}	7.41 ± 0.557^{A} 6.85 ± 0.502^{Bb}
Data are = chest c with diff	rep lepti eren	h; CC = chest of superscripts	$\pm SD. SD = \frac{1}{2}$ circumference	standard devia PBW = pin t Pilv different (P	tion; BL = bo one width; B < 0.05). ^{A,B} M	dy length; W T = backfat 1 eans with dif	/H = wither h thickness; UL fferent supers	eight; HH = hi A = ultrasound crints are signi	p height; RL loin muscle ficantly differ	= rump leng area; IF = in ent $(P < 0.0]$	(th; HW = hip tramuscular f	width; CD at. ^{a,b} Means
		J Jan a sec	0				Ito	0	C			

Genetics and Molecular Research 13 (4): 8834-8844 (2014)

addition, the CC, PBW, and BT values were also higher in AA carriers than GG carriers (P < 0.05). At the 7711 SNP locus, significant differences in BL and HH were observed between CC and CT genotypes (P < 0.01); individuals with the TT genotype had increased IF compared to those with CC and CT (P < 0.05). At the 17937 SNP locus, individuals with the AG genotype had significantly higher BL (P < 0.01), CC, and ULA values (P < 0.05) than those with the AA genotype. At the 20937 SNP locus, significant differences of BL and ULA were observed between GG and AA genotypes (P < 0.01), and the RL and BT values of individuals with genotype GG were higher than those of individuals with genotype AA (P < 0.05); compared with AA, individuals with the GA genotype showed increased IF (P < 0.01). However, no significant correlations were observed in the other indices for the four SNPs.

The effects of genetic variations of a gene could be demonstrated more readily by integrating haplotype combination analyses with the single-locus effects. The effects of the combinations of the four SNPs were evaluated, and a total of 14 haplotype combinations were identified; combinations with frequencies lower than 5% (data not shown) were not included, and the remaining combinations were selected for further analysis. As shown in Table 6, the H_1H_1 diplotype had significantly greater HH values than the H_5H_7 diplotype (P < 0.01), and similar results were found for BL (P < 0.01). In addition, individuals with the combined genotype H_2 - H_5 had significantly lower growth traits compared to other combinations (P < 0.01). For meat quality traits, H_1H_1 and H_4H_7 individuals had significantly greater IF values than those with the H_1H_2 diplotype (P < 0.05). In addition, association analyses showed highly significant differences between H_2H_5 and the other diplotypes (P < 0.05) in BT and ULA, in which the values of H_2H_5 individuals were lowest among all combinations.

DISCUSSION

In livestock breeding, body measurement and meat quality traits are affected by many factors including genotype, sex, age, breed, herd, location, and other random environmental factors, and are important indices for assessing the economic value of animals (Liu et al., 2010). Through marker-assisted selection studies, many important genes have been identified to be involved in controlling growth (Xue et al., 2011; Tian et al., 2011) and meat quality in livestock (Jiao et al., 2010; Fan et al., 2011). Studies in mammalian cells have suggested that SIRT2 may play a role in cell cycle regulation and cytoskeleton organization by targeting the cytoskeletal protein tubulin (North et al., 2003). Although ample evidence has demonstrated that SIRT2 plays an important role in lipid metabolism and bone growth in humans and mice, its effects on cattle growth and meat quality remain unclear. Interestingly, Jing et al. (2007) found that SIRT2 causes FoxO1 acetylation/deacetylation and reduces the expression of PPARy and C/EBP α in 3T3-L1 cells, leading to the inhibition of adipogenesis. Moreover, a recent study revealed that SIRT2 suppresses adipocyte differentiation by deacetylating FOXO1 and enhancing its repressive interaction with PPARy (Wang and Tong, 2009). Moreover, several previous studies have confirmed that SIRT2 plays an important role in the cell cycle, and overexpression of the wild-type SIRT2 gene can prolong the mitotic phase in the cell cycle (Dryden et al., 2003; North and Verdin, 2007; Nahhas et al., 2007). These studies indicate that the SIRT2 gene may mediate, directly or indirectly, meat production traits in animals.

Our results showed that individual cattle with the AA genotype of A7445G, CC genotype of C7711T, AG genotype of G17937A, and GG genotype of G20937A had superior growth and meat quality traits compared to individuals with other genotypes. Furthermore, we

Genetics and Molecular Research 13 (4): 8834-8844 (2014)

	ESD)	IF (%	
	quality trait (mean =	$ULA(cm^2)$	
	Meat	BT (cm)	
		PBW (cm)	
		CC (cm)	
		CD (cm)	
•	it (mean \pm SD)	HW (cm)	
	Body measuremer	RL (cm)	
,		HH (cm)	
*		WH (cm)	
		BL (cm)	

cattle.
inchuan
\mathcal{O}
in
traits
quality
meat
s and
traits
growth
with
lotypes
lapl
ff
ns c
atio
oci
Ass
6
ble (
Та

Genetics and Molecular Research 13 (4): 8834-8844 (2014)

	BL (cm)	WH (cm)	HH (cm)	RL (cm)	HW (cm)	CD (cm)	CC (cm)	PBW (cm)	BT (cm)	$ULA(cm^2)$	IF (%)
Hap1/1	$140.334 \pm 10.342^{\rm A}$	122.480 ± 9.587^{A}	$126.223 \pm 7.690^{\wedge}$	$43.625 \pm 3.587^{\Lambda}$	$39.964 \pm 3.011^{\rm A}$	61.929 ± 4.598^{A}	168.768 ± 11.258^{A}	19.694 ± 1.332^{A}	$0.924\pm0.060^{\rm A}$	50.677 ± 3.852^{A}	7.479 ± 0.659^{a}
Hap1/2	$135.763 \pm 11.587^{\rm B}$	$121.869 \pm 7.698^{\Lambda}$	$124.281\pm9.332A^{\rm B}$	$42.807 \pm 2.895^{\Lambda}$	39.597 ± 2.757^{A}	$60.535 \pm 5.693^{\Lambda}$	$167.035 \pm 10.369^{\Lambda}$	$19.281 \pm 0.824^{\mathrm{A}}$	$0.925 \pm 0.042^{\Lambda}$	$47.701 \pm 4.021^{\wedge}$	$6.882 \pm 0.425^{\rm b}$
Hap2/5	$122.867 \pm 9.256^{\circ}$	$113.711 \pm 10.051^{\rm B}$	$118.522 \pm 10.261^{\circ}$	39.467 ± 3.001^{B}	34.401 ± 2.251^{B}	53.524 ± 4.529^{B}	$148.133 \pm 8.654^{\rm B}$	$15.956 \pm 0.758^{\rm B}$	$0.720 \pm 0.046^{\rm B}$	39.050 ± 3.025^{B}	7.157 ± 0.521
Hap4/7	133.906 ± 11.526^{B}	121.011 ± 8.658^{A}	123.235 ± 9.440^{AB}	$42.625 \pm 4.011^{\wedge}$	$40.031 \pm 3.174^{\wedge}$	$60.444 \pm 4.526^{\wedge}$	$166.047 \pm 11.698^{\Lambda}$	$20.025 \pm 1.268^{\wedge}$	$0.880 \pm 0.058^{\Lambda}$	$48.945 \pm 3.558^{\Lambda}$	7.687 ± 0.658^{a}
Hap5/7	$133.511 \pm 9.587^{\rm B}$	$120.025 \pm 6.885^{\rm A}$	$122.884 \pm 7.241^{\rm B}$	42.233 ± 3.117^{A}	$39.045 \pm 2.770^{\Lambda}$	59.558 ± 4.552^{a}	164.116 ± 9.731^{A}	18.791 ± 1.335^{A}	$0.869 \pm 0.775^{\rm A}$	$46.351 \pm 2.255^{\rm A}$	7.281 ± 0.528
Data a	re reported as n	neans ± SD. SI	D = standard de	viation; BL =	body length;	WH = wither]	neight; HH = h	ip height; RL	= rump leng	th; HW = hip	width; CD
= ches	t depth; $CC = c_i$	hest circumfered	ence; PBW = pi	n bone width;	BT = backfat	thickness; UL	A = ultrasound	loin muscle a	trea; $IF = int$	ramuscular fa	t. ^{a,b,c} Means
with di	ifferent superso	ripts are signifi	icantly different	(P < 0.05). A,F	^{3,C} Means with	different supe	rscripts are sign	nificantly diffe	erent ($P < 0.0$	01).	

L.S. Gui et al.

also examined the association of haplotype combinations with some traits, including BL, RL, CC, CD, PBW, ULA, and IF. The proportion of individuals with diplotypes H_1H_1 (AA-CC-GG-CC), H_1H_2 (AA-CC-GG-CG), and H_4H_7 (AG-TT-GG-CC) was higher than that of other haplotype combinations. The high-frequency haplotypes have most likely been present in the population for a long time and may be regulated directly or indirectly by different rearing environments (Li et al., 2013). Individuals with the H_1H_1 haplotype showed superior growth and meat quality traits such as BT, BL, HH, ULA, and IF.

In addition, the A7445G mutation was intronic and C7711T was a synonymous mutation, and these two mutations did not change the structure of their encoded proteins. Earlier reports concluded that SNPs resulting in synonymous mutations can affect gene expression, phenotype, and, consequently, respective physiological functions (Van Laere et al., 2003; Krawczak et al., 2007). Therefore, these two variations may influence mitochondrial morphology and adipogenesis by affecting the transcription of the *SIRT2* gene in cattle. Further verification is needed to elucidate the underlying mechanism.

In summary, we reported four polymorphisms of the *SIRT2* gene in Qinchuan cattle and determined the association of the *SIRT2* gene with BMTs and meat quality traits. The present data suggest that the individuals of the combined genotype H_1H_1 have superior growth and meat quality traits. Our investigation provides evidence that the *SIRT2* gene could be used as a candidate gene for cattle breeding. Further research should be conducted in a large population before applying this gene to molecular marker-assisted selection.

ACKNOWLEDGMENTS

Research supported by the National "Five Year" Science and Technology Support Project (#2011BAD28B04-03), the China National "863" Program (#2011AA100307), the GMO New Varieties Major Project (#2011ZX08007-002), the National Beef and Yak Industrial Technology System (#CARS-38), the "13115" Scientific and Technological Innovation Program of Shaanxi Province, the Qinchuan Beef Cattle Breeding High-Quality and Efficient Breed Breeding Technology Research Extension Project (#2011KTCL02-07), the Chinese Beef Cattle Economically Important Traits Functional Genomics Studies Project (2013AA102505), and the Identification and Regulation of Qinchuan Cattle Meat Quality Traits Functional Gene Project (#31272411).

REFERENCES

- Ardlie KG, Kruglyak L and Seielstad M (2002). Patterns of linkage disequilibrium in the human genome. *Nat. Rev. Genet.* 3: 299-309.
- Barrett JC, Fry B, Maller J and Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265.

Blander G and Guarente L (2004). The Sir2 family of protein deacetylases. Annu. Rev. Biochem. 73: 417-435.

Dryden SC, Nahhas FA, Nowak JE, Goustin AS, et al. (2003). Role for human SIRT2 NAD-dependent deacetylase activity in control of mitotic exit in the cell cycle. *Mol. Cell Biol.* 23: 3173-3185.

Fan YY, Zan LS, Fu CZ, Tian WQ, et al. (2011). Three novel SNPs in the coding region of PPARγ gene and their associations with meat quality traits in cattle. *Mol. Biol. Rep.* 38: 131-137.

Gilbert RP, Bailey DR and Shannon NH (1993). Linear body measurements of cattle before and after 20 years of selection for postweaning gain when fed two different diets. J. Anim. Sci. 71: 1712-1720.

Guarente L (2007). Sirtuins in aging and disease. Cold Spring Harb. Symp. Quant. Biol. 72: 483-488.

Jiao Y, Zan LS, Liu YF, Wang HB, et al. (2010). A novel polymorphism of the MYPN gene and its association with meat

Genetics and Molecular Research 13 (4): 8834-8844 (2014)

quality traits in Bos taurus. Genet. Mol. Res. 9: 1751-1758.

- Jing E, Gesta S and Kahn CR (2007). SIRT2 regulates adipocyte differentiation through FoxO1 acetylation/deacetylation. *Cell Metab.* 6: 105-114.
- Krawczak M, Thomas NS, Hundrieser B, Mort M, et al. (2007). Single base-pair substitutions in exon-intron junctions of human genes: nature, distribution, and consequences for mRNA splicing. *Hum. Mutat.* 28: 150-158.
- Krishnan J, Danzer C, Simka T, Ukropec J, et al. (2012). Dietary obesity-associated Hiflalpha activation in adipocytes restricts fatty acid oxidation and energy expenditure via suppression of the Sirt2-NAD+ system. *Genes Dev.* 26: 259-270.
- Li M, Sun X, Hua L, Lai X, et al. (2013). SIRT1 gene polymorphisms are associated with growth traits in Nanyang cattle. Mol. Cell. Probes 27: 215-220.
- Liu Y, Zan L, Zhao S, Xin Y, et al. (2010). Molecular characterization, polymorphism of bovine ZBTB38 gene and association with body measurement traits in native Chinese cattle breeds. *Mol. Biol. Rep.* 37: 4041-4049.
- Marmorstein R (2004). Structure and chemistry of the Sir2 family of NAD+-dependent histone/protein deactylases. *Biochem. Soc. Trans.* 32: 904-909.
- Nahhas F, Dryden SC, Abrams J and Tainsky MA (2007). Mutations in SIRT2 deacetylase which regulate enzymatic activity but not its interaction with HDAC6 and tubulin. *Mol. Cell. Biochem.* 303: 221-230.
- Nei M and Roychoudhury AK (1974). Sampling variances of heterozygosity and genetic distance. Genetics 76: 379-390.
- North BJ and Verdin E (2007). Mitotic regulation of SIRT2 by cyclin-dependent kinase 1-dependent phosphorylation. J. Biol. Chem. 282: 19546-19555.
- North BJ, Marshall BL, Borra MT, Denu JM, et al. (2003). The human Sir2 ortholog, SIRT2, is an NAD+-dependent tubulin deacetylase. *Mol. Cell* 11: 437-444.
- Peck B, Chen CY, Ho KK, Di Fruscia P, et al. (2010). SIRT inhibitors induce cell death and p53 acetylation through targeting both SIRT1 and SIRT2. *Mol. Cancer Ther.* 9: 844-855.
- Rincon G, Farber EA, Farber CR, Nkrumah JD, et al. (2009). Polymorphisms in the STAT6 gene and their association with carcass traits in feedlot cattle. *Anim. Genet.* 40: 878-882.
- Sambrook J and Russell DW (2001). Molecular Cloning: a Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
- Tian WQ, Wang HC, Song FB, Zan LS, et al. (2011). Association between a single nucleotide polymorphism in the bovine chemerin gene and carcass traits in Qinchuan cattle. *Genet. Mol. Res.* 10: 2833-2840.
- Van Laere AS, Nguyen M, Braunschweig M, Nezer C, et al. (2003). A regulatory mutation in IGF2 causes a major QTL effect on muscle growth in the pig. *Nature* 425: 832-836.
- Wang F and Tong Q (2009). SIRT2 suppresses adipocyte differentiation by deacetylating FOXO1 and enhancing FOXO1's repressive interaction with PPARγ. *Mol. Biol. Cell* 20: 801-808.
- Xue M, Zan LS, Gao L and Wang HB (2011). A novel polymorphism of the myogenin gene is associated with body measurement traits in native Chinese breeds. *Genet. Mol. Res.* 10: 2721-2728.
- Zhou Y, Zhang H, He B, Du J, et al. (2012). The bicyclic intermediate structure provides insights into the desuccinylation mechanism of human sirtuin 5 (SIRT5). *J. Biol. Chem.* 287: 28307-28314.

Genetics and Molecular Research 13 (4): 8834-8844 (2014)