

Analysis of A Sequence Nucleotide Polymorphism of *STAT5A* Gene in Garganica Goat Breed

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Abstract— STATs (signal transducer and activator of transcription) are a group of transcription factors that mediate actions of a variety of peptide hormones and cytokines within target cells (for example prolactin and growth hormone). In particular, *STAT5A* gene is a candidate marker for quantitative traits in farm animals. In this study, the *STAT5A/Eco81I* polymorphism was investigated with PCR-RFLP in a sample of Garganica goats. Garganica breed is an Italian goat breed that originates in the Gargano promontory, in Apulia region, by crossing the autochthonous population of goat with west European goats. Garganica breed show an exceptional ability to adapt to particularly difficult environments, as well as an extraordinary capacity to utilize poor pasture that would not otherwise be used. The investigated polymorphism is a substitution C→T at position 6852 within the exon 7 of the *STAT5A* gene. Only two out of three possible genotypes were identified in the population. The allelic frequencies of alleles C and T were 0.863 and 0.137 respectively and the population was kept in Hardy-Weinberg equilibrium. Moreover, some population genetic indices were also reported.

Keywords— *STAT5A* gene; single nucleotide polymorphism; Garganica goat.

I. INTRODUCTION

The STAT (signal transducer and activator of transcription) family has seven members (*STAT1-4*, *STAT5A*, *STAT5B*, and *STAT6*). They play a prominent role as mediators of many hormone and cytokines within target cells. *STAT5*, also known as mammary gland factor, was discovered initially as a PRL-induced transcription factor [1]. *STAT5* is known as a main mediator of growth hormone action on target genes; it is a key intracellular mediator of prolactin signalling and can activate transcription of milk protein genes in response to prolactin [2], [3]. In different species, *STAT5* comprises two kinds of isoforms (*STAT5A* and *STAT5B*), encoded by two different genes which share a high degree of sequence homology (~90%). *STAT5A* and *STAT5B* demonstrate significant differences in the DNA binding specificities and tissue expression and distribution [4],[5]. *STAT5A* gene has been investigated in bovine species as a possible candidate gene affecting milk production and body growth traits [6]-[13]. The *STAT5A* gene has been mapped to chromosome 19 in goat and cattle, and to chromosome 11 in sheep [14]. It is well known that the autochthonous breeds have an economical weight in marginal areas of southern Italy. Most

of Italian goat breeds are represented by small populations with peculiar characteristics and productive traits which depend on the variability of environmental conditions and genetic makeup. Garganica breed show an exceptional ability to adapt to particularly difficult environments, as well as an extraordinary capacity to utilize poor pasture that would not otherwise be used. This breed originates in the Gargano promontory in Apulia region by crossing the autochthonous population of goat with west European goats [15]. Garganica goats are reared also in other regions in southern Italy (Basilicata, Campania, Calabria). This breed is medium-sized; animals have black glossy hair that may have some reddish shade and long twisted horns in both sexes. Nowadays, the Garganica goat breed is included in the list of Italian endangered breeds drawn up by the Italian Department for Environment, Food, and Rural Affairs (MiPAF, 2007). To date, only few polymorphisms of *STAT5A* gene were detected in goat species and few studies have been carried out on this concern [16], [17]. The aim of the present study was to investigate the *STAT5A/Eco81I* polymorphism in a sample of Garganica goat. This polymorphism is a substitution C→T at position 6852 within exon 7 [16]. Moreover, some genetic population indices were also reported.

II. MATERIALS AND METHODS

A. Determination of *STAT5A/Eco8II* polymorphism

A total of 40 goats belonging to Garganica breed were included in this study. Individual blood samples for DNA genotyping were collected on K3-EDTA tubes and stored at -25°C. Genomic DNA was isolated from whole blood using ZR Genomic DNA II Kit™ (Zymo Research). After genomic DNA isolation, all the samples were genotyped for *STAT5A/Eco8II* polymorphism by PCR-RFLP technique [16].

The following PCR primers were used:

-CTGCAGGGCTGTTCTGAGAG (forward)

-TGGTACCAGGACTGTAGCACAT (reverse)

to amplify a 215 bp PCR fragment, covering the seventh exon of the *STAT5A* gene.

The *STAT5A* gene fragment was amplified using thirty-five amplification cycles at the following conditions: 94°C/30 s 63°C/30 s and 72°C/30 s, with initial denaturation at 94°C for 5 min, and final extension at 72°C for 10 min.

The 215 bp PCR product was digested with *Eco8II* restriction endonuclease (Thermo Scientific, Waltham, MA, USA) at 37°C for 3 hours and then analyzed on a 3% agarose gel stained with ethidium bromide, in TBE buffer. The gels were examined under UV light. The nuclease cuts the 215 bp amplification product into 162 and 53 bp fragments for allele C, and 126, 53 and 36 bp for allele T.

B. Statistical analysis

The allele frequencies were calculated by simple allele counting according to the Hardy-Weinberg equilibrium [18]; the possible deviations of genotypic frequencies from expectation were tested by a Chi-square.

Population genetic indices, namely gene heterozygosity (H_e), gene homozygosity (H_o), effective allele numbers (N_e) and fixation index (F_{IS}) were performed by POPGENE32 software version 1.32 [19]. Moreover PIC (Polymorphism Information Content) were calculated according to Botstein et al. [20].

III. RESULTS AND DISCUSSION

In the investigated population, only two out of three possible genotypes were found; in particular the frequencies of CC and CT individuals were 72.50 and 27.50%, respectively and no individuals carrying TT genotype were found. Moreover, the frequencies of alleles C and T were 0.863 and 0.137, respectively (Table I). The distribution of genotypes was in Hardy-Weinberg equilibrium ($\chi^2=1.017$, $df=1$, $P=0.313$). The lack of TT genotype was also reported by An et al. [16] in Xinong Saanen and Guanzhong breeds. A very low frequency of TT individuals was subsequently reported by Wu et al. [17] in Xinong Saanen and Hainan black populations. In Table II, the calculated genetic indices at the *STAT5A/Eco8II* locus were reported. Gene homozygosity (H_o), gene heterozygosity (H_e), effective allele numbers (N_e) and fixation index (F_{IS}) were shown in Table II. F_{IS} is a measure of the deviation of genotypic frequencies from panmictic frequencies in terms of heterozygous deficiency or excess. Negative F_{IS} values indicate heterozygote excess and positive values indicate heterozygote deficiency compared with Hardy-Weinberg

equilibrium expectations. As reported in Table II, a slight excess of heterozygosity (negative F_{IS} value) was found. This result was also supported by the value of N_e ; in fact, the higher the value of N_e , the less homozygous are the alleles studied. The polymorphic information content (PIC) is a parameter indicative of the degree of informativeness of a marker. The PIC value may range from 0 to 1. In the studied population, PIC values was 0.209. According to the classification of PIC (low polymorphism if PIC value < 0.25, median if $0.25 < \text{PIC value} < 0.50$, and high if PIC value > 0.50), the investigated locus possessed low genetic diversity.

The genetic variability at *STAT5A/Eco8II* locus was previously investigated in four different populations from China, as showed in Table III. The allelic frequencies found in Garganica goat breed were similar to those observed in Hainan black breed and, interestingly, the frequency of the C allele is higher if compared with the frequency of allele T in all the investigated samples. An et al. [16] studied the association among genotypes at *STAT5A/Eco8II* locus and milk performance traits of animals: the does with CT genotype have greater milk yield than those carrying CC genotype ($P < 0.05$). Moreover, Wu et al. [17] found a positive relationship among *STAT5A/Eco8II* polymorphism and some phenotypic traits.

IV. CONCLUSIONS

The investigated polymorphism may play an important role in affecting milk production and growth traits of goats. However, further investigations are needed to help the investigating of the role of the studied polymorphism on production traits in goat species.

TABLE I
OBSERVED AND EXPECTED GENOTYPE FREQUENCIES AND ALLELIC FREQUENCIES AT *STAT5A/Eco8II* LOCUS IN GARGANICA GOAT BREED

SNP	Position	Genotypic frequencies (%)		Allelic frequencies		
		Observed	Expected			
<i>STAT5A/Eco8II</i>	Exon 7	CC	72.50	74.39	C	0.863
		CT	27.50	23.72	T	0.137
		TT	0	1.89		

TABLE III
POPULATION GENETIC INDICES CALCULATED AT THE *STAT5A/Eco8II* LOCUS

H_o	H_e	N_e	PIC	F_{IS}
0.725	0.275	1.311	0.209	-0.159

H_o , gene homozygosity; H_e , gene heterozygosity; N_e , effective allele number; PIC, polymorphism information content; F_{IS} , fixation index

TABLE IIIII
FREQUENCIES OF THE ALLELES *STAT5A T* AND *STAT5A C* IN DIFFERENT GOAT POPULATION AND GENETIC POPULATION INDICES OBSERVED BY OTHER AUTHORS

Breed	Allelic frequencies		H_e	N_e	PIC	References
	C	T				
Xinong Saanen	0.638	0.362	0.472	1.858	0.355	[17]
Hainan black	0.921	0.790	0.146	1.171	0.135	[17]
Xinong Saanen	0.700	0.300	0.610	-	0.330	[16]
Guanzhong	0.720	0.280	0.560	-	0.320	[16]

H_e , gene heterozygosity; N_e , effective allele number; PIC, polymorphism information content

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