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Research paper

# Association study and expression analysis of *MTNR1A* as a candidate gene for body measurement and meat quality traits in Qinchuan cattle

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ABSTRACT

Melatonin receptors, which mediate the functions of melatonin, play an important role in adipocyte differentiation, energy, and lipid metabolism. The aim of this study was to identify single nucleotide polymorphisms (SNPs) in bovine melatonin receptor 1A (MTNR1A) and to determine if these SNPs are associated with body measurement traits (BMTs) and meat quality traits (MQTs) in Qinchuan cattle. We identified three synonymous mutations (A455G, A497G, and C635T) and one missense mutation (G489A) p.Asp224Asn in *MTNR1A* gene in 420 Qinchuan cattle by sequencing. Association analysis indicated that these four SNPs were associated with some of the BMTs and MQTs ($P < 0.05$). Further, 6 combined haplotypes were constructed to guarantee the reliability of analysis results. Individuals with diplotypes H2H2 (AA-GG-GG-CC) had greater chest depth, heart girth, loin muscle area, and more back fat than the other combinations ($P < 0.05$). Pertaining to G489A mutation, RT-PCR study exhibited a higher mRNA expression of MTNR1A gene among individuals with SNP1/2/4-AG-GA-CT genotype than those with SNP1/2/4-AA-GG-CC genotype ($P < 0.05$). These results suggest that the genotype H2H2 could be used as a molecular marker of the combined genotype for future selection for BMTs and MQTs in Qinchuan cattle.



## 1. Introduction

Body measurement and meat quality traits play an important role in the evaluation of productivity in cattle, and they have received increasing attention in cattle selection and breeding (Jiang et al., 2005). In large livestock such as cattle, it is difficult to reach a more significant genetic progress using traditional breeding methods because the generation time is long, and the body measurement and meat quality traits are controlled by multiple genes (Boukha et al., 2011). However, application of DNA markers to improve these traits through marker-assisted selection (MAS) is shown to be a powerful and efficient strategy (Pedersen et al., 2009), and there are some commercially available markers for beef meat quality (Gao et al., 2007). Consequently, it would be reasonable to place considerable emphasis on sifting out numerous candidate genes and elaborating the significant associations between their genetic variations with body measurement and meat quality traits (Hirwa et al., 2011; Ribeca et al., 2014).

Melatonin is a derivative of tryptophan, an amino acid primarily generated in the pineal gland and secreted in a circadian and seasonal rhythmic manner (Brainard et al., 1982; Cozzi et al., 1991). Previous studies showed that melatonin is a negative regulator of adipocyte biology, energy, and lipid metabolism (Zhang et al., 2010; Majumdar et al., 2014). *In vivo* studies have indicated that pinealectomized rats have increased accumulation of adipose depots, which mimicked the withdrawal of melatonin (Alonso-Vale et al., 2004; Borges-Silva et al., 2005; Lardone et al., 2014). Daily nocturnal melatonin supplementation to rats decreased their intra-abdominal adipose accumulation, but did not alter their food intake (Wolden-Hanson et al., 2000). *In vitro* studies involving the long-term melatonin treatment resulted in decreased expression of the glucose transporter GLUT4 and glucose uptake in adipocytes (Brydon et al., 2001). Furthermore, melatonin was reported to inhibit triglyceride accumulation in the rat osteoblast-like ROS17/2.8 cell line (Sanchez-Hidalgo et al., 2007). Due to the important role of melatonin in adipocyte biology, energy and lipid metabolism, it was treated as a potential therapeutic targets for obesity (She et al., 2009), and has been used to improve the production performance and meat quality of pig (Tang et al., 2006).

Melatonin mediates its physiological functions through specific melatonin receptors, MTNR1A and MTNR1B. In mammals, MTNR1A and MTNR1B have been cloned in many species and it has been found that MTNR1A has a unique capacity for high-affinity binding to melatonin and that it mediates numerous physiological functions (Reppert et al., 1996; Lai et al., 2013; Yang et al., 2014a, 2014b). Based on this

*Abbreviations:* MTNR1A, melatonin receptor 1A; BMTs, body measurement traits; MQTs, meat quality traits; MAS, marker-assisted selection; 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; BFT, back fat thickness; IFC, intramuscular fat content; LMA, loin muscle area.

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background, it is rational to hypothesize that the MTNR1A gene may be correlated with body measurement traits (BMTs) and meat quality traits (MQTs). Sequencing was used to detect variants of the *MTNR1A* gene in cattle, and the relationships with BMTs and MQTs were evaluated for potential markers that could be used in selection process by breeders.

## 2. Materials and methods

### 2.1. DNA samples and data collection

All procedures involving animals were approved by the Animal Care and Use Committee of Northwest A&F University. A total of 420 animals of female Qinchuan cattle were selected to be unrelated for at least three generations from one experimental farm. These cattle were all fed on a diet of corn and corn silage after weaning at 6 months of age. The Qinchuan cattle breed is the most famous native breed for beef production in China and mainly reared in Shaanxi province (Liu et al., 2014b). The body measurement traits (BMT) 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 at 24 months of age as previously described (Ozkaya and Bozkurt, 2009). The meat quality traits (MQT), including back fat thickness (BFT), intramuscular fat content (IFC), and loin muscle area (LMA) at 24 months of age were measured using an ultrasound scanner (Brethour, 1994; Hamlin et al., 1995). Blood samples for association analysis were collected from the jugular vein. Genomic DNA samples were obtained from blood samples *via* standard methods (Sambrook and Russell, 2001).

### 2.2. SNP discovery and genotyping

The primers used to amplify the bovine *MTNR1A* gene were designed using Primer-BLAST, a primer designing tool available on the NCBI website (http://www.ncbi.nlm.nih.gov/tools/primer-blast/), based on the sequence of the chromosome 27 of *Bos taurus* breed Hereford (GenBank accession number: 539948). Three primer pairs covering all exons were used in PCR amplification (Table 1). Novel SNPs were detected and genotyped by direct sequencing using ABIPRIZM 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA).

### 2.3. Total RNA isolation and real-time PCR

Samples of back adipose tissue were collected at slaughter (10 female Qinchuan cattle at 24 months of age from National Beef Cattle Improvement Center and were fed under the same management condition) and snap frozen in liquid nitrogen and stored at −80 °C. Total RNA was extracted from each tissue by using RNAprep pure Tissue Kit (Tiangen, Beijing, China), according to the manufacturer's instructions. First strand cDNA was synthesized using reverse transcription kit (Fermentas Life Science, Hanover, USA). Quantitative real-time PCR (ABI7500) was carried out using SYBR Green (TaKaRa, Dalian, China). For evaluation of relative gene expression, β-actin was used as internal control. Primers were designed with the Primer 5.0 software (Table 2).

**Table 1**
Summary of PCR condition for the bovine *MTNR1A* gene.

| Name | Primer sequence | Position | Length | PCR Mg2 +, Ta |
|---|---|---|---|---|
| Primer 1 | F: CGCCAGTCCAAGTTTGATGC<br>R: TCTAGGAAAGAGCCAGTTGACAG | All of exon 1 | 788 bp | 1.5 Mm, 60 °C |
| Primer 2-1 | F: TTAGAATGCGGCAGAAGC<br>R: GACGGACTGCGTGAAGGT | Part of exon 2 | 735 bp | 1.5 Mm, 56 °C |
| Primer 2-2 | F: AGCCTCAGATACGACAAGC<br>R: TCCCACTCTGTTCCCTGA | Part of exon 2 | 910 bp | 1.5 Mm, 61 °C |

Ta = annealing temperature.

**Table 2**
Sequences of primer pairs and amplification conditions for real time PCR.

| Gene | Primer sequence | Length | Annealing temperature |
|---|---|---|---|
| β-actin | F: CACCAACTGGGACGACAT<br>R: ATACAGGGACAGCACAGC | 202 bp | 61 °C |
| *MTNR1A* | F: GGTGGTGTTCCATTTCATCG<br>R: CCAGCAAATGGCAAAGAGG | 181 bp | 61 °C |

Each experiment was performed at least three times in duplicate. The relative mRNA expression levels from real-time PCR were calculated using the $2^{-\Delta\Delta CT}$ method, as described elsewhere (Livak and Schmittgen, 2001).

### 2.4. Statistical analysis

Gene frequencies were determined through direct counting, and Hardy–Weinberg equilibrium was analyzed with the chi-square test using SAS 8.1 (SAS Institute Inc., Cary, NC, USA). The analyses for linkage disequilibrium (LD) and construction of the haplotypes that were obtained from the 420 animals were performed with the online SHEsis software (http://analysis.bio-x.cn/myAnalysis.php) (Shi and He, 2005).

The association analyses between the SNP marker genotypes of the *MTNR1A* gene and data for the meat quality traits (BL, WH, HH, RL, HW, CD, HG, PBW, BFT, LMA, IFC) were analyzed using the General Linear Model procedure and compared by Duncan's Multiple Range Test (SAS 8.1). The records of phenotypic traits of Qinchuan cattle aged 24 months were used for the following analysis. All analyses were done in two steps, first using a full animal model and then using a reduced animal model. The full animal model included fixed effects of marker genotype, season of birth (spring vs fall), birth year and random effects of animal. An effect associated with sex, birth year, and season of birth were not matched in the linear model, as the preliminary statistical analyses indicated that these effects did not have a significant influence on variability of traits in the analyzed population. So the reduced model was used in the final analysis. The linear model:

$$y_{ij} = u + G_i + e_j$$

where $y_{ij}$ is the phenotypic value of traits, u is the mean population mean, $G_i$ is the fixed effect of genotype, and $e_j$ is the random residual error.

The differential mRNA expression study in different genotypes was performed by ANOVA, with Tukey's post hoc test for subsequent individual group comparisons, using the SAS 8.1 software.

## 3. Results

### 3.1. Genotypic and allelic frequencies

We amplified and sequenced all the exons of the *MTNR1A* gene from 25 animals of Qinchuan cattle. Obtained sequences were compared with the previously reported sequence (GenBank accession number: 539948) and four SNPs, located at nucleotides g.A455G (exon2), g.G489A (exon2), g.A497G (exon2), and g.C635T (exon2). The SNP2 g.G489A was missense mutations Asp224Asn, and SNP1 g.A455G, SNP3 g.A497G, and SNP4 g.C635T were synonymous mutations (Fig. 1). These four SNPs were genotyped by sequencing in Qinchuan cattle, and found three genotypes at each locus (Fig. 1). The $\chi 2$ test indicated that these three mutations of g.A455G, g.G489A, and g.C635T were not in Hardy–Weinberg equilibrium ($P < 0.05$) (Table 3).

### 3.2. Linkage disequilibrium and haplotype analyses

To reveal the linkage relationships among SNP1, SNP2, SNP3, and SNP4, linkage disequilibrium between these four SNPs was estimated.