Preliminary study on the relationship between a SNP in exon 16 of the STAT5A gene and some milk composition traits in Jersey cows

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Abstract

In mammals, the STAT proteins (signal transducers and activators of transcription) are a group of cytoplasmic transcription factors that mediate the actions of many peptide hormones and cytokines within target cells. 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. In this study, the T→C nucleotide polymorphism at position 12743 in exon 16 of the bovine STAT5A gene was investigated with PCR-RFLP in a sample of Jersey cows. The T→C transition at position 12743 changes the amino acid sequence in the STAT5A protein and it has already been shown to modify the STAT5A DNA-binding properties. The objectives of this study were to estimate the allele and genotype frequencies of this polymorphism in Jersey cows and to determine associations between this SNP and some milk production traits. All the three possible genotypes were identified in the studied population. The observed frequencies of *C* and *T* alleles were 0.14 and 0.86. The TT genotype was the most frequent followed by TC and CC genotype. No association between genotype and fat and protein content were found. However it may be necessary to carry out further studies about this polymorphism to better clarify the role of this SNP on production traits in cattle.

Key words: STAT5A, gene polymorphism, Jersey breed, milk

1. Introduction

Molecular markers can play an important role for animal genetic improvement through conventional breeding strategies. Availability of information, particularly for those *loci* which affect the performance traits may be important tools in breeding programs. The use of molecular genetics technologies potentially offer a way to select a breeding animal for a wide range of traits and to enhance reliability in predicting the mature phenotype of the individual.

The STATs proteins (signal transducers and activators of transcription) are a 7-member family of latent cytoplasmic transcription factors that mediate actions of many peptide hormones and cytokines within target cells (Darnell et al., 1994; Schindler & Darnell, 1995). 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 localisation, and this has been shown to occur in response to a wide range of hormones and cytokines. STAT5, also known as mammary gland factor (MGF), was initially discovered as a PRL-induced transcription factor (Wakao et al., 1994). STAT5 is known as a main mediator of growth hormone (GH) action on target genes (Argetsinger & Carter-Su, 1996); it is a key intracellular mediator of prolactin signalling and can activate transcription of milk protein genes in response to prolactin (Wakao *et al.* 1994). The STAT5 factors interact and functionally synergize with receptors for glucocorticoid and insulin (Lechner et al., 1997). Initially a single STAT5 gene was identified in sheep but subsequently two forms of STAT5 (STAT5A and STAT5B), encoded by two different genes, have been identified in mouse, human, rat and cattle cells (Hou et al., 1995; Kazansky, et al., 1995; Liu et al., 1995; Mui et al., 1995; Ripperger et al., 1995; Lin et al., 1996; Silva et al., 1996; Goldammer et al., 1997). The

genes encoding STAT5A and STAT5B 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 (Moriggl et al., 1996). Moreover they exhibit differences both in their DNA binding specificities (Boucheron et al., 1998; Verdier et al., 1998) and with respect to their tissue distribution (Liu et al., 1995; Mui et al., 1995). In cattle the STAT5A gene has been assigned to chromosome 19q17. The STAT *locus* also contains STAT3 and STAT5B genes (Seyfert et al., 2000; Moleenar et al., 2000). The STAT5A gene consists of 19 exons encoding 794 amino acids chain (Seyfert et al., 2000).

Several nucleotide sequence polymorphisms of the bovine STAT5A gene (GenBank AJ242522 and AJ237937) have been detected: McCracken et al. (1997) found TG repeats of different length within STAT5A intron 12; Antoniou et al. (1999) described two SSCP variants of the gene fragment that encodes SH2 domain in bovine STAT5A protein; Brym et al. (2004) detected a new SNP (A/G) located in intron 9 at position 9501; Khatib et al. (2008) studied many SNPs in STAT5A gene and their association with embryonic survival and milk composition. Flisikowski & Zwierzchowski (2002) reported a new single nucleotide polymorphism in exon 7 of the bovine STAT5A gene also investigated by other Authors (Dario et al., 2009a; Dario et al., 2009b; Selvaggi et al., 2009; Sadeghi et al., 2009; Selvaggi & Dario, 2011). Moreover, Flisikowski & Zwierzchowski (2003) reported the deletion of CCT in intron 15; subsequently the same authors described the substitution T/C at position 12743 within exon 16, which changed the amino acid sequence (Val/Ala at position 686) (Flisikowski et al., 2003; Flisikowski et al., 2004). The latter mutation was shown to modify DNA-binding properties of the STAT5A. In the DNA-protein binding assays (electrophoretic mobility shift assay – EMSA) nuclear proteins extracted from livers of *CC* genotype animals always showed less DNA protein complexes than those of animals TT (Flisikowski et al. 2003).

In this study, this single nucleotide polymorphism (SNP) was investigated with PCR-RFLP in Jersey cows. The increasing interest for the Jersey breed is mainly due to the characteristics of the milk produced by these animals: it is excellent in terms of quality because of the high fat and protein content, the superior calcium concentration and the low non-protein nitrogen content.

Over the last years there has been a fairly good increase in the number of Jersey breeders in Italy even if most of the Jersey cows are bred in mixed herds with Holsteins or other breeds.

The objectives of this study were to estimate the allele and genotype frequencies of the different alleles of T→C nucleotide polymorphism at position 12743 in exon 16 of the bovine STAT5A gene in a sample of Jersey cows and to determine the association between this polymorphism and some milk production traits.

2. Materials and Methods

A total of sixty-four Jersey cows were included in the study. The animals were all primiparous and were milked twice a day. The cows, calved from September 2008 to April 2009, belonged to 6 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 64 Jersey cows on K_3-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 the gene polymorphism in exon 16 of STAT5A gene.

The T→C polymorphism at position 12743 in exon 16 of the bovine STAT5*A* gene was determined as previously described by Flisikowski et al. (2003). 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, encompassing parts of intron 15 and exon 16 of the STAT5*A* gene. The polymerase chain reactions (PCR) were performed using a PCR-mix with: primers STAT5A_F and STAT5A_R each 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°C, 1 min at 61°C, and 1 min at 72°C – 34 cycles.

The yield and specificity of the PCR reactions were both evaluated by electrophoresis of the products 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°C. 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 (Falconer & Mackay, 1996). The differences between observed and expected frequencies of genotypes were tested using a Chi-square test in order to verify if the population was in Hardy–Weinberg equilibrium. Data concerning the first lactation of each cow were obtained from the local breeders association.

Effects of polymorphic variants of the STAT5A gene on milk production traits were analyzed using the GLM procedure of SAS (Sas/Stat User's Guide Statistics) 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 the cow (fat and protein content); 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,…,3); F_k is the fixed effect of k^{th} farm (1,...,6); e_{ijkl} is the random error.

Due to the low number of CC cows found in the studied 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.

3. 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 0.141 and 0.859 respectively being quite similar to those reported in other breeds (see Table 1). The TT genotype was the most frequent in the studied population (75.00%) followed by TC (21.88%) and CC genotype (3.12%). The calculated $\chi 2$ value was 0.58 (d.f.=1), indicating Hardy-Weinberg equilibrium in the population (P=0.45).

No association between this SNP and the considered production traits (fat and protein content) were found significant at statistical analysis (see Table 2). A similar result was found by Flisikowski et al. (2004) on Polish Friesian cows. In this paper, the Authors investigated the association among the STAT5A/*Msl*I polymorphism and some milk production traits finding no relationship 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≤0.05) more milk and FCM than TT homozygotes, with higher (P≤0.05) content of lactose. Moreover, the daily yield of value corrected milk (VCM), milk total solids, solids-non-fat, protein, and lactose was higher (P≤0.01) in cows with TC if compared to those genotyped as

TT. However, it may be necessary to carry out further studies about this polymorphism to better clarify the role of this SNP on production traits in cattle.

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Table 1. 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., 2003
Aberdeen Angus	10	0.900	0.100	Flisikowski et al., 2003
Hereford	16	0.875	0.125	Flisikowski et al., 2003
Jersey	64	0.859	0.141	Present work
Polish Friesian	150	0.850	0.150	Flisikowski et al., 2004
Limousine	16	0.812	0.188	Flisikowski et al., 2003
Charolaise	18	0.805	0.195	Flisikowski et al., 2003
Polish Friesian	37	0.756	0.244	Flisikowski et al., 2003

Table 2. Means and standard error of milk composition traits in Jersey cows with different STAT5A/*Msl*I genotypes

Genotype	Fat	Protein
	%	%
TC	4.50±0.19	3.89±0.08
TT	4.82±0.09	3.88±0.04