

References

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A single strand conformational polymorphism in the bovine gene *STAT5A*

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Accepted 10 November 1998

Source/Description: Milk protein gene expression in mammary epithelial cells is regulated by the action of prolactin mediated through the *STAT5A* (Signal Transduction and Activator of Transcription 5A) protein. While *STAT5A*-deficient mice developed normally, mammary lobulo-alveolar development during pregnancy was impeded, and females failed to lactate after parturition because of a failure of terminal differentiation¹. Since the *STAT5A* protein has a central role in this pathway, the *STAT5A* gene is a candidate gene for dairy cattle quantitative traits. A microsatellite was described in an intron of the gene². However, there are major alleles (frequencies from 0.78 to 0.94) in several breeds² which are likely to limit the utilization of this marker. Additional polymorphism is necessary to help to investigate the role of *STAT5A* in milk production trait variation. A PCR fragment of 795 bp was amplified from genomic DNA and cloned. The fragment was sequenced and

encodes the entire SH2 domain of the *STAT5A* protein (GenBank number AF079568). Primers were designed that amplify 379 bp of this SH2 domain and a Single Strand Conformational polymorphism (SSCP) was characterized.

PCR primers:

STAT5-5': CTGGGAGAACCTAACATCACT

STAT5-R: AGACCTCATCCTTGGGCC

PCR condition and SSCP analysis: The polymerase chain reactions were performed using 50 ng of DNA in a buffer containing 10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.001% gelatin (w/v), 1.5 mM MgCl₂, 0.5 units of *Taq* polymerase (Promega, Madison, WI.), 30 μM of dGTP, dCTP and dTTP, 3 μM of dATP and 3.3 μM of ³²P dATP (3000 Ci/mm), and 2 μM of STAT5-5' and STAT5-R primers. A touchdown PCR protocol was used: 2 min at 94 °C, then 14 cycles for 30 s at 94 °C, 30 s at 72 °C and decreasing of 0.5 °C every cycle, 30 s at 72 °C, followed by 24 cycles for 30 s at 94 °C, 30 s at 65 °C, 30 s at 72 °C. A final extension step was performed at 72 °C for 10 min. The PCR fragments were loaded on polyacrylamide gels (0.5× TBE buffer, 6% T, 5% C, 5% glycerol) and run at room temperature at five watts constant for at least 16 h.

Polymorphism: DNA of 20 offspring from seven families and their respective parents were used in PCR reactions to confirm the Mendelian inheritance of this polymorphism (Fig. 1).

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Three PCR/RFLPs identified in the promoter region 1-1 of the bovine aromatase gene (*CYP19*)

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Accepted 19 November 1998

Source/description: The *CYP19* gene encodes for the key enzyme of estrogen biosynthesis, aromatase cytochrome P450^{1,3}. The expression of this gene is directed by separate, tissue specific promoter regions^{4,5}. In the bovine one of those is promoter region 1-1 which is mainly active in the placenta. Two primer pairs were derived from this region (EMBL accession number Z69241). Amplicons were generated from different animals and screened for polymorphic sites by direct sequencing (ABI 310C, Perkin Elmer, Weiterstadt, Germany). Three polymorphisms located within restriction enzyme recognition sites could be identified (Table 1).

Chromosomal location: The bovine *CYP19* gene, including the placenta specific promoter 1-1, has been physically mapped to band q2.6 of chromosome 10².

PCR/RFLP assays: PCR-amplifications were performed in 25 μl reaction mixture containing the following: 20–100 ng genomic DNA; primers, 500 nM each; dNTPs, 200 μM each; 10 mM Tris-HCl, pH 9.0; 50 mM KCl; 1.5 mM MgCl₂; Triton X100, 0.1%; BSA, 0.2 mg/ml; *Taq* DNA Polymerase (Appligene/ONCOR, Heidelberg, Germany), 0.25 units. The PCR mix was incubated at 94 °C for 2 min. This was followed by 30 cycles of 94 °C for 15 s, 55 °C for 30 s and 70 °C for 2 min. The last PCR step was 70 °C for 5 min. 10 μl of the PCR products were subsequently added to 10 μl of one of the following restriction enzyme master mixes: (1) *PvuII*, 2 units per assay; 33 mM Tris-acetate, pH 7.8; K-acetate, 66 mM; Mg-acetate, 10 mM; DTT, 5 mM; 37 °C for 14 h. (2) *BseNI*, 2 units per assay; 10 mM Tris-HCl, pH 7.4; MgCl₂, 10 mM; BSA, 0.1 mg/ml; 65 °C for 6 h. (3) *Cfr13I*, 2 units per assay; 33 mM Tris-acetate, pH 7.9; Mg-acetate, 10 mM; K-acetate, 66 mM; BSA, 0.1 mg/ml; 37 °C for 14 h. Restriction fragments were subsequently

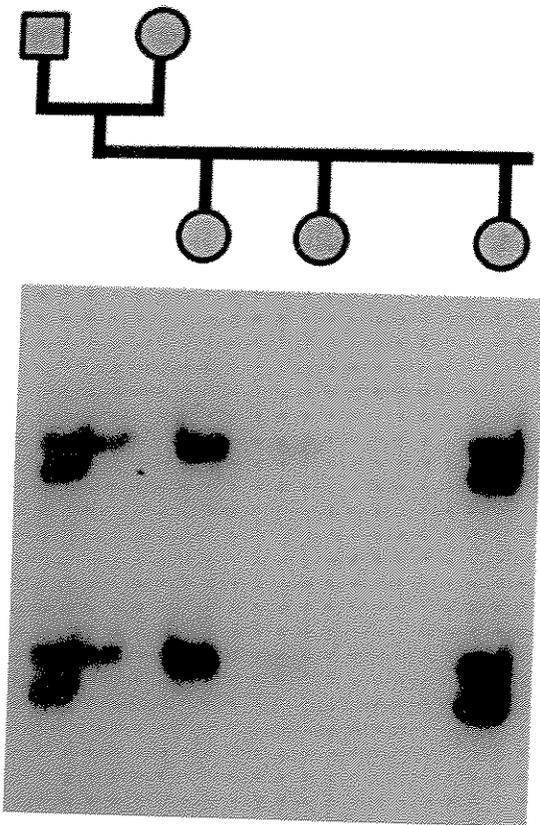


Fig. 1. SSCP in the genomic sequence of the bovine *STAT5A* gene