Brief Gene Mapping Report

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Assignment[1] of Signal Transducer and Activator of Transcription 5A *(STAT5A)* gene to porcine chromosome 12p13→p11 by radiation hybrid panel mapping

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[1] To our knowledge this is the first time this gene has been mapped in swine.

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Rationale and significance

Signal Transducers and Activators of Transcription (STATs) are a family of latent cytoplasmatic proteins that participates in the regulation of gene expression when cells encounter various extracellular polypeptides (Darnell, 1997). Seven mammalian STAT genes have been identified in three chromosomal clusters. The genes encoding STATs 1/4, STATs 2/6 and STATs 3/5A-5B are tightly linked on mouse chromosomes 1, 10 and 11 respectively (human chromosomes 2, 12 and 17 respectively) (Darnell, 1997). These chromosome locations suggest the existence of an evolutionary primordial gene that was duplicated and that this site was involved subsequently in gene duplication events. More recently, the *STAT5* gene was further duplicated. Since the *Drosophila* gene appears to be most related to *STAT5*, the STAT 3/5A-5B encoding site may represent the ancestral gene (Ihle, 1996; Darnell, 1997).

STAT5A and *STAT5B* are expressed in most tissues and they can be activated in tissue cells by prolactin (PRL), growth hormone (GH) and many cytokines. In the mammary gland, *STAT5A* and *STAT5B* are activated by PRL and probably placental lactogen (Hennighausen et al., 1997).

Two isoforms of STAT5 (A and B) have also been identified in bovine mammary glands as described in mouse. STAT5 activity is absent in non-lactating and non-pregnant cows and is present in late pregnancy and throughout lactation suggesting a key role in the regulation of milk protein gene expression (Yang et al., 2000).

Materials and methods

Nucleotide sequence

Pig genomic DNA was isolated from blood samples by the phenol-chloroform method. The porcine *STAT5A* mRNA sequence (GenBank accession No. NM_214290) was used to design primers in exon 14 and exon 16, according to the conservation of the structure of human (NM_003152) and mouse (NM_011488) *STAT5A* genes:

STAT5-E14F: 5′-TGC CTG ACA AAG TCC TGT GG-3′
STAT5A-E16R: 5′-CCG ATT TCT GAG TCG CTA AAG-3′

PCR amplification was performed in a 25-μl reaction mixture containing 0.625 units Taq DNA polymerase (Invitrogen SA, Barcelona, Spain), 1 × PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.5 μM of each primer and approximately 50 ng genomic DNA. The thermal cycling profile was 95°C for 5 min, 35 cycles of 95°C for 30 s, 60°C for 1 min and 72°C for 2.5 min, with a final extension of 72°C for 7 min.

The amplified product was analysed on agarose gel and sequenced in both directions using BigDye™ Terminator v3.1 Cycle Sequencing Kit and a 3730 DNA Analyser (Applied Biosystem, CA, USA) obtaining a 930-bp nucleotide sequence (GenBank accession no. DQ136147) including 135 bp of the 3′ end of exon 14, intron 14 (554 bp), exon 15 (95 bp), intron 15 (93 bp) and 53 bp of the 5′ end of exon 16.

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Radiation hybrid mapping

From the previously described nucleotide sequence, we designed a pig-specific primer located in intron 15:

STAT5A-I15F: 5′-GAA TGA CGG GTA AGG AAT GG-3′

Chromosome location was identified using the INRA-University of Minnesota porcine radiation hybrid panel (IMpRH) (Yerle et al., 1998), using STAT5A-I15F and STAT5A-E16R primers.

The PCR was performed in 12.5-μl reactions containing 25 ng genomic DNA, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 200 μM of each dNTP, and 0.625 units Taq DNA polymerase (Invitrogen SA, Barcelona, Spain). The cycling profile was 95°C for 5 min, 35 cycles of 95°C for 30 s, 58°C for 1 min and 72°C for 1.5 min, with a final extension of 72°C for 5 min. Data was analysed with the IMpRH mapping tool (Milan et al., 2000).

Results

The IMpRH panel was employed to determine the precise location of *STAT5A*. We found that the porcine *STAT5A* gene is located on chromosome 12 at 72 cR of the SW957 marker (LOD score 5.06). The assignment of *STAT5A* to SSC12p13→p11 is consistent with the pig and human comparative maps and supports the conservation of synteny between the SSC12 and the HSA17 chromosomes.

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