

Preliminary study on the STAT5A/AvaI polymorphism in Jersey cows: relation with milk production traits

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Abstract. In this study the STAT5A/AvaI polymorphism was investigated with PCR-RFLP in a sample of 126 Jersey cattle reared in southern Italy. This polymorphism is localized in the coding region of the bovine STAT5A gene. It is a substitution C→T at position 6853 within the exon 7. Only two out of three possible genotypes for the C/T SNP were identified. The overall frequencies of alleles C and T were 0.76 and 0.24 respectively. In order to study the relationship between STAT5A/AvaI polymorphism and milk performance traits, the data for a 305-day milk production that include milk yield (MY), protein and fat yield (PY and FY respectively), fat and protein percentage (FC and PC respectively) were used. Significant differences between the two genotypes were found in MY, FY and PY (P<0.01). The CC cows produced more milk than CT ones (6218.09 vs. 5751.72 kg). Moreover CC cows yielded more fat and protein than CT. No significant difference was found in fat and protein content.

Keywords: STAT5A, Gene polymorphism, Jersey cattle, Milk production traits.

1. Introduction

The STATs 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 [1, 2]. STAT5, also known as mammary gland factor (MGF), was discovered initially as a PRL-induced transcription factor. STAT5 is known as a main mediator of growth hormone (GH) 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. The STAT5 factors interact and functionally synergize with receptors for glucocorticoid and insulin [3, 4]. 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. 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. Moreover they exhibit differences both in their DNA binding specificities and with respect to their tissue distribution [4].

In the cattle the STAT5A gene has been assigned to chromosome 19q17. The STAT locus also contains STAT3 and STAT5B genes. The STAT5A gene consists of 19 exons encoding 794 amino acid chains [5]. Several nucleotide sequence polymorphisms of the bovine STAT5A gene (GenBank AJ242522 and AJ237937) have been detected [6,7, 8, 9, 10; 11]. Flisikowski & Zwierzchowski [12] reported a new single nucleotide polymorphism in exon 7 of the bovine STAT5A gene. Exon 7 codes for amino acids 250-480 of the STAT5A molecule in the DNA-binding domain responsible for binding of this transcription factor to promoters of the target genes [13]. The SNP studied by Flisikowski *et al.* [14] was found in the coding region of the bovine STAT5A gene. It is a substitution C→T at position 6853 within the exon 7. This mutation is detectable by *AvaI* enzymatic restriction. Analysis of a reading frame has not shown a change in

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the amino acid sequence in the protein coded by the polymorphic gene; CCC and CCT triplets both code for the amino acid proline.

The objectives of this study were to estimate the allele and genotype frequencies of the different alleles of the STAT5A/AvaI polymorphism in Jersey cows and to determine associations between this polymorphism and the milk production traits.

2. Materials and Methods

2.1. Animals

Over the last years there has been a fairly good increase in the number of Jersey cows breeders in Italy even if most of the Jersey cows are bred in mixed herds with Holsteins and some with other breeds. 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. Furthermore Jersey breed showed an extreme adaptability to all breeding types and to different environmental conditions being very competitive in terms of longevity, health resistance, heat and cold tolerance.

A total of one-hundred and twenty-six unrelated cows belonging to Jersey breed were included in the study. The animals, calved from October 2004 to June 2005, belonged to 12 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 all primiparous and were milked twice a day. The data for a 305-day milk production include milk yield (MY), protein and fat yield (PY and FY respectively) were obtained from the local breeder association; while fat and protein percentage (FC and PC respectively) were calculated.

2.2. Determination of STAT5A/AvaI polymorphism

Individual blood samples for DNA genotyping were collected from 126 Jersey cows on 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 STAT5A/AvaI 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.* [14]. The PCR product was digested with AvaI restriction endonuclease (Sigma; 3h, 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 expected: 181 and 34bp for the CC genotype; 215, 181 and 34bp for the CT and 215bp for the TT genotype.

2.3. Statistical analysis

The STAT5A allele frequencies were calculated by simple allele counting [15]. In order to verify if the population was in Hardy–Weinberg equilibrium, the differences between observed and expected frequencies of genotypes were tested using a Chi-square test.

Effects of the variants of the STAT5A gene on milk production traits were analysed using the MIXED procedure of SAS [16] according to the following statistical model: $Y_{ijklm} = \mu + G_i + M_j + F_k + A_l + e_{ijklm}$; where: Y_{ijklm} is the analysed trait of the 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,...,3); F_k is the fixed effect of k^{th} farm (1,...,12); A_l is the random effect of the animal and e_{ijklm} is the random error.

3. Results

3.1. Gene frequency

The observed frequencies of C and T alleles were 0.76 and 0.24 respectively. As reported in table 1, the STAT5A CC genotype was the most frequent in the studied population (52.38%) followed by the CT genotype (47.62%), while no TT animals were found. The calculated χ^2 value was 12.30 (d.f.=1), indicating

Hardy-Weinberg disequilibrium in the population ($P < 0.01$). In Table 2 were reported the frequencies of the two alleles in Jersey breed and in different cattle breeds as observed by other authors.

3.2. Polymorphism and milk performances traits

Data reported in Table 3 show the effects of the STAT5A/*Ava*I polymorphism on milk production traits. Significant differences between the CC and CT genotypes were found in MY, FY, and PY ($P < 0.01$). In particular CT cows yielded less milk than CC animals (5751.72 vs. 6218.09 kg). No significant differences were found about FC (4.76 vs. 4.76%) and PC (3.93 vs. 3.97% for CC and CT respectively). Consequently, due to the higher quantity of milk yielded by CC cows, the quantity of fat yielded by CC animals during the lactation was higher if compared to that of CT genotype (293.80 vs. 261.37kg respectively). Similar result was found by comparing the quantity of protein yielded in the same period, in fact CC cows yielded more protein than the CT ones (243.39 vs. 224.92kg respectively). Both these results were found meaningful at the statistical analysis. On the basis of these results, CC cows produce more milk than CT without differences in PC and FC and it is possible to suppose a positive effect of the C allele on the considered parameters. On the other hand, the absence of TT cows in the studied population does not permit a direct evaluation of the performances of the TT cows. However the lack of TT cows maybe indirectly due to the dairy selection.

4. Discussion

Because of their role as mediators of the action of peptide hormones and cytokines such as GH and prolactin, transcription factors STAT5 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 only few reports about QTLs affecting milk production traits on BTA19: fat yield and fat percent in German and French Holstein [17], fatty acids composition of milk produced by pasture-fed dairy cattle [18], fat percent in French Holstein [19]. This report also points towards the fact that there is no high selection pressure on BTA19, unlike on BTA06. Due 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.

The STAT5A/*Ava*I polymorphism had not been previously reported for Jersey cattle breed and the frequencies of alleles obtained in this study were similar to those reported in other milk purpose breeds by other authors as illustrated in Table 1. Flisikowski *et al.* [14] 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 [20] observed a lower frequency of the STAT5A T allele in many breeds, particularly in beef cattle. The T allele was very frequently detected in the Podolica breed by Dario *et al.* [21].

In spite of the importance of the STAT5A protein in milk production and growth processes, only few reports were carried out regarding the association between the STAT5A/*Ava*I polymorphism and the production traits in farm animals. Recently Dario *et al.* [21] studied the effect of STAT5A/*Ava*I 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 were consistent with those reported by Flisikowski *et al.* [14] that found the live body weight at the age of 9 and 15 months, the dressing percentage and four carcass traits in the animals of the CC genotype more favourable than in CT animals. On the other hand, Suguisawa *et al.* [22] reported no effect of this polymorphism on beef cattle growth, carcass and meat quality traits. Sadeghi *et al.* [23] studied the association between this polymorphism and the breeding values of milk production traits in 134 Iranian Holstein bulls. In this breed they found significant higher quantity of protein yielded by CT animals if compared to the CC even if no significant differences were found in protein content. Our results are consistent with those reported by Selvaggi *et al.* [24] who studied the same SNP in Italian Brown cattle breed. In this paper they reported that CC animals showed higher MY, FY, PY and PC than CT cows assuming a superiority of the C allele.

However it is necessary to carry out further studies about the STAT5A/*Ava*I polymorphism 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 [25]. Moreover it may be interesting to study the changes in STAT5B gene because of the closeness of the two genes.

5. References

- [1] J.E. Darnell Jr, I.M. Kerr, G.R. Stark. JAK-STAT pathways and transcriptional activation in response to IFNs and other extracellular signalling proteins. *Science*. 1994, **264** (5164): 1415-1421.
- [2] C. Schindler and J.E. Darnell Jr. Transcriptional responses to polypeptide ligands. The JAK-STAT pathway. *Annu. Rev. Biochem.* 1995, **64** 621-651.
- [3] H. Wakao, F. Gouilleux, B. Groner. Mammary gland factor (MGF) is a novel member of the cytokine regulated transcription factor gene family and confers the prolactin response. *EMBO J.* 1994, **13** (9):2182-2191.
- [4] L.S. Argetsinger and C. Carter-Su. Growth hormone signalling mechanisms: involvement of the tyrosine kinase JAK2. *Horm. Res.* 1996, **45** (Suppl. 1): 22-24.
- [5] A. Moleenar, T.T. Wheeler, J.Y. McCracken, H. Seyfert. The STAT3-encoding gene resides within the 40 kbp gap between the STAT5A- and STAT5B-encoding genes in cattle. *Anim. Genet.* 2000, **31** (5): 333– 346.
- [6] J.Y. McCracken, A.J. Molenaar, R.J. Snell, H.W. Davey, R.J. Wilkins. A polymorphic TG repeat present within the bovine STAT5A gene. *Anim. Genet.* 1997, **28** 453-464.
- [7] E. Antoniou, B.J. Hirts, M. Grosz, J. Skidmorec. A single strand conformation polymorphism in the bovine gene STAT5A. *Anim. Genet.* 1999, **30** (3): 232.
- [8] P. Brym, S. Kamiński, A. Ruśc. New SSCP polymorphism within bovine STAT5A gene and its associations with milk performance traits in Black-and-White and Jersey cattle. *J. Appl. Genet.* 2004, **45** (4): 445-452.
- [9] K. Flisikowski and L. Zwierzchowski. Polymerase chain reaction-heteroduplex (PCR-HD) polymorphism within the bovine STAT5A gene. *J. Appl. Genet.* 2003, **44** (2): 185–189.
- [10] K. Flisikowski, N. Strzałkowska, K. Słoniewski, J. Krzyżewki, L. Zwierzchowski. Association of a sequence nucleotide polymorphism in exon 16 of the STAT5A gene with milk production traits in Polish Black-and-White (Polish Friesian) cows. *Anim. Sci. Pap. Rep.* 2004, **22** (4): 515-522.
- [11] K. Flisikowski, M. Szymanowska, L. Zwierzchowski. The DNA binding capacity of genetic variants of the bovine STAT5A transcription factor. *Cell. Mol. Biol. Lett.* 2003, **8** (3): 831–840.
- [12] K. Flisikowski and L. Zwierzchowski. Single-strand conformation polymorphism within exon 7 of the bovine STAT5A gene. *Anim. Sci. Pap. Rep.* 2002, **20** (2): 133-137.
- [13] S. Pellegrini and I. Dusanter-Fourt. The structure, regulation and function of the Janus kinases (JAKs) and signal transducers and activators of transcription (STATs). *Eur. J. Biochemistry* 1997, **248** (15): 615-633.
- [14] K. Flisikowski, J. Oprzdek, E. Dymnicki, L. Zwierzchowski. New polymorphism in bovine STAT5A gene and its association with meat production traits in beef cattle. *Anim. Sci. Pap. Rep.* 2003, **21** (3)147–157.
- [15] D.S. Falconer and T.F.C. Mackay. *Introduction to Quantitative Genetics*. 4th ed, Longman Group Ltd, Essex,1996
- [16] SAS User's Guide Statistics, Version 8.0 Edition 1999, SAS Inst. Inc. Cary, NC.
- [17] J. Bennewitz, N. Reinsch, C. Grohs, H. Leveziel, A. Malafosse, H. Thomsen, N. Xu, C. Looft, C. Kuhn, G.A. Brockmann, M. Schwerin, C. Weimann, S. Hiendleder, G. Erhardt, I. Medjugorac, I. Russ, M. Forster, B. Brenig, F.Reinhardt, R. Reents, G. Averdunk, J. Blumel, D. Boichard, E. Kalm. Combined analysis of data from two granddaughter designs: A simple strategy for QTL confirmation and increasing experimental power in dairy cattle. *Genet. Sel. Evol.* 2003, **35** (3): 319-338.
- [18] C.A. Morris, N.G. Cullen, B.C. Glass, D.L. Hyndman, T.R. Manley, S.M. Hickey, J.C. McEwan, W.S. Pitchford, C.D. Bottema, M.A. Lee. Fatty acid synthase effects on bovine adipose fat and milk fat. *Mamm. Genome.* 2007, **18** (1):64–74.
- [19] D. Boichard, C. Grohs, F. Bourgeois, F. Cerqueira, R. Faugeras, A. Neau, R. Rupp, Y. Amigues, M.Y. Boscher, H. Leveziel. Detection of genes influencing economic traits in three French dairy cattle breeds. *Genet. Sel. Evol.* 2003, **35** (1): 77-101.
- [20] L. Suguisawa Identificação de genótipos superiores para crescimento e qualidade de carcaça em bovinos de corte submetidos ao modelo biológico superprecoce. 2005, Tese (Doutorado em Zootecnia). Faculdade de Medicina Veterinária e Zootecnia, Universidade Estadual Paulista, Botucatu.
- [21] C. Dario, M. Selvaggi, D. Carnicella, G. Bufano. STAT5A/*Ava*I polymorphism in Podolica bulls and its effect on growth performances traits. *Livest. Sci.* 2009, **123** (1): 83-87.

- [22] L. Suguisawa, A.A. Souza, H.N. Oliveira, R.A. Curi, A.C. Silveira. Relationship between growth hormone and STAT5A genes polymorphisms and beef cattle growth, carcass and meat quality traits. *In: 8th World Congress on Genetics Applied to Livestock Production*, August 13-18, 2006, Belo Horizonte, MG, Brasil.
- [23] M. Sadeghi, M. Moradi Shahrabak, G. Rahimi Mianji, A. Nejati Javaremi. Polymorphism at *locus* of STAT5A and its association with breeding values of milk production traits in Iranian Holstein bulls. *Livest. Sci.* 2009, **123** (1):97-100.
- [24] M. Selvaggi, C. Dario, G. Normanno, G.V. Celano, M. Dario. Genetic polymorphism of STAT5A protein: relationships with production traits and milk composition in Italian Brown cattle. *J. Dairy Res.* 2009, 76 (4): 441-445.
- [25] H. Khatib, R.L. Monson, V. Schutzkus, D.M. Kohl, G.J. Rosa, J.J. Rutledge. Mutations in the STAT5A gene are associated with embryonic survival and milk composition in cattle. *J. Dairy Sci.* 2008, **91** (2):784-793.

Table1. Observed and expected numbers and percentages (in brackets) of STAT5A genotypes detected by *AvaI* RFLP analysis and allele frequencies in the sample of Jersey cows

NUMBER	STAT5A GENOTYPE			ALLELE FREQUENCY	
	CT	CC	TT	C	T
OBSERVED	60 (47.62%)	66 (52.38%)		0.76	0.24
EXPECTED	45.72 (36.28%)	73.14 (58.05%)	7.14 (5.67%)		
$\chi^2=12.30$	P<0.01				

Table 2. Frequencies of the alleles STAT5A *T* and STAT5A *C* in Jersey breed and in different cattle breeds observed by other authors. Allele frequencies are shown in increasing order for the *T* allele

BREED	No.	ALLELIC FREQUENCIES		REFERENCES
		<i>T</i>	<i>C</i>	
Canchim	29	0.00	1.00	Suguisawa [20]
Nelore	36	0.00	1.00	Suguisawa [20]
Brangus	18	0.00	1.00	Suguisawa [20]
Hereford	16	0.07	0.93	Flisikowski <i>et al.</i> [14]
Limousine	16	0.13	0.87	Flisikowski <i>et al.</i> [14]
Iranian Holstein	134	0.13	0.87	Sadeghi <i>et al.</i> [23]
Charolaise	18	0.14	0.86	Flisikowski <i>et al.</i> [14]
Red Angus	10	0.15	0.85	Flisikowski <i>et al.</i> [14]
Italian Brown	233	0.17	0.83	Selvaggi <i>et al.</i> [24]
Black and White	30	0.17	0.83	Flisikowski <i>et al.</i> [14]
Simmental	11	0.19	0.81	Flisikowski <i>et al.</i> [14]
Polish White-Back	15	0.20	0.80	Flisikowski <i>et al.</i> [14]
JERSEY	126	0.24	0.76	PRESENT WORK
Polish Red	30	0.27	0.73	Flisikowski <i>et al.</i> [14]
Angus	10	0.35	0.65	Suguisawa [20]
Podolica	108	0.56	0.44	Dario <i>et al.</i> [21]

Table 3. Means and standard error of milk production traits in Jersey cows with different STAT5A/*AvaI* genotypes

	MILK YIELD	FAT		PROTEIN	
	kg	%	kg	%	kg
CC	6218.09±147.17 ^A	4.76±0.08	293.80±5.58 ^A	3.93±0.03	243.39±4.94 ^A
CT	5751.72±135.25 ^B	4.76±0.07	261.37±5.13 ^B	3.97±0.03	224.92±4.54 ^B

A, B= P< 0.01