Gene 559 (2015) 16–21

Contents lists available at ScienceDirect

Gene

journal homepage: www.elsevier.com/locate/gene

Sequence variants in the bovine silent information regulator 6, their linkage and their associations with body measurements and carcass quality traits in Qinchuan cattle

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ARTICLE INFO

Article history:
Received 9 November 2014
Received in revised form 20 December 2014
Accepted 5 January 2015
Available online 7 January 2015

Keywords:
SIRT6 gene
Molecular markers
Haplotype
Beef quality

ABSTRACT

Silent information regulator 6 (SIRT6) belongs to the family of class III nicotinamide adenine dinucleotide (NAD)-dependent deacetylase and plays an essential role in DNA repair and metabolism. This study was conducted to detect potential polymorphisms of the bovine SIRT6 gene and explore their relationships with body measurement and carcass quality in Qinchuan cattle. Four sequence variants (SVs) were identified in intron 6, exon 7, exon 9, and 3′ UTR, via sequencing technology conducted in 468 individual Qinchuan cattle. Eleven different haplotypes were identified, of which two major haplotypes had a frequency of 45.7% (–CACT–) and 14.8% (–CGTC–). Three SVs (SV2, SV3 and SV4) were significantly associated with some of the body measurements and carcass quality traits ($P < 0.05$ or $P < 0.01$), and the H_2H_7 (CC–GA–TT–TC) diplotype had better performance than other combinations. Our results suggest that some polymorphisms in SIRT6 are associated with production traits and may be used as candidates for marker-assisted selection (MAS) and management in beef cattle breeding programs.

1. Introduction

Silent information regulator genes, also referred to as sirtuins, belong to the family of class III nicotinamide adenine dinucleotide (NAD)-dependent deacetylase (Luft, 2014). They control key cellular and physiological processes, including apoptosis, energy homeostasis, mitochondrial function, and longevity. Mammals contain seven sirtuins (SIRT1–7) with different localization patterns and biological functions (Haigis and Sinclair, 2010; Haigis and Guarente, 2006). Nowadays, sirtuins are recognized as potential therapeutic targets for energy metabolism (Guarente, 2006).

Mammalian SIRT6 is predominantly localized in the nucleus, which is consistent with its role in DNA repair and metabolism (Silberman et al., 2014). Recent evidence suggests that SIRT6 regulates animal body growth through central control, while modulating glucose and lipid metabolism at both systemic and local levels (Li and Kazgan, 2011). Mostoslavsky et al. (2006) demonstrated that mice with a SIRT6 gene deletion were undersized and developed a number of abnormalities, including profound lymphopenia, fat depletion, and severe metabolic defects. Similarly, liver-specific deletion of SIRT6 causes altered expression of genes involved in glycolysis and lipid metabolism, such as ACC1 (acetyl-CoA carboxylase-1), FAS (fatty acid synthase) and GPAT (mitochondrial glycerol 3-phosphate acyltransferase), leading to striking phenotypes, such as body weight of mice (Kim et al., 2010). In turn, overexpression of SIRT6 attenuated adipogenesis in adipose tissue, which was associated with down regulation of a selective set of peroxisome proliferator activated receptor responsive (PPARγ) genes (Kanfi et al., 2010). Taken together, these findings lend credence to the hypothesis that SIRT6 is an excellent candidate gene for growth-related traits in livestock.

So far, there are no reports on associations between the variations of SIRT6 and body measurement and carcass quality traits in herbivore breeding. The aim of this study was to contribute to the understanding of the role of SIRT6 in body measurement and carcass quality trait

Abbreviations: SVs, sequence variants; BMTs, body measurement traits; SNPs, single nucleotide polymorphisms; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphisms; MAS, marker-assisted selection; LD, linkage disequilibrium; He, gene heterozygosity; Ne, effective allele numbers; PIC, polymorphism information content.

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http://dx.doi.org/10.1016/j.gene.2015.01.008
0378-1119/

Table 1
Primers used for polymerase chain reaction (PCR) amplification of the SIRT6 gene in *Qinchuan* cattle.

Primer	Primer sequence (5′ to 3′)	Length	Location	Tm (°C)
P1 (SV1–SV2)	5′-TGGGAACCAGCAGTGAGC-3′ 5′-CTATTACGAGCAAAGTGAGGC-3′	435 bp	Part of exon 5, intron 6, intron 7 and part of exon 7	58.5
P2 (SV3–SV4)	5′-GCCCAGAAGAAGGGAAGCAT-3′ 5′-TGGGCAAGGTTGTGGATGGT-3′	1411 bp	Part of exon 7, intron 8, intron 9 and part of 3′ UTR	62.9

variation in cattle by identifying single nucleotide polymorphisms (SNPs) in the bovine SIRT6 gene and conducting haplotype construction and association analysis. The findings may contribute to beef cattle breeding and genetics programs.

2. Materials and methods

2.1. DNA samples and data collection

A total of 468 adult animals (that were female, 18–24 months old, and unrelated for at least three generations) were randomly selected from the National Beef Cattle Improvement Center's experiment farm (Yangling, Shaanxi, China) and were fed under the same management condition. Body measurement traits, including body length, withers height, hip height, rump length, hip width, chest depth, chest circumference, and pin bone width, were measured as previously described (Gilbert et al., 1993). Meanwhile, the carcass quality traits were obtained corresponding to each individual, including back fat thickness, ultrasound loin muscle area and intramuscular fat content (Rincon et al., 2009). In order to minimize the systematic error, a single person was assigned to measure each trait.

DNA samples were extracted from blood samples collected from the jugular vein and stored at −80 °C according to the standard phenol chloroform protocol (Sambrock and Russell, 2001). DNA content was estimated by a spectrophotometer. Genomic DNA was diluted to 50 ng/μL. All DNA samples were stored at −20 °C for subsequent analysis.

2.2. SNP detecting

Primers used to amplify the bovine SIRT6 gene were designed from a published gene sequence (XM_005208961.1). Primers, annealing temperatures, fragment sizes, and location are given in Table 1. The cycling protocol was as follows: denaturation for 5 min at 95 °C; 35 cycles of 94 °C for 30 s; annealing for 30 s; primer extension at 72 °C for 30 s, with a final extension performed at 72 °C for 10 min. All PCR products were then sequenced using an ABI 3730 sequencer (ABI, Foster City, CA, USA), in both the forward and reverse directions.

Thirty random DNA samples were mixed to form a single DNA pool, from which mutations of SIRT6 were detected. The PCR products were amplified from genomic DNA and directly sequenced in both directions.

2.3. Genotyping

Four SVs were found by DNA sequencing; one non-coding mutation and three coding mutations in the exons (Fig. 1). Sequence variant (SV) 2 was a missense mutation (Alu → Ays), while SV3 resulted in a synonymous mutation (Ala → Ala). According to the sequence mutations, the PCR products were digested with *Taq1*, *Mbo11*, *Bsl1* and *Mbo1* restriction enzymes to genotype individuals.

For SV2, new primers were designed to create an *Mbo11* restriction site (GAAGAN$_8^{\wedge}$) in the 148-bp PCR product fragment. The forward primer was 5′-GGACCCCAGGGAGGGCCGCCA-3′ and the reverse primer was 5′-CACTGGGGTCAGACCTGCTGTCT-3′. The naturally occurring "G" nucleotide was substituted with "T" in the forward primer (boxed) in order to introduce a new recognition site (GAAGAN$_8^{\wedge}$).

SV1, SV3 and SV4 were genotyped by PCR–PFLP methods. According to the sequence mutations, the PCR products were digested with *Taq1*, *Mbo11*, *Bsl1* and *Mbo1* restriction enzymes to genotype individuals.

Aliquots of 10 μL PCR products were digested with 10 U *Taq1* (SV1)/*Mbo11* (SV2)/*Bsl1* (SV3)/*Mbo1* (SV4) for 8 h at 37 °C, respectively. The digested products were detected by electrophoresis on a 3.5% agarose gel stained with ethidium bromide.

3. Statistical analysis

Genotypic and allelic frequencies were determined by direct counting. Hardy–Weinberg disequilibrium (HWE), gene heterozygosity (He), gene homozygosity (Ho) and effective allele numbers (Ne) were statistically analyzed according to the approaches described by Nei and Roychoudhury (1974). The polymorphism information content (PIC) was calculated by Botstein's methods (Botstein et al., 1980).

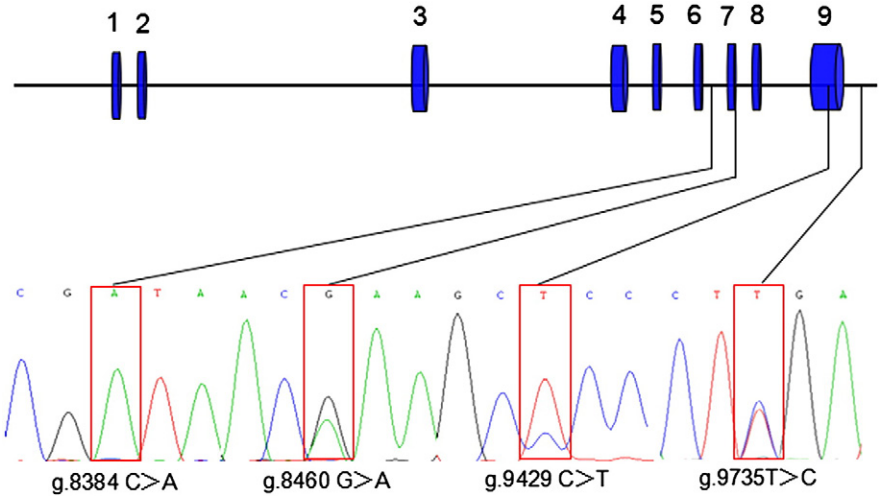

Fig. 1. Schematic representation of the SIRT6 gene with the localization of the four identified SVs. For the g.8384 C>A, the heterozygote CA was not observed in the sampled animals.