Novel Nucleotide Variations, Haplotypes Structure and Associations with Growth Related Traits of Goat AT Motif-Binding Factor (ATBF1) Gene

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ABSTRACT: The AT motif-binding factor (ATBF1) not only interacts with protein inhibitor of activated signal transducer and activator of transcription 3 (STAT3) (PIAS3) to suppress STAT3 signaling regulating embryo early development and cell differentiation, but is required for early activation of the pituitary specific transcription factor 1 (Pit1) gene (also known as POU1F1) critically affecting mammalian growth and development. The goal of this study was to detect novel nucleotide variations and haplotypes structure of the ATBF1 gene, as well as to test their associations with growth-related traits in goats. Herein, a total of seven novel single nucleotide polymorphisms (SNPs) (SNP 1-7) within this gene were found in two well-known Chinese native goat breeds. Haplotypes structure analysis demonstrated that there were four haplotypes in Hainan black goat while seventeen haplotypes in Xinong Saanen dairy goat, and both breeds only shared one haplotype (hap1). Association testing revealed that the SNP2, SNP5, SNP6, and SNP7 loci were also found to significantly associate with growth-related traits in goats, respectively. Moreover, one diplotype in Xinong Saanen dairy goats significantly linked to growth related traits. These preliminary findings not only would extend the spectrum of genetic variations of the goat ATBF1 gene, but also would contribute to implementing marker-assisted selection in genetics and breeding in goats. (Key Words: ATBF1 Gene, Single Nucleotide Polymorphisms, Haplotypes, Growth-related Traits, Association)

INTRODUCTION

As the global economy is rapidly expanding, the demand for goat products is increasing in numerous developed and developing countries, such as China, India and South Africa. However, these goat products are experiencing serious shortage in those countries. Therefore, the question of how to improve goat growth and development has aroused interests in goat selection and breeding (Choudhary et al., 2007). The growth-related traits (e.g. body weight, body length, body height) are controlled by multiple genes, so it is difficult to rapidly improve growth traits using traditional methods. Consequently, an effective DNA marker-assisted selection (MAS) would speed up the development and improvement goat products. Besides, it is more realistic to focus on some important genes and explore their nucleotide variations with growth-related traits. Thereby, identifying, mapping, and analyzing novel nucleotide variations of the candidate genes and detecting their associations with economic traits are required for an effective MAS system.

AT motif-binding factor (ATBF1, also known as Zinc finger homeobox 3 [ZFHX3]) gene was firstly isolated as an AT (adenine and thymine)-binding factor of human α-fetoprotein (AFP) and was mapped in human Chr.16q22.3-q23.1 (Morinaga et al., 1991). Human ATBF1 is found to have two different transcripts: ATBF1-A and ATBF1-B. Function experiments show that ATBF1-A inhibits the enhancer of AFP and induces cell differentiation and death, ...
while ATBF1-B promotes AFP expression by activating its enhancer (Ninomiya et al., 2002; Nojiri et al., 2004; Junge et al., 2005; Sun et al., 2007; Cleton-Jansen et al., 2008; Kai et al., 2008). From the available studies, ATBF1 is responsible for suppressing AFP transcription by binding with its enhancer competing with hepatocyte nuclear factor-1 (HNF-1) (Yasuda et al., 1994), thereby it plays an important role in cell differentiation and death (Ishii et al., 2003; Junge et al., 2011; Perea et al., 2013), tumour genesis (Sun et al., 2012; Sun et al., 2014), atrial fibrillation and embryonic development (Benjamin et al., 2009; Gudbjartsson et al., 2009; Perea et al., 2013). Furthermore, ATBF1 interacts with Smads to regulate thyroid-stimulating hormone beta (TSH-β) signaling pathway (Massagué, 2005; Moustakas et al., 2012), thus it represses AFP expression (Sakata et al., 2014). Besides, ATBF1 regulates estrogen receptor signaling, functioning mammary gland (Li et al., 2012) and as well as in progesterone receptors signaling gland (Li et al., 2013) as and as well as in progesterone receptors signaling (Li et al., 2013).

To date, ATBF1 is described as the biggest anti-transcription factor for regulating expression of many critical genes, such as signal transducer and activator of transcription 3 (STAT3) (also known as POU1F1) and protein inhibitor of activated STAT3 (PIAS3) by forming ATBF1-PIAS3 complex and combining with active STAT3, thereby inhibiting expression of proliferative genes by reducing STAT3-DNA binding activity (Nojiri et al., 2004; Nishio et al., 2012; Jiang et al., 2014). Importantly, ATBF1 not only activates expression of Pit1 gene though interacting with Pit1 enhancer (Qi et al., 2008), but also potentially synergizes with PROP1 that can bind to the enhancer of Pit1 gene and regulate the expression levels of growth hormone, prolactin, and TSH-β (Carvalho et al., 2006; Davis et al., 2010; Araujo et al., 2013). STAT3, Pit1, and PROP1 genes play an important role in embryo early development and cell differentiation (Zhong et al., 1994; Schindler et al., 1995; Darnell, 1997; Heinrich et al., 1998; Shuai et al., 1999; Kamohara et al., 2000; Fang et al., 2012; Gudi et al., 2012; Akcay et al., 2013; Pan et al., 2013; Navardauskaite et al., 2014), so ATBF1 gene was hypothesized to produce important effects on early development and cell differentiation, thus it would affect the grow traits in animals.

To date, few studies about the nucleotide variations of goat ATBF1 gene and its effects on growth traits have been reported. To improve understanding of goat ATBF1 gene, this work firstly explored the novel nucleotide variations, haplotypes structure of goat ATBF1 gene, and analyzed its associations with growth related traits. These findings would not only extend the spectrum of genetic variations of the goat ATBF1 gene, but also would contribute to implementing MAS in genetics and breeding in goats.

**MATERIALS AND METHODS**

**Animals and data collection**

In this study, a total of 707 goats from two well-known Chinese native goat breeds (Hainan Black goats [HNBG] = 284; Xinong Saanen dairy goats [XNSN] n = 423) were used. All selected individuals were healthy and unrelated. The HNBG goats were 2 to 3 years old and reared in native breeding farms, in Zanzhou County, Hainan province, China. All XNSN individuals were 2 to 6 years old, among which 21.3%, 50.8%, 8.9%, 12.7%, and 6.3% were 2 years old, 3 years old, 4 years old, 5 years old, and 6 years old, respectively. The XNSN goats were reared on Chinese native dairy goat breeding farm in Qianyang County, Shaanxi Province, China (Zhao et al., 2013).

Body measurement traits for all selected individuals were measured, including body weight (BW), body height, body length (BL), chest circumference (ChC), chest depth, chest width, hucklebone width (HuW), hip width, and cannon circumference (CaC), according to the method of Gilbert et al. (1993). Consequently, body length index (BLI), chest circumference index (ChCI), cannon circumference index (CaCI), hucklebone width index (HuWI) and trunk index (TI) were also calculated on the basis of our reported description (Fang et al., 2010).

**DNA isolation and DNA pool construction**

Extraction of DNA samples from ear tissues and blood leukocytes (Sambrock et al., 2001; Green et al., 2012) were diluted to working concentration (50 ng/μL) according to our previous report (Lan et al., 2013). A total of 50 DNA samples from two breeds were randomly selected to construct DNA pools, which were used as templates for polymerase chain reaction (PCR) amplification to explore SNPs of ATBF1 gene.

**Primers design and DNA sequencing**

The 5’ UTR, exons, introns and 3’ UTR regions of the goat ATBF1 gene were amplified from the constructed DNA pools. Fourteen pairs of primers were designed to amplify the goat ATBF1 gene using Primer Premier Software (version 5.0) based on the sheep ATBF1 gene sequence (GenBank Accession No. NC_019471) as the goat was not available (Table 1). PCR reactions were performed in 25 μL volume containing 50 ng genomic DNA, 0.5 μM of each primer, 1× Buffer (including 1.5 mM MgCl2, 200 μM dNTPs and 0.625 units of Taq DNA polymerase [MBI, Vilnius, Lithuania]). The Touch-Down PCR protocol was as follows: denatured at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 68°C to 51°C for 30 s, and 72°C for 2 min, finally extended at 72°C for 10 min. Then to sequence
accurately, the products were sequenced only when they had a single objective band of each pair of primers.

Genotyping using PCR-based amplification-created restriction site—restriction fragment length polymorphism (PCR-ACRS-RFLP) and PCR-RFLP

The primers were selected to amplify and genotype the variants of goat \textit{ATBF1} gene only if mutations were found after DNA pool sequencing and Blastn analyses. In this work, seven novel SNPs were detected, namely NC_019471:g.25504G>A (SNP1), g.25748G>A (SNP2), g.26902A>G (SNP3), g.32001C>G (SNP4), g.32029A>G (SNP5), g.163442C>G (SNP6), g.163517A>G (SNP7).

| Loci     | Primer sequences (5’→3’) | Tm (°C) | Sizes (bp) | Detection methods                                      |
|----------|--------------------------|---------|------------|-------------------------------------------------------|
| P1       | Forward: AAGGCAATGCTGGTCGCTAT (nt24226-24245) Reverses: AGGGTGGAACTAAGGGGA (nt25435-25454) | 60      | 1,229      | Pool DNA sequencing                                   |
| P2 (SNP1)| Forward: CTGTGCTGTTCCGATCACCT(TG) (nt25718-25747) Reverses: TGGACGAAACCAGAAATGATG (nt26321-26337) | 62.5    | 1,202      | TaqI PCR-RFLP (AA = 824+159+112+105 bp; AG = 824+517+307+159+112+105 bp; GG = 517+307+159+112+105+ bp) |
| P2       | (mis-match-SNP2)         |         |            | MspI PCR-RFLP (AA = 365 bp; AG = 365+337+28 bp; GG = 337+28 bp) |
| P3       | Forward: GATGCGCAACTGCTCTG(TG) (nt26321-26337) Reverses: GATGCGCAACTGCTCTG(TG) (nt26903-26932) | 62.5    | 1,406      | Pool DNA sequencing                                   |
| P3       | (mis-match-SNP3)         |         |            | Hinfl PCR-RFLP (AA = 172 bp; AG = 172+30 bp; GG = 140+32 bp) |
| P4 (SNP4)| Forward: GTGTTCGGTTCCATAGGC (nt31849-31850) Reverses: AATGCACTGCTCTGAGTA (nt33215-33263) | 62.8    | 1,146      | AvaI PCR-RFLP (CC = 1082+71 bp; CG = 1082+574+508+71 bp; GG = 574+508+71 bp) |
| P4       | (mis-match-SNP5)         |         |            | Scall PCR-RFLP (AA = 172 bp; AG = 172+30 bp; GG = 140+32 bp) |
| P5       | Forward: AGTCAGGCAACATCGACCA (nt31849-31850) Reverses: GATGCACTGCTCTGAGTA (nt33215-33263) | 59.5    | 879        | Pool DNA sequencing                                   |
| P6       | Forward: GCTTACGGGACACCCAGAAG (nt31849-31850) Reverses: AATGCACTGCTCTGAGTA (nt33215-33263) | 59.5    | 1,086      | Pool DNA sequencing                                   |
| P7 (SNP6,SNP7)| GACTCTTACCGAACCAGCTA(TG) (nt162942-162964) Reverses: TAACGAGACCACACCACCCCA (nt164391-164413) | 55.9    | 1,472      | PstI PCR-RFLP (CC = 1,260+212 bp; CG = 1,260+575+503+212 bp; GG = 575+503+212 bp) Smpl PCR-RFLP (AA = 1064+203+135+70 bp; AG = 1064+898+203+135+70 bp; GG = 898+203+135+70 bp) |
| P8       | Forward: GTGTAGCCGACCGGCAAC (nt31849-31850) Reverses: GATGCACTGCTCTGAGTA (nt33215-33263) | 58      | 1,811      | Pool DNA sequencing                                   |
| P9       | Forward: GCTTACGGGACACCCAGAAG (nt31849-31850) Reverses: AATGCACTGCTCTGAGTA (nt33215-33263) | 50      | 3,611      | Pool DNA sSequencing                                  |
| P10      | Forward: GTACCGCGACATCGACCA (nt31849-31850) Reverses: AATGCACTGCTCTGAGTA (nt33215-33263) | 64      | 3,898      | Pool DNA sequencing                                   |
| P11      | Forward: AACCGTCTTACTGACATGC (nt31849-31850) Reverses: AATGCACTGCTCTGAGTA (nt33215-33263) | 60      | 1,415      | Pool DNA sequencing                                   |

PCR, polymerase chain reaction; \textit{ATBF1}, AT motif-binding factor 1; SNP, single nucleotide polymorphism; TaqI, \textit{Thermus aquaticus} YT-1; MspI, \textit{Moraxella species}; Hinfl, \textit{Haemophilus influenzae} RF; AvaI, \textit{Bacillus megaterium} T110; Scall, \textit{Streptomyces achromogenes}; PstI, pancreatic secretory trypsin inhibitor; PCR-RFLP, PCR- restriction fragment length polymorphism.

1 N showed a mismatch of forward or reverse primer for creating a restriction site.
locus, created restriction endonuclease Moraxella species (MspI) site (CCGG) was formed when the forward primer actual nucleotide “T” was induced to “C” at NC_019471: g.25746 locus. Thus the MspI could recognize the SNP of g.25748 G with induced point mutation g.25746 C, not with g.25746 T. iii) For the NC_019471: g.26902 A>G (SNP3) locus, new restriction endonuclease Haemophilus influenzae Rf (HinII) site (GANTC) was established by changing the reverse primer actual nucleotide “A” to “T” at NC_019471: g.26905 locus. Then the SNP of g.26902 G with induced point mutation g.26905 T could be genotyped by HinII PCR-ACRS-RFLP, rather than g.26905 A. iv) For the NC_019471: g.32001 C>G (SNP4) locus, the endonuclease Bacillus megaterium T110 (Aval) site (CCCGTG) was used to genotype the allele of g. 32001 G, not the g. 32001 C. v) Since the NC_019471: g.32029 A>G (SNP5) also could not be genotyped by the natural restriction or economic restriction endonuclease, the other reverse primer was designed to form new restriction endonuclease Streptomyces achromogenes (ScaII) (CCCGG) point. The actual nucleotide “A” was induced into “G” at the NC_019471: g.32031, so the Streptomyces achromogenes (ScaII) could genotype the SNP of g.32029 G with induced point mutation g.32031G, not with g.32031 A. vi) For the NC_019471: g.163442 G>A (SNP6) locus, the endonuclease pancreatic secretory trypsin inhibitor (PstI) (CTGCAG) was used to genotype the SNP of g. 163442 G, not g. 163442 A. vii) For the NC_019471: g.163517A>G (SNP7) locus, the endonuclease MspI (CCGG) was used to genotype the SNP of g.163517 G, not g.163517 A. For the above loci, the 8 μL PCR products were digested with 3 U TaqI, MspI, HinII, Aval, ScaII, PstI, MspI, respectively, for 12 h at 37°C except TaqI and HinII, at 65°C. The digested products were detected by electrophoresis of 1.5% to 3.5% agarose gel stained with ethidium bromide.

Statistical analysis

Genotypic frequencies, allelic frequencies and Hardy-Weinberg equilibrium (HWE) were analyzed by the SHEsis program (http://analysis.bio-x.cn) (Li et al., 2009), as well as linkage disequilibrium (LD) structure and haplotypes across seven SNPs loci in HNBG and XNSN breeds (Wang et al., 2013). According to PopGene version 1.3.1 (Yeh et al., 2000), population parameters, such as gene heterozygosity (He), effective allele numbers (Ne) and polymorphism information content (PIC) were calculated.

The associations of the genetic variations and growth-related traits were calculated according to the general linear model by the SPSS software (version 18.0) (International Business Machines [IBM] Corporation, New York, USA) for Windows. Statistical testing was carried on the records of growth traits of HNBG and XNSN goats. The mixed statistical of the linear model analysis, not including the effects of farm, sex, season of birth (spring versus fall), age of dam and sire, which had no significant effects on the variation of traits in the mammal populations (Lan et al., 2007; Zhao et al., 2013). Therefore, the statistical linear model was: $Y_{ij} = \mu + A_i + G_j + e_{ij}$, where $Y_{ij}$ is the observation of the body measurement traits, $\mu$ is the overall mean of each trait, $A_i$ is the fixed effect of age, $G_j$ is the fixed effect of genotype or combined genotype, and $e_{ij}$ is the random residual error (He et al., 2014; Wang et al., 2014). Thus the fixed effect of genotypes and age was a major source of variation and the p-value for the difference between the least squares means was less than 0.05. Diplotypes of combined haplotypes of SNPs with growth traits correlation analysis were carried out to explore the possible interactions between the SNPs. The model was similar to above model analysis, except that the interaction between two SNPs was treated as a fixed effect.

RESULTS

Novel nucleotide variations within goat ATBF1 gene

After DNA sequencing and alignment analysis, seven SNPs loci were firstly found, namely, SNP1-7 (Figure 1). The SNP1-TaqI locus (25504 G>A) was located at exon 2 and mutated from G to A, resulting in a missense mutation, CGA (372 R) to CAA (372 Q), which could be genotyped by the TaqI PCR-RFP method (Figure 2a). The SNP2-MspI locus (25748 G>A) was located at exon 2 and mutated from G to A, resulting in a synonymous change, TCG (453 Ser) to TCA (453 E), which could be genotyped by the MspI PCR-ACRS-RFLP method (Figure 2b). The SNP3-HinII locus (26902 A>G) was located at exon 3 and mutated from A to G, resulting in a synonymous change, TCG (453 Ser) to TCA (453 E), which could be genotyped by the HinII PCR-ACRS-RFP method (Figure 2c). The SNP4-Aval locus (32001 C>G) was located at exon 3 and mutated from C to G, which could be genotyped by the Aval PCR-RFP method (Figure 2d). The SNP5-ScaII locus (32029 A>G) was located at exon 3 and mutated from A to G, which could be genotyped by the ScaII PCR-ACRS-RFP method (Figure 2e). The SNP6-PstI locus (163442 C>G) was located at exon 8 and mutated from C to G, which could be genotyped by the PstI PCR-RFP method (Figure 2f). The SNP7-MspI locus (163517A>G) was located at intron 8 and mutated from A to G, which could be genotyped by the MspI PCR-RFP method (Figure 2g).

Frequencies of genotypes and alleles within goat ATBF1 gene

Statistics analysis showed that the frequencies of genotypes and main alleles are different at different SNP
loci in two goat breeds (Table 2). For example, only one genotype of SNP4-\textit{Ava}I, SNP5-\textit{Sac}II, and SNP6-\textit{Pst}I was found in HNBG, but three genotypes were found in XNSN dairy goat. The frequencies of two alleles of each SNP locus in XNSN dairy goat, SNP4-\textit{Ava}I and SNP5-\textit{Sac}II loci were approximately same except the SNP6-\textit{Pst}I locus. As shown in Table 2, the frequencies of the two alleles of SNP2-\textit{Msp}I were similar in both HNBG and XNSN dairy goats, as well as SNP7-\textit{Msp}I locus. The classification of PIC values demonstrated that all SNPs loci were medium genetic

![Figure 1. Sequence chromatograms of seven novel SNPs loci of the goat ATBF1 gene. a to g represented the pooling sequence chromatograms of NC_019471:g.25504G>A (SNP1), g.25748G>A (SNP2), g.26902 A>G (SNP3), g.32001 C>G (SNP4), g.32029 A>G (SNP5), g.163442 C>G (SNP6), g.163517A>G (SNP7), respectively. SNPs, single nucleotide polymorphisms; ATBF1, AT motif-binding factor 1.](image-url)
diversity except those that had only one kind of genotype and most SNPs loci were at HWE except SNP2-MspI and SNP5-SacII loci in XNSN dairy goat and SNP7-MspI locus in HNBG.

Haplotype structure and linkage disequilibrium analysis

Four haplotypes were found in HNBG while seventeen haplotypes in XNSN dairy goat (Table 3). Only 1 haplotype (hap 1) was simultaneously found in both breeds, but the frequency was low (8.5%). The frequency of the hap 4 (27.5%) was highest in HNBG, and the hap 13 (14.1%) was the highest in XNSN dairy goat.

The LD of seven SNPs in two populations was analyzed. As shown in Table 4 and Figure 3, the D’ and r² values of

Figure 2. Electrophoresis pattern of seven novel genetic variations of goat ATBF1 gene. a to g represented the electrophoresis pattern of the SNP1-7 loci, respectively. ATBF1, AT motif-binding factor 1; SNPs, single nucleotide polymorphisms.
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HNBG were very low (approximately zero), except the $D'$ values (0.861) and $r^2$ values (0.02) between SNP6 and SNP7 loci. As shown in Table 5 and Figure 4, the $r^2$ values of XNSN were very low as well as the $D'$ values, except the $D'$ values between SNP4 and SNP5 (0.670), SNP4 and SNP6 (0.574), SNP4 and SNP7 (0.642), SNP6 and SNP7 loci.

Table 2. Genotypes, alleles, He, Ne, and PIC for the SNPs of the goat ATBF1 gene

| Breeds/loci | Sizes (N) | Genotype numbers and frequencies (%) | Allele frequencies (%) | HWE p values | Population parameters |
|------------|-----------|--------------------------------------|------------------------|--------------|-----------------------|
| SNP1-TaqI  |           |                                      |                        |              |                       |
| HNBG       | 284       | AA 0 0 284(100) 0 100               | $>0.05$                | 0 1 0        |                       |
| XNSN       | 423       | AA 0 0 423(100) 0 100               | $>0.05$                | 0 1 0        |                       |
| SNP2-MspI  |           |                                      |                        |              |                       |
| HNBG       | 284       | AA 0 0 144(50.7) 50 50             | $>0.05$                | 0.500 2.000 0.375 |
| XNSN       | 423       | AA 0 0 204(48.2) 41.9 58.1         | $<0.01$                | 0.487 1.950 0.368 |
| SNP3-HinfI |           |                                      |                        |              |                       |
| HNBG       | 284       | AA 0 0 100 0                     | $>0.05$                | 0 1 0        |                       |
| XNSN       | 423       | AA 0 0 100 0                     | $>0.05$                | 0 1 0        |                       |
| SNP4-Aval  |           |                                      |                        |              |                       |
| HNBG       | 284       | CC 0 0 100 0                     | $>0.05$                | 0 1 0        |                       |
| XNSN       | 423       | CC 0 0 100 0                     | $>0.05$                | 0.496 1.986 0.373 |
| SNP5-SacII |           |                                      |                        |              |                       |
| HNBG       | 284       | AA 0 0 100 0                     | $>0.05$                | 0 1 0        |                       |
| XNSN       | 423       | AA 0 0 100 0                     | $>0.05$                | 0.492 1.968 0.371 |
| SNP6-PstI  |           |                                      |                        |              |                       |
| HNBG       | 284       | CC 0 0 99(33.4) 99.8 0.2         | $>0.05$                | 0.500 2.000 0.375 |
| XNSN       | 423       | CC 0 0 20(4.7) 78.6 21.4         | $>0.05$                | 0.460 1.851 0.354 |
| SNP7-MspI  |           |                                      |                        |              |                       |
| HNBG       | 284       | AA 0 0 100 0                     | $>0.05$                | 0 1 0        |                       |
| XNSN       | 423       | AA 0 0 100 0                     | $>0.05$                | 0.496 1.983 0.373 |

He, gene heterozygosity; Ne, effective allele numbers; PIC, polymorphism information content; SNPs, single nucleotide polymorphisms; ATBF1, AT motif-binding factor 1; HWE, Hardy-Weinberg equilibrium; HNBG, Hainan Black goat; XNSN, Xinong Saanen dairy goat.

Table 3. Haplotype frequency within the ATBF1 gene in goat breeds

| Different haplotypes | SNP1-SNP2-SNP3-SNP4- SNP5-SNP6-SNP7 | Haplotype frequency |
|----------------------|--------------------------------------|---------------------|
|                      |                                      | HNBG | XNSN |
| Hap1                | G A A C A C A                         | 0.225 | 0.085 |
| Hap2                | G A A C A G                           | 0.272 | 0     |
| Hap3                | G A A C A C                           | 0.228 | 0     |
| Hap4                | G G A C A G                           | 0.275 | 0     |
| Hap5                | G A A C A G G                         | 0     | 0.015 |
| Hap6                | G A A C G C A                         | 0     | 0.134 |
| Hap7                | G A A G A C A                         | 0     | 0.046 |
| Hap8                | G A A G A C G                         | 0     | 0.020 |
| Hap9                | G A A G G A G                         | 0     | 0.027 |
| Hap10               | G A A G G C G                         | 0     | 0.139 |
| Hap11               | G A A G G A G                         | 0     | 0.016 |
| Hap12               | G A A G G G G                         | 0     | 0.077 |
| Hap13               | G A A G A C A                         | 0     | 0.141 |
| Hap14               | G G A A C A G                         | 0     | 0.027 |
| Hap15               | G G A A C G G                         | 0     | 0.020 |
| Hap16               | G G A A G C A                         | 0     | 0.019 |
| Hap17               | G G A A C A C                         | 0     | 0.016 |
| Hap18               | G G A A C G C                         | 0     | 0.058 |
| Hap19               | G G A A G C G                         | 0     | 0.036 |
| Hap20               | G G A A G G G                         | 0     | 0.124 |

ATBF1, AT motif-binding factor 1; SNP, single nucleotide polymorphism; HNBG, Hainan Black goat; XNSN, Xinong Saanen dairy goat; Hap, haplotype.
(0.737).

Relationships between the genetic variations and related-growth traits

The associations of the genetic variations with growth related traits except SNP1 and SNP3 loci were determined (Table 6). In the SNP2-MspI locus, the genotype of AG had demonstrated significantly superior HuWI traits than genotype GG in HNBG, while genotype GG was found to have significantly superior BL, ChC, and ChCI traits when compared with genotype AA, as well as genotype GG and AG had significantly superior BLI traits in XNSN dairy goat. The different genotypes of SNP5-ScaII locus had significantly associated with BW, demonstrating that the genotype AA and GG was superior to AG in XNSN dairy goat. The different genotypes of SNP6-PstI locus had significant associate with BL, which demonstrated that the genotype CC and GG was superior to CG in XNSN dairy goat. In SNP7-MspI locus, the different genotypes were found to be significantly associate with CaC and CaCI traits in HNBG and TI trait in XNSN dairy goat. For the locus, the genotype GG was superior in HNBG and genotype AA and AG in XNSN dairy goat.

Effects of the interaction of each two single nucleotide polymorphisms to growth traits

Though the $r^2$ values of HNBG between SNP6 and SNP7 were low, but at the same time, the D’ values were

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**Table 4.** D’ and $r^2$ values of pairwise linkage disequilibrium of the *ATBF1* gene in HNBG goat

| HNBG-locus/ D’ | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | SNP7 |
|---------------|------|------|------|------|------|------|------|
| SNP1          | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP2          | 0.00 | 0.00 | 0.001 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP3          | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP4          | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP5          | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP6          | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP7          | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

**Table 4.** D’ and $r^2$ values of pairwise linkage disequilibrium of the *ATBF1* gene in HNBG goat

| HNBG-locus/ $r^2$ | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | SNP7 |
|-------------------|------|------|------|------|------|------|------|
| SNP1              | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP2              | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP3              | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP4              | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP5              | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP6              | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP7              | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

*ATBF1*, AT motif-binding factor 1; HNBG, Hainan Black goat; SNP, single nucleotide polymorphism.

**Figure 3.** Linkage disequilibrium (LD) plot of *ATBF1* gene in HNBG. *ATBF1*, AT motif-binding factor 1; HNBG, Hainan Black goat.
high (0.861), so we analyzed the effects of the interaction between SNP6 and SNP7 of HNBG with growth traits as well as between SNP4 and SNP5 (0.670), SNP4 and SNP6 (0.574), SNP4 and SNP7 (0.642), SNP6 and SNP7 (0.737) of XNSN. As shown in Table 7, the diplotypes of SNP6 and SNP7 were found to have significant effects on ChC (p = 0.025). The phenotype ChC trait of combined genotypes CC-AA, CC-AG, CC-GG, CG-AG, and GG-GG was greater than CG-GG in XNSN.

**DISCUSSION**

As a cancer suppressor gene, *ATBF1* gene not only regulates cell proliferation and differentiation (Ninomiya et al., 2002; Ishii et al., 2003; Jung et al., 2011), but also interacts with PIAS3 to suppress STAT3 signaling way (Nishio et al., 2012; Jiang et al., 2014). Most importantly, ATBF1 is necessary for the *Pit1* gene activation, indicating that ATBF1 could indirectly participate in the regulative roles of *Pit1* gene, including regulating Wnt/beta-catenin pathway and POU1F1 pathway (Carvalho et al., 2006; Qi et al., 2008; Davis et al., 2010). All these functional experiments suggested that the *ATBF1* gene would affect growth traits of livestock. Therefore, this work studied the relationship between the nucleotide variations of this gene and growth related traits in goats.

**Table 5.** D′ and r² values of pairwise linkage disequilibrium of the *ATBF1* gene in XNSN goat

| Locus/D' | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | SNP7 |
|----------|------|------|------|------|------|------|------|
| SNP1     |      | -    | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP2     |      |      | -    | 0.00 | 0.26 | 0.190| 0.073|
| SNP3     |      |      |      | -    | 0.00 | 0.00 | 0.00 |
| SNP4     |      |      |      |      | -    | 0.670| 0.574|
| SNP5     |      |      |      |      |      | -    | 0.461|
| SNP6     |      |      |      |      |      |      | -    |
| SNP7     |      |      |      |      |      |      |      |

| Locus/r² | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | SNP7 |
|----------|------|------|------|------|------|------|------|
| SNP1     |      |      | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| SNP2     |      |      |      | -    | 0.00 | 0.029| 0.002|
| SNP3     |      |      |      |      | -    | 0.00 | 0.00 |
| SNP4     |      |      |      |      |      | -    | 0.256|
| SNP5     |      |      |      |      |      |      | -    |
| SNP6     |      |      |      |      |      |      | -    |
| SNP7     |      |      |      |      |      |      |      |

*ATBF1*, AT motif-binding factor 1; XNSN, Xinong Saanen dairy goat; SNP, single nucleotide polymorphism.

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**Figure 4.** Linkage disequilibrium (LD) plot of *ATBF1* gene in XNSN. *ATBF1*, AT motif-binding factor 1; XNSN, Xinong Saanen dairy goat.
We found seven novel SNPs, of which two were missense mutations (SNP1 and SNP3), two were synonymous changes (SNP2 and SNP6) and three SNPs loci (SNP4, SNP5, and SNP7) were located at several introns. The missense mutation loci (SNP1 and SNP3) only had one kind of genotype of each locus, meaning that the mutation frequency was very low. The missense mutation with amino acid change could affect protein structure, resulting in loss of normal function, which might cause embryonic lethality. We detected haplotypes structure and found the common haplotype (hap1) had a relatively high frequency in two breeds, for the haplotype was present in the population for a long time. The haplotypes of highest frequencies in HNBG and XNSN dairy goat were different, probably caused by variety distinctiveness.

Association testing revealed that the SNP2, SNP5, SNP6 and SNP7 loci were also found to significantly associate with growth-related traits in goats. Among them, although SNP2 and SNP6 were synonymous mutations, they might affect transcriptional efficiency for codon preference and stability of mRNA (Chamary et al., 2005). Many studies have shown that no change of amino acid sequence could still affect gene performance, for example, two synonymous SNPs of bovine NUCB2 gene were significantly associated with growth traits (Li et al., 2010). Although SNP5 and SNP7 were intronic mutations, they also might affect alternatively spliced transcripts of mRNA or transcription factor binding, thus affecting phenotype. A famous example of intronic mutation was located at intron 3 of the porcine IGF2 gene. This mutation lead to a significant effect in skeletal muscle (Van et al., 2003). Besides, the combined genotypes of SNP6 and SNP7 in Xinong Saanen dairy goats was significantly linked to growth related traits. Therefore, this association data reflected that these nucleotide variations within ATBF1 gene produced significant effects on growth related traits.

| Table 6. Relationship between the novel SNPs of the goat ATBF1 gene and growth traits |
|-----------------------------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Locus/growth traits       | Observed genotypes (LSM±SE) | p value |
|---------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| SNP2-MspI                 |                 |                 |                 |                 |                 |
| XNSN breed                |                 |                 |                 |                 |                 |
| BL                        | AA              | 75.21±0.79b     | 77.50±0.90ab     | 77.58±0.61a     | 0.039           |
| ChC                       | AG              | 87.58±0.91b     | 90.57±0.90ab     | 90.35±0.71a     | 0.045           |
| BLI                       | GG              | 109.40±1.80ab   | 111.72±1.20a     | 105.39±1.25b    | 0.007           |
| HNBG breed                |                 |                 |                 |                 |                 |
| HuWI                      | AA              | 68.25±0.47a     | 66.82±0.45b      | 69.33±0.59a     | 0.004           |
| SNP5-ScaII                |                 |                 |                 |                 |                 |
| XNSN breed                |                 |                 |                 |                 |                 |
| BW                        | AA              | 77.66±0.43a     | 76.23±0.63b      | 80.65±1.42a     | 0.016           |
| SNP6-PstI                 |                 |                 |                 |                 |                 |
| XNSN breed                |                 |                 |                 |                 |                 |
| BL                        | AA              | 116.20±0.75a    | 116.92±0.69a     | 113.84±0.68b    | 0.018           |
| HNBG breed                |                 |                 |                 |                 |                 |
| CaC                       | AA              | 7.70±0.09b      | 7.65±0.08b       | 7.96±0.07a      | 0.009           |
| SNP7-MspI                 |                 |                 |                 |                 |                 |
| XNSN breed                |                 |                 |                 |                 |                 |
| TI                        | AA              | 14.59±0.19b     | 14.51±0.21b      | 15.06±0.13b     | 0.046           |
| HNBG breed                |                 |                 |                 |                 |                 |
| CaCI                      | AA              | 89.04±0.80a     | 89.96±0.74a      | 91.00±1.03a     | 0.025           |
| SNP5, ScaII, MspI, XNSN, HNBG, BW, CaC, CaCI, BL, ChC, BLI, ChCI, TI |

SNPs, single nucleotide polymorphisms; ATBF1, AT motif-binding factor 1; LSM, lease squares means; SE, standard error; MspI, Moraxella species; XNSN, Xinong Saanen dairy goat; BL, body length; ChC, chest circumference; BLI, body length index; ChCI, chest circumference index; HNBG, Hainan Black goat; HuWI, hucklebone width index; ScaII, Streptomyces achromogenes; BW, body weight; MspI, Moraxella species; TI, trunk index; CaC, cannon circumference; CaCI, cannon circumference index. The values with different letters (a and b) within the same row differ significantly at p<0.05 and p<0.01, respectively.

Table 7. Associations between diplotypes (combined genotypes and haplotype) of SNPs and growth traits in XNSN

| Growth traits | Diploype loci (SNP6+SNP7) | p value |
|---------------|-----------------|---------|
| ChC (cm)      |                 |         |
| (n = 53)      |                 |         |
| CC-AA         | 89.04±0.80a     |         |
| (n = 50)      |                 |         |
| CC-AG         | 89.96±0.74a     |         |
| (n = 13)      |                 |         |
| CC-GG         | 91.00±1.03a     |         |
| (n = 36)      |                 |         |
| CG-AG         | 89.94±1.05a     |         |
| (n = 20)      |                 |         |
| CG-GG         | 85.85±1.17b     |         |
| (n = 8)       |                 |         |
| GG-GG         | 92.62±1.51a     |         |
| SNPs, single nucleotide polymorphisms; XNSN, Xinong Saanen dairy goat; ChCI, chest circumference index. The values with different letters (a and b) within the same row differ significantly at p<0.05 and p<0.01, respectively.
suggesting that this gene can be used as a marker gene in improving goat growth traits.

Briefly, seven novel SNPs mutations were firstly found, and four of them significantly affected goat growth related traits, which extends the known genetic variations spectrum of goat ATBF1 gene and is a benefit towards implementing MAS in genetics and breeding of goats.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

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