Gene 538 (2014) 354–360

Contents lists available at ScienceDirect

Gene

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

Linkage disequilibrium and haplotype distribution of the bovine *LHX4* gene in relation to growth

Gang Ren [a,1], Yong-Zhen Huang [a,1], Tian-Bao Wei [a], Jun-Xia Liu [b], Xian-Yong Lan [a], Chu-Zhao Lei [a], Chun-Lei Zhang [c], Zhi-Ying Zhang [a], Xing-Lei Qi [d], Hong Chen [a,*]

[a] College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, Shaanxi 712100, China
[b] College of Animal Science and Technology, Shihezi University, Shihezi, Xinjiang 832002, China
[c] Institute of Cellular and Molecular Biology, Xuzhou Normal University, Xuzhou, Jiangsu 221116, China
[d] Xianan cattle Technology & Development Company, Biyang, Henan 463700, China

ARTICLE INFO

Article history:
Accepted 12 December 2013
Available online 23 January 2014

Keywords:
Association
Cattle
Haplotype
LHX4
Growth traits

ABSTRACT

LIM homeobox transcription factor 4 (*LHX4*) is a LIM homeodomain transcription factor involved in pituitary gland and nervous system development. The aim of this study was to examine the association of the *LHX4* polymorphisms with growth traits in beef cattle breed. A total of 7 single nucleotide polymorphyisms (SNPs) have been identified in the coding region and noncoding region of the bovine *LHX4* by sequencing pooled DNA samples (Pool-Seq) and PCR-single strand conformation polymorphism (PCR-SSCP) methods. The linkage disequilibrium was assessed in 871 individuals representing four main cattle breeds from China. The SNPs 2–5 and 7–8 were found to be in complete linkage disequilibrium, respectively. The result of haplotype analysis of 13 SNPs showed that 31 haplotypes were found in four Chinese cattle breeds, and 20 genotypes were only found in Nanyang cattle. The statistical analyses indicated that the SNP1–5, and 6 are associated with the body weight at 18, and 6 months of age in Nanyang cattle population ($P < 0.05$), but no significant associations between their twenty combined genotypes. Our results provide evidence that some polymorphisms in *LHX4* are associated with growth traits at certain ages, and may be used as candidates for marker-assisted selection and management in cattle.

1. Introduction

LIM homeobox transcription factor 4 (LHX4) is one of the LIM-homeodomain (LIM-HD) transcription factors, which is essential in the pituitary gland and nervous system development. The LIM domain, a multifunctional protein/protein interaction domain, was first recognized in several other members of this class of transcription factors: LIN11, ISL1, and MEC3 (Hunter and Rhodes, 2005). The large LIM protein superfamily also includes cytoskeletal proteins, signaling cascade transducers, and transcriptional coactivators. In mammals there are at least twelve LIM-HD genes encoding developmental regulatory proteins featuring two LIM domains and a DNA-binding HD (Mullen et al., 2007).

In humans, the mutations in the LIM-HD family were found to be of major importance for combined pituitary hormone deficiency (CPHD), which denoted impaired production of *growth hormone* (*GH*) and one or more of the other five anterior pituitary hormones (Sloop et al., 2000). A heterozygous mutation in *LHX4* had been identified in a family affected with *CPHD* leading to *GH*, *thyroid-stimulating hormone* (*TSH*), and *adrenal corticotropic hormone* (*ACTH*) deficiencies (Machinis et al., 2001). Further study explained the mechanism involved: the splice site mutation made LHX4 protein failed to bind and subsequently to activate the *pituitary-specific transcription factor 1* (*POU1F1*) regulatory sequence (Machinis and Amselem, 2005). Functional studies showed that this mutation induced a complete loss of transcriptional activity of *POU1F1* promoter and thus impaired the transactivation of *prolactin (PRL)* and *GH* promoters (Castinetti et al., 2008). Up to now, at least eight mutations in *LHX4* gene had been reported responsible for CPHD in humans (Pfaeffle et al., 2008; Tajima et al., 2007).

In cattle, *LHX4* gene is located on chromosome 16, and contains six exons. In our previous studies it has been shown that six mutations are associated with body weight and body length in cattle (Liu et al.,

Abbreviations: bp, base pair(s); BW, body weight; CH, Chinese Holstein; CPHD, combined pituitary hormone deficiency; ACTH, adrenal corticotropic hormone; GLM, general linear models; He, heterozygosity; Ho, homozygosity; HWE, Hardy–Weinberg equilibrium; JX, Jiaxian cattle; LD, linkage disequilibrium; LSM, least square means estimates; LHX4, LIM homeobox transcription factor 4; LIM-HD, LIM-homeodomain; Ne, effective allele numbers; NY, Nanyang; PAGE, polyacrylamide gel electrophoresis; PCR-SSCP, polymerase chain reaction-single stranded conformational polymorphism; PIC, polymorphism information content; POU1F1, pituitary-specific transcription factor 1; PRL, prolactin; Pool-Seq, sequencing pooled DNA samples; QC, Qinchuan; SNV, single nucleotide variant; SNPs, single nucleotide polymorphisms; SPSS, statistical product and service solutions; TBE, is a buffer solution containing a mixture of Tris base, boric acid and EDTA.

* Corresponding author at: No.22 Xinong Road, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China. Tel.: +86 29 87092120; fax: +86 29 87092164.
E-mail addresses: rengang1213@gmail.com (G. Ren), hyzsci@126.com (Y.-Z. Huang), chenhong1212@263.net (H. Chen).
[1] Gang Ren and Yong-Zhen Huang contribute equally to this work.

0378-1119/$ – see front matter © 2014 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.gene.2013.12.037

2011; Ren et al., 2010). The purpose of this study was to identify 13 single nucleotide polymorphisms (SNPs) in the bovine *LHX4* gene, and to carry out haplotype construction and association analysis so as to contribute to the understanding of the role of *LHX4* in variation of growth traits in cattle, which possibly contributed to animal breeding and genetics.

2. Materials and methods

2.1. Animal source, DNA preparation and growth data

We investigated the genetic variation in bovine LHX4 among a set of 817 individuals representing four cattle breeds in China: Nanyang cattle (NY, $n = 276$), Qinchuan cattle (QC, $n = 308$), Jiaxian cattle (JX, $n = 141$), and Chinese Holstein (CH, $n = 92$). Nanyang, Qinchuan, and Jiaxian are three important breeds for beef production in China; while Chinese Holstein is a dairy breed. These four breeds are the main breeds of China and they are reared in the provinces of Henan and Shaanxi. Calves were weaned on average at 6 months of age and raised from weaning to slaughter on a diet of corn and corn silage. The animals of each breed were selected to be unrelated for at least three generations, with the aim of having diverse lineages within each breed. The Nanyang animals were from the Nanyang Cattle breeding center (Nanyang City, Henan Province, P.R. China); the Jiaxian animals were from the Jiaxian Cattle breeding farm (Jiaxian County, Henan Province, P.R. China); Qinchuan cattle were from the reserve farm (Fufeng County, Shaanxi province, P.R. China) and the Qinchuan Cattle fineness breeding center (Yangling, Shaanxi province, P.R. China); the Chinese Holstein animals were from a milk cattle breeding farm (Xi'an City, Shaanxi Province, P.R. China).

Genomic DNA of 817 cattle were isolated from 2% heparin-treated blood samples and stored at −80 °C, by following the standard procedures (Sambrook and Russell, 2002). 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.

We quantified the growth traits of 273 Nanyang animals, these cattle used for the association study came from their common ancestors, the samples were collected from the same generation and pedigrees of core breeding population animals were traced back three generations. The animals were weaned at an average of 6 months of age and raised from weaning to slaughter on a corn–corn silage diet. The traits under study were the weight at birth (BW0), 6 months (BW6), 12 months (BW12), 18 months (BW18), and 24 months (BW24). These traits were measured following the description in Gilbert et al. (1993).

2.2. Variants detection (primer design, PCR amplification, and sequencing)

Primers used to amplify the bovine *LHX4* gene were designed from a published gene sequence (GenBank accession number: NC_007314.3). Primers and fragment sizes are given in Table 1. The detection results of allelic variation at the SNPs were based on the electrophoretic PCR-SSCP pattern of the PCR products.

PCR was performed in 25 μL of reaction volume, containing 50–100 ng genomic DNA, 10 pM of each primer, 1 × buffer (including 1.5 mM $MgCl_2$), 200 μM dNTPs and 1.5 units of *Taq* DNA polymerase (MBI, Vilnius, Lithuania).

Six pairs of primers (Table 1) were designed for PCR amplification of the *LHX4* gene from cattle genomic DNA. PCR products were commercially sequenced for genetic variants discovery. Generally, PCR products amplified from genomic DNA were directly sequenced in both directions (Shanghai Sangon Biotech Co., Ltd., P. R. China; Applied Biosystems 3730xl DNA sequencer, Foster City, CA, USA).

In an effort to discover SNP in a cost-effective manner, SNP discovery was implemented by sequencing of pooled DNA samples (Pool-Seq). We first use 25 samples from each of four cattle breeds for Pool-Seq, and then we analyzed all the samples using PCR-SSCP, which includes sequenced 25 samples. The sequences were imported into the BioXM software 2.6 and were analyzed and searched for SNPs.

2.3. Genotyping of LHX4 allele by means of PCR-SSCP

PCR primers were redesigned to facilitate genotyping of the thirteen single nucleotide polymorphisms (SNPs) using PCR-SSCP technique in four study populations. The reduction in size of these amplicons can facilitate the examination and analysis of these SNPs by improving amplification efficiency.

Aliquots of 5 μL PCR products were mixed with 5 μL denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene-cyanol, and 0.025% bromophenol blue), heated at 98 °C for 10 min, and immediately chilled

Table 1
Genetic variants distribution and primer sets for polymerase chain reaction (PCR) amplification in the bovine *LHX4* gene.

SNVs	Primer location	Primer sequences F/R (5′ → 3′)	AT (°C)[a]	SAF (bp)[b]	SNPs	Variant type	Variant location	Mutations type	DPS (nt) [c]
no	Exon 1	5′ GTAGCCATGGCGCTTCATCT 3′ 5′ TCGGAAGCTCAGCTCACTGTCAC 3′	56.7	243					
no	Exon 2	5′ GTGGAATCCTGCTGGAGAAACG 3′ 5′ TCGGAAGCTCAGCTCACTGTCAC 3′	56.7	243					
SNV1	Exon 3	5′ TTCAGGAGGATAAACAT 3′ 5′ TAGAAGTGGTAGGAGGT 3′	68.0	322	SNP1	g.34924G>A	Exon 3	Synonymous	0
					SNP2	g.34933C>T	Exon 3	Synonymous	9
					SNP3	g.34993C>T	Exon 3	Synonymous	60
					SNP4	g.35011G>A	Exon 3	Synonymous	18
					SNP5	g.35014T>C	Exon 3	Synonymous	3
SNV2	Exon 4	5′ GCCGACAGATTCTTTCCA 3′ 5′ AGACTTACTGGCAGAGTCCTG 3′	61.8	214	SNP6	g.42243G>A	Exon 4	Synonymous	7229
SNV3	Intron 4 and Exon 5	5′ GTAGCCATGGCGCTTCATCT 3′ 5′ TCGGAAGCTCAGCTCACTGTCAC 3′	68.5	416	SNP7	g.42542G>A	Intron 4	–	299
					SNP8	g.42553A>G	Intron 4	–	11
					SNP9	g.42631A>G	Intron 4	–	78
					SNP10	g.42702T>C	Exon 5	Synonymous	
SNV4	Exon 6	5′ TAGGCTTCCCACCATACA 3' 5′ TCCCTCCAGTTTCTTACC 3′	68.5	382	SNP11	g.45014 C>T	Exon 6	Synonymous	2312
					SNP12	g.45294G>A	Exon 6	Synonymous	280
					SNP13	g.45311G>A	Exon 6	Synonymous	17

SNV1: SNP1–5 (); SNV2: SNP6; SNV3: SNP7–10; SNV4: SNP11–13.
[a] AT: Annealing temperature.
[b] SAF: Size of amplification fragment.
[c] DPS: Distance from previous SNV (nt).