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ASSOCIATION ANALYSIS BETWEEN POLYMORPHISM OF *STAT5A* GENE AND GROWTH TRAITS IN CHINESE GUIZHOU BLACK GOATS

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STAT5A gene plays a significant role in growth traits by affecting JAK/STAT pathway. We identified two SNPs which were related to growth traits from 206 healthy and adult purebred individuals of Guizhou Black goats using the methods of direct sequencing and PCR-SSCP. The association analysis between genotypes and growth traits showed that the individuals of genotype TC were superior than CC in chest girth significantly ($P<0.05$), and the individuals of GA were significant superior than GG and AA in chest girth and body weight ($P<0.05$). Besides that, the heterozygotes had higher growth traits than those of homozygotes in general. Therefore, we made a conclusion that the two SNPs of *STAT5A* gene will serve as marker-assisted selection for improving growth traits in goats, but we should still continue to prove the validity of the conclusion by enlarging the total samples and breeds.
Keywords: STAT5A gene, growth traits, Guizhou black goats, association analysis, quantitative traits.

INTRODUCTION

STAT5A is one of the significant members of STAT (signal transduction and activator of transcription, STAT) family, which contains seven members called STAT1-4, STAT5A, STAT5B and STAT6 (Zhang *et al*., 2012). STAT5 with plenty of functions, not only it can regulate the cell cycle, cell proliferation, cell differentiation and cell apoptosis (Du *et al.*, 2012), but also it plays an important role in regulating organism's growth and immune response (Lin *et al.*, 2012). The implementation of above functions is dependent on the signal transduction pathways of JAK2-STAT5 (Janus Kinase2-Signal Transducer and Activator of Transcription5), JAK3-STAT5 and STAT5-Foxp3 (Signal Transducer and Activator of Transcription5-Forkhead box P3) (Gu *et al*. 2013; Li *et al*., 2010). The protein structure of STAT5A is comprised of five conserved domains, the N-terminal domain, SH2 domain, coiled-coil domain, DNA-binding domain and amino-terminal region. Every part of them has unique feature in structure and function. For instance, N-terminal domain and SH2 domain are higher conserved than other domain, the coiled-coil domain plays a role in promoting the interaction of proteins, the DNA-binding domain which containing some β-sheets is situated in the centre of the protein, and the last domain has the important effects on associating two separate STAT5A dimers (Bradshaw and Dennis, 2011; Kramer and Moriggl, 2012).
STAT5A, which plays a significant role in cell activity, immune system, cancer regulation mechanism and so on (Liang *et al*., 2009; Vafaizadeh *et al*., 2010; Peck *et al*., 2011), is discovered as a MGF (mammary gland factor, MGF) regulating the milk protein initially. However, in recent years, a large number of studies have showed that STAT5A has close connection with growth performance of animals. For example, STAT5A is found to be able to control the growth traits by influencing the growth hormone in pigs (Fang *et al.*, 2012), STAT5A is also proved to play a negative regulation role in cell proliferation with STAT5B in mice (Yu *et al*., 2010), two linked mutation sites in the intron 9 of *STAT5A* gene may affect the growth traits of Laoshan dairy goats in body weight, body height and chest girth significantly.
Guizhou Black goat is a breed of Chinese goat whose origins can be traced back to the Han Dynasty. The breed is known as its crude feed tolerance, higher disease resistance, delicious and tender meat, which superior than normal goats. However, it has some shortcomings in growth rate and growth traits. Compared with the traditional feeding and breeding, Marker-assisted selection (MAS) has unique advantages of improving the growth traits on molecular level, such as shorter cycle, more obvious effect and higher profit. The elementary research is aimed to determine the useful SNPs in the intron of *STAT5A* gene and their effects on the growth traits in Guizhou Black goats.

MATERIALS AND METHODS

***Sample and date collection*:** A total of 206 healthy and adult purebred individuals of Guizhou Black goats obtained from the Pengteng Ecological Agriculture and Animal Husbandry

Comprehensive Development Co., Ltd., all the goats were included in the research under the basic same feeding management condition. Collecting the blood and recording the date of growth traits including body weight, body height, body length, hucklebone width and chest girth of total individuals separately.

The DNA was extracted by using the genomic DNA kit (Sangon Biotech Co., Ltd. Shanghai), then the concentration of DNA was adjusted to the same concentration and used to construct DNA pool.

***Primer design and PCR amplification*:** On the basis of *STAT5A* gene sequence (GenBank accession No: NC_019468.1) from NCBI database, the PCR Primer Design Software of Primer Premier 5.0 was used to design 2 pairs of specific primers, Exon 7 and 10 were amplified to detect the SNPs. 5' of *STAT5A* in the Exon 7: CAGGGCCTTCTTCCTCATGG, 3' of *STAT5A* in the Exon 7: GACTGTAGCACATCCAGGCT. 5' of *STAT5A* in the Exon 10: CCAGGGTGCATACAGGACAG, 3' of *STAT5A* in the Exon 10: GAGGAGAACGCAGCAGGTTA.

Primers above were synthesized by Sangon Biotech (Shanghai) Co., Ltd. PCR reaction mixture: 2.0μl DNA template, 1.5μl 10 pmol/μl upstream primer, 1.5μl 10 pmol/μl reverse primer, 10.0μl 2xEs Taq MasterMix, and ddH_2O to a final volume 20.0μl. PCR producer system: initial denaturation at 95°C for 5 min, 30 cycles of denaturation at 95°C for 30 s, annealing at 65.0/58.0°C for 30 s, extension at 72°C for 30 s, and one final extension at 72°C for 10 min. The PCR products were examined by 1% agarose gel electrophoresis (Fig. 1), then used to conduct the bi-directional sequencing.

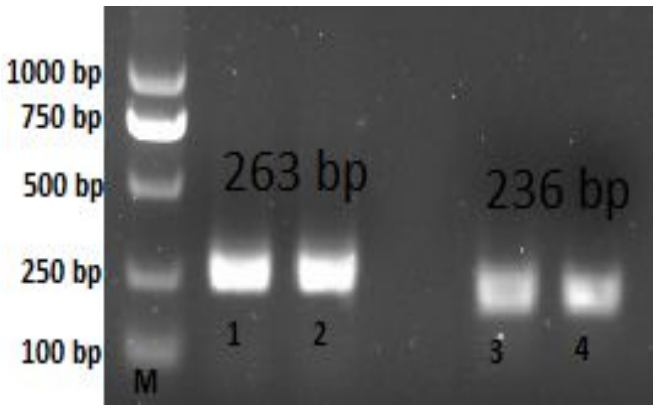

Figure 1. Amplification results of two pairs of primers in Guizhou Black Goats. M: DL1000 DNA Marker; 1,2: PCR product of Exon 7; 3,4: PCR product of Exon 10.

***Statistical analysis*:** Firstly, the date of genotype frequency was recorded, then the Chi-Square Goodness-of-Fit Test was conducted to build the model of the least square method. The phenotypic value of Individual traits (Y_{ijk}) was calculated by:

$$Y_{ijk} = \mu + marker_k + e_{ijk.}$$

Where μ was the general mean, $marker_k$ was the effect of marker genotype, and e_{ijk} was the random error. The association analysis between different genotypes and growth traits were analyzed by the least square method in SPSS 19.0 GLM (general liner model), the statistical results were present as means ± standard error.

RESULTS

By the sequence analysis of DNAstar and BLAST softwares, the two SNPs T11289C and A14320G (Fig. 2) were detected in intro 7 and 10 separately

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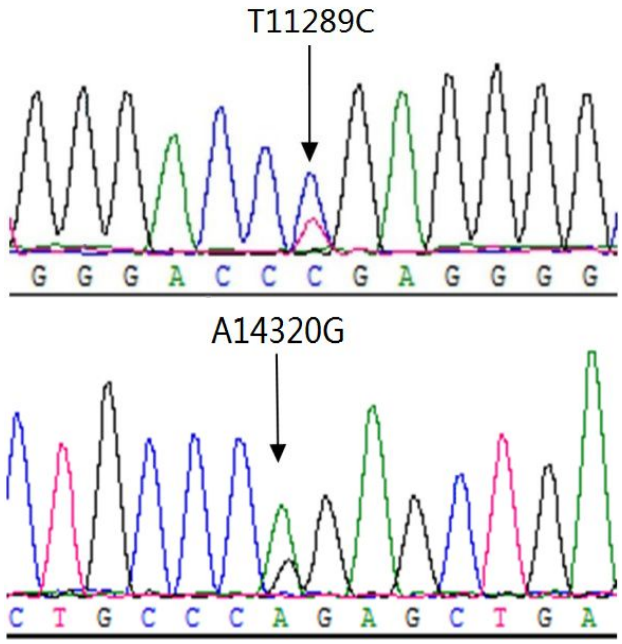

Figure 2. The sequencing atlas of two SNPs with DNA pool. The peak under arrow means the mutation site.

***Genotyping and Sequencing*:** The sequencing results showed that the T11289C mutation included two genotypes called TT and TC, but the A14320G mutation includes three genotypes named GG, GA and AA (Fig. 3). The genetic parameters of two SNPs were both analyzed, the data showed that the frequency of dominant genotype CC and GG were 0.7294 and 0.9029, and the polymorphism information content (PIC) were 0.2062 and 0.0959, which were both low polymorphism (Table 1). The T11289C locus conformed to Hardy-Weinberg equilibrium ($P>0.05$), but the A14320G locus deviated from it ($P<0.05$).

Table 1. The analysis of genetic characters of T11289C and A14320G loci.

SNPs	Genotype	Individual Number	Genotype frequency	Allele	Allele frequency	Effective number of alleles (Ne)	Homo-zygosity (Ho)	Hetero-zygosity (He)	Poly-morphism information content (PIC)	χ^2
T11289C	CC	150	0.7282	C	0.8641	1.3070	0.7651	0.2349	0.2073	1.4066
	TC	56	0.2718	T	0.1359					
A14320G	GG	186	0.9029	A	0.0534	1.1123	0.8990	0.1010	0.0959	1.8436
	AA	2	0.0097	G	0.9466					
	GA	18	0.0874							

Note: *PIC*>0.5 means high diversity, 0.25<*PIC*<0.5 means moderate diversity, *PIC*<0.25 means low diversity. The experimental animal is Guizhou Black goat, and the study was carried in Key Laboratory of Animal Genetics, Breeding and Reproduction in the Plateau Mountainous Region, Ministry of Education, College of Animal Sciences, Guizhou University.

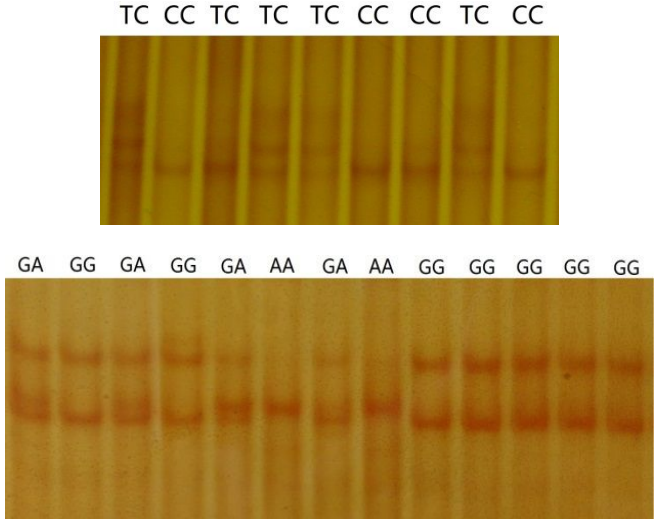

Figure 3. The electrophoresis patterns of PCR-SSCP of T11289C and A114320G loci. Different electrophoresis band types indicate different genotypes.

***Association analysis*:** Five growth traits including body weight, body height, body length, hucklebone width and chest girth, were analyzed by SPSS 19.0 GLM according to the data of different genotypes. Table 2 and Table 3 shows the relationship between two variation loci and growth traits in 206 healthy and adult purebred Guizhou Black Goats. From the tables, it was obvious that the individuals of genotype TC are superior than CC in chest girth significantly, and the individuals of GA are superior than GG and AA in body weight and chest girth significantly too. Furthermore, the heterozygotes (TC and GA) had higher growth traits than those of homozygotes (CC and GG, AA) in general. Meanwhile, the two SNPs were also proved to have important influence on growth traits.

Table 2. Association analysis between two genotypes and growth traits of T11289C locus.

Growth Traits	Genotypes	
	CC(150)	TC(56)
Body Weight/kg	35.13±0.73	37.33±1.20
Body Height /cm	58.46±0.55	58.78±0.91
Body Length /cm	63.69±0.55	64.49±0.90
Hucklebone Width /cm	8.45±0.07	8.37±0.12
Chest Girth /cm	79.94 ± 0.56^{a}	82.85 ± 0.92^{b}

According to three genotypes of A14320G locus, we concluded the results of genetic effects of quantitative traits. Table 4 shows the calculated value of five quantitative traits including homozygote additive effect (a), heterozygote dominant effect (d), genotype value mean (μ), the average effect of gene (α), and breeding value (A). The results suggested that the genotype GA has higher breeding value.

Table 3. Association analysis between three genotypes and growth traits of A14320G locus.

Growth Traits	Genotypes		
	AA(2)	GA(18)	GG(186)
Body Weight/kg	32.50 ± 6.25^{ab}	39.70 ± 2.09^{b}	35.20 ± 0.65^{a}
Body Height /cm	52.40±4.62	59.84±1.54	58.45±0.48
Body Length /cm	61.50±4.78	64.61±1.59	63.68±0.50
Hucklebone Width /cm	8.50±0.63	8.54±0.21	8.42±0.07
Chest Girth /cm	77.00 ± 4.87^{ab}	83.89 ± 1.62^{b}	80.31 ± 0.51^{a}

Note: Above data stands for "mean ±SE". Figures in brackets denote the individual number of each genotype. Within the same line, values with different superscripts lowercase letters differ (*P*<0.05).

Table 4. Genetic analysis of quantitative traits at G14320A locus of *STAT5A* gene.

SNPs	Growth traits	Genotype	Allele	a	d	μ	α	A
A14320G	Body weight	GG	G	1.21	0.59	1.80	3.67	3.46
		GA	A				-0.21	7.34
		AA						-0.41
	Chest girth	GG	G	1.48	0.53	2.01	2.86	2.70
		GA	A				-0.16	5.72
		AA						-0.32

DISCUSSION

STAT5A has many biological functions as one of the important member of STAT family. STAT5A can not only affect the tumorigenesis, mammogenesis and immune system mechanism (Huang et al., 2013), but also influence the cell proliferation, cell differentiation and cell cycle with STAT5B (Shain *et al*., 2009). Meanwhile, the researches in domestic pigs, cows and Laoshan dairy goats have proved that STAT5A has significant effect on growth performance of animal. Intron is a stretch of DNA that interrupts a gene and does not contribute to the specification of a protein. The function of intro is still not clear, but some of them can observably enhance the efficiency of gene expression. Such as the intron of tobacco mosaic virus 35S is able to enhance the promoter activity in monocotyledons and dicotyledons. A previous research has proved that non-coding RNA (ncRNA) can also act a guiding part in gene expression repression, suggesting that intronic transcripts have some effects on transcriptional regulatory machineries (Heo and Sung, 2011).

In this research, we used the means of Polymerase Chain Reaction-Single Strand Conformation Polymorphism (PCR-SSCP) and direct sequencing to screen the SNPs, which associated with growth traits. The two SNPs had different effects on growth traits in Guizhou Black Goats, T11289C had two genotypes CC and TC, the relevance of the results showed the individuals of TC were superior than CC in chest girth significantly, meanwhile the numerical value of other growth traits but hucklebone width in individuals of genotype TC were higher too. However, A14320G had three genotypes GA, GG and AA, the relevance of the results showed the individuals of genotype GA were superior than GG and AA in chest girth and body weight, meanwhile the numerical value of other growth traits in individuals of genotype GA were higher too. In addition, the results of genetic effects of quantitative traits also showed that the breeding value of individuals of genotype GA was higher, which suggested the genotype GA has higher value in animal breeding.

In conclusion, our research has proved that the two SNPs can promote growth traits by affecting *STAT5A* gene in Guizhou Black Goats. The individuals of genotype TC and GA are superior than other individuals of genotypes in growth traits, so it seemed that *STAT5A* gene can serve as a candidate gene for goat breeding in further research. However, more goat breeds and data of growth traits need to be collected for the further study to confirm its function.

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