Gene 561 (2015) 219–224

Contents lists available at ScienceDirect

Gene

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

Exploring genotype–phenotype relationships of the LHX3 gene on growth traits in beef cattle

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ARTICLE INFO

Article history:
Received 29 August 2014
Received in revised form 6 January 2015
Accepted 12 February 2015
Available online 15 February 2015

Keywords:
Association analyses
Cattle
Haplotype
LHX3
Growth trait

ABSTRACT

The *LIM-homeobox gene 3* (*LHX3*) plays an essential role in pituitary gland and nervous system development. Sequence variants (SVs) in coding and non-coding regions of *LHX3* gene have an impact on *LHX3* transcription and growth traits in cattle. Previously, we have identified 3 single nucleotide polymorphisms (SNPs: 1–3) in all exons and intron 2 regions of the *LHX3* gene in cattle. Here, 7 novel SNPs (SNPs: 4-10) were identified by DNA sequencing and polymerase chain reaction single-stranded conformational polymorphism (PCR-SSCP) methods. In the present study, a total of 10 SNPs were assessed linkage disequilibrium (LD) in 802 cows representing four main cattle breeds from China (Nanyang, Qinchuan, Jiaxian, and Chinese Holstein). The assessment results demonstrated that 17 haplotypes and 18 diplotypes were revealed in these cattle populations. Moreover, association analysis indicated that the genotypes of SNPs 1–6 are associated with the body weight at 6, 12 and 18 months of age in Nanyang cattle ($P < 0.01$ or $P < 0.05$), whereas no significant association was found between the 18 diplotypes and growth traits. Our results provide evidence that some SNPs in *LHX3* gene may be associated with body weight at certain age, and *LHX3* gene may be used as candidate gene for marker-assisted selection (MAS) in beef cattle breeding.

1. Introduction

LIM-homeodomain 3 (*LHX3*) is a transcription factor required for the development of the pituitary and motor neurons, and is also expressed in the auditory system (Kristrom et al., 2009). In mammals, there are at least 12 LIM-homeodomain (LIM-HD) genes encoding developmental regulatory proteins featuring two LIM domains and a DNA-binding HD (Hunter and Rhodes, 2005). LHX3 involves regulated interactions with the histone acetyltransferase complex subunits LANP and TAF-1β to modulate pituitary gene regulation (Hunter et al., 2013).

In humans, the *LHX3* gene maps to chromosome 9, it encodes a transcription factor that is expressed in early fetal life and is a key regulator of pituitary development (Sheng et al., 1996). In mice, the *LHX3* is required for both structural development and cellular differentiation of the pituitary gland (Sloop et al., 1999). In some cases, congenital combined pituitary hormone deficiency (CPHD) has been found to be associated with defects in transcription factors *LHX3* and *4* (Kelberman and Dattani, 2007). In dog, a DNA repeat in intron 5 of *LHX3* in dogs is associated with pituitary dwarfism (Netchine et al., 2000).

In cattle, *LHX3* gene has been located on chromosome 11 and it has 6 exons, which encoded 403 amino acids. Stone et al. (1999) reported the QTL on chromosomes 7, 11, 14, 18 and 26 affecting carcass and growth traits in cattle. To date, only few studies on *LHX3* gene in cattle have been reported and no association analysis has been detected (Jing et al., 2008). Herein, the purpose of this study was to detect single nucleotide polymorphisms (SNPs) of the bovine *LHX3* gene in four Chinese cattle breeds (Nanyang, Qinchuan, Jiaxian, and Chinese Holstein), and assessed the association of genotypes with growth traits in Nanyang cattle. This study will provide some useful information on the genomic study of candidate gene and may have important benefits for cattle breeding and genetics.

Abbreviations: bp, base pair(s); CH, Chinese Holstein; CPHD, congenital combined pituitary hormone deficiency; GLM, general linear models; He, heterozygosity; HWE, Hardy–Weinberg equilibrium; JX, Jiaxian; LD, linkage disequilibrium; LHX3, LIM homeobox 3; QTL, quantitative trait loci; Ne, effective allele numbers; NY, Nanyang; PAGE, polyacrylamide gel electrophoresis; PCR-SSCP, polymerase chain reaction–single stranded conformational polymorphism; PIC, polymorphism information content; QC, Qinchuan; SNPs, single nucleotide polymorphisms.

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http://dx.doi.org/10.1016/j.gene.2015.02.030
0378-1119/

2. Materials and methods

2.1. Resource population, DNA preparation and growth data

In this study, in order to explore the genetic variation of the bovine *LHX3* gene, a total of 802 blood samples including from four Chinese cattle breeds: Nanyang cattle (NY, $n = 263$, Henan province), Qinchuan cattle (QC, $n = 302$, Shaanxi province), Jiaxian cattle (JX, $n = 143$, Henan province), and Chinese Holstein (CH, $n = 94$, Shaanxi province). Nanyang, Qinchuan and Jiaxian cattle represent the main breeds for beef production in China; whereas 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, respectively. 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, PR China); the Jiaxian animals were from the Jiaxian Cattle breeding farm (Jiaxian County, Henan Province, PR China); Qinchuan cattle were from the reserve farm (Fufeng County, Shaanxi province, PR China) and the Qinchuan Cattle fineness breeding center (Yangling County, Shaanxi province, PR China); the Chinese Holstein animals were from a milk cattle breeding farm (Xi'an City, Shaanxi Province, PR China).

Genomic DNA was extracted from leukocytes of 2% heparin-treated whole blood samples following standard procedures (Sambrook and Russel, 2002). The genomic DNA diluted to 50–100 ng/μL and stored at −80 °C.

We measured the growth traits in Nanyang cattle for the association analysis. The traits under study were the body weight at 6, 12, 18, and 24 months old. Statistical analysis was performed as described in Gilbert et al. (1993).

2.2. Primer design and PCR amplification

According to the nucleotide sequence of the bovine *LHX3* gene (GenBank accession number: AY923832). The PCR primers were designed to amplify the *LHX3* gene using the Primer software (Version 5.0).

The PCR amplification reaction was performed according to the manufacturer's protocol (MBI, Vilnius, Lithuania).

PCR amplification protocol as follows: The cycling protocol was 5 min at 94 °C, 30 cycles of 94 °C for 25–30 s, annealing for 30 s (annealing temperature show in Table 1), 72 °C for 30 s, with a final extension at 72 °C for 10 min.

2.3. SNPs' identification and genotyping

Sequencing pooled DNA samples is the most cost-effective approach to screen polymorphisms within bovine *LHX3* gene. The pooled DNA samples consisted of 25 individual genomic DNA samples selected randomly from each cattle breed. The PCR amplification was first carried out with pooled DNA samples as template, and then the PCR products were directly sequenced in both directions for genetic variants discovery (Sham et al., 2002). Sequences were analyzed with BioXM software (Version 2.6). The genotyping of the novel SNPs was successfully implemented using PCR-SSCP technique according to Zhang et al. (2007).

2.4. Statistical analysis

Genotypic and allele frequencies were direct counting. Population genetic indexes such as gene homozygosity (*Ho*) and effective allele numbers (*Ne*) were computed by POPGENE software (Version 3.1) (Yeh et al., 1999; Nei, 1973). Polymorphism information content (*PIC*) was calculated according to Botstein's methods (Botstein et al., 1980). The linkage disequilibrium (LD) structure was performed with the HAPLOVIEW software (Version 3.32) (Barrett et al., 2005; Amandine et al., 2010; Zhao et al., 2007). Haplotype was obtained using the PHASE software (Version 2.1) (Stephens et al., 2001; Huang et al., 2011).

The association analysis between single and combined genotypes and growth traits was performed by the general linear models (GLM) procedure of SPSS software (Version 16.0) (Holzer and Precht, 1992; Derecka et al., 2009; Huang et al., 2010). The reduced model was used in the final analysis (Boldman et al., 1993; Hickford et al., 2009).

3. Results and discussion

3.1. SNPs' genotyping and genetic diversity

Ten SNPs including 7 novel SNPs and 3 previously reported SNPs were found by DNA sequencing, they were distributed in exon 2, exon 6, intron 2 and intron 6. There were 4 synonymous SNPs in exon 2 (SNP 1) and exon 6 (SNPs 4, 5 and 6) (Fig. 1, Table 1).

The SV1 (SNPs 1–3) locus in exon 2 and intron 2, three SSCP genotypes SV1-AA, SV1-AB and SV1-BC were observed. The PCR products of different genotypes were sequenced, three novel SNPs were revealed: g.7553 G > A (SNP 1; exon 2), g.7631 C > T (SNP 2; intron 2) and g.7668 C > G (SNP 3; intron 2) (Table 1). Three haplotypes were described as: A (G-C-C), B (G-T-G) and C (A-T-G) respectively. With the sequence data from different individuals, three different SSCP genotypes were described as: AA (G-C-C/G-C-C), AB (G-C-C/G-T-G) and BC (G-T-G/A-T-G).

Table 1
Primer information for the detection of sequence variants (SVs) in the bovine *LHX3* gene.

SNPs	Variant type	Variant location	Mutations type	DPS (nt)[a]	Sequences of primers (F/R)	AT (°C)[b]	SAF (bp)[c]
1[d]	g.7553 G > A	Exon 2	Silent	0	5′ CTGGGAGCTGGGTGGGATGG 3′	67.5	368
2[d]	g.7631 C > T	Intron 2	–	78	5′ TGTTTGGGGAAAAGGAAGGGTG 3′		
3[d]	g.7668 C > G	Intron 2	–	37			
4	g.10385 G > T	Exon 6	Silent	2717	5′ TTCCACCGCAGACGAGCCAGC 3′	66.5	261
5	g.10427 T > C	Exon 6	Silent	42	5′ GCATCGGGGTACACCAAGC 3′		
6	g.10478 A > C	Exon 6	Silent	51			
7	g.10684 T > G	3′UTR	–	206	5′ GCCCCTCCTATCCAGCTT 3′	65.7	333
8	g.10686 C > T	3′UTR	–	2	5′ CCCAGAAATGACCCTCCCG 3′		
9	g.10734 G > A	3′UTR	–	48			
10	g.10777 C > A	3′UTR	–	43			

[a] DPS: Distance from previous SNP (nt).
[b] AT: Annealing temperature.
[c] SAF: Size of amplification fragment.
[d] Obtained from Jing et al. (2008).