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Polymorphisms in adrenergic receptor genes in Qinchuan cattle show associations with selected carcass traits

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ABSTRACT

The beta-adrenergic receptors coded by the *ADRB1*, *ADRB2* and *ADRB3* genes play important roles in mediating metabolic effects, especially lipolysis, insulin resistance and energy balance. This study investigated the expression levels of these three genes in different tissues of Qinchuan cattle by real-time polymerase chain reaction (RT-PCR). Expressed levels of RNA from the *ADRB2* gene were generally much higher than for *ADRB1* and *ADRB3*. *ADRB1* and *ADRB2* expression levels were highest in subcutaneous fat and lower in muscle, whereas *ADRB3* expression was higher in muscle tissue. Eight single nucleotide polymorphisms (SNPs) were discovered in 503 Qinchuan cattle by DNA sequencing, containing three missense mutations (g.1148G > C in *ADRB1*, g.1293C > T and g.1311T > C in *ADRB2*), four synonymous mutations (g.1054T > C, g.1122C > T and g.1143G > T in *ADRB1* and g.506A > G in *ADRB3*), as well as one mutation in 3′untranslated region (3′UTR) (g.2799G > A in *ADRB3*). Interestingly, five of them were located in regions predicted to contain multiple repeats of CG nucleotides (CpG islands). Association analysis showed relationships between most of those SNPs or combined haplotypes and carcass traits of Qinchuan cattle. This study association analysis suggests that polymorphisms in these genes might be useful for selection in beef cattle breeding.

1. Introduction

Recently, fatty acid composition has become a concern because of its relationship with human health (Zhang et al., 2009). In animals, fat depots, especially in the intramuscular fat layers, are closely related to meat quality characteristics such as tenderness, color, flavor and juiciness (Cameron et al., 2000; Fiems et al., 2000; Ventanas, Ruiz, García, & Ventanas, 2007). An important group of genes encode the proteins involved in energy metabolism, adipokines, cellular respiratory system elements, the proteins that regulate the process of food intake or factors controlling adipocyte proliferation and differentiation (Lands, Arnold, McAuliff, Luduena, & Brown, 1967).

As we well know, one of the key components of the energy balance system is the β-adrenergic receptors (Lowell & Bachman, 2003). The three known beta-adrenergic receptors, coded by genes *ADRB1*, *ADRB2* and *ADRB3*, belong to the class of G protein-coupled receptors, which have been described as involved in mediating metabolic effects, especially lipolysis in adipose tissue (Carpene, Bousquet-Melou, Galitzky, Berlan, & Lafontan, 1998; Chorostowska-Wynimko, 2002; Lowell & Bachman, 2003), insulin resistance (Mottagui-Tabar et al., 2008) and energy balance (Webber, 2003). Previous research also confirmed that ADRBs knockout mice have reduced metabolic rate and altered fat deposition (Bachman, 2002). These three genes were suggested as crucial players in controlling the lipid metabolism process, including assisting in fat assimilation in the digestive tract, influencing triglyceride storage and mobilization, blunting increase in circulating free fatty acid and decreasing fat oxidation in adipose tissues (Jocken et al., 2006; Krief et al., 1993).

In humans, polymorphisms in the *ADRB1*, *ADRB2* and *ADRB3* genes have been associated with a wide range of medical conditions, some of which are associated with lipolysis in adipose tissue (Erhardt, Czakó, Csernus, Molnár, & Kosztolányi, 2005; Kim et al., 2006; Lange et al., 2005). Gly389Arg in *ADRB1* is associated with body weight and body mass index (BMI) (Dionne et al., 2002). *ADRB2* plays important roles in glucose tolerance, insulin sensitivity and fat distribution (Gonzalez et al., 2003; Rauhio et al., 2013). *ADRB3* is associated with waist

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circumference in human (Malik et al., 2011), intramuscular fat content and fatty acid composition in pigs (Xue, Wang, Jin, Zhang, & Xu, 2015), and birth weight, growth rate, carcass composition and meat quality in sheep (Byun, Fang, Zhou, & Hickford, 2008; Forrest, Hickford, & Frampton, 2007; Forrest, Hickford, Hogan, & Frampton, 2003; Wu, Qiao, Liu, Yuan, & Liu, 2012).

Considering the important functions of *ADRB1*, *ADRB2* and *ADRB3* in energy and fat acid metabolism, as well as the limited research on those genes in cattle, the aims of this study were to investigate the expression patterns and polymorphisms of these genes and to evaluate the associations between polymorphisms and meat quality traits in the Qinchuan cattle population.

2. Material and methods

2.1. RNA samples collection and expression pattern analyses

Three Qinchuan cattle about 20 months of age were purchased from the farm of the National Beef Cattle Improvement Center (NBCIC) in Yangling, China. They were fed under the same condition and euthanized with a captive bolt gun, then exsanguinated according to the guidance of the Institutional Animal Care and Use Committee (College of Animal Science and Technology, Northwest A & F University, China). Twelve different tissues, including subcutaneous fat, muscle, heart, spleen, liver, kidney, lung, rumen, reticulum, omasum, small intestine and large intestine of each animal were obtained and frozen immediately in liquid nitrogen, and then kept at − 80 °C until analysis. Total RNA was extracted from the tissues using a Total RNA kit with DNase I (Tiangen, Beijing, China) and then reverse-transcribed using a PrimeScript™ RT reagent Kit (Perfect Real Time) (TaKaRa, Dalian, China). cDNA from the reverse transcription of each tissue were used for RT-PCR which was performed using a SYBR Green PCR Master Mix kit (TaKaRa, Dalian, China) and 7500 System SDS V 1.4.0 (Applied Biosystems, USA). In our expression pattern analyses, β-actin (AY141970.1), and GAPDH (NM_001034034) were used as housekeeping genes, and all of the primers used in the real-time PCR experiment were listed in Table 1. ADRBs levels were quantified relative to the geometric mean of β-actin and GAPDH via the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). All the measurements were performed in triplicate.

2.2. DNA samples and data collection

A total of 503 Qinchuan cattle from 18 to 24 months of age were randomly selected from the NBCIC farm, and their intramuscular fat content (IMF), backfat thickness (BF) and ultrasound loin muscle area (ULA) were measured by ultrasonography (Aquila Vet, Esaote Piemedical, Köln, Germany) as previously described (Brethour, 1994; Hamlin, Green, Cundiff, Wheeler, & Dikeman, 1995).

At the same time, blood samples were collected from the jugular vein of each cattle, then the genomic DNA was extracted from these samples according to the standard phenol chloroform protocol (Chong, 2001). The purity and concentration of each genomic DNA sample was detected by the NanoDrop™ 1000 spectrometer (Thermo Scientific, Waltham, MA, USA).

Table 1
Primers used in RT-PCR analysis of expression patterns.

Gene	Primer sequence (5′ to 3′)	Tm (°C)	Product length (bp)
β-actin	CACCAACTGGGACGACAT ATACAGGGACAGCACAGC	61.0	202
GAPDH	CCAACGTGTCTGTTGTGGAT CTGCTTCACCACCTTCTTGA	61.0	80
ADRB1	TGCTGGTAGTGCCGTTTGGA TGAAGATGGGCAGGAAGGACA	61.0	261
ADRB2	AAGGAAACCTGCTGTGAC CACCCTGGAGTAGACGAAG	61.0	108
ADRB3	CGCCCATCATGAGCAAATG AACGAGACCGAGGAGGAGAG	61.0	127

2.3. PCR amplification, sequencing and variants discovery

According to the sequences of bovine *ADRB1* (AC_000183.1), *ADRB2* (AC_000164.1) and *ADRB3* (AC_000184.1), seven primer pairs which include the majority of the whole coding and non-coding regions of these three genes were designed for PCR amplification using Primer Premier 5.0 software (Premier Biosoft International, Palo Alto, CA), and the subset of primers which retrieved SNPs are shown in Table 2. PCR amplification was performed in a 20-μL reaction mixture, containing 10 pM of each primer, 0.3 mM dNTPs, 2.5 mM $MgCl_2$, 20 ng genomic DNA, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). A total of 35 cycles were used with denaturation at 94 °C for 30 s, annealing at Tm °C (Table 2) for 30 s, and extended at 72 °C for 30 s, with a final extension at 72 °C for 10 min. PCR products were detected via electrophoresis in 1.5% agarose gel (containing 200 ng/mL ethidium bromide) and purified with Axygen kits (MBI Fermentas, Amherst, NY, USA), and then sequenced in an ABI PRIZM 377 DNA sequencer (Perkin-Elmer, Waltham, MA, USA). To explore the variants existing in these three genes, sequence maps were aligned to the Bos_taurus_UMD_3.1.1 reference genome using DNASTAR SeqMan program (DNASTAR Inc., Madison, WI, USA).

2.4. Bioinformatics study

The amino acid sequences of the *ADRB1*, *ADRB2* and *ADRB3* genes for different species (cattle, human, sheep and mouse) were acquired using BLAST provided by NCBI. Multiple sequence alignment was performed using Clustalx2.0 software (Larkin et al., 2007). MethPrimer program (http://www.urogene.org/methprimer/) was used to predict CpG islands according to a previously published study (Li & Dahiya, 2002).

2.5. Statistical analyses

Genotypic frequencies and allelic frequencies were directly calculated for all SNPs during the sequence map alignments. Hardy-Weinberg disequilibrium (HWE), gene heterozygosity (He) and effective allele numbers (Ne) were calculated according to the previously described approaches (Nei & Li, 1979; Nei & Roychoudhury, 1974). Polymorphism information content (PIC) was calculated based on Botstein's methods (Botstein, White, Skolnick, & Davis, 1980). The linkage disequilibrium (LD) and haplotype distributions of the SNPs were analyzed with the HAPLOVIEW software (Ver. 3.32) (Barrett, Fry, Maller, & Daly, 2005) and PHASE program (Ver. 2.1) (Stephens & Scheet, 2005).

Associations between each SNP and the measured traits (IMF, BF and ULA) were performed using the SPSS 16.0 software (version17.0). The applied linear model was: $Y_{ijk} = \mu + S_i + G_j + A_k + E_{ijk}$, where Y_i is the trait value, μ is the overall mean for each trait, S_i is the random effect with the ith sire, G_j is the fixed effect associated with the jth genotype, A_k is the fixed effect of the kth age, and E_{ijk} is the random error.

For the association analysis between combined genotypes and the traits, the statistical model was similar to the model above with a slight modification; in this analysis, G_j represented the fixed effect associated with the jth combined genotypes.