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NOVEL 16-BP INSERTION/DELETION VARIANT OF *ZNF132* GENE AND ITS INFLUENCE ON GROWTH TRAITS IN GOATS

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ABSTRACT

Zinc finger protein 132 (ZNF132) is a transcriptional repressor and plays an important role in animal production traits. Insertion/deletion (indel) polymorphisms are relatively simple and effective DNA markers. The objective of this study was to explore the potential novel indel variants within the goat *ZNF132* gene, as well as to evaluate their effects on growth traits. A novel 16-bp indel was first verified in a 5′ untranslated region (UTR) of the *ZNF132* gene, and three genotypes were detected. The minor allelic frequencies were 0.160 and zero in the Hainan black goat (HNBG) and Guanzhong dairy goat (GZ) breeds, respectively. Additionally, there were significant differences in the distribution of genotypic and allelic frequencies between these two breeds ($P< 0.01$). An association analysis revealed that the 16-bp novel indel was significantly associated with the body length of the HNBG breed ($P=0.046$), and that the individuals with genotype II exhibited the shortest body length, suggesting that the 16-bp indel significantly affected growth traits. These findings could extend the spectrum of genetic variations of the *ZNF132* gene and provide a valuable theoretical basis via indel markers for marker-assisted selection (MAS) in goat breeding and genetics.

Keywords: Goat; Zinc finger protein 132 (*ZNF132*) gene; insertion/deletion (indel) polymorphism; Growth traits; Association.

INTRODUCTION

The zinc finger (ZNF) protein family is known to exist in almost all eukaryotes, and constitutes a major subset of eukaryotic transcription factors (Seetharam and Stuart *et al*., 2013). Owing to the arrangement characteristics of the ZNF motifs, the ZNF proteins can be divided into at least seven categories, namely, Cys2His2 (C2H2), Cys4, Cys6, Cys4HisCys3, Cys3HisCys4, Cys2HisCys and Cys3His, which play key roles as either subunits of transcription proteins, splicing factors, or DNA damage repair proteins (Pennisi., 2003). The C2H2 type remains the largest group of the ZNF motifs and has been proven to affect RNA binding and protein-protein interactions (Brayer and Segal, 2008).

ZNF132 is abbreviated for Zinc finger protein 132, which is conserved in the human, chimpanzee, rhesus monkey, dog, cow, and goat. According to the NCBI website (www.ncbi.nlm.nih.gov), a total of 57 organisms have orthologs with the human *ZNF132*gene. Human ZNF132, which contains four types of conserved domains, whereas cattle have two and goats have three, is mapped in Chr.19q13.4. Additionally, it is expressed in the heart, kidney, lymph, bone, and other tissues and organs. Furthermore, the predicted structure of ZNF132 includes 18 C2H2 ZNF motifs and one Kruppel-associated box domain, suggesting that it functions as a transcriptional repressor, whereas other cellular functions remain undetermined (Abildgaard *et al*., 2012).

Based on our current results of whole genome sequencing in dairy goats (data not shown), we verified large insertion/deletion (indel) polymorphisms in the *ZNF132* gene. From the NCBI (rs645641082) database, the 16-bp indel was predicted to be in the 5′ UTR of the *ZNF132* gene in the Yunnan black goat. Moreover, the *ZNF132* gene was a related candidate gene for dairy cow mastitis and human prostate cancer (Mömke *et al*., 2005; Abildgaard *et al*., 2012). However, to date, the biological effects of the goat *ZNF132* gene on growth traits was unknown.

During the past decade, the application of indel has become increasingly popular for marker-assisted selection (MAS). As an initial source of variationsin biodiversity, indelsare widely distributed in the genome, are short-length polymorphisms characterized by low mutation rates, high inter-population diversity, short amplicon strategy, and simplicity of laboratory analysis (Tian *et al*., 2008; Zhang *et al*., 2015). Therefore, the indel marker is a useful tool for MAS (Hayashi *et al*., 2006), which could be widely and effectively applied in animal and plant sciences. To our knowledge, until date, a study of indel variations within the goat *ZNF132* gene and the effects on growth traits of these variations on growth traits has not been conducted. This study aimed to first verify the potential indel within the goat *ZNF132* gene and to analyze the relationship between the possible indel and growth traits,which would not only extend the spectrum of genetic variations of the goat *ZNF132* gene,

but would also contribute to the implementation of MAS via indel markers in the genetics and breeding of goats.

MATERIALS AND METHODS

All experiments performed in this study were approved by the International Animal Care and Use Committee of the Northwest A&F University. Furthermore, the care and use of animals completely complied with local animal welfare laws, guidelines, and policies.

DNA samples and data collection: Genomic DNA samples were isolated from 519 healthy and unrelated female individuals belonging to two indigenous Chinese goat breeds, namely, the Hainan black goat (HNBG, n = 284) and the Guanzhong dairy goat (GZ, n = 235). The HNBG goats were approximately 2 - 3 years old and were reared in native breeding farms in Zanzhou County, Hainan Province, China, and the GZ breed was from the Sanyuan GZ breeding base in Shaanxi Province (Deng *et al*., 2010; Pan *et al*., 2011; Zhang *et al*., 2015). Additionally, body measurement traits for all selected individuals were recorded, including body weight, body height, body length, chest circumference, chest depth, chest width, hucklebone width, hip width, and cannon circumference; consequently, body length index, chest circumference index, chest width index, cannon circumference index, huckle bone width index and trunk index, were calculated on the basis of our reported descriptions (Fang *et al*., 2010; Jia *et al*., 2015).

DNA isolation and genomic DNA pool construction: DNA samples were extracted and diluted to an established concentration (50 ng/μL) based on our previous report (Lan *et al*. 2007; Lan *et al*., 2013; Jia *et al*., 2015; Zhang *et al*., 2015). Fifty DNA samples were randomly selected from the two breeds to construct genomic DNA pools, which were used as templates for polymerase chain reaction (PCR) amplification to explore the potential variations within the *ZNF132* gene.

Primer design and PCR amplification: Nine pairs of primers were designed to amplify the goat *ZNF132* gene using Primer Premier software (version 5.0) based on *Capra hircus* (GenBank Accession No. NC_022310.1) (Table 1).The 5' UTR, all exons and the first intron regions were enclosed. The PCR reactions were performed using 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 (TD-PCR) protocol was as follows: 5 min at 95 °C; 2 cycles of 94 °C for 30 s, annealing from 68 °C to 50 °C by a 3 °C decrease for 30 s, 72 °C for 80 s; 35 cycles of 94 °C for 30 s, 50 °C annealing for 30 s, 72 °C for 80 s; a final extension at 72 °C for 10 min; and subsequent cooling to 4 °C. Following this, PCR products were sequenced (Zhang *et al*., 2015). The products were detected by electrophoresis on a 1.5% - 3.5% agarose gel stained with ethidium bromide.

Based on DNA sequencing, a pairs of primers (P9) was selected to detect the novel indel within the *ZNF132* gene (Table 1). The PCR amplification procedure had an initial denaturation at 95 °C for 5 min; 94 °C for 30 s; 55.9 °C annealing for 20 s; 35 cycles of 94 °C for 30 s; a final extension at 72 °C for 10 min; and subsequent cooling to 4 °C. The products were detected by electrophoresis on a 3.5% agarose gel stained with ethidium bromide.

Statistical analyses: Genotypic frequencies, allelic frequencies and Hardy-Weinberg equilibrium (HWE) were analyzed using the SHEsis program (http://analysis.bio-x.cn) (Li *et al*., 2009). Polymorphism information content (PIC) was calculated by Nei's method via our established website online *(http://www.msrcall.com/Gdicall.aspx)* (Wu *et al*., 2014; Jia *et al*., 2015). Distribution differences for genotypic and allelic frequencies between the HNBG and GZ were analyzed using the χ^2 test, which was carried out using SPSS software (Version 18.0) (International Business Machines (IBM) Corporation,New York, USA) (Pan *et al*., 2013).

Only HNBG breed was utilized for the association analyses, as the GZ demonstrated mono-polymorphism. Association tests of the 16-bp indel of the *ZNF132* gene growth related traits of the HNBG were conducted. These association analysis were performed with the procedure of procedure using SPSS software (Version 18.0) if the data conformed to the characteristic of normality and homogeneity of variances. If not, the nonparametric test (Kruskal-Wallis) was conducted using SPSS software (Version18.0). The ANOVA applied the general linear model and the statistical linear model was: $Y_{ij} = \mu+G_i+e_{ij}$, where Y_{ij} is the observation of the body measurement traits, μ is the overall mean of each trait, G_i 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).

RESULTS

By using the pool sequencing and bioinformatics analysis, the novel 16-bp indel within the goat *ZNF132* gene was first found in the 5' UTR, which was described as

"NC_022310:g.60928619insGGGGGGTTCGGGTG

AG" (also referred to as "-53 nt to -38 nt from the ATG"). The 16-bp indel of the *ZNF132* gene could be easily and clearly genotyped by direct 3.5% agarose gel detection. During the analysis, the genotype II exhibited

one band (204 bp), the DD genotype exhibited one band (188 bp), and the ID genotype exhibited two bands (204 bp, 188 bp) (Fig.1.).

As displayed in Table 1, the genotype frequencies and allelic frequencies of the 16-bp indel locus in the HNBG and GZ breeds were evaluated. The major "D" allelic frequencies were 0.840 and 1.000 for HNBG and GZ breeds, respectively. The effective allele values of this indel were 1.190 for the HNBG breed and 1.000 for the GZ breed. In addition, these loci were not at HWE in all studied breeds ($P < 0.05$).

Genotypic and allelic frequency distributions were significantly different between the dairy breed (GZ) and meat breed (HNBG) based on the χ^2 test (χ^2=16.50,P=3.0×10^{-4} for genotypic distribution; χ^2 =16.33, P=5.0×10^{-5} for allelic distribution).

The associations between the 16-bp indel and the goat growth traits were investigated (Table 2). A significant relationship was observed between this indel locus and body length in the HNBG breed (P=0.046). Moreover, the individuals with DD and ID genotypes were longer than those with genotype II. These indel loci also appear to have significant effects on certain traits such as hip width (P = 0.057), chest width (P = 0.073), and chest width index (P = 0.099). Moreover, this mutation locus has no influence on other growth related traits based on the current study.

Table 1.PCR primer sequences of the goat *ZNF132* gene for applification.

Loci	Primer sequences (5′–3′)	T_m (°C)	Product size (bp)	region	Notes
ZNF132 -1F	GCCTTCGTCCCAAGTTCCA	60.5		Exon1	clone
P1	F: GCCTTCGTCCCAAGTTCCA R: CCATCTCATCCTCTGTTGTCCC	TD-PCR	1316	Exon 1	
P2	F: GATCGGTCCTGGGTGTTC R: CCTCCCTTCTTGGCTCTGT	TD-PCR	1082	Intron 1	
P3	F: GAGCAACAGAGCCAAGAAG R: AAACCTGGGTAGGAAGCA	TD-PCR	926	Exon 2,3	
P4	F: TGGCATAATGTTGTTGAGGT R: TCTGGTGATGAATAAGGGTG	TD-PCR	1224	Exon 4	Pool DNA sequencing
P5	F: AGCACAAAGGGCAGGGAG R: GCAGAGGCGGCTGAAGA	TD-PCR	1464	Exon 4	
P6	F: ACCTCCTTCGCCATCAG R: GGACATCCCGCATATTTT	TD-PCR	1461	Exon 4	
P7	F-inner:CAGCCTTATCTATCACTGGAGAGTTCATAA R-inner:ACTGCACTCGTAAGGCCTTTCCCTAG	TD-PCR	209/188/ 341	Exon 4	
P8	F-outer:AAAGGCCTTATGAGTGTAATGAATGTGG R-outer:CCTTTCTCTGGTGTGAACTATCTGGTGT				
P9	F: GCCTTCGTCCCAAGTTCCA R:GGTCCATCAGCGACTCTGCA	55.9	188/204	5' UTR	for indel detecting

*__TD-PCR:__ Touch-down polymerase chain reaction.

Table 2. Genotypes, alleles, He, Ne, and PIC for the novel indel of the goat *ZNF132* gene.

Breeds	Sizes	Genotypicfrequencies			Allelic frequencies		[a]HWE	Population parameters			
	N	DD	ID	II	I	D	*P* values	Ho	He	Ne	PIC
HNBG	284	0.847	0.131	0.021	0.088	0.912	P<0.05	0.840	0.160	1.190	0.147
GZ	235	1.000	0	0	0	1.000	P<0.05	1.000	0	1.000	0

Note: HWE, Hardy-Weinberg equilibrium; **Ho**, homozygosity;**He**,heterozygosity; **Ne**, effective allele numbers; **PIC**, polymorphism information content;

Table 3. Relationship between the 16-bpindel of ZNF132 gene and growth related traits in HNBG breed.

Growth traits	Observed genotypes (LSM[a]±SE)			*p* values
	DD	ID	II	
Body length (cm)	**[a]56.34±0.42**	**[a]56.43±3.77**	**[b]51.37±2.31**	**0.046**
Chest width (cm)	15.06±0.18	14.80±0.47	12.97±1.13	0.073
Hip width (cm)	13.62±0.13	13.51±0.34	12.00±0.69	0.057
Chest width index	57.21±0.57	57.68±1.85	51.20±5.69	0.099

Note: The values with different letters (a and b) within the same row differ significantly at $p<0.05$.

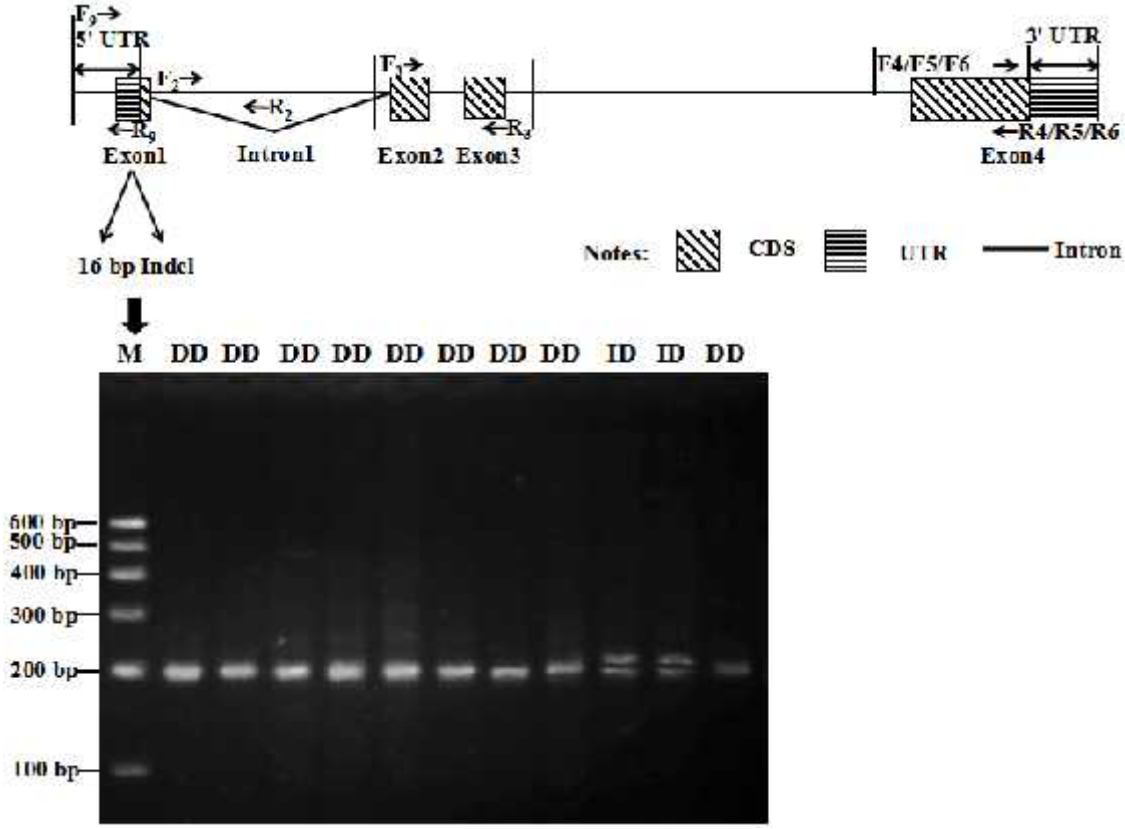

Fig. 1 Electrophoresis pattern of the novel indel variants of the *ZNF132* gene in goats.

DISCUSSION

Herein, a novel 16-bp indel (NC_022310:g.60928619insGGGGGGTTCGG GTGAG) within the 5′ UTR of the *ZNF132* gene in goats was first confirmed, and was consistent with the predicted indel region of this gene in the 5′ UTR in the Yunnan black goat based on the NCBI (rs645641082) database. The HNBG, a meat goat breed, is well-known due to its tolerance to local high temperatures and wet weather. However, this breed of goat has some notable flaws, including slow growth rates and small body sizes (Wang *et al*., 2015). In contrast, the GZ has a strong tolerance to crushed feed and local harsh weather; however, it has high milk yields and large body sizes (Deng *et al*., 2010). It is notable that there was a preponderance of genotype DD in all GZ individuals, and inferior genotypes II and ID existed in HNBG individuals, which could indicate consistency between genotype and phenotype. Moreover, the genotypic and allelic distributions of the 16-bp indel locus were significantly different between the two breeds, implying that the genotypic and allelic distributions of the novel indel were significantly associated with breed utility.

The associations between the indel locus and goat growth related traits were also analyzed. Individuals with genotype II exhibited shorter body length than those with other genotypes, which might be related to the particular structure of ZNF132 as a transcriptional repressor. The mechanism might be that the individuals with genotype II enhanced the high expression of the *ZNF132* gene, and then further inhibition effects led to low growth traits (Tovar *et al*., 2015). Many studies have also shown that indels within certain crucial genes were

associated with growth traits in livestock. For example, the 5-bp indel within the 5' UTR of the *MSTN* gene altered the functional structure of the MSTN protein so that the growth traits in goats were affected (Zhang *et al*., 2012). A promoter with the 23-bp insertion of the *PRNP* gene binding strongly to RP58 (repressor protein 58) affected production traits in cattle (Sander *et al*., 2005). The 13-bp deletion from porcine *DGAT2* gene 3' UTR appeared to stimulate its expression and led to an increase in porcine backfat thickness (Zang *et al*., 2016). Therefore, it could be speculated that the 16-bp indel was significantly associated with growth traits in goats by affecting the expression of the *ZNF132* gene, which would extend the spectrum of genetic variations of the *ZNF132* gene and provide a valuable theoretical basis for MAS via indel markers in goat breeding and genetics.

Briefly, a novel 16-bp indel located within the *ZNF132* gene significantly affected growth traits, suggesting that this indel could potentially be a useful DNA marker for the selection of high quality individuals in MAS breeding with relation to growth traits in goats.

Conflict of interest: We confirm that this manuscript has not been published in whole or in part and is not being considered for publication elsewhere. There are no any ethical conflicts of interest for all authors. The corresponding authors, Dr. XY Lan and H Chen, take responsibility on behalf of all authors for the authorship, authenticity and integrity of this manuscript, and affirms that all authors and acknowledged contributors have read and approved this manuscript.

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