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Association studies on the bovine lipoprotein lipase gene polymorphism with growth and carcass quality traits in Qinchuan cattle

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ABSTRACT

Lipoprotein lipase (LPL) is considered as an essential enzyme in lipid deposition and tissue metabolism. It has been proposed to be a lead candidate gene for genetic markers of lipid deposition and energy balance. In this paper, polymorphisms in the LPL gene were investigated in 554 Chinese Qinchuan cattle by PCR-RFLP and DNA sequencing. Seven single nucleotide polymorphisms (SNPs) were identified, which included one mutation (g.91C > T) in the 5′untranslated region (UTR), four synonymous mutations (g.17015A > G, g.18362G > A, g.18377T > C and g.19873T > C) and two mutations (g.25225A > G and g.25316T > G) in the 3′UTR. The frequencies of SNP g.18377T > C and g.25316T > G were skewed from Hardy–Weinberg equilibrium in all the samples (chi-square test, $P < 0.05$). An association analysis showed that five loci (except for g.91C > T and g.18377T > C) were significantly correlated with some growth and carcass quality traits. These results demonstrate that LPL might be a potential candidate gene for marker-assisted selection (MAS).

1. Introduction

Lipoprotein Lipase (LPL) is the rate-limiting enzyme that plays an important role in lipoprotein metabolism [1], and is described as a “gatekeeper” for its function in partitioning lipoprotein-derived free fatty acids (FFAs) in peripheral tissues [2,3]. Once hydrolyzed, the lipoprotein-derived FFAs are available for up-take by extrahepatic tissues for storage or oxidation [4]. Up-regulation of LPL in the skeletal muscle of transgenic mice fed a high-fat diet resulted in significantly decreased plasma triglyceride (TG) and FFA levels [5]. LPL specifically acts to hydrolyze the core TG of circulating TG-rich lipoproteins, releasing non-esterified fatty acids for tissue uptake and metabolism [6]. Down-regulation of LPL activity resulted in a significant reduction in the lipolytic rate of chylomicrons and very-low-density lipoproteins (VLDL) [7,8].

In humans and rodents, several studies have confirmed that elevated LPL is associated with hypertension [9], obesity [10], and insulin resistance [11] in metabolic syndrome. For example, overexpression of LPL in skeletal muscle led to excessive intramyocellular lipid deposition, which suggests there is a connection between lipid storage and insulin sensitivity [12]. Such interactions have been well described, and peripheral regulation of energy substrate utilization by interactions between leptin and insulin appear to contribute through cross-talk between leptin and insulin signaling pathways in muscle [13]. In addition, neurons which lacked LPL exhibited a significant change in the expression of genes involved in glycolysis and lipid metabolism, such as sterol regulatory element-binding protein-1c (Sterol-1c), pyruvate dehydrogenase kinase (Pdk4), and agouti-related protein (AgRP), leading to the most striking phenotypes (body weight and energy balance) in mice [14].

LPL is synthesized by a variety of tissues, but mostly in adipose tissue and skeletal muscle, and regulates fat deposition, energy balance, body weight, and growth traits [15,16]. Based on the location and biological properties in mammals, it is hypothesized that LPL is an attractive candidate gene to assist in the selection for

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growth and carcass traits in cattle. Qinchuan cattle were the focus of the present study as it is known as one of the outstanding indigenous breeds in China. However, carcass traits, including marbling and intramuscular fat (IMF) content, are significantly lower than those of imported cattle selected for high levels of intramuscular fat, such as Wagyu. Until recently, there has been a lack of information about the association of bovine LPL genotypes with growth and carcass traits in Qinchuan cattle. In the present study, we performed extensive LPL screening by direct sequencing and polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) to detect polymorphisms and examined their associations with growth and carcass traits. Here, we present 7 genetic polymorphisms found in LPL, and the results of an association study in Chinese Qinchuan cattle.

2. Materials and methods

2.1. Animals and phenotypic data

A total of 554 female Qinchuan cattle aged from 18 to 24 months were the progeny of 12 sires and reared at the Experimental Farm of National Beef Cattle Improvement Center (Yangling Shaanxi, China). Calves were weaned at an average age of six months age and raised on a diet of corn and corn silage, according to nutrient requirements of growing heifers (NRC, 2000). Heifers had free access to the diet and fresh water. All heifers were fed with the same ratio of roughage to concentrate (6:4) and under similar management and rearing environment (similar temperature, humidity).

Growth traits, including body length (BL), withers height (WH), and chest circumference (CC) were measured according to Gilbert's method [17]. Carcass traits, including ultrasound back fat thickness (BT), ultrasound loin-eye area (ULA), and intramuscular fat content (IMF) were measured using the Rincon method [18].

2.2. Genotyping

DNA was extracted from whole samples using a Blood DNA Kit (OMGAM Bio-Tek, Doraville, USA). The DNA content was estimated by spectrophotometer. Genomic DNA was diluted to 50 ng/μL and stored at −20 °C for subsequent analysis.

Primers for polymerase chain reaction (PCR) were designed from the LPL gene sequence available at GenBank (NCBI Reference Sequence: AC_000165.1) using Primer Premier Software (Version 5.0). PCR amplification product was a 20 μL mixture composed of 50 ng DNA, 10 pM of each primer, 0.2 mM dNTP, 2.5 mM $MgCl_2$, and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The cycling protocol was as follows: an initial denaturation for 5 min at 95 °C; 35 cycles of 94 °C for 30 s; annealing for 30 s at optimum temperature (Table 1); primer extension at 72 °C for 30 s, and the final extension was performed at 72 °C for 10 min.

To optimize the SNP identifying method used to detect the mutations in LPL, DNA was pooled from 30 randomly selected samples and amplified by PCR. Finally, the products were sequenced on an automated sequencer (BioXM software, Version 2.6) to search for DNA polymorphisms.

2.3. Data analysis

Allele and genotype frequencies were estimated by direct counting. The Chi-squared test for Hardy–Weinberg equilibrium (HWE) was applied to assess the deviations in the number of observed versus expected genotypes. Population genetic indices, including gene heterozygosity and polymorphism information content (PIC) were calculated according to Nei's methods [19].

The association between SNPs and growth and carcass traits in Qinchuan cattle was analyzed using general linear models (GLM) with SPSS software (Version 18.0). The linear model was: $Y_{ijk} = u + G_i + A_j + S_k + E_{ijk}$, where Y_{ijk} were the traits measured in each individual animal, μ was the overall population mean for the traits, G_i was the fixed effect associated with the single-locus SNP genotype, A_j was the fixed effect due to the age (2 classes), S_k was the random effect to sire (12 classes), and E_{ijk} was the standard error. Bonferroni corrections were applied, which uses a modified criterion for significance (a/k, where a = 0.05, and k is the overall number of independent statistical tests conducted on the given data). In this study, we analyzed six traits and seven different SNPs, resulting in an adjusted *P*-value of 0.12×10^{-2} for the 5% significance threshold. Software TASSEL (Version 2.1) was used to calculate the explained phenotypic variation of the SNPs.

3. Results

3.1. SNP discovery and genotyping of selected SNPs

The bovine LPL gene maps on chromosome 8 and consists of 10 exons (http://www.ncbi.nlm.nih.gov/nuccore/NC_007306.5). In the current study, the constructed DNA pools were used to amplify specific regions of the LPL gene, including 5′UTR, exon 1, 3, 4, 5, 6, 7, and 3′UTR. The PCR products were sequenced and seven SNPs were identified as shown in Fig. 1: g.91C > T (5′UTR), g.17015A > G (Exon 5), g.18362G > A (Exon 6), g.18377T > C (Exon 6), g.19873T > C (Exon 7), g.25225A > G (3′UTR), and g.25316T > G (3′UTR), which were cleaved by restriction enzymes HhaI, MspI, PvuII, PcsI, DdeI, BfmI, and DdeI, respectively (Table 1).

Table 1
Primer sets for PCR and PCR-RFLP used for genotyping sequence variants that were detected in the bovine LPL gene.

Locus	Position	AA coded	Primer (5′–3′)	Tm (°C)	Production size	Restriction enzyme	Genotype pattern (bp)
g.91C > T	5′UTR	–	TTCCAATCACAGAAGCAG ACAGAACAGTAGACCCGC	58.6	189bp	Hha1/GCG^C	CC: 157 and 32 CT: 189, 157 and 32 TT: 189
g.17015A > G	Exon 5	Pro244Pro	GCCCACATACGTCAACTAG GGCACTTGGTCAGTAAGAG	60.5	448bp	Msp1/C^CGG	AA: 448 AG; 448, 331 and 117 GG: 331 and 117
g.18362G > A	Exon 6	Leu266Leu	CTTAAACAGGACCTACGATC CCTTACTAGCAGTGGGAAT	55.5	585bp	Pvu11/CAG^CTG	AA: 585 AG: 585, 440 and 145 GG: 440 and 145
g.18377T > C	Exon 6	His271His	CTTAAACAGGACCTACGATC CCTTACTAGCAGTGGGAAT	55.5	585bp	Pcs1/ WCGN$_4^N_3$CGW	CC: 417 and 168 CT: 585, 417 and 168 TT: 585
g.19873T > C	Exon 7	Thr355Thr	GCTTGTTTGTGCTGCGGT GCCTGGTTGGTGTATGTA	56.0	129bp	Dde1/C^TNAG	CC: 129 CT: 129, 102 and 27 TT: 102 and 27
g.25225A > G	3′UTR	–	CTTGAAGGTGGTGAGGGAC TAAACGGGTCCTACTCACG	61.0	537bp	Bfm1/C^TRYAG	AA: 441 and 96 AG: 537, 441 and 96 GG: 537
g.25316T > G	3′UTR	–	GCTACAGAAGAAAGAACA TAGAAAACGCAGAATCCC	61.0	366bp	Dde1/C^TNAG	GG: 366 GT: 366, 270 and 96 TT: 270 and 96