Small Ruminant Research 129 (2015) 25–36

Contents lists available at ScienceDirect

Small Ruminant Research

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

Novel genetic variants associated with mRNA expression of signal transducer and activator of transcription 3(*STAT3*) gene significantly affected goat growth traits

Wenchao Jia[a], Xianfeng Wu[b], Xiangchen Li[c], Tian Xia[b], Chuzhao Lei[b], Hong Chen[b], Chuanying Pan[a,b,*], Xianyong Lan[b,*]

[a] College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, PR China
[b] College of Animal Science and Technology, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Northwest A&F University, Yangling, Shaanxi 712100, PR China
[c] Institute of Beijing Animal Science and Veterinary, Chinese Academy of Agricultural Science, Beijing 100194, PR China

ARTICLE INFO

Article history:
Received 12 September 2014
Received in revised form 8 February 2015
Accepted 15 May 2015
Available online 28 May 2015

Keywords:
Goat
Signal transducer and activator of transcription 3 (*STAT3*) gene
Single nucleotide polymorphism (SNP)
mRNA expression
Associated with growth traits

ABSTRACT

The signal transducer and activator of transcription 3 (STAT3) has been shown to play key roles in cell development and apoptosis. In this study, we reported three novel single nucleotide polymorphisms (SNPs) within the *STAT3* gene: AC_000176:g.45204C > T (SNP1), g.62058A > G (SNP2), and g.62230C > T (SNP3) in the second, tenth, and eleventh introns, among two Chinese native goat breeds. By using the forced polymerase chain reaction–restriction fragment length polymorphism technique, the SNPs were genotyped and we determined the minor allelic frequencies of Xinong Saanen dairy (XNSN) goats and Hainan black (HN) goats. Haplotype frequencies and linkage disequilibrium (LD) coefficients demonstrated that the CAC and CGT haplotypes had the highest frequencies, and the SNP2 and SNP3 loci had strong LD values in these breeds. Statistical analyses indicated a positive association between either a single SNP locus or and combined genotypes of two- or three-loci associations with some growth traits ($P < 0.05$ or $P < 0.01$). Next, we found the different genotypes were significantly associated with mRNA expression levels of the *STAT3* gene. Together, we show that the expression of *STAT3* is associated with goat growth traits, which will be beneficial for animal breeding in the future.

1. Introduction

Signal transducers and activators of transcription (STATs) are an important class of transcription factors that play an essential role in the cellular response to cytokines (Qin et al., 2012). The family of *STATs* comprises seven structurally and functionally related members: *STAT1*, *STAT2*, *STAT3*, *STAT4*, *STAT5a*, *STAT5b*, and *STAT6*, which all contain the Src homology domain 2 (SH2) domain, the Src homology domain 3 (SH3) domain, and a tyrosine phosphorylation site at their carboxyl-terminal region (Alvarado et al., 2010; Kurochkina and Guha, 2013). While most STAT proteins have been studied extensively, STAT3 remains to be fully characterized (Stark and Darnell, 2012).

STAT3 has been shown to be an important mediator of the signal imparted by the IL-6 (interleukin-6) family by binding to the phosphorylated receptor through its SH2 domain, and in turn, is phosphorylated by JAK (Janus

* Corresponding author. Current Address: College of Life Sciences, Northwest A&F University, No. 22, Xinong Road, Yangling, Shaanxi 712100, PR China.
E-mail addresses: jiawenchaozyq@163.com (W. Jia), panyu1980@126.com (C. Pan), lanxianyong79@nwsuaf.edu.cn (X. Lan).

http://dx.doi.org/10.1016/j.smallrumres.2015.05.014
0921-4488/

kinase) (Timofeeva et al., 2012; Zhuang, 2013; Demaria et al., 2014). Recently, STAT3 has been implicated in many cellular functions including the regulation of hepatic acute-phase proteins (Hilfiker-Kleiner et al., 2005; Subramaniam et al., 2012), the growth arrest of monocytic cells (Demaria et al., 2014), the regulation of autophagy (Kamran et al., 2013), and cancer biology since changes in its expression levels can cause cells to transform into tumor-like cells (Siveen et al., 2014). Knocking out the *STAT3* gene causes early embryonic death (Ohkubo et al., 2013). Since *STAT3* is critical for epithelial cell apoptosis, post lactating mammary gland development, skin reconstructed, keratinocyte migration, and macrophage inactivation (Gober et al., 2012), it could directly affect growth traits via stimulating the expression of prolactin (PRL) and growth hormone (GH), making it an ideal candidate gene for goat breeding and genetics (Richard and Stephens, 2014).

To date, few studies of this gene focused on livestock animals such as goat. It is difficult to obtain superior growth traits using the conventional breeding methods unless marker assisted selection (MAS) is widely used in an animal breeding program (Pan et al., 2013; Zhao et al., 2013). A critical initial step, therefore, is to identify and evaluate the genetic effects of candidate genes associated with growth and development.

The Xinong Saanen dairy goat (XNSN) and Hainan black goat (HN), reared mainly in Northwest and Southeast China. Both, however, do not meet the standards of the development plan of Chinese goat industry since they are both slow-growth breeds. To improve the Chinese goat industry, MAS can be implemented to increase the rearing size of these breeds by overcoming their negative traits. The aim of this study was to screen novel genetic variations and evaluate their effects on growth traits in two famous Chinese native goat breeds. As well, we aimed to determine if the different genotypes affected the mRNA expression levels of the *STAT3*. Together, this work will improve our understanding of the role of *STAT3* in the growth of commercial goat breeds and contribute to goat breeding and genetics.

2. Materials and methods

2.1. Animals and data collection

This study was conducted in compliance with the requirements of the Animal Ethics Committee of Northwest A&F University. XNSN and HN were used for DNA isolation, and XNSN was used for RNA isolation.

In order to explore the genetic variations of the goat *STAT3* gene, the two Chinese native goat breeds XNSN ($n = 365$) and HN ($n = 220$), were used. Individuals within each breed were unrelated for a minimum of three generations to ensure diverse genetic lineages. A total of 365 blood samples were from the healthy and unrelated female XNSN individuals, which were reared on the Chinese native dairy goat breeding farm in Qianyang county (East Longitude 106–107°; North Latitude 34°), Shaanxi, PR China, respectively. All XNSN individuals were 2–6 years old adults. Among them, the XNSN individuals that are two years old, three years old, four years old, five years old and six years old were about 21.3%, 50.8%, 8.9%, 12.7% and 6.3%, respectively (Wu et al., 2014). A total of 220 healthy and unrelated female HN goats was investigated and measured in seven growth traits, which were reared in native breeding farms, in Zanzhou County (East Longitude 109.5°; North Latitude 19.5°), Hainan province, China, respectively. All HN goats were 2–3 years old (Wu et al., 2014). The following growth traits of the XNSN and HN goats were measured: body weight (BW), body height (BH), body length (BL), body slanting length (BSL), chest girth (CG), chest depth (CD), chest width (CW), hip width (HW), cannon bone circumference (CB), body trunk index (BTI), body length index (BLI), chest girth index (CGI), cannon bone circumference index (CBI), chest width index (CWI), and thurl width index (TWI) (Wu et al., 2014).

For studying whether the single nucleotide polymorphisms (SNPs) were significantly associated with the mRNA expression of *STAT3* gene, 18 ram (Xinong Saanen dairy goat, XNSN) were selected and slaughtered at 360 days of age. Six fresh tissue samples, including adipose tissues (subcutaneous fat), spleen, kidney, heart, liver, and muscle, were collected for RNA extraction and these samples were collected for each of organs.

2.2. DNA/RNA isolation and DNA/cDNA pool construction

For the genetic variation analysis of the goat *STAT3* gene, genomic DNA was isolated from tissue samples using a phenol-chloroform-based extraction protocol and stored at −80 °C (Lan et al., 2007). The concentration of the isolated DNA was determined using a spectrophotometer, diluted to 50 ng/μL, and stored at −20 °C for subsequent analysis. Fifty DNA samples were randomly selected from the XNSN and HN breeds to construct two different genomic DNA pools. The genomic DNA pools were used as templates for PCR amplification and determining the genetic variations of the *STAT3* gene.

To determine the mRNA expression levels of *STAT3*, total RNA was isolated from fresh tissue samples using the TaKaRa MiniBEST Universal RNA Extraction Kit (TaKaRa Biotech Co. Ltd.) according to the manufacturer's instructions. RNA quality was confirmed by The PrimeScript™ RT reagent Kit with gDNA Eraser (Perfect Real Time) (TaKaRa Biotech Co. Ltd.) and was used to remove any genomic DNA contaminations. The above-mentioned reverse transcriptase reagent Kit was used to synthesize cDNA according to the manufacturer's instructions. RT conditions for each cDNA synthesis were 42 °C for 15 min, 85 °C for 5 s, and maintained at 4 °C. cDNA pools were constructed, as previously described for the genomic DNA pools, to investigate *STAT3* mRNA expression levels different tissues using quantitative real-time PCR (qRT-PCR).

2.3. Primer design and PCR amplification for detecting SNPs and mRNA expression

Ten pairs of primers were used to amplify the coding and non-coding regions within the goat *STAT3* gene based on the published bovine *STAT3* gene sequence (NCBI: AC_000176) (Table 1). PCR was performed in 25 μL reaction volumes 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 U of *Taq* DNA polymerase (MBI Fermentas, USA). PCR reactions were carried out in a PCR thermal cycler (Bio-Rad, USA) using the following program; 3 min initial denaturation at 95 °C; 35 cycles of 94 °C for 30 s following by a 35 s annealing at specific annealing temperatures (AT) (Table 1); and a 45 s extension at 72 °C. The final extension was performed at 72 °C for 10 min.

Since the goat *STAT3* mRNA sequence has not been published, primers for the mRNA expression analysis were designed based on the bovine *STAT3* gene sequence (NCBI: AC_000176) (Table 1). To enhance the mRNA-specificity of the amplification products and to reduce any genomic DNA contamination, gene-specific primers for exons 2 and 3 were designed by Beacon Designer 7.0. *GAPDH* was chosen as the housekeeping gene as is it stably expressed in various tissues. qRT-PCR reactions were performed in a CFX96 Real Time PCR Detection System (Bio-Rad, USA), and SYBR Green was used as the detection dye. Each 10-μL reaction contained 5 μL SYBR® Premix Ex Taq™ II (TaKaRa Biotech Co. Ltd.) and 1 μL of cDNA. The thermal cycle conditions were as follows: 95 °C for 3 min, followed by 39 cycles of 95 °C for 10 s, and 55 °C for 30 s.

2.4. SNP Genotyping and qRT-PCR analysis

To screen for *STAT3* genetic variants, PCR products amplified from genomic DNA were commercially sequenced in both directions (GenScript, Nanjing, China). To improve the cost-effectiveness of the single nucleotide polymorphisms (SNPs) discovery screen, sequencing was performed in pooled DNA samples (Pool-Seq). The sequences were imported into the BioXM software (Version 2.6) and were analyzed by Chromas software (Version 2.22). To facilitate the genotyping of the novel SNPs from the analyzed Pool-Seq populations, forced PCR-RFLP primers were designed. Interestingly, the three SNPs (SNP1-*Dde*I:AC_000176:g.45204C > T in intron 2; SNP2-*Rsa*I: g.62058A > G in