A single strand conformational polymorphism in the bovine gene STAT5A

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Source/Description: Milk protein gene expression in mammary epithelial cells is regulated by the action of prolactin mediated though 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 differentiation\(^1\). 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 gene\(^2\). However, there are major alleles (frequencies from 0.78 to 0.94) in several breeds\(^3\) 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 AF079858). Primers were designed that amplify 375 bp of this SH2 domain and a Single Strand Conformational polymorphism (SSCP) was characterized.

PCR primers:

STAT5-5:\(5'(CTGCGGACGACCATCACTACAC')\)

STAT5-3:\(5'(AGACCTCAGCCTTGGGCGG')\)

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, 0.01% gelatin (w/v), 1.5 mm MgCl\(_2\), 0.5 units of Taq polymerase (Promega, Madison, WI), 30 mm dGTP, dCTP and dTTP, 3 mm of dATP (3000 Ci/mm), and 2 mm of STAT5-5' and STAT5-3' 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).

References


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

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Source/description: The CYP19 gene encodes the key enzyme of estrogen biosynthesis, aromatase cytochrome P450 12. 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). Amplifications 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).

Cytogenetic location: The bovine CYP19 gene, including the placenta specific promoter 1-1, has been physically mapped to band q2-q6 of chromosome 10 8.

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 MgCl\(_2\); Triton X100, 0.1%; BSA, 0.2 mg/ml; Taq DNA Polymerase (Appligene/ONCOR, Heidelberg, Germany), 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 7.6; K-acetate, 66 mm; Mg-acetate, 10 mm; DTT, 5 mm; 37°C for 14 h. (2) RsalI, 2 units per assay; 10 mm Tris-HCl, pH 7.4; MgCl\(_2\), 10 mm; BSA, 0.1 mg/ml; 65°C for 6 h. 3) ClalI, 2 units per assay; 33 mm Tris-acetate, pH 7.6; 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

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