Arch. Anim. Breed., 60, 285–296, 2017
https://doi.org/10.5194/aab-60-285-2017
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Archives Animal Breeding

Evaluation of novel SNPs and haplotypes within the *ATBF1* gene and their effects on economically important production traits in cattle

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Received: 14 April 2017 – Revised: 10 July 2017 – Accepted: 24 July 2017 – Published: 29 August 2017

Abstract. AT motif binding factor 1 (*ATBF1*) gene can promote the expression level of the growth hormone 1 (*GH1*) gene by binding to the enhancers of the *POU1F1* and *PROP1* genes; thus, it affects the growth and development of livestock. Considering that the *ATBF1* gene also has a close relationship with the Janus kinase–signal transductor and activator of transcription (JAK–STAT) pathway, the objective of this work was to identify novel single-nucleotide polymorphism (SNP) variations and their association with growth traits in native Chinese cattle breeds. Five novel SNPs within the *ATBF1* gene were found in 644 Qinchuan and Jinnan cattle for first time using 25 pairs of screening and genotyping primers. The five novel SNPs were named as AC_000175:g.140344C>G (SNP1), g.146573T>C (SNP2), g.205468C>T (SNP3), g.205575A>G (SNP4) and g.297690C<T (SNP5). Among them, SNP1 and SNP2 were synonymous coding SNPs, while SNP5 was a missense coding SNP, and the other SNPs were intronic. Haplotype analysis found 18 haplotypes in the two breeds, and three and five closely linked loci were revealed in Qinchuan and Jinnan breeds, respectively. Association analysis revealed that SNP1 was significantly associated with the height across the hip in Qinchuan cattle. SNP2 was found to be significantly related to chest circumference and body side length traits in Jinnan cattle. SNP3 was found to have significant associations with four growth traits in Qinchuan cattle. Moreover, the different combined genotypes, SNP1–SNP3, SNP1–SNP4 and SNP2–SNP5 were significantly associated with the growth traits in cattle. These findings indicated that the bovine *ATBF1* gene had marked effects on growth traits, and the growth-trait-related loci can be used as DNA markers for maker-assisted selection (MAS) breeding programs in cattle.

1 Introduction

With the fast improvement in living standards in developing countries, especially China, the demand for beef consumption has increased quickly. Although cattle breed resources are very abundant in China, poor quality and low growth rate of many breeds are still barriers to an increase in cattle production. It is difficult to meet our needs and improve the breeding speed by using traditional methods; thus, the efficient genetical methods, such as DNA marker-assisted selection (MAS), should be used to improve the efficiency of production and lay the foundation for breeding new breeds (Pedersen et al., 2009). As the most practical and economic method, the MAS strategy relies on the numerous single-nucleotide polymorphisms (SNPs) associated with production traits. Therefore, more functional SNPs, which could be applied in MAS breeding of domestic livestock, should be discovered. An example is the single A-to-G substitution near the ovine *CLPG* gene, which has been used for double-muscle livestock breeding (Cockett et al., 1994).

The AT motif binding factor 1 (*ATBF1*) gene encodes a transcription factor with multiple homeodomains and zinc finger motifs; thus, it is also named zinc finger homeobox 3 (*ZFHX3*). *ATBF1* was first isolated as an AT (adenine and thymine)-binding factor of human α-fetoprotein (AFP) (Morinaga et al., 1991). It was reported to function as a tu-

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mor suppressor in several cancers (Kawaguchi et al., 2016; Sun et al., 2014, 2015). More importantly, it plays an important role in regulating myogenesis, adipose tissue development and transactivating the cell cycle inhibitor (Jung et al., 2005; Postigo and Dean, 1997, 1999; Richard and Stephens, 2014).

Furthermore, *ATBF1* could promote the expression level of the growth hormone 1 (*GH1*) gene by binding to the enhancers of the *POU1F1* and *PROP1* genes (Araujo et al., 2013; Qi et al., 2008), which are the key genes in mammalian growth, development and the lactation-related hypothalamic–pituitary–adrenal (HPA) axis pathway (*PITX2/PITX1 – HESX1 – LHX3/LHX4 – PROP1 – POU1F1*) (Davis et al., 2010; Ma et al., 2017). In addition, another key gene in the HPA axis pathway, *PITX2*, has a positive regulation relationship with *ATBF1* under the participation of miR-1 (Huang et al., 2015).

Meanwhile, *ATBF1* has close relationships with STAT family genes, which are growth-related genes. *STAT3* and *STAT5A* are two key genes in Janus kinase–signal transducer and activator of transcription pathway (JAK–STAT), and JAK–STAT is responsible for promoting the secretion of a variety of cytokines, growth factors and *GH1* (Herrington et al., 2000; Liongue and Ward, 2013; Trovato et al., 2012). *ATBF1* could enhance the suppression of STAT3 signaling by interaction with *PIAS3*, which is a protein inhibitor of the activated STAT family (Lao et al., 2016; Nojiri et al., 2004; S. F. Yang et al., 2016). Thus, it was surmised that the *ATBF1* gene plays an important role in regulating mammalian growth and development.

SNP research is a crucial step for the application of the genome project in human and MAS breeding in mammals. The genes mentioned above, *CLPG*, *STAT3*, *STAT5A*, *POU1F1* and *PROP1*, all have SNPs associated with growth traits, and some SNP genotypes were found to be significantly associated with mRNA expression levels (Jia et al., 2015; Lan et al., 2007, 2009; Wu et al., 2014; Zhao et al., 2013). In humans, functional SNPs that were associated with disease were found in the *ATBF1* gene (Liu et al., 2014; Tsai et al., 2015). Moreover, four SNPs that were significantly associated with goat growth traits were identified in the goat *ATBF1* gene (Zhang et al., 2015b).

Considering the important roles of the *ATBF1* gene in the HPA axis and JAK–STAT pathways, which are related to mammalian growth and development, and the significance of SNP in biological process and livestock breeding, the purpose of this study was to identify crucial SNP variations within the *ATBF1* gene in native Chinese cattle breeds. This will also help to promote the understanding of *ATBF1* gene function and better apply the excellent local cattle germplasm resources in cattle MAS breeding.

2 Materials and methods

2.1 Animal samples and data collection

Experimental animal samples used in this study were approved by the Faculty Animal Policy and Welfare Committee of Northwest A&F University under contract. The care and use of experimental animals fully complied with local animal welfare laws, guidelines and policies.

A total of 644 blood samples were collected from healthy and unrelated adult cattle belonging to two well-known Chinese native cattle breeds, Qinchuan cattle (459) and Jinnan cattle (185). All Qinchuan individuals were reared in a native breeding farm in Fufeng County, Shaanxi Province. Jinnan individuals were reared on a Yuncheng cattle farm in Shanxi Province.

The growth trait data of the Qinchuan cattle were collected from the Qinchuan breeding farm, including body weight, body height, body length, chest circumference, hucklebone width, height across the hip, chest width, chest depth, rump length and hip width. The growth trait data of the Jinnan cattle, including body height, height across the hip, chest circumference, rump length and body side length, were collected from the Jinnan cattle farm. All growth trait data were measured as described by Zhang et al. (2015a).

2.2 DNA isolation and genomic DNA pool construction

Genomic DNA samples were extracted from the leukocytes of the blood samples as described by Dang et al. (2014). All genomic DNA samples were diluted to the working concentration 50 ng µL^{-1} for the DNA pool construction and polymerase chain reaction (PCR) amplification. To construct DNA pools, 30 DNA samples were randomly and selected from the Qinchuan and Jinnan cattle. The two DNA pools were used as templates for PCR amplification, and the product of amplification was used to sequence and explore genetic variations in the *ATBF1* gene.

2.3 Primer design and PCR amplification for SNP screening

To expose novel SNPs in the bovine *ATBF1* gene, 20 pairs of primer were designed using the Primer Premier 5 software based on the bovine *ATBF1* gene DNA sequence (NCBI reference sequence: AC_000175.1) (Table 1). The 25 µL PCR reaction volume includes 50 ng of genomic DNA from the DNA pool, 0.5 µM of each primer and 12.5 µL 2 × EcoTaq PCR SuperMix (+dye) (Beijing TransGen Biotech Co., Ltd., Beijing, China). The touchdown PCR program was executed as follows: pre-denaturation at 95 °C for 4 min, followed by 18 cycles of denaturation at 94 °C for 30 s, annealing at 68 °C for 30 s (decreased by 1 °C per cycle) and extending at 72 °C for 1 kb min^{-1}, then another 22 cycles at 94 °C for 30 s, 50 °C for 30 s and 72 °C for 1 kb min^{-1}, finally extending

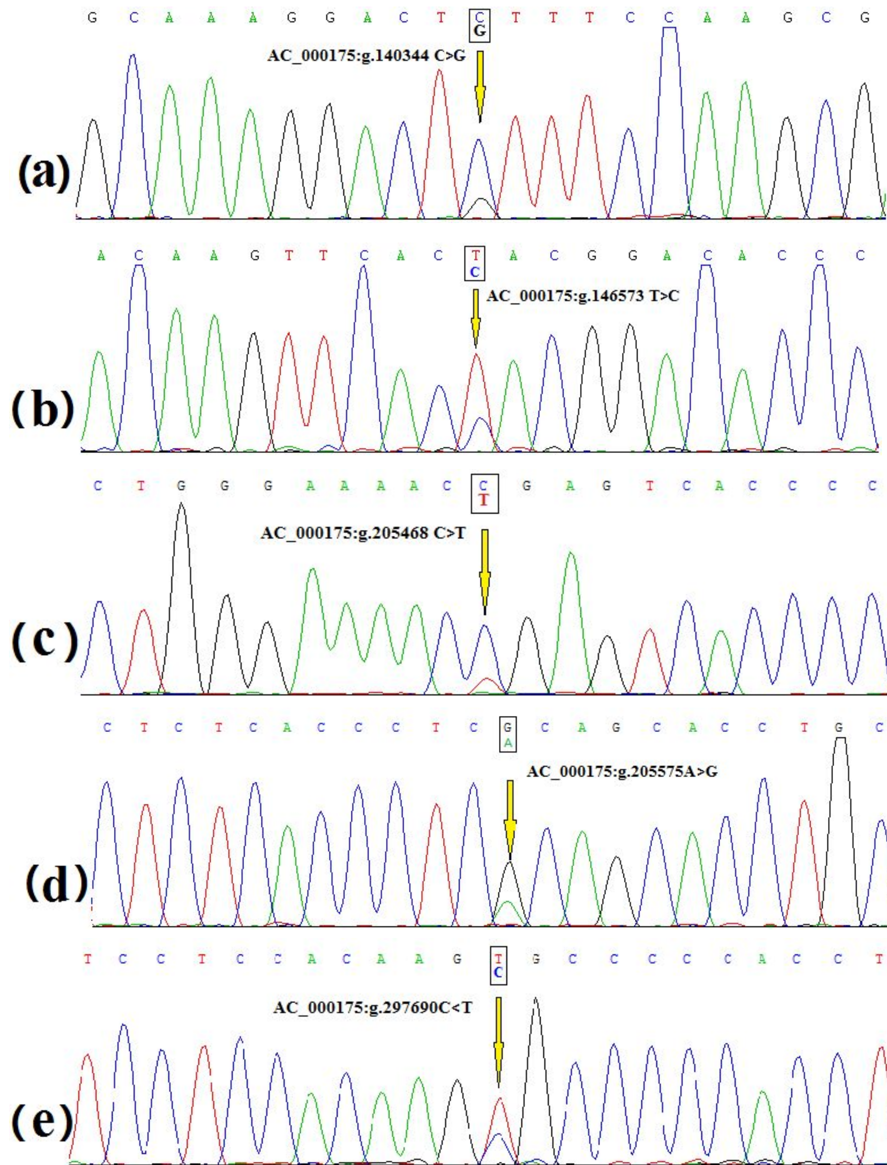

Figure 1. Sequence chromas of five novel SNP loci in the bovine *ATBF1* gene. Note: panels **(a)**, **(b)**, **(c)**, **(d)** and **(e)** represent the pooling sequence chromas of SNP1, SNP2, SNP3, SNP4 and SNP5, respectively.

at 72 °C for 10 min. The products of PCR amplification were sequenced to screen the SNP loci.

2.4 Primer design and genotyping by PCR-RFLP, forced PCR-RFLP and T-ARMS-PCR methods

DNA pool sequencing and sequence analysis identified five novel SNPs within the Qinchuan and Jinnan bovine *ATBF1* gene, namely, AC_000175:g.140344C>G (SNP1), g.146573T>C (SNP2), g.205468C>T (SNP3), g.205575A>G (SNP4) and g.297690C<T (SNP5) (Fig. 1). According to the sequencing results, PCR restriction fragment length polymorphism (PCR-RFLP), forced PCR-RFLP and tetra-primer amplification refractory mutation system PCR (T-ARMS-PCR) methods were used to detect genotypes of Qinchuan and Jinnan individuals (Table 2). The primers of PCR-RFLP and forced PCR-RFLP were designed by the Primer Premier 5 software, and T-ARMS-PCR primers were designed on the Primer1 website (http://primer1.soton.ac.uk/primer1.html) (Collins, 2012; Ye et al., 2001). The genotyping methods used on different SNP loci were introduced as below:

SNPs genotyped with the PCR-RFLP and forced PCR-RFLP methods: SNP3 and SNP5 were detected using the PCR-RFLP method, and the PCR amplification products were digested with DdeI and HhaI restriction enzymes. SNP2

Table 1. Amplification PCR primers for screening the novel SNPs within the bovine *ATBF1* gene; bp: base pair.

Names	Primer sequences ($5' \rightarrow 3'$)	Product sizes (bp)	Location
P1	F: GAAAGGGCTTCTCCTGACG R: GATACCGCACCCATTGTCC	367	Intron 1
P2	F: CCTGACTCTAACGCTGTGCT R: GGATGGGCTTCCTCTTGC	1264	Exon 2
P3	F: CTTTCCACATAGCCTCATCCTT R: TTTATTGGCACTTTCATCAGCA	1202	Exon 2
P4	F: TGCTGATGAAAGTGCCAATA R: GAGCATCCAGTCGTCCCTT	1116	Exon 2
P5	F: GTGTCAGGTGTCCCATAGCC R: AATGCCAGTCCCTCCAGTTA	1153	Exon 3
P6	F: GATTATTGTGCCAGGAAGCC R: GATCTGAACCCAAAGACTGAA	714	Exon 4
P7	F: GCTCAGGCACCACGAAG R: CAGGACACCAGGGATACAAA	1080	Exon 5
P8	F: GACTCTTACCCAGCACGTACCCT R: TAACAGAAACCCACCATCCACAA	1461	Exon (6+7)
P9	F: CAGGACACCCTCTGGGCTAC R: ATGGAGACATCATAAGGGAG	1454	Exon 8
P10	F: CATTGGGCTTGATTTCTAT R: GGTGGCATTCCTACACTTT	1138	Exon 9
P11	F: CACCTTTACCACCACCAAC R: TACGAGGCCGCTTATTCT	1442	Exon 9
P12	F: GCCCATCTTCTCGCCACT R: ATCCTGCCCTTCCTCGTC	802	Exon 9
P13	F: CAGGATGACAGCCAGAATG R: CTTGCCAGCAGTGGGTTA	680	Exon 9
P14	F: TACAGCATCCTCTGCGTTCT R: CCGTGCCTTCCACCTTGA	1235	Exon 9
P15	F: GATGGCAATGTCTGAGTATGA R: ACCCTGGTCTGTGCTGAA	1031	Exon 9
P16	F: AACCGTCCTCAGCATCGC R: CGTGTCAGACTCCTCCGAAT	1457	Exon 10
P17	F: CGCTCACTCAAACGACAG R: AATCTACTCAACACCGAAAA	1221	Exon 10
P18	F: TTCTCAGGTCAATCGCTCAC R: CACCGCTCAGACTGCCTA	1261	Exon 10
P19	F: TGTTAGGCAGTCTGAGCG R: TTCTGGGTTAATGTGGAG	1409	Exon 10
P20	F: TTCTCCACATTAACCCAG R: TCAGTCAGCTCCATCACC	1314	Exon 10

was genotyped using the forced PCR-RFLP method, and the C nucleic acid on g.146575 was changed to T to make a locus that can be recognized by the EcoT14I (StyI) restriction enzyme. The PCR reaction volume for the two methods was 13 µL, including 25 ng of genomic DNA, 0.2 µM of each primer and 6.5 µL of $2 \times$ EcoTaq PCR SuperMix (+dye). The amplification system was as follows: pre-denaturation at 95 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 30 s, optimal annealing temperature for 30 s and extending at 72 °C for 1 kb min^{-1}, finally extending at 72 °C for 10 min. Then the amplification products were digested with a special restriction enzyme and special temperature for 12 to 16 h. The volume of digestion contains 2 U (U is unit of restriction enzyme) restriction enzyme, 2 µL of $10 \times$ buffer, 10 µL of PCR product and 6 µL of distillation H_2O. Then the enzyme-digested products were genotyped using agarose gel electrophoresis. The electrophoretic band size and genotyping information are shown in Table 2.

SNPs genotyped with the T-ARMS-PCR method: SNP1 and SNP4 were genotyped using the T-ARMS-PCR method for failing to search for a suitable restriction enzyme. The special primers and genotyping information are exhibited in Table 2. The PCR reaction volume was 13 µL. The touch-down PCR program (from 68 to 50 °C; decreased by 1 °C

Table 2. PCR primer sequences for *ATBF1* gene genotyping in cattle; bp: base pairs.

Loci	Primer sequence (5′ → 3′)	AT* (°C)	Size (bp)	Detection method
P21-SNP1 (g.140344C>G)	F inner: AAGAGGAGGAGGAGGGCTGCAAAGGAGTC R inner: AGCTCGTCGTCCAGCTCGCTTGGATAC F outer:GGGGCAGCAGAAGGAGAGAAGCAAGAAG par R outer: TCGACAGGGTCTGGAGCACATTAGGCAT	Touchdown PCR	180/200/325	T-ARMS-PCR C allele: 180 bp; G allele: 200 bp; Product size of two outer primers: 325 bp
P22-SNP2 (g.146573T>C)	F: GGGCAGTGCCTCAGGTAGGA R: CAGCAGGTCCAGGGTGTCCAT	61.7	231	Forced PCR-RFLP (EcoT14 I (StyI)) (TT=231 bp; TC=231+209+22 bp; CC=209+22 bp)
P23-SNP3 (g.205468C>T)	F: GATTATTGTGCCAGGAAGCC R: GATCTGAACCCAAAGACTGAA	60	714	PCR-RFLP (DdeI) (CC=589 bp; CT=589+440 bp; TT=440 bp)
P24-SNP4 (g.205575A>G)	F-inner: AGGGCACGTCCCTCTCTCTCACCCGCA R-inner: CACTCTCGTGCTGCTGCAGGTGCGGC F-outer: GGAAGGGCCCCCTGGGAAACCGAGTCAC R-outer: TCCTCGTCCTCCTCGGGGAGGCCCTTCT	Touch down PCR	216/153/316	T-ARMS-PCR A allele: 216 bp G allele: 153 bp Product size of two outer primers: 316 bp
P25-SNP5 (g.297690C<T)	F: TACAGCATCCTCTGCGTTCT R: CCGTGCCTTCCACCTTGA	60	1235	PCR-RFLP (HhaI) (TT=594 bp; CT=594+564 bp; CC=564 bp)

Note: the single nucleic acid that is underlined is different from the reference sequence, and the change is required for forced PCR-RFLP and T-ARMS-PCR primer designing.
* AT: annealing temperature.

per cycle) was executed for PCR amplification. Then the products were genotyped using agarose gel electrophoresis directly.

2.5 Statistical analysis

Genotypic frequencies and allelic frequencies were calculated according to Botstein's method (Botstein et al., 1980). Population genetic diversity index, homozygosity (Ho), effective allele number (Ne) and polymorphism information content (PIC) were calculated successively on the MSRcall website (http://www.msrcall.com/). Hardy–Weinberg equilibrium (HWE), linkage disequilibrium (LD) structure and haplotypes of the five SNP loci in Qinchuan and Jinnan breeds were calculated using the SHEsis program (http://analysis.bio-x.cn) (Li et al., 2009; Q. Yang et al., 2016).

The relationship between genotypes, haplotypes and the growth traits in Qinchuan and Jinnan populations were analyzed using the SPSS software (version 17.0) (IBM Corp., Armonk, NY, USA). Since all cattle were adult females and each breed was fed the same nutritional diet on their respective farms, the basic linear model $Y = \mu + G + e$ was used to determine the relationship between genotypes, haplotypes and growth traits for each breed. In the formulate, Y denotes the trait data of each animal, μ the overall mean for each trait, G the effect of genotype and e the random error (Dang et al., 2014; Zhang et al., 2015a).

3 Results

3.1 Novel SNP identification and genotyping of the bovine *ATBF1* gene

According to the sequence chromas, five novel SNPs (SNP1 to SNP5) were identified within the Qinchuan and Jinnan cattle *ATBF1* gene (Fig. 1). Among them, SNP1 and SNP2 were synonymous mutations, which were located at exon 2 and exon 3, respectively. SNP1 and SNP2 loci code the 503th leucine and the 963th threonine of the cattle ATBF1 protein, respectively. SNP3 and SNP4 were located at intron 3, and SNP4 was close to the exon 4 splicing site (four base distances). SNP5 was a missense coding SNP at exon 9, resulting in the 2488th amino acid valine to alanine. The genotyping results can be seen from the agarose gel electrophoresis photos, which shows that SNP1–SNP5 were successfully genotyped by their own methods (Fig. 2).

3.2 Genetic diversity analysis of the bovine *ATBF1* gene

Genotype frequency and allelic frequency were calculated according to the genotyping results. At the SNP1 locus, the frequency of allele C was distinctly higher than allele G in the two breeds. Genotype CC is the most prevalent. At the SNP2 locus, the frequency of TT was significantly higher than the other genotypes. At the SNP3 locus, the frequency of allele T is higher than C. At the SNP4 locus, there was no

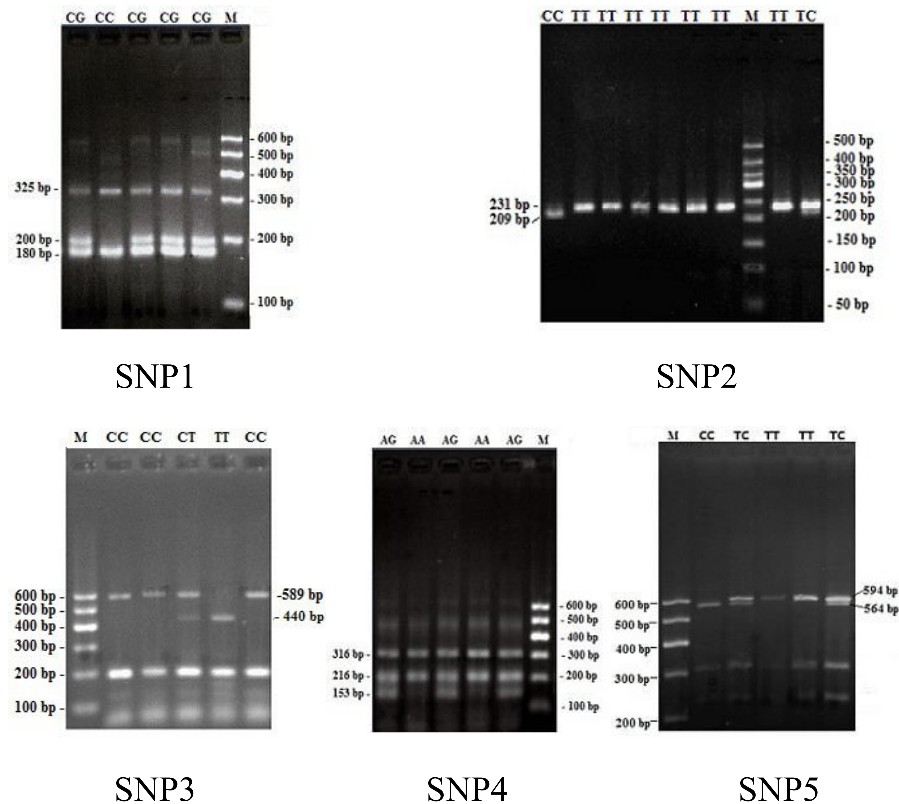

Figure 2. Agarose gel electrophoresis patterns of five novel SNPs of the bovine *ATBF1* gene. Note that the letter "M" above the figure represents the DNA marker.

GG genotype, and the frequency of AG was higher than AA. At the SNP5 locus, genotype frequency of the heterozygote was higher than the other genotypes, and the frequency of allele T was higher than C (Table 3).

The genetic diversity parameters Ho, Ne and PIC of the five loci of the Qinchuan and Jinnan populations were calculated and are shown in Table 3. These results suggest that these loci are polymorphic in these two cattle breeds. However, the values of PIC suggest that these loci are low polymorphisms ($0 < \mathrm{PIC} < 0.25$) and intermediate polymorphisms ($0.25 < \mathrm{PIC} < 0.5$) (Table 3) (Pan et al., 2013). The Hardy–Weinberg equilibrium P value shows that some loci were at Hardy–Weinberg equilibrium ($P > 0.05$) and some were in disequilibrium ($P < 0.05$).

3.3 Haplotype and linkage disequilibrium analysis of the five SNP loci

Haplotype pairwise linkage disequilibrium analysis indicated that there were a total of 18 haplotypes in Qinchuan and Jinnan cattle. Among these haplotypes, seven were shared by these two populations. Nine haplotypes were unique to Qinchuan cattle and two haplotypes were unique to Jinnan cattle. The frequencies of the haplotypes showed that Hap 7 (CTCGT) and Hap 5 (CTCAT) were the main haplotypes in the Qinchuan and Jinnan cattle populations, respectively (Table 4). Based on the D' and r^2 values, three closely linked loci were revealed in the Qinchuan breed and five were revealed in the Jinnan breed (Fig. 3). The D' values were 0.756 (SNP1 and SNP3), 0.608 (SNP1 and SNP4) and 0.624 (SNP2 and SNP5) in the Qinchuan cattle breed. In the Jinnan cattle breed, the D' values were 0.640 (SNP1 and SNP3), 0.999 (SNP1 and SNP4), 0.997 (SNP2 and SNP4), 1.000 (SNP3 and SNP4) and 0.696 (SNP4 and SNP5) (Fig. 3). Thus, we further analyzed the effects of the combined genotypes above and growth traits in cattle.

3.4 Relationships between the genetic variations and growth-related traits

Association analysis found that different genotypes of the SNP1 locus were similar, with a significant association with the height across the hip in Qinchuan cattle ($P = 0.05$), and the heterozygote carriers had the highest value (Table 5). At the SNP2 locus, the different genotypes were significantly associated with chest circumference and body side length traits in Jinnan cattle, and the CC genotype carriers had the highest growth trait index (Table 5). For SNP3, the different genotypes were found to have a significant association with chest width, chest depth and hucklebone width growth traits,

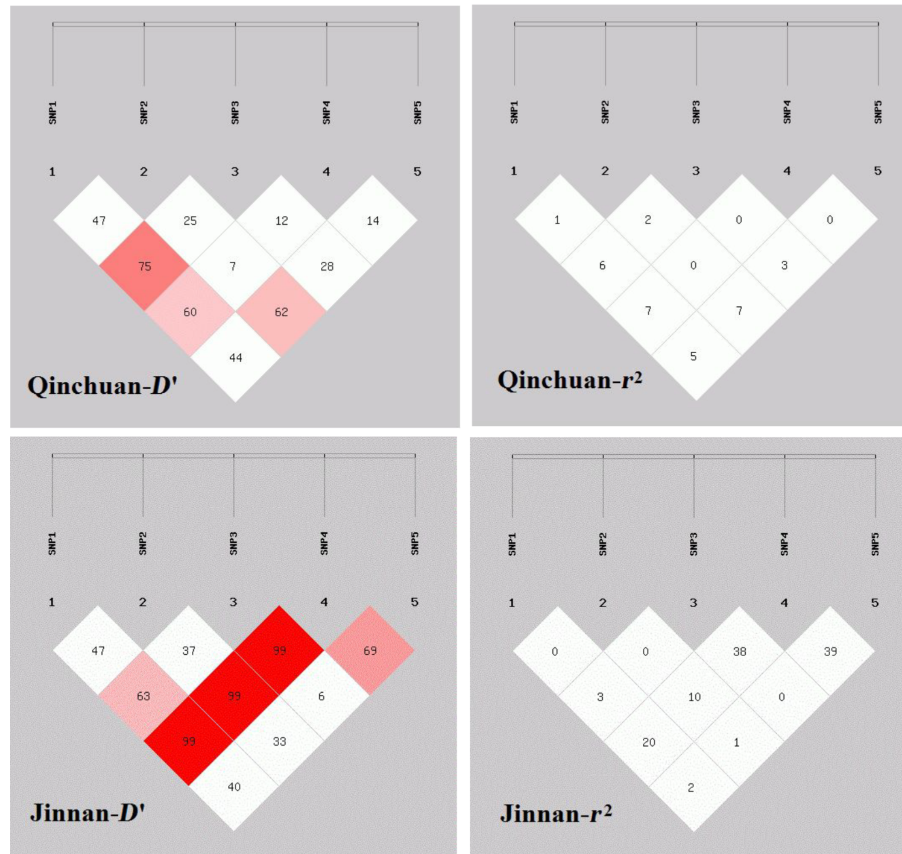

Figure 3. Linkage disequilibrium (LD) plot (D' and r^2) of five novel SNP loci within the *ATBF1* gene in Qinchuan and Jinnan cattle.

and CC carriers had the best growth trait index in Qinchuan cattle. Moreover, the genotypes had a similar significant association with body height ($P = 0.05$), and the heterozygote carriers had the best value in Qinchuan cattle (Table 5). However, no significant association between different genotypes of SNP4 and SNP5 loci and growth-related traits were found.

The association analysis found that three and one combined genotypes were associated with growth traits in Qinchuan and Jinnan breeds, respectively. At the SNP1–SNP3 loci, the combined genotype CG–CC carriers had significantly wider chests than the CC–TT carriers in the Qinchuan breed ($P<0.05$) (Table 6). At the SNP1–SNP4 loci, the CG–AG carriers had a higher height across the hip than CC–AA carriers in the Qinchuan breed ($P = 0.05$) (Table 6). For the SNP2–SNP5 loci in Qinchuan cattle, TC–CC had the smallest chest circumference, chest width and hucklebone width values among all the combined genotypes ($P<0.05$) (Table 6). For the SNP1–SNP3 loci in Jinnan cattle, CG–CT carriers had the smallest body height, height across the hip and body side length, and CG-CC had the largest chest circumference and rump length, among all the combined genotypes (Table 6). In addition, no significant association was found between the other combined genotypes and growth-related traits.

4 Discussion

Due to the important roles of *ATBF1* in regulating myogenesis and adipose tissue development and its close relationship with the HPA axis and JAK–STAT pathways in livestock science (Huang et al., 2015; Jiang et al., 2014; S. F. Yang et al., 2016; Zhao et al., 2016), *ATBF1* was chosen as the candidate gene. In this study, we recovered five SNPs in the bovine *ATBF1* gene for the first time. Three novel SNPs were exonic, while the other two novel SNPs were intronic. For individual genotype identification, three methods, namely PCR-RFLP, forced PCR-RFLP and T-ARMS-PCR, were applied. For the SNP locus, where the nucleotide sequence could be recognized using restriction enzymes, the PCR-RFLP method can be used to identify individual genotypes (Wang et al., 2013). For the SNP locus without the sequence that could be recognized using restriction enzymes, the forced PCR-RFLP primers were designed. This method needs to change one or two nucleotides, which are close to the SNP locus, to make this sequence recognizable using restriction enzymes and genotyping (Huang et al., 2014). For the SNP locus, which is difficult to genotype using the two methods above, T-ARMS-PCR is available (Li et al., 2014; Wang et al., 2014; Zhang et al., 2015a).

Table 3. Genotype, allelic distribution and genetic diversity of five SNP loci of the bovine *ATBF1* gene.

Locus/ Breed	Genotype frequency (Number of animals)			Allelic frequency		P value (HWE)[a]	Diversity parameters[b]		
							Ho	Ne	PIC
SNP1	CC (n)	CG (n)	GG (n)	C	G				
Qinchuan	0.556 (60)	0.407 (44)	0.037 (4)	0.759	0.241	$P>0.05$	0.634	1.576	0.299
Jinnan	0.721 (111)	0.221 (34)	0.058 (9)	0.831	0.169	$P<0.05$	0.719	1.390	0.241
SNP2	TT (n)	TC (n)	CC (n)	T	C				
Qinchuan	0.794 (247)	0.196 (61)	0.010 (3)	0.892	0.108	$P>0.05$	0.808	1.238	0.174
Jinnan	0.836 (153)	0.148 (27)	0.016 (3)	0.910	0.090	$P>0.05$	0.836	1.196	0.151
SNP3	CC (n)	CT (n)	TT (n)	C	T				
Qinchuan	0.528 (171)	0.457 (148)	0.015 (5)	0.756	0.244	$P<0.05$	0.631	1.584	0.301
Jinnan	0.486 (90)	0.459 (85)	0.054 (10)	0.716	0.284	$P>0.05$	0.595	1.680	0.323
SNP4	AA (n)	AG (n)	GG (n)	A	G				
Qinchuan	0.420 (21)	0.580 (29)	0 (0)	0.710	0.290	$P<0.05$	0.588	1.700	0.327
Jinnan	0.227 (40)	0.773 (136)	0 (0)	0.614	0.386	$P<0.05$	0.526	1.902	0.362
SNP5	CC (n)	TC (n)	TT (n)	C	T				
Qinchuan	0.202 (58)	0.453 (130)	0.345 (99)	0.429	0.571	$P>0.05$	0.510	1.960	0.370
Jinnan	0.227 (42)	0.459 (85)	0.314 (58)	0.457	0.543	$P>0.05$	0.504	1.986	0.373

Note: [a] P value (HWE): Hardy–Weinberg equilibrium P value.
[b] Diversity parameters: Ho: gene homozygosity; Ne: effective allele numbers; PIC: polymorphism information content.

Table 4. Haplotype frequency within the *ATBF1* gene in Qinchuan and Jinnan cattle.

Haplotype name	SNP1	SNP2	SNP3	SNP4	SNP5	Haplotype	Haplotype frequencies	
							Qinchuan cattle	Jinnan cattle
Hap 1	C	C	C	A	C	CCCAC	0.052	0
Hap 2	C	C	T	A	T	CCTAT	0.030	0
Hap 3	C	C	T	G	C	CCTGC	0.040	0.050
Hap 4	C	T	C	A	C	CTCAC	0.129	0.059
Hap 5	C	T	C	A	T	CTCAT	0.129	0.391
Hap 6	C	T	C	G	C	CTCGC	0.049	0.100
Hap 7	C	T	C	G	T	CTCGT	0.163	0
Hap 8	C	T	T	A	C	CTTAC	0.111	0
Hap 9	C	T	T	G	C	CTTGC	0.001	0.100
Hap 10	G	C	C	A	T	GCCAT	0.022	0.150
Hap 11	G	C	C	G	C	GCCGC	0.014	0
Hap 12	G	C	T	A	T	GCTAT	0.018	0
Hap 13	G	T	C	A	C	GTCAC	0.048	0.050
Hap 14	G	T	C	A	T	GTCAT	0.135	0
Hap 15	G	T	C	G	C	GTCGC	0.044	0
Hap 16	G	T	T	A	T	GTTAT	0.017	0
Hap 17	G	T	T	G	C	GTTGC	0	0.041
Hap 18	G	T	T	G	T	GTTGT	0	0.059

The preferred methods are PCR-RFLP and forced PCR-RFLP because they are more accurate and mature than T-ARMS-PCR (Cai et al., 2013; Sun et al., 2013). T-ARMS-PCR is easy to operate, using less time and money, but the accuracy is lower than the other two methods. This study performed both PCR-RFLP and T-ARMS-PCR methods to calculate the accuracy of them (Li et al., 2014). The result showed that the consistency of these two methods is 98.8 %, 40 % of inconsistency was caused by PCR-RFLP and 60 % of inconsistency was caused by the T-ARMS-PCR method (Li

Table 5. Association of *ATBF1* gene SNP3 genotypes and cattle growth traits.

Locus/breed	Growth trait	Observed genotypes (LSM ± SE)*			P value
SNP1		CC	CG	GG	
Qinchuan	height across the hip	$124.75^{ab} \pm 1.07$	$127.34^{a} \pm 1.13$	$122.67^{b} \pm 1.45$	$P = 0.05$
SNP2		TT	TC	CC	
Jinnan	chest circumference	$183.77^{b} \pm 1.26$	$185.04^{ab} \pm 2.84$	$201.33^{a} \pm 5.55$	$P < 0.05$
Jinnan	body side length	$151.50^{ab} \pm 0.99$	$150.88^{b} \pm 1.63$	$158.00^{a} \pm 2.00$	$P < 0.05$
SNP3		CC	CT	TT	
Qinchuan	body height	$128.88^{ab} \pm 0.51$	$129.27^{a} \pm 0.64$	$124.00^{b} \pm 2.72$	$P = 0.05$
Qinchuan	chest width	$38.25^{a} \pm 0.44$	$38.37^{a} \pm 0.44$	$33.90^{b} \pm 0.95$	$P < 0.05$
Qinchuan	chest depth	$64.20^{A} \pm 0.51$	$64.21^{A} \pm 0.60$	$59.60^{B} \pm 0.93$	$P < 0.01$
Qinchuan	hucklebone width	$43.21^{a} \pm 0.43$	$41.96^{b} \pm 0.48$	$41.80^{ab} \pm 1.83$	$P = 0.05$

Note: *(LSM ± SE), LSM: least squares mean; SE: standard error. The LSM values with different superscripts within the same row differ significantly at $P < 0.05$ for a and b and $P < 0.01$ for A and B.

Table 6. Associations between combined genotypes and growth traits in Qinchuan and Jinnan cattle.

Loci/breed	Growth traits	Combined genotypes (number)/observed genotypes (LSM ± SE)*						P value
SNP1–SNP3		CC–CC (24)	CC–CT (24)	CC–TT (4)	CG–CC (21)	CG–CT (13)		
Qinchuan	chest width	$39.69^{ab} \pm 1.22$	$38.00^{ab} \pm 1.26$	$34.38^{b} \pm 1.07$	$39.83^{a} \pm 1.18$	$38.54^{ab} \pm 1.29$		$P < 0.05$
SNP1–SNP4		CC–AA (6)	CC–AG (12)		CG–AA (6)	CG–AG (11)		
Qinchuan	height across the hip	$122.67^{b} \pm 2.32$	$124.25^{ab} \pm 1.99$		$125.00^{ab} \pm 2.45$	$129.09^{a} \pm 1.90$		$P = 0.05$
SNP2–SNP5		TT–CC (26)	TT–CT (76)	TT–TT (70)	TC–CC (17)	TC–CT (26)	TC–TT (7)	
Qinchuan	chest circumference	$178.40^{A} \pm 2.99$	$177.86^{A} \pm 3.06$	$177.41^{A} \pm 3.11$	$151.76^{B} \pm 14.62$	$180.23^{A} \pm 3.07$	$181.43^{A} \pm 5.22$	$P < 0.01$
Qinchuan	chest width	$37.94^{ab} \pm 0.91$	$38.07^{ab} \pm 0.74$	$38.43^{ab} \pm 0.59$	$35.82^{b} \pm 1.43$	$39.31^{a} \pm 1.04$	$38.29^{ab} \pm 1.25$	$P < 0.05$
Qinchuan	hucklebone width	$23.90^{a} \pm 0.91$	$23.68^{a} \pm 0.74$	$23.10^{ab} \pm 0.59$	$21.53^{b} \pm 1.43$	$23.87^{a} \pm 1.04$	$22.79^{ab} \pm 1.25$	$P < 0.05$
SNP1–SNP3		CC–CC (40)	CC–CT (56)	CC–TT (7)	CG–CC (17)	CG–CT (12)	GG–CC (6)	
Jinnan	body height	$127.70^{ab} \pm 0.93$	$129.41^{ab} \pm 0.85$	$129.57^{ab} \pm 1.76$	$131.18^{a} \pm 2.02$	$125.83^{b} \pm 1.63$	$127.33^{ab} \pm 1.45$	$P < 0.05$
Jinnan	height across the hip	$130.43^{ab} \pm 1.23$	$132.04^{ab} \pm 0.94$	$134.00^{ab} \pm 2.06$	$133.76^{a} \pm 2.37$	$127.58^{b} \pm 1.89$	$128.50^{ab} \pm 2.60$	$P < 0.05$
Jinnan	body side length	$186.00^{b} \pm 1.97$	$151.68^{ab} \pm 1.59$	$151.71^{ab} \pm 4.83$	$155.47^{a} \pm 2.35$	$144.67^{b} \pm 3.60$	$148.33^{ab} \pm 4.29$	$P < 0.05$
Jinnan	chest circumference	$152.60^{ab} \pm 2.67$	$185.50^{ab} \pm 1.86$	$184.29^{ab} \pm 5.59$	$188.59^{a} \pm 4.13$	$177.08^{b} \pm 4.09$	$177.83^{ab} \pm 5.24$	$P < 0.05$
Jinnan	rump length	$47.86^{ab} \pm 0.90$	$47.83^{b} \pm 0.63$	$46.43^{ab} \pm 1.82$	$50.71^{a} \pm 0.99$	$45.36^{b} \pm 0.89$	$47.00^{ab} \pm 3.61$	$P < 0.05$

Note: *(LSM ± SE), LSM: least squares mean; SE: standard error. The LSM values with different superscripts within the same row differ significantly at $P < 0.05$ for a and b and $P < 0.01$ for A and B.

et al., 2014). Our previous study also identified that the accuracy of T-ARMS-PCR and PCR-RFLP reached 99.07 and 99.69 %, respectively, based on the sequencing result (Zhang et al., 2015a).

Genetic diversity analysis found that three loci were not at Hardy–Weinberg equilibrium. The disequilibrium of SNP4 in the two breeds may be caused by the deficiency of genotype GG. A possible explanation for the disequilibrium is that artificial selection promotes the mutation of these loci, and these mutations only happened a few generations ago.

The association analysis found that the synonymous coding SNPs, SNP1 and SNP2, were associated with three growth-related traits. There are studies that have the same results as this study, which are that coding SNPs are associated with economically important production traits. For example, the synonymous mutation AC_000163:g.18161C>G SNP in the goat *PITX2* gene is associated with milk density in the Guanzhong dairy goat (Zhao et al., 2013). The synonymous mutation might produce codon usage bias, thereby influencing the production traits (Lan et al., 2007).

Furthermore, the different genotypes of intronic variation SNP3 were significantly associated with four growth-related traits in Qinchuan cattle. The combined genotypes containing the SNP3 locus were significantly associated with chest width in the Qinchuan breed and five other traits in the Jinnan breed. Studies showed that the intronic mutation G3072A in sheep *IGF2* was associated with skeletal muscle development (Cockett et al., 1994) and the intronic mutation AC_000163:g.18353TNC in the goat *PITX2* gene was associated with more than 10 milk production traits (Zhao et al., 2013). The intronic mutation might affect the binding of the DNA sequence and DNA binding factors, such as transcrip-

tion factors and splicing factors. Moreover, intronic mutation might influence the transcriptional efficiency as well as stability of mRNA (Zhao et al., 2013).

Furthermore, association analysis of genotypes of single SNP loci and growth-related traits is an important way to evaluate the effects of a gene in animal breeding. However, the association analysis between combined genotypes and growth related traits will be more reliable and efficient for evaluating the effects of genetic variations in a gene (Akey et al., 2001; Schaid, 2004). Thus, we analyzed the association between the combined genotypes with higher D' value and growth straits. A total of three different combined genotypes were found to have effects on four and five different growth traits in Qinchuan and Jinnan cattle, respectively. These results demonstrated the important roles of *ATBF1* single-nucleotide variations in cattle.

5 Conclusions

In the present study, three novel SNPs (SNP1, SNP2 and SNP3) and three combined genotypes (SNP1–SNP3, SNP1–SNP4 and SNP2–SNP5) in the *ATBF1* gene were significantly associated with growth-related traits in cattle. SNP1 was similarly significantly associated with the height across the hip in Qinchuan cattle ($P = 0.05$), and the heterozygote carriers had the highest value. SNP2 was significantly associated with chest circumference ($P < 0.05$) and body side length traits ($P < 0.05$) in Jinnan cattle, and the CC genotype carriers had the highest growth trait index. For SNP3, associations between *ATBF1* genotypes and body height ($P = 0.05$), chest width ($P < 0.05$), chest depth ($P < 0.01$) and hucklebone width ($P = 0.05$) of Qinchuan cattle were found, and CC carriers had the best growth trait indexes for the first three traits. Moreover, a total of three different combined genotypes (SNP1–SNP3, SNP1–SNP4 and SNP2–SNP5) were found to have effects on four and five different growth traits in Qinchuan and Jinnan cattle, respectively. Thus, SNP1, SNP2 and SNP3 have the potential to be useful DNA markers for the improvement of growth-related traits in cattle.

Data availability. The original data are available upon request to the corresponding authors.

Competing interests. The authors declare that they have no conflict of interest.

Acknowledgements. This work was supported by the National Natural Science Foundation of China (no. 31672400), Science and Technology Coordinator Innovative engineering projects of Shaanxi Province (2014KTZB02-02-02-02) and the Program of National Beef Cattle and Yak Industrial Technology System (no. CARS-37).

Edited by: Steffen Maak
Reviewed by: Faruk Balci and one anonymous referee

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