Genetic polymorphism of STAT5A protein: relationships with production traits and milk composition in Italian Brown cattle

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STATs 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). Therefore, STAT5A gene is a candidate marker for quantitative traits in farm animals with respect to milk production traits. In this study the STAT5A/Ava polymorphism was investigated with PCR-RFLP in a sample of 233 Italian Brown cattle. This polymorphism is localized in the coding region of the bovine STAT5A gene. It is a substitution C→T at position 6853 within exon 7. All three possible genotypes for the C/T polymorphism were identified. The overall frequencies of alleles C and T were 0.83 and 0.17 respectively; the Hardy-Weinberg equilibrium was verified. In order to study the relationship between STAT5A/Ava polymorphism and milk performance traits, the data for a 305-d milk production that included milk yield, protein and fat yield, fat and protein percentage were used. Significant differences between the two genotypes were found in yields of milk, fat and protein and protein percentage (P<0.01). CC cows produced more milk than CT (5418.68 vs 5149.54 kg). Protein content was higher in milk from CC compared with CT genotypes (3.40 vs 3.21%). No significant difference was found in fat content. Owing to the low number of TT cows in the studied population, this genotype was not included in the statistical analysis; in fact the number of TT cows was not enough to provide an accurate statistical analysis. Although more studies are needed to better clarify the role of this SNP on production traits, STAT5A/Ava polymorphism appears to be a promising indirect marker to improve milk production traits in cattle.

Keywords: STAT5A, gene polymorphism, Italian Brown cattle, milk production traits.

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

STAT proteins (signal transducers and activators of transcription) are a 7-member family of latent cytoplasmic transcription factors that mediate actions of a variety of peptide hormones and cytokines within target cells (Darnell et al. 1994; Schindler & Darnell, 1995). 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 occurs in response to a range of hormones and cytokines.

STAT5, also known as mammary gland factor (MGF), was discovered initially 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 (Argestinger & 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). 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.

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proves the manufacturing of dairy products by affecting cheesemaking as it gives a higher cheese yield and it improves the biological and organoleptic qualities. In Italy there are >100 cheeses produced with the milk from Brown cows.

A total of 233 Italian Brown cows were included in the study. The cows were progeny of 46 sires with the number of half-sibs ranging from 2 to 6. The animals, which calved from September 2006 to May 2007, belonged to eight different farms located in southern Italy. All the cows were fed the same lactation diet according to energy recommendations for lactating cows. The animals which calved from September 2006 to May 2007, belonged to eight different farms located in southern Italy. All the cows were fed the same lactation diet according to energy recommendations for lactating cows. The animals were of different parities (from 1st to 4th) and were milked twice a day. The data for a 305-d milk production including milk yield (MY), protein and fat yield (PY and FY respectively) were collected from 233 Italian Brown cows in K3-EDTA tubes and stored at −25 °C. Genomic DNA was isolated from whole blood using GFX Genomic Kit (Amersham, Germany). After genomic DNA isolation the cows were genotyped for the bovine STAT5A gene was determined as previously described by Flisikowski et al. (2003b). The sequences of primers used for amplification of a part of the seventh exon

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number</th>
<th>Allele Frequencies</th>
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<tbody>
<tr>
<td>Canthin</td>
<td>29</td>
<td>0.00 1.00</td>
</tr>
<tr>
<td>Nelore</td>
<td>36</td>
<td>0.00 1.00</td>
</tr>
<tr>
<td>Brangus</td>
<td>15</td>
<td>0.20 0.80</td>
</tr>
<tr>
<td>Hereford</td>
<td>16</td>
<td>0.07 0.93</td>
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<tr>
<td>Limousine</td>
<td>16</td>
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<tr>
<td>Iranian Holstein</td>
<td>134</td>
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<tr>
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</tr>
<tr>
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<td>30</td>
<td>0.17 0.83</td>
</tr>
<tr>
<td>White</td>
<td>15</td>
<td>0.20 0.80</td>
</tr>
<tr>
<td>Simmental</td>
<td>11</td>
<td>0.19 0.81</td>
</tr>
<tr>
<td>Polish White-Back</td>
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</tr>
<tr>
<td>Polish Red</td>
<td>30</td>
<td>0.27 0.73</td>
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<td>Angus</td>
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<td>0.35 0.65</td>
</tr>
<tr>
<td>Podolica</td>
<td>108</td>
<td>0.56 0.44</td>
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</table>

The objectives of the present study were to estimate the allele and genotype frequencies of the different alleles of the bovine STAT5A gene and to determine associations between this polymorphism and milk production traits.

**Materials and Methods**

**Animals**

Milk from Italian Brown cattle is particularly suitable for cheesemaking as it gives a higher cheese yield and it improves the manufacturing of dairy products by affecting also their biological and organoleptic qualities. In Italy there are >100 cheeses produced with the milk from Brown cows.

**Table 1. Frequencies of the alleles STAT5A T and STAT5A C in Italian Brown breed and in different cattle breeds reported by other authors. Allele frequencies are shown in increasing order for the T allele**

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**Determination of STAT5A/Aval polymorphism**

Individual blood samples for DNA genotyping were collected from 233 Italian Brown cows in K$_3$-EDTA tubes and stored at −25 °C. Genomic DNA was isolated from whole blood using GFX Genomic Kit (Amersham, Germany). After genomic DNA isolation the cows were genotyped for STAT5A/Aval gene polymorphism.

The C→T polymorphism at position 6853 in exon 7 of the bovine STAT5A gene was determined as previously described by Flisikowski et al. (2003b). The sequences of primers used for amplification of a part of the seventh exon
of STAT5A gene containing the polymorphic sites for C and T alleles were:

STAT5AF (forward): 5'-CTG CAG GGC TGT AGC AG-3';
STAT5AR (reverse): 5'-TGG TAC CAG GAC TGT AGC ACA T-3'

The 215 bp gene fragment was amplified using 34 amplification cycles at the following conditions: 94 °C/1 min and 72 °C/1 min.

The PCR product was digested with AvaI restriction endonuclease (Sigma; 3 h, 10 units/20 μl, 37 °C) and analysed on a 2% agarose gel stained with ethidium bromide, in TBE buffer. The nuclease cuts the 215 bp amplification product into 181 and 34 bp fragments for allele C, while allele T remains uncut. The following DNA restriction fragment were obtained: 181 and 34 bp for the CC genotype; 215, 181 and 34 bp for the CT and 215 bp for the TT genotype.

Statistical analysis

STAT5A allele frequencies were calculated by simple allele counting (Falconer & Mackay, 1996). Differences between observed and expected frequencies of genotypes were tested using a Chi-square test in order to verify whether the population was in Hardy-Weinberg equilibrium.

Effects of polymorphic variants of the STAT5A gene on milk production traits were analysed using the MIXED procedure of SAS (Sas/Stat User’s Guide Statistics, 1999) according to the following statistical model:

\[ Y_{ijklmno} = \mu + G_i + S_j + M_k + F_l + P_m + (P \times G)_m + A_n + e_{ijklmno} \]

where \( Y_{ijklmno} \) is the analysed trait of the cow (MY, FY, PY, PC); \( \mu \) is the overall mean; \( G_i \) is the fixed effect of the ith genotype (1, 2); \( S_j \) is the random effect of the jth sire (1, …, 46); \( M_k \) is the fixed effect of kth season of calving (1, …, 3); \( F_l \) is the fixed effect of lth farm (1, …, 8); \( P_m \) is the fixed effect of mth parity (1, …, 4); \( P \times G_m \) is the interaction between the mth parity and the ith genotype (1, …, 8); \( A_n \) is the random effect of the animal and \( e_{ijklmno} \) is the random error. Owing to the low number of TT cows in the studied population, this genotype was not included in the statistical analysis; in fact the number of TT cows was too small to provide an accurate statistical analysis.

### Results

#### Gene frequency

Observed frequencies of C and T alleles were 0.83 and 0.17 respectively. STAT5A CC genotype was the most frequent in the studied population (68.67%) followed by the CT genotype (29.18%) while the TT genotype had the lowest frequency (2.15%). The calculated \( \chi^2 \) value was 0.52 (df=1), indicating Hardy-Weinberg equilibrium in the population.

Table 1 gives the frequencies of the two alleles in Italian Brown breed and in different cattle breeds reported by other authors.

### Polymorphism and milk performance traits

Data reported in Table 2 show the effects of the STAT5A/ AvaI polymorphism on lactation production traits. Significant differences between the CC and CT genotypes were found for MY, FY, PY and PC (\( P<0.01 \)). In particular CT cows yielded less milk than CC animals (5149.54 kg v. 5418.68 kg). Moreover the milk from CC cows was significantly richer in protein (+0.19%) and consequently the quantity of protein yielded by CC animals during the lactation was higher than for CT genotype. On the other hand no significant difference was found in fat percentage; in spite of this, due to the strong difference in MY between the two genotypes, FY was significantly higher in CC than in the heterozygotes.

On the basis of these results, CC cows produce more milk than CT and protein content is higher in milk from CC compared with CT genotypes. These results are consistent with the higher frequency of CC genotype as result of dairy selection.

### Discussion

Hormones, growth factors, and other regulatory proteins associated with the ‘somatotropic axis’ are candidate markers for quantitative traits in farm animals. Genes encoding for prolactin (PRL), prolactin receptor (PRLR), transcription factor Pit-1, insulin-like growth factor 1 (IGF1), and perhaps presently unknown genes involved in milk production may contribute to progress in genetic selection of farm animals. Transcription factors STAT5A may be considered as members of the somatotropic axis.
Therefore genes encoding for STAT5A and STAT5B are candidate markers for quantitative traits in cattle.

There are a few reports of QTLs affecting milk production traits on BTA19: fat yield and fat percent in German and French Holstein (Bennewitz et al. 2003), fatty acids composition of milk produced by pasture-fed dairy cattle (Morris et al. 2007), fat percent in French Holstein (Boichard et al. 2003). This report also points towards the fact that there is no high selection pressure on BTA19, unlike on BTA06. Moreover a QTL in BTA19 influences the fore-udder attachment and the front-teat placement (Schrooten et al. 2000). Owing to the localization of the STAT5A gene on BTA19 it is possible to assume that the effect of the synonymous mutation studied on the phenotype results from linkage disequilibrium with other closely linked genes on BTA19.

STAT5A/Aval polymorphism had not been reported previously for Italian Brown cattle and the frequencies of alleles obtained in this study were similar to those reported in other dairy breeds (Table 1).

Flisikowski et al. (2003b) reported allelic frequencies only in beef or dual-purpose animals and they found the TT genotype only in Polish native breeds (Polish Red and Polish White-Back). Suguisawa (2005) observed a lower frequency of the STAT5A Pol allele in many breeds, particularly in beef cattle. The T allele was very frequently detected in the Podolica breed by Dario et al. (2009). Allelic frequencies reported in Italian Brown are similar to those recently reported by Sadeghi et al. (2009) for Iranian Holstein bulls.

Despite the importance of the STAT5A protein in milk production and growth, there are only a few reports on the association between STAT5A/Aval polymorphism and production traits in farm animals.

Recently Dario et al. (2009) studied the effect of STAT5A/Aval polymorphism on growth performance traits in Podolica bulls and they suggest the superiority of C allele for growth performances because both CC and CT bulls tended to show a higher live weight and a faster growth in comparison with TT animals. These results are consistent with those reported by Flisikowski et al. (2003b) which show the live body weight at age 9 and 15 months, the dressing percentage and four carcass traits in the animals of the CC genotype to be more favourable than in CT animals. Moreover CC individuals used less feed for maintenance and meat production. On the other hand, Suguisawa et al. (2006) reported no effect of this polymorphism on beef cattle growth, carcass or meat quality traits.

Sadeghi et al. (2009) studied the association between this polymorphism and the breeding values of milk production traits in 134 Iranian Holstein bulls. In this breed they found a significant higher quantity of protein yielded by CT animals when compared with CC individuals even though no significant differences were found in protein content. Our results disagree with those reported by Sadeghi et al. (2009) although both allelic and genotypic frequencies were similar in Italian Brown and in Iranian Holstein breeds.

Further studies of STAT5A/Aval polymorphism are needed also in other breeds to better clarify the role of this SNP on production and reproductive traits in cattle. In fact the STAT5A gene is the first found to be associated with both milk production and fertility (Khattib et al. 2008). Moreover it may be interesting to study also changes in STAT5B gene because of the closeness of the two genes.

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