Gene 563 (2015) 136–141

Contents lists available at ScienceDirect

Gene

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

Research paper

Haplotype combination of the bovine *CFL2* gene sequence variants and association with growth traits in Qinchuan cattle

Yujia Sun [a,1], Xianyong Lan [a], Chuzhao Lei [a], Chunlei Zhang [b], Hong Chen [a,b,*]

[a] College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, Shaanxi 712100, China
[b] Institute of Cellular and Molecular Biology, Jiangsu Normal University, Xuzhou, Jiangsu 221116, China

ARTICLE INFO

Article history:
Received 14 November 2014
Received in revised form 24 January 2015
Accepted 10 March 2015
Available online 12 March 2015

Keywords:
CFL2 gene
Haplotype combination
Sequence variants
Growth traits
Qinchuan cattle

ABSTRACT

The aim of this study was to examine the association of *cofilin2* (*CFL2*) gene polymorphisms with growth traits in Chinese Qinchuan cattle. Three single nucleotide polymorphisms (SNPs) were identified in the bovine *CFL2* gene using DNA sequencing and (forced) PCR-RFLP methods. These polymorphisms included a missense mutation (NC_007319.5: g. C 2213 G) in exon 4, one synonymous mutation (NC_007319.5: g. T 1694 A) in exon 4, and a mutation (NC_007319.5: g. G 1500 A) in intron 2, respectively. In addition, we evaluated the haplotype frequency and linkage disequilibrium coefficient of three sequence variants in 488 individuals in QC cattle. All the three SNPs in QC cattle belonged to an intermediate level of genetic diversity ($0.25 < PIC < 0.5$). Haplotype analysis of three SNPs showed that 8 different haplotypes were identified in all, but only 5 haplotypes were listed except for those with a frequency of <0.03. Hap4 (-GTC-) had the highest haplotype frequencies (34.70%). However in the three SNPs there were no significant associations between the 13 combined genotypes of the *CFL2* gene and growth traits. LD analysis showed that the SNP T 1694 A and C 2213 G loci had a strong linkage ($r^2 > 0.33$). Association analysis indicated that SNP G 1500 A, T 1694 A and C 2213 G were significantly associated with growth traits in the QC population. The results of our study suggest that the *CFL2* gene may be a strong candidate gene that affects growth traits in the QC cattle breeding program.

1. Introduction

Qinchuan cattle are a local breed belonging to the central plains group and found in central Shaanxi in China. They are usually red, but individuals with yellow coloration are also found. Qinchuan cattle are one of the most important local breeds in China because of its meat quality and appearance.

Cofilin is an actin-binding protein of 20 kDa, and was originally discovered in porcine brain as an actin monomer-binding protein (Maekawa et al., 1984; Nishida et al., 1984). Numerous cofilin homologues have been characterized genetically and biochemically in various organisms including mammals (Abe et al., 1990; Maekawa et al., 1984; McKim et al., 1994; Ono et al., 1994). In mice and human, an ubiquitously expressed cofilin1 (CFL1) and a skeletal muscle specific cofilin2 (*CFL2*) have been demonstrated (Gillett et al., 1996; Ono et al., 1994). In embryonic mammal muscle, *CFL1* was predominantly expressed. During subsequent muscle development, *CFL1* decreases, while *CFL2* expression increases to become the predominant isoforms in mature mammal skeletal muscle (Mohri et al., 2000). In chicken, there is only one cofilin isoform that is highly homologous with mammalian cofilin-2 (Abe and Obinata, 1989; Ono et al., 1994). Cofilin expression is elevated significantly in denervated (Shinagawa et al., 1993) and dystrophic (Hayakawa et al., 1993) skeletal muscles, which show degeneration of the contractile apparatus including disassembly of actin filaments.

CFL2 is a small actin-binding protein of 18.7 kDa, encoded by the *CFL2* gene (Yonezawa et al., 1985). Human and mice *CFL2* genes were spliced by the alternative use of exon1a and exon1b, and form two transcripts *CFL2*a and *CFL2*b. The *CFL2*a transcript was found in various tissues, while *CFL2*b was expressed predominantly in mature skeletal muscle and involved in myofibrilogenesis, muscle fiber growth and development (Zhao et al., 2009).

The exact function of *CFL2* is unknown. But a critical role for *CFL2* in skeletal muscle function was demonstrated by identification of recessive *CFL2* mutations in two families with congenital myopathies characterized by the presence of nemaline rods and minicores (Agrawal et al., 2007). Based on *CFL2* expression in mice, it has been postulated that *CFL2* may be involved in regulation of actin assembly during myofibrilogenesis and actin dynamics in the mature muscle (Agrawal et al., 2012). So far, mutations in seven different genes have been

Abbreviations: SNP, single nucleotide polymorphism; PCR-RFLP, restriction fragment length polymorphism; UTR, untranslated region; LD, linkage disequilibrium; QC, Qinchuan; PIC, polymorphism information content.

* Corresponding author at: No. 22 Xinong Road, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China.
E-mail addresses: sunyujia1988@163.com (Y. Sun), chenhong1955@126.com (H. Chen).
[1] Address: No. 22 Xinong Road, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China.

http://dx.doi.org/10.1016/j.gene.2015.03.016
0378-1119/

Table 1
Primer information for PCR amplification of bovine *CFL2* gene.

Primers	Primer sequences	Length/Loci
P1	1F: 5′ CCCTCCTTCTCCTCGCAG 3′ 1R: 5′ AGGAAGCGAAGGGCAAAG 3′	449 bp/exon 1
P2	2F: 5′ CCAGACTAAAGTGAATCCTC 3′ 2R: 5′ AACTACTACATTGCCTCCC 3′	767 bp/exon 2
P3	3F: 5′ TGTAGGGCTCCTGAAAGTGCG 3′ 3R: 5′ CCAAGGCAGGTGAGGTGTATG 3′	723 bp/exon 3
P4	4F: 5′ GCCAAAACAACTGGGTAT 3′ 4R: 5′ CAAGGAAAACAAGGCAAC 3′	651 bp/exon 4

reported to cause nemaline myopathy, including one instance of a missense mutation in *CFL2*, a gene encoding the actin-binding protein *CFL2* (Agrawal et al., 2007; Sambuughin et al., 2010; Sanoudou and Beggs, 2001). A recent study showed that *CFL2* is not critical for muscle development, but essential for muscle maintenance (Agrawal et al., 2012). There are reports that *CFL2* causes an autosomal recessive form of congenital myopathy with features of both nemaline and myofibrillar myopathy, and given the clinical variability and the multitude of histological features of congenital myopathies, *CFL2* sequence analysis should be considered in patients presenting with an autosomal recessive form of congenital myopathy (Ockeloen et al., 2012). In terms of these important roles, *CFL2* could be a key candidate in the development and metabolism of muscle and adipose tissue.

However, the polymorphisms and function of the bovine *CFL2* gene have not been elucidated completely. Therefore, the objective of this study was to identify the single nucleotide polymorphism (SNP) in the bovine *CFL2* gene using DNA sequencing and (forced) PCR-RFLP analysis, and to carry out haplotype structure and establish an association between haplotype combination of the bovine *CFL2* gene and growth traits, which possibly contributed to animal breeding.

2. Materials and methods

2.1. Animal population, DNA extraction and data statistics

Blood samples were obtained from 488 healthy and unrelated female bovines (36 months old). They were from the reserved farm of Qinchuan cattle (Fufeng County, Shaanxi Province, China) and the finest breeding center of Qinchuan cattle (Yangling, Shaanxi Province, China). Genomic DNA was extracted from 1 mL of 2% heparin-treated blood samples and stored at −80 °C following the standard procedures (Green and Sambrook, 2012). The content of DNA was estimated spectrophotometrically, and then the genomic DNA was diluted to 50 ng/μL. All DNA samples were stored at −20 °C for subsequent analysis. Data of growth traits (wither heights, height at hip cross, body length, heart girth, chest breadth, chest depth, rump length, hucklebone width, hip width, body mass) of 488 individuals were recorded and used for association analysis.

2.2. Primer design, PCR amplification, and commercial sequences

Based on the sequence of the bovine *CFL2* gene (GenBank accession number: NC_007319.5), four pairs of primers were designed (Table 1) for PCR amplification of the *CFL2* gene from cattle genomic DNA. Each amplification reaction was carried out in a 25 μL mixture containing 50 ng genomic DNA, 1 μmol/L of each primer, 1 × buffer (including 1.5 mmol/L $MgCl_2$), 200 μmol/L dNTPs (dATP dTTP dGTP and dCTP), and 0.6 U of *Taq* DNA polymerase (MBI Fermentas, Vilnius, Lithuania). PCR reactions were carried out using a Touchdown PCR System Thermal Cycler Dice (TaKaRa, Dalian, China). The Touchdown PCR protocol consisted of two phases: phase 1 included an initial step of 95 °C for 4 min, followed by 20 cycles of denaturation at 94 °C for 30 s, annealing at variable temperatures for 30 s, and extension at 72 °C for 1 min. In the first cycle, the annealing temperature was set to 70 °C, and at each of the 19 subsequent cycles, the annealing temperature was decreased by 1 °C (i.e. it varied from 70 °C to 50 °C at 1 °C decrements along the 20 cycles). Phase 2 consisted of 20 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 1 min. The final extension was performed at 72 °C for 10 min. The PCR products were stored at 4 °C.

2.3. PCR-RFLP and forced PCR-RFLP

PCR primers were redesigned to facilitate genotyping of the three SNPs using PCR, PCR-RFLP, and forced PCR-RFLP, and 3% agarose gel electrophoresis (AGE) analysis in studied populations. In the study, the fragments of the bovine *CFL2* gene were amplified by the three pairs of enzyme primers. G 1500 A (SNP1) was genotyped by the PCR-RFLP, and T 1694 A (SNP2) and C 2213 G (SNP3) were genotyped by the forced PCR-RFLP. Enzyme primers, restriction enzymes selected (TaKaRa), and fragment sizes are given in Table 2. For forced RFLP, specifically modified primers for each of the three SNPs were used so that the PCR product in each case had an internal restriction enzyme site in one allelic sequence and not the other. To detect the three SNPs, aliquots of 10 μL of PCR products were digested with 10 U of *Hinf*I, *Vsp*I and *Hae*III (TaKaRa) for 8 h at 37 °C, 37 °C and 37 °C, respectively. The digested products were detected by electrophoresis in 3.0% agarose gel stained with 200 ng/mL ethidium bromide, and the gels were run at a constant voltage (120 V) for 0.5–1.0 h.

2.4. Statistical analysis

Gene frequencies were determined in cattle by direct counting, and the Hardy–Weinberg equilibrium (HWE) was tested based on the likelihood ratio for different locus-population combinations by the POPGENE software (Version 3.2). Population genetic indexes, such as *He* (gene heterozygosity), *Ho* (gene homozygosity; $Ho + He = 1$), *Ne* (effective allele numbers; reciprocal of homozygosity) and *PIC* (polymorphism information content) were calculated according to Nei's methods (Nei and Roychoudhury, 1974), implemented in POPGENE software (version 1.3.1). The analyses for linkage disequilibrium (LD), as measured by D' and r^2, and construction of the haplotypes that were obtained from the 488 animals were performed with the online SHEsis software (http://analysis2.bio-x.cn/myAnalysis.php) (Shi and He, 2006). The association analysis between single SNP marker genotypes and growth traits were performed by the least squares method as applied in the general linear models procedure of the SPSS (Ver. 17.0) software. The reduced

Table 2
Primers used for (forced)-PCR-RFLP analysis of bovine *CFL2* gene.

Sequence variants	Amino acid change	Primer sequences	Restriction enzymes and fragment sites (bp)
G 1500 A		1F: 5′ GACCTAGTATTTATATTCTGG 3′ 1R: 5′ AACTACTACATTGCCTCCC 3′	*Hinf*I 389, 279, 110
T 1694 A	Ile 131 Ile	2F:5′ TGTAGGGCTCCTGAAAGTGCG 3′ 2R:5′ GCCATTTACTTGCCACTCATGATT 3′	*Vsp*I 197, 172, 25
C 2213 G	Pro 312 Ala	3F: 5′ TGCACTTGACTGCAGTTCTGTGGG 3′ 3R: 5′ CACTCAATGGGGGAAAAAAGGC 3′	*Hae*III 276, 252, 24