Bovine STAT5A Gene Polymorphism Analysis and Its Association with Milk Composition Traits in Jersey Cows

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***Abstract*—In mammals, the STAT proteins (signal transducers and activators of transcription) are a family of cytoplasmic transcription factors mediating the actions of many peptide hormones and cytokines within target cells. In particular, STAT5A is a crucial mediator in the lactogenic hormone response being a candidate marker for milk traits in farm animals. In the present paper, the T→C nucleotide polymorphism at position 12743 in exon 16 of the bovine STAT5A gene was analyzed with PCR-RFLP in a sample of Jersey cows. The purposes of this investigation were to determine the frequencies of the variant alleles and the genotypes of this SNP in Jersey cows and to verify its association with some milk production traits. All the three possible genotypes were identified in the studied population. The observed frequencies of *C* and *T* alleles were 0.147 and 0.853 repectively. The TT genotype was the most frequent followed by TC and CC ones. No significant differences between the TT and TC genotypes were found considering MY, FY PC and PY. On the other side, the difference concerning the fat content of milk produced by cows belonging to TC and TT groups was found significant at the statistical analysis: in particular, milk from TT animals had a higher fat content in comparison with that of TC ones (4.55 *vs*. 4.14%, respectively; *P*<0.05). However it may be necessary to carry out further investigations about this SNP to better clarify its role on milk production traits in cattle.**

***Index Terms*—Jersey breed, milk production, SNP, STAT5A gene.**

I. Introduction

The recent advances of molecular genetics in the identification of loci affecting production traits of domestic animals have opened new and interesting opportunity for their genetic improvement. With the use of molecular technologies, it may be possible to select a breeding animal for a wide range of traits and to enhance reliability in predicting the mature phenotype of the individual. Milk production traits, which are under control of several genes, are very important in dairy cattle due to their economic relevance: to improve milk yield and composition is of great significance for breeders.

The STATs proteins (signal transducers and activators of transcription) comprise a 7-member family of latent cytoplasmic transcription factors that mediate actions of many peptide hormones and cytokines within target cells [1], [2]. They act as signal transducers in the cytoplasm and transcription activators in the nucleus. The DNA-binding capacity of STATs is induced by phosphorylation of a tyrosine residue at the C-terminus of the protein, which leads to dimerization and nuclear localization, and this has been shown to occur in response to a wide range of hormones and cytokines. STAT5 was initially discovered as a PRL-induced transcription factor and named mammary gland factor (MGF) [3]. STAT5 is also known as a main mediator of growth hormone (GH) action on target genes [4]; it is a key intracellular mediator of prolactin signalling and can activate transcription of milk protein genes in response to prolactin [3]. Initially, a single STAT5 gene was identified in sheep but subsequently two forms of STAT5 (A and B), encoded by two different genes, have been identified in mouse, human, rat and cattle cells [5]-[12]. The genes encoding STAT5A and STAT5B are derived from a single ancestral gene [13]; they are highly homologous, being ~90% identical in coding sequence. The two isoforms differ by few amino acids in the carboxylic end of the protein molecule [14]. Moreover, STAT5A and STAT5B show differences both in their DNA binding specificities [15], [16] and with respect to their tissue distribution [7], [8]. In cattle the STAT5A gene has been assigned to chromosome 19q17 and consists of 19 exons encoding 794 amino acids chain [13]. The STAT *locus* also contains STAT3 and STAT5B genes [13], [17].

Several nucleotide sequence polymorphisms of the bovine STAT5A gene (GenBank AJ242522 and AJ237937) have been detected: McCracken *et al.* [18] found TG repeats of different length within intron 12; Antoniou *et al.* [19] described two SSCP variants of the gene fragment that encodes the SH2 domain in bovine STAT5A protein; Brym et al. [20] reported a new SNP (A/G) located in intron 9 at position 9501. Khatib *et al.* [21] studied many SNPs in STAT5A gene and their association with embryonic survival and milk composition. Flisikowski and Zwierzchowski [22] reported a new single nucleotide polymorphism in exon 7 of the bovine STAT5A gene also investigated by other authors [23]-[27]. Moreover, Flisikowski and Zwierzchowski [28] described the substitution T→C at position 12743 within exon 16, which changed the amino acid sequence (Val→Ala at position 686) and a deletion of CCT in intron 15, also investigated by He *et al.* [29] in Holstein cows. The SNP T→C at position 12743 was shown to modify the DNA-binding properties of the protein. In the DNA-protein binding assays (electrophoretic mobility shift assay – EMSA), nuclear proteins derived from CC genotype animals always

Manuscript received January 15, 2013; revised March 22, 2013.

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showed less DNA protein complexes than those of a TT animals [30].

In this study, the substitution T→C at position 12743 within exon 16 was investigated with PCR-RFLP method in Jersey cows. There is an increasing interest for the Jersey breed that is mainly due to the characteristics of milk produced: it is excellent in terms of quality for the high fat, protein and calcium content and the low non-protein nitrogen content. Moreover, Jersey cattle ability to adapt to different breeding types and to different environmental condition contributed greatly to the breed's current success worldwide. In Italy, over the last years there has been a fairly good increase in the number of Jersey breeders even if most of the Jersey cows are bred in mixed herds with Holsteins or other breeds. That being so, the purposes of this investigation were to determine the frequencies of the variant alleles and the genotypes of the SNP in exon 16 of the bovine STAT5A gene in a sample of Jersey cows and to verify the association between this SNP and some milk production traits.

II. Materials and Methods

A total of ninety-five unrelated cows belonging to Jersey breed were included in the study. The animals were all primiparous and were milked twice a day. The cows, calved in two different seasons, belonged to 3 different farms located in southern Italy. They were fed with the same lactation diet, according to the energy recommendations for lactating cows, and they had free access to water.

Individual blood samples for DNA genotyping were collected from all cows on K_3-EDTA tubes and stored at -25 ℃. 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 the gene polymorphism in exon 16 of STAT5A gene as previously described by Flisikowski et al. [30].

The following PCR primers were used STAT5A_F – 5'-AGC CCT ACA GCT CCA ATC CT-3' and STAT5A_R – 5'-GGG TGT ACC CGC TGC TTA G-3 to amplify a 281-bp PCR fragment harboring parts of intron 15 and exon 16 of the STAT5A gene. The polymerase chain reactions (PCR) were performed using a PCR-mix with: each primers at a final concentration of 2 pmol/µl, 1 U Taq polymerase (SIGMA), 1 µl Taq polymerase buffer, dNTPs of 2.0 mM/µl, ca. 100 ng of genomic DNA, and H2O up to 10 µl. The following PCR protocol was used: 1 min at 94 ℃, 1 min at 61 ℃, and 1 min at 72 ℃ – 34 cycles. The yield and specificity of the PCR reactions were both evaluated by electrophoresis of the amplified fragments in 2% agarose gel stained with ethidium bromide in TBE buffer. The PCR products were digested in 10-µl aliquots with 10 U of *Msl*I restriction nuclease (BioLabs, New England, USA) for 3 hours at 37 ℃. The restriction fragments were subjected to electrophoresis in 2% agarose/ethidium bromide gels in TBE buffer. The gels were examined under UV light.

The STAT5A allele frequencies were calculated by simple allele counting [31]. The polymorphism was tested for deviation from Hardy-Weinberg equilibrium (HWE) by comparing the observed and expected genotype frequencies using the χ^2 test. Data for a 305-day milk production including milk yield (MY), protein (PC) and fat (FC) content were obtained from the local breeder association; fat and protein yielded (FY and PY, respectively) were calculated.

Effects of polymorphic variants of the STAT5A gene on milk production traits were analyzed using the GLM procedure of SAS [32] according to the following statistical model:

$$Y_{ijkl} = \mu + G_i + M_j + F_k + e_{ijkl}$$

where: Y_{ijkl} is the analysed trait of each cow; µ is the overall mean; G_i is the fixed effect of the i^{th} genotype (1, 2); M_j is the fixed effect of j^{th} season of calving (1, 2); F_k is the fixed effect of k^{th} farm (1,...,3); e_{ijkl} is the random error. Due to the low number of CC cows found in the population, this genotype was not included in the statistical analysis; in fact the number of CC cows is not enough to provide an accurate statistical analysis.

III. Results and Discussion

The 281-bp fragment contains two *Msl*I restriction sites, only one of these appears to be polymorphic. The T→C substitution deletes one cutting site of *Msl*I. All the three possible genotypes were identified. The observed frequencies of C and T alleles were 0.147 and 0.853 respectively being quite similar to those reported in other breeds (see Table I). The TT genotype was the most frequent in the studied population (73.68%) followed by TC (23.16%); only three animals were genotypes as CC (3.16%). As reported in Table II, the distribution of the genotypes was kept in Hardy-Weinberg equilibrium: the calculated $\chi 2$ value was 0.59 (P=0.45; d.f.=1).

TABLE I: Frequencies of C and T Alleles in Jersey Breed and in Different Cattle Breeds as Reported by Other Authors. Allele Frequencies Are Shown in Decreasing Order for the T Allele

Breed	No.	Allelic Frequencies		References
		T	C	
Simmental	11	0.909	0.091	Flisikowski *et al.* [30]
Aberdeen Angus	10	0.900	0.100	Flisikowski *et al.* [30]
Hereford	16	0.875	0.125	Flisikowski *et al.* [30]
Jersey	95	0.853	0.147	Present work
Polish Friesian	150	0.850	0.150	Flisikowski *et al.* [33]
Limousine	16	0.812	0.188	Flisikowski *et al.* [30]
Charolaise	18	0.805	0.195	Flisikowski *et al.* [30]
Polish Friesian	37	0.756	0.244	Flisikowski *et al.* [30]

TABLE II: Observed and Expected Numbers and Percentages (in brackets) of STAT5A Genotypes Detected by MslI RFLP Analysis and Allele Frequencies in the Sample of Jersey Cows

Number	STAT5A/*Msl*I Genotype		
	TT	TC	CC
Observed	70 (73.68%)	22 (23.16%)	3 (3.16%)
Expected	69.06 (72.70%)	23.87 (25.13%)	2.06 (2.17%)
X^2 =0.59 P=0.44			

Data reported in Table III show the effects of the STAT5A/*Msl*I polymorphism on milk production traits. No significant differences between the TT and TC genotypes were found concerning MY (5888.20 vs. 5896.74 kg), FY (261.81 vs. 242.54 kg), PC (3.77 vs. 3.79%) and PY (219.45 vs. 223.17 kg for TT and CT respectively). On the other side, the difference concerning the fat content of milk produced by cows belonging to TC and TT groups was found significant at the statistical analysis: in particular, milk from TT animals had a higher fat content in comparison with that of TC ones (4.55 *vs*. 4.14%, respectively; $P<0.05$).

TABLE III: MEANS AND STANDARD ERROR OF MILK PRODUCTION TRAITS IN JERSEY COWS WITH DIFFERENT STAT5A/MSLI GENOTYPES

Production Traits	Genotypes	
	tt	tc
Milk yield (MY) (kg)	5888.20 ±96.63	5896.74 ±141.33
Fat (FC) (%)	4.55 ±0.04a	4.14 ±0.10b
Fat (FY) (kg)	261.81 ±2.84	242.54 ±6.33
Protein (PC) (%)	3.77 ±0.02	3.79 ±0.06
Protein (PY) (kg)	219.45 ±1.49	223.17 ±4.78

a, *b*=*P* <0.05

Even if, on the basis of these results, it is possible to suppose a positive effect of the T allele on milk fat content, the low number of CC cows in the studied population does not permit the evaluation of the performances of this genotype and a real comparison among genotypes. However, the reason why the frequency of CC individuals in Jersey breed is very low may be indirectly due to the selective pressure.

In a previous study conducted on Polish Friesian cows, Flisikowski *et al.* [33] investigated the association among the STAT5A/*Msl*I polymorphism and some milk production traits finding results that partially agree with those reported in the present paper. In particular, they found no relationship between the studied SNP and protein and fat content of milk. On the other side, the same authors observed that cows carrying TC genotype produced daily significantly ($P\leq0.05$) more milk and fat corrected milk (FCM) than TT homozygotes, with higher ($P\leq0.05$) content of lactose. Moreover, the daily yield of value corrected milk (VCM), milk total solids, solids-non-fat, protein, and lactose was higher ($P\leq0.01$) in cows with TC if compared to those genotyped as TT.

However, taking into account the relatively small size of the studied population, the present results should be interpreted as an association between the investigated SNP and milk traits in this population. In order to confirm these results, further investigations, including also other breeds, are necessary to better clarify the role of this SNP on production traits in cattle.

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