Open Access
Asian Australas. J. Anim. Sci.
Vol. 28, No. 10 : 1394-1406 October 2015
http://dx.doi.org/10.5713/ajas.14.0860
www.ajas.info
pISSN 1011-2367 eISSN 1976-5517

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

Xiaoyan Zhang, Xianfeng Wu, Wenchao Jia[1], Chuanying Pan, Xiangcheng Li[2],
Chuzhao Lei, Hong Chen, and Xianyong Lan*
College of Animal Science and Technology, Northwest A&F University,
Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, Shaanxi 712100, China

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,

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* Corresponding Author: Xianyong Lan. Tel: +86-29-87092102, Fax: +86-29-87092164, E-mail: lanxianyong79@nwsuaf.edu.cn
[1] College of Life Sciences, Northwest A&F University, Yangling, Shaanxi 712100, China.
[2] Institute of Beijing Animal Science and Veterinary, Chinese Academy of Agricultural Science, Beijing 100194, China.
Submitted Nov. 6, 2014; Revised Mar. 11, 2015; Accepted Apr. 1, 2015

while ATBF1-B promotes AFP expression by activating its enhancer (Ninomiya et al., 2002; Nojiri et al., 2004; Jung 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; Jung 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., 2009; Massagué 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 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*), pituitary specific transcription factor 1 (*Pit1*) (also known as *POU1F1*) and prophet of Pit-1 (*PROP1*) genes. ATBF1 interacts with 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; Godi 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 $MgCl_2$, 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

Table 1. PCR primer sequences of the goat *ATBF1* gene for amplification

Loci	Primer sequences (5'→3')	Tm (°C)	Sizes (bp)	Detection methods
P1	Forward: AAGGACAATGGGTGCGGTAT (nt24226-24245) Reverse: AGCGGTGGAAACTAAAGGGA (nt25435-25454)	60	1,229	Pool DNA sequencing
P2 (SNP1)	Forward: CTTTCCACATAGCCTCATCCTT(nt24979-25000) Reverse: TTTATTGGCACTTTCATCAGCA (nt26159-26180)	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)	Forward: CAAGAAGTGGGTGATCCAGACTGTTTC$^{1}\boxed{C}$C (nt25718-25747) Reverse: TCGCACCATCAAAGACAAC(nt26064-26082)	55		MspI PCR-RFLP (AA = 365 bp; AG = 365+337+28 bp; GG = 337+28 bp)
P3	Forward: TGCTGATGAAAGTGCCAATA (nt26159-26178) Reverse: TTGACGAAACCCGAAAGTAG (nt27525-27564)	62.5	1,406	Pool DNA sequencing
P3 (mis-match-SNP3)	Forward: ATGCGACACGGTCCTGG(nt26321-26337) Reverse: GGATGCGCAGGTTCCGGGCCACGTTGG$^{1}\boxed{A}$CT (nt26903-26932)	61.3		HinfI PCR-RFLP (AA = 533 bp; AG = 533+503+30 bp; GG = 503+30 bp)
P4 (SNP4)	Forward: GTGTCAGGTGTCCCATAGCC (nt31489-31508): Reverse: AATGCCAGTCCCTCCAGTTA (nt32615-32634)	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)	Forward: AGCAGTGGATAGCACCTTG(nt31888-31905) Reverse: GCATGTCTAGGGGGATTTCACCGCCCAC$^{1}\boxed{C}$G (nt32030-32059)	58.3	172	ScaII PCR-RFLP (AA = 172 bp; AG = 172+140+32 bp; GG = 140+32 bp)
P5	Forward: ATGGACGATGCACGAACC (nt88882-88899) Reverse: GATCTGAACCCAAAGACTGAA (nt89740-89760)	59.5	879	Pool DNA sequencing
P6	Forward: GCTCAGGCACCACGAAG (nt144646-144662) Reverse: CAGGACACCAGGGATACAAA (nt145712-145731)	59.5	1,086	Pool DNA sequencing
P7 (SNP6,SNP7)	Forward: GACTCTTACCCAGCACGTACCCT(nt162942-162964) Reverse: TAACAGAAACCCACCATCCACAA(nt164391-164413)	55.9	1,472	PstI PCR-RFLP (CC = 1,260+212 bp; CG = 1,260+757+503+212 bp; GG = 757+503+212 bp) MspI PCR-RFLP (AA = 1064+203+135+70 bp; AG = 1064+898+203+166+135+70 bp; GG = 898+203+166+135+70 bp)
P8	Forward: TGTTAGTTCAGGGTCAGTTC(nt172005-172022) Reverse: ATGGAGACATCATAAGGGAG(nt173796-173815)	58	1,811	Pool DNA sequencing
P9	Forward: TCCTCCCTTATGATGTCTCCA(nt173794-173814) Reverse: GGTAGTTCAAGTTGCTCGTTC(nt177384-177404)	50	3,611	Pool DNA sSequencing
P10	Forward: GTACCGCGAGCACTACGACA(nt176420-176439): Reverse: GGACCTCAGGGAACAGCAAA(nt180298-180317)	64	3,898	Pool DNA sequencing
P11	Forward: AACCGTCCTCAGCATCGC (nt184007-184024) Reverse: CGTGTCAGACTCCTCCGAAT (nt185402-185421)	60	1,415	Pool DNA sequencing

PCR, polymerase chain reaction; *ATBF1*, AT motif-binding factor 1; SNP, single nucleotide polymorphism; *Taq*I, *Thermus aquaticus* YT-1; MspI, *Moraxella species*; HinfI, *Haemophilus influenzae* Rf; AvaI, *Bacillus megaterium* T110; *Sca*II, *Streptomyces achromogenes*; PstI, pancreatic secretory trypsin inhibitor; PCR-RFLP, PCR- restriction fragment length polymorphism.

[1] $\boxed{N}$ showed a mismatch of forward or reverse primer for creating a restriction site.

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 *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.26902 A>G (SNP3), g.32001 C>G (SNP4), g.32029 A>G (SNP5), g.163442 C>G (SNP6), g.163517A>G (SNP7).

In order to detect these SNPs, the PCR-restriction fragment length polymorphism (RFLP) and PCR-amplification-created restriction site (ACRS)-RFLP were carried out. i) For the NC_019471:g.25504 G>A (SNP1) locus, the endonuclease *Thermus aquaticus* YT-1 (*Taq*I) (TCGA) was used to genotype the SNP of g.25504 G, not g.25504 A. ii) For the NC_019471: g.25748 G>A (SNP2) locus, created restriction endonuclease *Moraxella species* (*Msp*1) site (CCGG) was formed when the forward primer actual nucleotide "T" was induced to "C" at NC_019471: g.25746 locus. Thus the *Msp*1 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 (*Hin*fI) 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 *Hin*fI PCR -ACRS-RFLP, rather than g.26905 A. iv) For the NC_019471: g.32001 C>G (SNP4) locus, the endonuclease *Bacillus megaterium* T110 (*Ava*I) site (CYCGRG) 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* (*Sca*II) (CCGCGG) point. The actual nucleotide "A" was induced into "G" at the NC_019471: g.32031, so the *Streptomyces achromogenes* (*Sca*II) 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 C>G (SNP6) locus, the endonuclease pancreatic secretory trypsin inhibitor (*Pst*I) (CTGCAG) was used to genotype the SNP of g. 163442 G, not g. 163442 C. vii) For the NC_019471: g.163517A>G (SNP7) locus, the endonuclease *Msp*I (CCGG) was used to genotype the SNP of g. g.163517 G, not g. g.163517 A.

For the above loci, the 8 μL PCR products were digested with 3 U *Taq*I, *Msp*I, *Hin*fI, *Ava*I, *Sca*II, *Pst*I, *Msp*I, respectively, for 12 h at 37°C except *Taq*I and *Hin*fI, 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: $Y_{ijk}= \mu+A_i+G_j+e_{ijk,}$, where Y_{ijk} is the observation of the body measurement traits, μ 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_{ijk} 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-*Taq*I 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 *Taq*I PCR-RFP method (Figure 2a). The SNP2-*Msp*I 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 Ser), which could be genotyped by the *Msp*I PCR-ACRS-RFP method (Figure 2b). The SNP3-*Hin*fI locus (26902 A>G) was located at exon3 and mutated from A to G, resulting in a missense change, AAA (453 K) to TCA (453 E), which could be genotyped by the *Hin*fI PCR-ACRS-RFP method (Figure 2c).The SNP4-*Ava*I locus (32001 C>G) was located at intron 3 and mutated from C to G, which could be genotyped by the *Ava*I PCR-RFP method (Figure 2d). The SNP5-*Sca*II locus (32029 A>G) was located at intron 3 and mutated from A to G, which could be genotyped by the *Sca*II PCR-ACRS-RFP method (Figure 2e). The SNP6-*Pst*I locus (163442 C>G) was located at exon 8 and mutated from C to G, which could be genotyped by the *Pst*I PCR-RFP method (Figure 2f). The SNP7-*Msp*I locus (163517A>G) was located at intron 8 and mutated from A to G, which could be genotyped by the *Msp*I 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

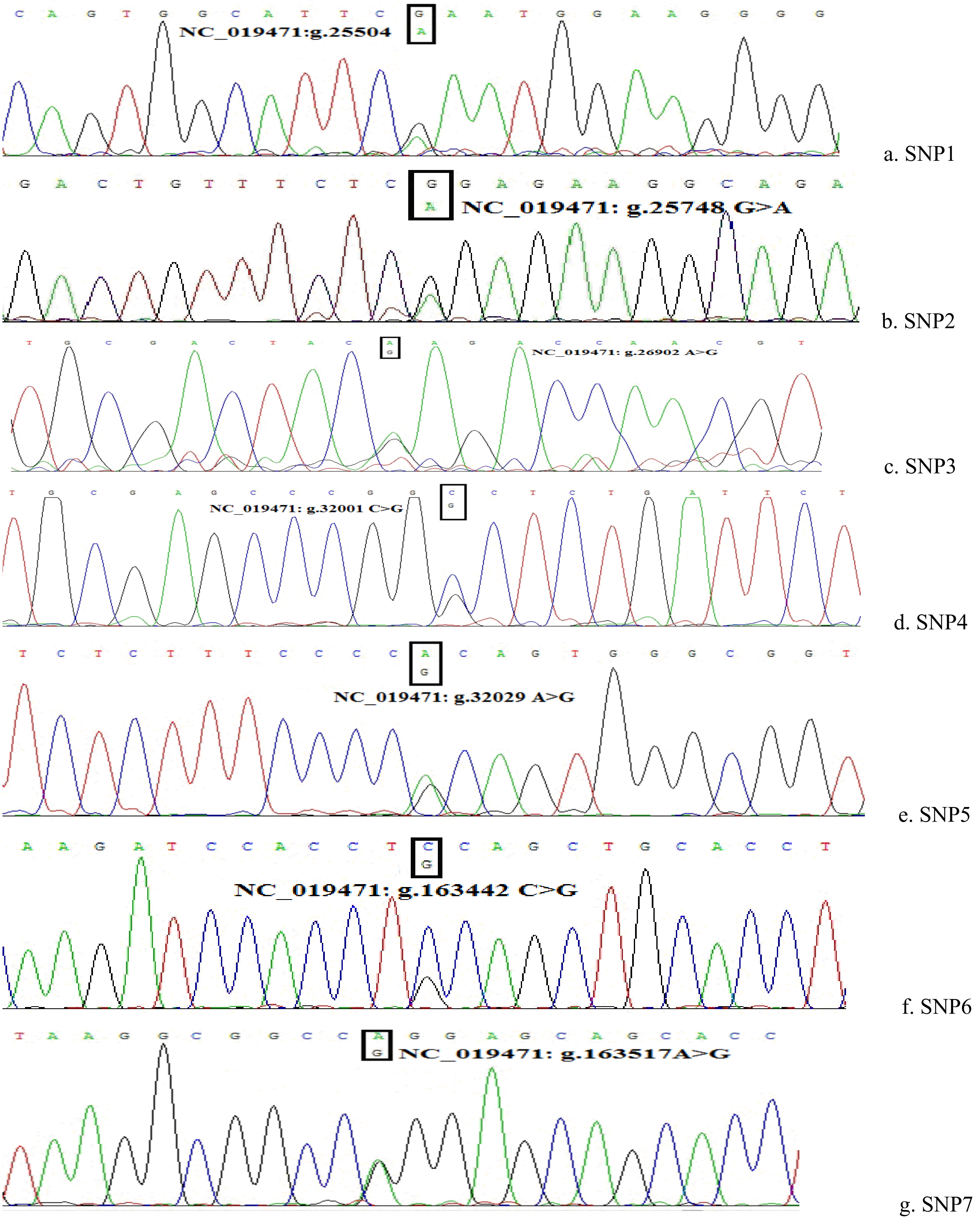
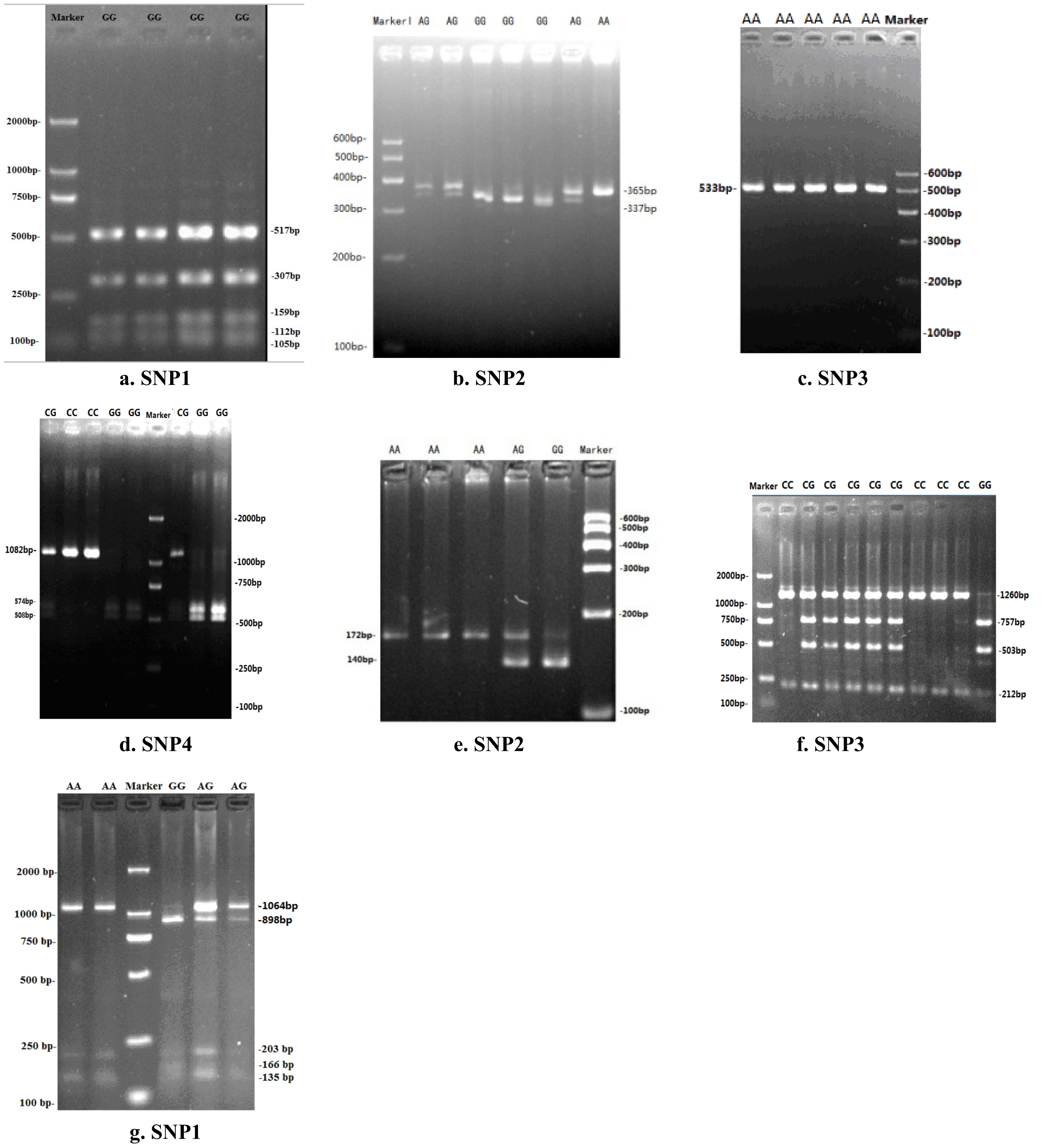


Figure 1. Sequence chromas of seven novel SNPs loci of the goat *ATBF1* gene. a to g represented the pooling sequence chromas of NC_019471:g.25504G>A (SNP1), g.25748G>A (SNP2), g.26902 A>G (SNP3), g.32001 C>G (SNP4), g.32029 A>G (SNP5), g.163442 C>G (SNP6), g.163517A>G (SNP7), respectively. SNPs, single nucleotide polymorphisms; *ATBF1*, AT motif-binding factor 1.

loci in two goat breeds (Table 2). For example, only one genotype of SNP4-*Ava*I, SNP5-*Sac*II, and SNP6-*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-*Ava*I and SNP5-*Sac*II loci were approximately same except the SNP6-*Pst*I locus. As shown in Table 2, the frequencies of the two alleles of SNP2-*Msp*I were similar in both HNBG and XNSN dairy goats, as well as SNP7-*Msp*I locus. The classification of PIC values demonstrated that all SNPs loci were medium genetic



a. SNP1 b. SNP2 c. SNP3

d. SNP4 e. SNP2 f. SNP3

g. SNP1

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.

diversity except those that had only one kind of genotype and most SNPs loci were at HWE except SNP2-*Msp*I and SNP5-*Sac*II loci in XNSN dairy goat and SNP7-*Msp*I 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 r^2 values of

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		
								He	Ne	PIC
SNP1- TaqI		AA	AG	GG	A	G				
HNBG	284	0	0	284(100)	0	100	>0.05	0	1	0
XNSN	423	0	0	423(100)	0	100	>0.05	0	1	0
SNP2-MspI		AA	AG	GG	A	G				
HNBG	284	70(24.6)	144(50.7)	70(24.6)	50	50	>0.05	0.500	2.000	0.375
XNSN	423	136(32.2)	83(19.6)	204(48.2)	41.9	58.1	<0.01	0.487	1.950	0.368
SNP3-*Hinf*I		AA	AG	GG	A	G				
HNBG	284	284(100)	0	0	100	0	>0.05	0	1	0
XNSN	423	423(100)	0	0	100	0	>0.05	0	1	0
SNP4-*Ava*I		CC	CG	GG	C	G				
HNBG	284	284(100)	0	0	100	0	>0.05	0	1	0
XNSN	423	102(24.2)	183(43.3)	138(32.5)	45.8	54.2	<0.05	0.496	1.986	0.373
SNP5-SacII		AA	AG	GG	A	G				
HNBG	284	284(100)	0	0	100	0	>0.05	0	1	0
XNSN	423	171(40.4)	153(36.2)	99(23.4)	58.5	41.5	<0.01	0.492	1.968	0.371
SNP6-*Pst*I		CC	CG	GG	C	G				
HNBG	284	283(99.6)	1(0.4)	0	99.8	0.2	>0.05	0.500	2.000	0.375
XNSN	423	263(62.2)	140(33.1)	20(4.7)	78.6	21.4	>0.05	0.460	1.851	0.354
SNP7-*Msp*I		AA	AG	GG	A	G				
HNBG	284	72(25.4)	102(35.9)	110(38.7)	43.3	56.7	<0.01	0.491	1.965	0.370
XNSN	423	137(32.5)	188(44.4)	98(23.1)	54.7	45.3	>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.

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

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 C G	0.272	0
Hap3	G G A C A C A	0.228	0
Hap4	G G A C A C 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 A G G	0	0.027
Hap10	G A A G G C G	0	0.139
Hap11	G A A G G G A	0	0.016
Hap12	G A A G G G G	0	0.077
Hap13	G G A C A C A	0	0.141
Hap14	G G A C A C G	0	0.027
Hap15	G G A C A G G	0	0.020
Hap16	G G A C G C A	0	0.019
Hap17	G G A G A C A	0	0.016
Hap18	G G A G G C A	0	0.058
Hap19	G G A G G C G	0	0.036
Hap20	G G A G 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.

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
SNP2	-	-	0.000	0.00	0.00	0.00	0.001
SNP3	-	-	-	0.00	0.00	0.00	0.00
SNP4	-	-	-	-	0.00	0.00	0.00
SNP5	-	-	-	-	-	0.00	0.00
SNP6	-	-	-	-	-	-	0.861
SNP7	-	-	-	-	-	-	-
HNBG-locus/r^2							
SNP1	-	0.00	0.00	0.000	0.000	0.00	0.00
SNP2	-	-	0.000	0.000	0.000	0.00	0.00
SNP3	-	-	-	0.000	0.000	0.00	0.00
SNP4	-	-	-	-	0.000	0.00	0.00
SNP5	-	-	-	-	-	0.00	0.00
SNP6	-	-	-	-	-	-	0.02
SNP7	-	-	-	-	-	-	-

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

(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-*Msp*I 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-*Sca*II 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-*Pst*I locus had significant associate with BL, which demonstrated that the genotype CC and GG was superior to CG in XNSN dairy goat. In SNP7-*Msp*I 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

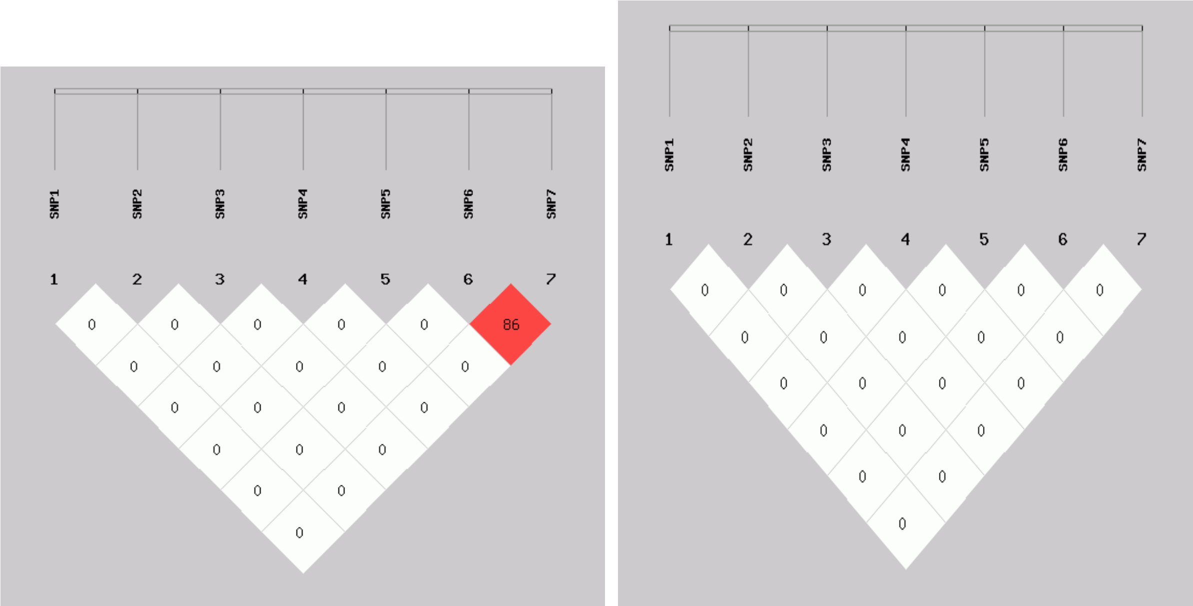


Figure 3. Linkage disequilibrium (LD) plot of *ATBF1* gene in HNBG. *ATBF1*, AT motif-binding factor 1; HNBG, Hainan Black goat.

Table 5. D′ and r^2 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	0.00
SNP2	-	-	0.00	0.026	0.190	0.073	0.005
SNP3	-	-	-	0.00	0.00	0.00	0.00
SNP4	-	-	-	-	0.670	0.574	0.642
SNP5	-	-	-	-	-	0.461	0.306
SNP6	-	-	-	-	-	-	0.737
SNP7	-	-	-	-	-	-	-
Locus/r^2							
SNP1	-	0.00	0.00	0.00	0.00	0.00	0.00
SNP2	-	-	0.00	0.00	0.029	0.002	0.00
SNP3	-	-	-	0.00	0.00	0.00	0.00
SNP4	-	-	-	-	0.256	0.086	0.301
SNP5	-	-	-	-	-	0.077	0.081
SNP6	-	-	-	-	-	-	0.186
SNP7	-	-	-	-	-	-	-

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

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.

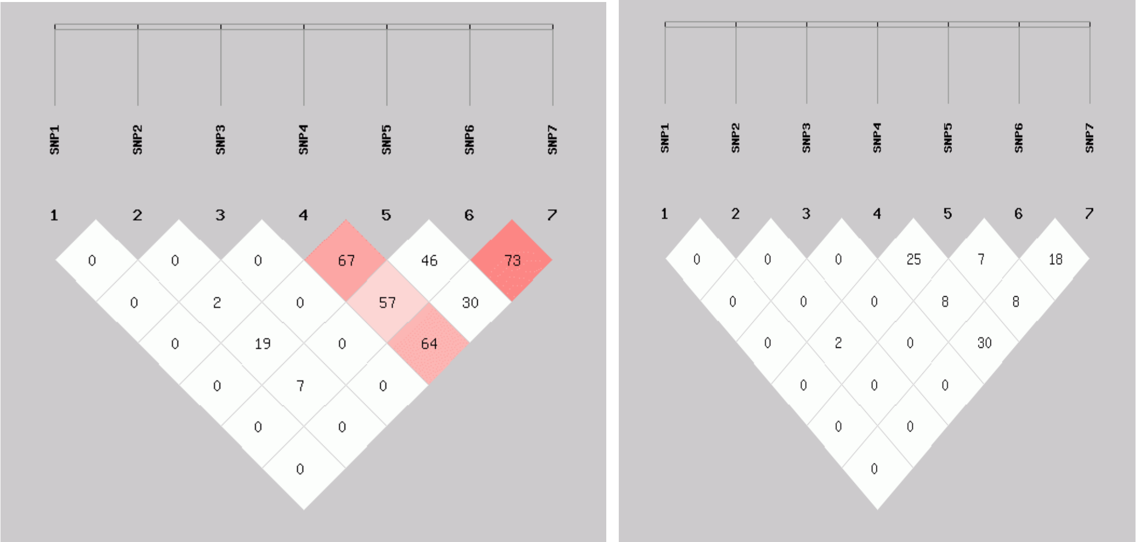


Figure 4. Linkage disequilibrium (LD) plot of *ATBF1* gene in XNSN. *ATBF1*, AT motif-binding factor 1; XNSN, Xinong Saanen dairy goat.

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-*Msp*I				
XNSN breed	AA	AG	GG	
BL	75.21±0.79^{b}	77.50±0.90$^{a b}$	77.58±0.61^{a}	0. 039
ChC	87.58±0.91^{b}	89.57±0.90$^{a b}$	90.35±0.71^{a}	0. 045
BLI	111.01±1.34^{b}	114.98±0.97^{a}	115.14±0.99^{a}	0. 021
ChCI	129.22±1.46^{b}	132.96±1.23$^{a b}$	134.12±1.23^{a}	0. 027
HNBG breed	AA	AG	GG	
HuWI	109.40±1.80^{ab}	111.72±1.20^{a}	105.39±1.25^{b}	0.007
SNP5-*Sca*II				
XNSN breed	AA	AG	GG	
BW	68.25±0. 47^{a}	66.82±0.45^{b}	69.33±0. 59^{a}	0. 004
SNP6-*Pst*I				
XNSN breed	CC	CG	GG	
BL	77.66±0.43^{a}	76.23±0.63^{b}	80.65±1.42^{a}	0. 016
SNP7-*Msp*I				
XNSN breed	AA	AG	GG	
TI	116.20±0.75^{a}	116.92±0.69^{a}	113.84±0.68^{b}	0. 018
HNBG breed	AA	AG	GG	
CaC	7.70±0. 09^{b}	7.65±0. 08^{b}	7.96±0. 07^{a}	0. 009
CaCI	14.59±0.19$^{a b}$	14.51±0.21^{b}	15.06±0.13^{a}	0. 046

SNPs, single nucleotide polymorphisms; *ATBF1*, AT motif-binding factor 1; LSM, lease squares means; SE, standard error; *Msp*I, *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; *Sca*II, *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.

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

Growth traits	Diplotype loci (SNP6+SNP7)						p value
ChC (cm)	CC-AA	CC-AG	CC-GG	CG-AG	CG-GG	GG-GG	
	(n = 53)	(n = 50)	(n = 13)	(n = 36)	(n = 20)	(n = 8)	
	89.04±0.80^{a}	89.96±0.74^{a}	91.00±1.03^{a}	89.94±1.05^{a}	85.85±1.17^{b}	92.62±1.51^{a}	0.025

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.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (No.31172184), the Young New Star Project on Science & Technology of Shaanxi Province (No.2011kjxx64) and Technology Foundation for Selected Overseas Chinese Scholar of Shaanxi Province (Xianyong Lan, 2014). We greatly thank the staff of the dairy goat breeding farm, Sanyuan country, Shaanxi province, P.R. China, the meat goat breeding farm, Zanzhou, Hainan province, P.R. China, for their collecting dairy goat samples and meat goat samples.

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