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SIRT1 gene polymorphisms associated with carcass traits in Luxi cattle

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Abstract. *SIRT1* is the gene that codes for Sirtuin 1, an NAD (nicotinamide adenine dinucleotide)-dependent class III histone deacetylase. This gene plays a key role in adipose tissue and muscle development in animals. Chinese Luxi cattle ($n = 169$) were selected to identify *SIRT1* SNPs (single nucleotide polymorphisms) and investigate the relationship of these SNPs with carcass traits. Five SNPs (g.-382G > A, g.-274C > G, g.17324T > C, g.17379A > G, and g.17491G > A) were identified by direct sequencing. SNPs g.-382G > A and g.-274C > G were located within the promoter region of this gene. SNP g.-382G > A was significantly associated with dressing percentage, meat percentage, and striploin and ribeye weights, and the g.-274C > G polymorphism had a strong effect on carcass, tenderloin, and high rib weights in Luxi cattle. These findings will provide possible clues for the biological roles of *SIRT1* underlying beef cattle carcass traits.

1 Introduction

Sirtuin 1, also known as NAD (nicotinamide adenine dinucleotide)-dependent deacetylase Sirtuin 1, is an NAD+-dependent protein deacetylase; it has many established protein substrates and is thought to regulate an impressive list of biological functions (McBurney et al., 2013). Sirtuin 1 has an important function in endocrine signaling, specifically in glucose and fat metabolism in mammals (Zillikens et al., 2009; Picard et al., 2004). Increased hepatic *SIRT1* activity enhances gluconeogenesis and inhibits glycolysis (Rodgers et al., 2005; Zillikens et al., 2009). In the pancreatic β cells, *SIRT1* positively regulates insulin secretion in response to glucose (Bordone et al., 2006). It is also involved in cellular differentiation, apoptosis, metabolism, and aging (Shakibaei et al., 2012; Sasaki et al., 2014; Luna et al., 2013; Gueguen et al., 2014).

Previous studies have suggested that SNPs (single nucleotide polymorphisms) within *SIRT1* increase the risk of obesity (De Oliveira et al., 2012), type 2 diabetes, and Parkinson's disease (Schug and Li, 2011; Inamori et al., 2013; Shiota et al., 2012; Civelek et al., 2013; Dong et al., 2011; Rai et al., 2012; Figarska et al., 2013). In adipose tissue, Sirtuin 1 inhibits fat storage and increases lipolysis via the repression of peroxisome proliferator-activated receptor-γ (PPAR-γ). PPAR-γ is a key regulator in adipogenesis and fat storage, controlling the expression of many adipocyte-specific genes (Picard et al., 2004). These studies suggested that Sirtuin 1 is a key regulator of whole-body energy balance and plays a role in human health (Sasaki et al., 2014).

The bovine *SIRT1* gene, which includes nine exons on chromosome 28, is highly expressed in the liver and adipose tissue (Ghinis-Hozumi et al., 2011). *SIRT1* may play an important role in the development of bovine adipose tissue in vivo. Although *SIRT1*, forkhead box O1 (*FOXO1*), and

Table 1. PCR primers and conditions for identification of SNPs in *SIRT1* (NM_001192980).

SNPs	Primers (5′–3′)	Genotyping methods	Temp (°C)	Restriction enzyme	Genotype pattern (bp)
g.G-382A	F: GTTTAGCCTTAACGCCGTTCAGGAAATT* R: GTCTTTCAGAGTCTTCAAATCAGTGCCC	ACRS	56	Vsp I	166/136 + 30
g.C-274G	F: GTATAGTCCACGGGGTTACAG R: CCAAACTTGTCTTTCAGAGTC	PCR-RFLP	59	Sma I	273/235 + 38
g.T17324C	F (inner): GTTAGTAAACTTCAGAATTGCTTTgCT R (inner): TAATTTTTCCTACAAAACTAATATAAgGG F (outer): CTAGATGCTTTGAGATTGTCGTGTGTTG R (outer): ACTAAGCACACTATTTGAAACTTGAGTG	T-ARMS-PCR	58		550 bp (outer) 270 bp (alleteT) 330 bp (alleteC)
g.A17379G	F: TTCCAACCATCTCTTTGTCAC R: AATAATAAGGCTTAATCTGAATT*	ACRS	57	EcoR I	235/211 + 24
g.G17491A	F (inner): AAATACTGGCCTCAACTCTTAATTtTA R (inner): AAATCCAAATTAACATCTGACATTTtAC F (outer): TACTTCGCAACTATACTCAGAACATAGA R (outer): GTTTGATCTCTAGGTTAGGAAGATCCT	T-ARMS-PCR	58		477 bp (outer) 296 bp (alleteG) 236 bp (alleteA)

Note: * purposeful mismatch was introduced in the sequence to create a restriction site.

PPAR-γ expression appear to be nonlinear during the stages of preadipocyte differentiation, these genes play an important role during bovine adipocyte development in Lilu cattle (Liu et al., 2014). The study examined the variations of *SIRT1* in Luxi beef cattle by identified SNPs, and explored possible associations between *SIRT1* variants and carcass traits. These molecular markers will provide some theoretical basis for improving cattle carcass characteristics.

2 Materials and methods

2.1 Animals and genomic DNA isolation

In the Shandong province, 169 Chinese Luxi cattle were reared in same conditions. The animals were slaughtered at the age of 24 months according to Chinese national law (China Administration Rule of Laboratory Animal; Operating Procedure of Cattle Slaughtering GB/T 19477-2004). Carcass traits were recorded and blood samples were collected. Genomic DNA containing nucleotides from leukocytes was isolated from blood samples and stored at −20 °C following standard procedures (QIAamp DNA Blood Mini Kit, Qiagen, Germany).

2.2 SNP detection

We used the primer sequences from M. X. Li et al. (2013) (Table 1) to detect SNPs. The 30 µL reaction volume included 15 µL Taq 2 × PCR MasterMix (QUANSHIJIN, Beijing, China), 3 µL DNA template (20 ng µL^{-1}), 9.6 µL ddH_2O, and 1.2 µL of each primer (10 pmol µL^{-1}).

The g.-382G > A and g.17379A > G polymorphisms were genotyped using the amplification-created restriction site (ACRS) method (Figarska et al., 2013). The tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) was carried out to genotype SNPs g.17324T > C and g.17491G > A (Haliassos et al., 1989). The PCR reactions were performed in a total volume of 10 µL, containing 10 pmol of each of the inner primers, 1 pmol of each of the outer primers, 200 mM of each dNTP, 2 mM of $MgCl_2$, 1 × PCR buffer, 50 ng of DNA, and 0.2 U of Taq DNA polymerase (MBI, Fermentas, Waltham, MA, USA). To increase the specificity of the reaction, a touchdown profile was followed.

2.3 Statistical analysis

DNA sequences were assembled and aligned for mutation analysis with DNASTAR (DNAS Inc., Madison, WI, USA). Allele and genotype frequencies were directly calculated. Heterozygosity, effective number of alleles, and polymorphic information content (PIC) were estimated based on Botstein et al. (1980). A chi-square test assessed conformance with Hardy–Weinberg equilibrium (HWE). Association of genotype with performance traits was analyzed with the general linear model (GLM) procedure of SPSS 16.0.

3 Results

3.1 Identification of SNPs

Five SNPs were detected in the exons, flanking introns, and promoter sequences of *SIRT1*, including four transi-

Table 2. Genotypic and allelic frequencies (%), value of χ^2 test, and diversity parameters of the bovine *SIRT1* gene.

SNPS	Genotype	Number	GF	Allele	AF	χ^2 (HWE)	He	Hom	PIC	Ne
g.-382G > A	GG	96	0.568	G	0.722	9.230	0.402	0.598	0.321	1.671
	GA	52	0.308	A	0.278					
	AA	21	0.124							
g.-274C > G	CC	108	0.639	C	0.769	12.018	0.355	0.645	0.292	1.550
	CG	44	0.260	G	0.231					
	GG	17	0.101							
g.17324T > C	TT	113	0.669	T	0.805	3.031	0.314	0.686	0.265	1.458
	TC	46	0.272	C	0.195					
	CC	10	0.059							
g.17379A > G	AA	122	0.722	A	0.831	8.121	0.280	0.720	0.241	1.390
	AG	37	0.219	G	0.169					
	GG	10	0.059							
g.17491G > A	GG	139	0.823	G	0.888	20.434	0.200	0.800	0.180	1.249
	AG	22	0.130	A	0.112					
	AA	8	0.047							

Note: GF: genotypic frequency; AF: allelic frequency; χ^2 (HWE): Hardy–Weinberg equilibrium χ^2 value; χ^2 0.05 (df = 2) = 5.99, χ^2 0.01 (df = 2) = 9.21; He: gene heterozygosity; Hom: gene homozygosity; PIC: polymorphism information content; Ne: effective allele number.

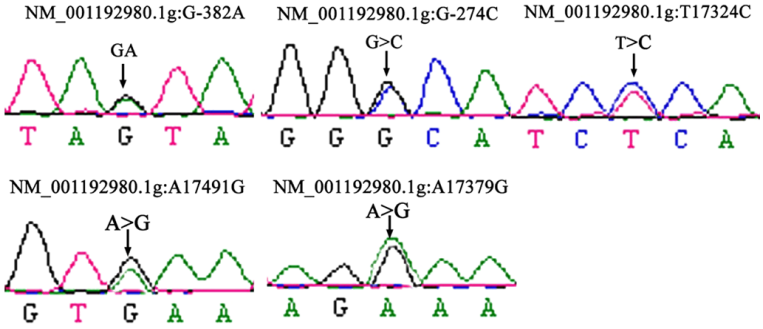

Figure 1. Schematic representation of the *SIRT1* gene with the localization of the five identified SNPs.

tions (G/A at g.-382G > A, T/C at g.17324T > C, A/G at g.17379A > G, and G/A at g.17491G > A) and one transversion (g.-274C > G) (Fig. 1). The nomenclature adopted for the SNPs was based on the convention described by the Human Genome Variation Society (Den Dunnen et al., 2016). No SNPs were found in the coding sequence from the set of animals used in this study. SNPs g.-382G > A and g.-274C > G were located in the promoter region and could cause disruption of several transcription factor binding sites, as predicted by MatInspector release 8.0 (Cakir et al., 2009). The other three SNPs were found in intron five. All five SNPs were successfully genotyped.

Genotypic and allelic frequencies, value of χ^2 test, and PIC of the bovine *SIRT1* gene have been shown in Table 2. The g.-382G > A, g.-274C > G, and g.17324T > C loci had moderate polymorphism and thus genetic diversity, which implies that these SNPs have a potential for selection. The g.17379A > G and g.17491G > A loci had low genetic diversity and selection potential.

3.2 The relationship between SNPs and carcass traits

Significant differences between genotypes and carcass traits of beef cattle are shown in Table 3. In g.-382G > A, AA genotypes have a more significant difference ($P < 0.05$) in dressing percentage, meat percentage, and striploin than the GG and GA genotypes; however, there is no difference in ribeye. In g.-274C > G, AA genotypes have a more significant difference in carcass, tenderloin and high rib weight than GG and GC genotypes. However, no differences between SNPs and carcass traits were found when focusing on 17379A > G and g.17491G > A.

Based on these results, we predicted potential differential transcription factor (TF) binding sites according to the presence of different alleles using MatInspector Release 8.0. At

Table 3. Significant SNP, genotype, and carcass trait associations.

SNPs	Traits	Genotypes (mean ± SE)			*P* value
		GG	GA	AA	
g.-382	Dressing percentage/%	53.184 ± 1.258^{a}	53.627 ± 0.951^{a}	49.880 ± 1.165^{b}	0.043
	Meat percentage/%	47.990 ± 1.632^{a}	48.008 ± 1.234^{a}	42.824 ± 1.511^{b}	0.029
	Striploin/kg	8.113 ± 0.449^{a}	6.898 ± 0.567^{a}	6.367 ± 0.634^{b}	0.041
	Ribeye/kg	8.618 ± 0.751	6.174 ± 0.312	5.029 ± 0.394	0.039
g.-274		GG	GC	CC	
	Carcass weight/kg	326.118 ± 12.910^{a}	300.455 ± 16.049^{a}	269.778 ± 17.743^{b}	0.015
	Tenderloin/kg	4.47 ± 0.324^{a}	4.213 ± 0.293^{a}	3.608 ± 0.324^{b}	0.039
	High rib/kg	10.605 ± 0.448^{a}	9.579 ± 0.557^{a}	8.85 ± 0.616^{b}	0.027
g.17379		GG	GA	AA	
	Striploin/kg	7.703 ± 0.411	8.058 ± 0.232	6.968 ± 0.300	0.036
g.17324		TT	TC	CC	
	Bone weight/kg	18.269 ± 1.858	19.832 ± 2.220	12.248 ± 2.304	0.048

g.-382G > A, a myocyte-specific enhancer factor 2 (MEF2) binding site was generated on substitution to the A allele. At g.-274C > G, in the presence of the C allele, a binding site for a CDE (cell-cycle-dependent element) was generated, whereas the same binding site was abolished in the presence of the G allele.

4 Discussion

There are several variants associated with body mass index and risk of obesity in human *SIRT1* gene (Zillikens et al., 2009). Recent studies have found possibly useful SNPs in the *SIRT1* gene and explored the relationships between these SNPs and ultrasound-measured carcass traits in Qinchuan cattle (Gui et al., 2015). We identified five SNPs in bovine *SIRT1* and estimated the extent of associations between these SNPs and carcass traits in Chinese Luxi cattle.

Association analysis showed that SNP g.17379A > G was significantly associated with tenderloin, striploin, and ribeye and that polymorphisms with g.17324T > C had a strong effect on bone weight (these effects became non-significant following the Bonferroni correction). This SNP did not result in changes in amino acids. Such associations may be a result of linkage disequilibrium between *SIRT1* and other genes on the same chromosome that have a significant effect on these carcass traits. It is interesting to note that the SNP g.17379A > G was severely out of HWE. Subsequent sequencing showed that this was not due to technical error. We considered two possible explanations: (1) Luxi cattle have experienced high selection pressure. Artificial selection led to the loss of non-favored alleles. (2) The analyzed breed has an insufficiently large population size.

Five SNPs (g.-382G > A, g.-274C > G, g.17324T > C, g.17379A > G, and g.17491G > A) were identified in the Luxi cattle and are similar to previous research results (Ye et al., 2001; M. Li et al., 2013). The role of *SIRT1* as an inhibitor of adipogenesis and the recent demonstration of its involvement in white adipose tissue "browning" (M. X. Li et al., 2013) as well as the roles played by *SIRT1* in muscle metabolism (Qiang et al., 2012) have motivated us to further investigate the effects of the identified SNPs on beef cattle carcass traits. Our results showed that SNP g.-382G > A was significantly associated with dressing percentage, meat percentage, and striploin and ribeye weights, and g.-274C > G polymorphism had a strong effect on carcass, tenderloin, and high rib weights in Luxi cattle.

At g.-382G > A, a MEF2 binding site was generated on substitution to the A allele. At g.-274C > G, in the presence of the C allele, a binding site for a CDE was generated, whereas the same binding site was abolished in the presence of the G allele. These indicated that g.-382G > A and g.-274C > G polymorphisms might affect the binding affinity of the surrounding sequences with TF and further influence the activity of the *SIRT1* promoter that was associated with growth trait regulation.

Carcass traits are regulated by multiple genes and are influenced by interactions among them; thus, the effects of these SNPs should be further validated before they can be incorporated into beef cattle breeding practices.

Competing interests. The authors declare that they have no conflict of interest.

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