J. Anim. Breed. Genet. ISSN 0931-2668

ORIGINAL ARTICLE

Polymorphism and chromosomal localization of the porcine signal transducer and activator of transcription 5B gene (*STAT5B*)

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Received: 5 October 2005;
accepted: 24 February 2006

Summary

Signal transducers and activators of transcription (STATs) are a family of transcription factors. STAT5A and 5B are two highly related proteins encoded by two distinct genes. Transgenic knockout mice studies have indicated the importance of STAT5 proteins for the regulation of both lactation and growth performance. Moreover, different studies determine the role of STAT5 proteins in the modulation of adipocyte function. In this study, we sequenced one fragment of *STAT5B* gene from animals of six breeds (Duroc, Iberian, Landrace, Large White, Piétrain and Meishan) to identify genetic variants. A G/A single nucleotide polymorphism in intron 14 creates a polymorphic *Pst*I restriction site and was genotyped by polymerase chain reaction restriction fragment length polymorphism in the six breeds. Allele *G* was only present in Large White, Piétrain and Meishan populations, detecting only *G* allele in this last pig breed. The *STAT5B* gene was located by radiation hybrid mapping to porcine chromosome 12, within the confidence interval for the fatty acid composition quantitative trait loci, previously identified in an Iberian × Landrace cross.

Introduction

Signal transducers and activators of transcriptions (STATs) are a family of latent cytoplasmic proteins that are involved in the signal transduction pathways of multiple cytokines and peptide hormones. STAT proteins become activated through phosphorylation when extracellular signalling polypeptides bind with specific cell surface receptors. They dimerize and enter the nucleus to regulate transcription of many different genes (Darnell 1997).

Seven distinct mammalian *STAT* genes have been identified: *STAT1*, *STAT2*, *STAT3*, *STAT4*, *STAT5A*, *STAT5B* and *STAT6*, and have been mapped to three different mouse chromosomes (Copeland *et al.* 1995). This data suggests that the STAT family has arisen from a common ancestral gene by repeated gene duplication events followed by dispersion of the linked loci to different chromosomes (Copeland *et al.* 1995).

The STAT5 proteins were first identified as mammary gland factors binding to the β-casein gene promoter, suggesting a key role in the regulation of milk protein gene expression (Schmitt-Ney *et al.* 1991). STAT5A and STAT5B are two highly related proteins (96% amino acid similarity) encoded by two distinct genes that are expressed in most tissues (Darnell 1997). Different studies with STAT5-deficient mice have been performed to determine the specific function of these two proteins (Liu *et al.* 1997;

Udy *et al.* 1997). *STAT5A* knockout mice have a defect in mammary gland development and lactation, while *STAT5B* knockout mice phenotype was associated with the loss of growth hormone (GH) responses. Moreover, STAT5A and 5B double deficient mice confirmed the role of these proteins in the GH and prolactin (PRL) signalling (Teglund *et al.* 1998). This double deficient mice model presented also a significant reduced epidermal fat pads size (Teglund *et al.* 1998); suggesting a role for STAT5 proteins in the modulation of adipocyte function. Recently, Hogan & Stephens (2005) have determined the mechanism by which STAT5 proteins and PRL have an antilipogenic function in adipocytes. These authors have demonstrated the role of STAT5 proteins in the regulation of fatty acid synthase gene expression, contributing to the regulation of energy balance.

Given the importance of STAT5 proteins in the regulation of energy homeostasis, lactation and growth performance, the aim of this work was to study the genetic polymorphism in the porcine *STAT5B* gene and to determine its chromosomal location.

Materials and methods

Nucleotide sequence and detection of polymorphisms

Pig genomic DNA was isolated by the phenol–chloroform method (Ausubel *et al.* 1987) from blood samples corresponding to Duroc (n = 19), Iberian (n = 14), Landrace (n = 20), Large White (n = 20), Piétrain (n = 24) and Meishan (n = 17) pigs. The pig *STAT5B* mRNA sequence (GenBank accession number NM_214168) was aligned with the genomic DNA sequence of human *STAT5B* (NM_012448) and mouse *STAT5B* (NM_011489) genes to determine the likely exon–intron boundaries of the porcine gene. Then, a primer pair (Table 1) was designed in exon 14 (F14) and exon 16 (R16). The polymerase chain reaction (PCR) was performed in a 25-μl reaction mixture containing 0.625 units of *Taq* DNA polymerase (Invitrogen SA, Barcelona, Spain), 1x PCR buffer, 2 mM $MgCl_2$, 200 μM of each dNTP, 0.5 μM of each primer and approximately 50 ng of genomic DNA. The thermal cycling profile was: 95°C for 5 min, 35 cycles of 95°C for 30 s, 64°C for 1 min, and 72°C for 2.5 min, with a final extension of 72°C for 7 min.

Table 1 Primer sequences

Primer name	Primer sequence (5′–3′)	Primer localization
F14	TGCCTGACAAAGTCCTGTGG	Exon 14
F15	ACACTTTCTGGCAGTGGTTTG	Exon 15
R14	GGAAATGTGGCCTTCATTGG	Intron 14
R16	TGCCACCAATTTCAGAGTCA	Exon 16

Polymerase chain reaction products from two animals per breed were directly sequenced in both directions using BigDye™ Terminator v3.1 Cycle Sequencing Kit in an 3730 DNA Analyser (Applied Biosystems, Foster City, CA, USA). To detect polymorphisms, MultAlin software (Corpet 1988) and Seq-Scape™v2.1.1 software (Applied Biosystems) were used to analyse the nucleotide sequences.

PCR restriction fragment length polymorphism genotyping

Polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) technique was used to genotype the single nucleotide polymorphism (SNP) found at position 191 (using as reference the GenBank DQ144238 sequence). A 580-bp fragment encompassing 188 bp of exon 14 and 392 bp of intron 14 was amplified using primers F14 and R14. The PCR reaction was performed in a 25-μl reaction mixture containing 0.625 units of *Taq* DNA polymerase (Invitrogen SA), 1x PCR buffer, 1.5 mM $MgCl_2$, 200 μM of each dNTP, 0.5 μM of each primer and approximately 50 ng of genomic DNA. Thermocycling was carried out under the following conditions: 95°C for 5 min, 35 cycles of 95°C for 30 s, 62°C for 1 min, and 72°C for 90 s, with a final extension of 72°C for 5 min.

Amplification products (5 μl) were digested with 5 U of the *Pst*I restriction enzyme in a 20-μl volume, and the genotypes were determined by analysis of the digestion products on 2% agarose gels. In total 114 DNA samples of unrelated animals from six different pig breeds (Duroc, Iberian, Landrace, Large White, Piétrain and Meishan) were genotyped.

Chromosomal localization

Radiation hybrid mapping was performed with the INRA-University of Minnesota porcine radiation hybrid panel (IMpRH) (Yerle *et al.* 1998). We designed a pig-specific primer (F15) in exon 15 of the *STAT5B* gene, assuming the conservation of the exon–intron boundaries between the porcine and the human (NM_012448) and mouse (NM_011489) *STAT5B* genes. F15 and R16 primers were used to amplify a 271-bp fragment. The PCR mixture included 25 ng of genomic DNA, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 200 μM of each dNTP, and 0.625 units of *Taq* DNA

polymerase (Invitrogen SA) in a 12.5-μl reaction. 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 90 s, with a final extension of 72°C for 5 min. The PCR product was size separated on 2.5% agarose gels stained with ethidium bromide. Data was analysed with the IMpRH mapping tool (Milan *et al.* 2000).

Results and discussion

One fragment of approximately 1.5 kb was amplified and sequenced in animals from Duroc, Iberian, Landrace, Large White, Meishan and Piétrain breeds using primers F14 and R16 (Table 1). This fragment was sequenced from both ends obtaining two sequences of 639 bp (DQ144238) and 449 bp (DQ144239). The first sequence contains from exon 14 to intron 14, while the second sequence spans from intron 14 to exon 16. The alignment and analysis of these sequences allowed the identification of five nucleotide substitutions. These mutations were located in exon 14 at nucleotide positions 120 and 144 for Piétrain, and in the 5′-end of intron 14 at nucleotide positions 191 for Meishan and Piétrain, 326 for Duroc, Large White and Meishan, and 439 for Meishan. The two SNPs occurring in the coding region were synonymous.

The SNP found at position 191 creates a polymorphic *Pst*I site when a G is present. In order to perform the genotyping by PCR-RFLP, the primer pair F14 and R14 (Table 1) was used. The digested products showed fragments of 580 bp for the *A* allele, in which the polymorphic site is absent, and 210 + 370 bp for the allele *G* (Figure 1).

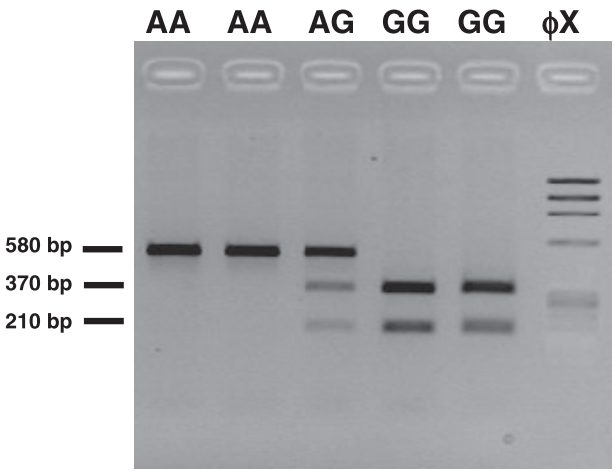

Figure 1 PCR-RELP (*Pst*I) genotypes of porcine *STAT5B* gene. The genotypes (*GG*, *AG* and *AA*) are shown at the top. φX: marker IX (Roche, Barcelona, Spain).

Allele frequencies were determined by genotyping a total of 114 unrelated animals of six different pig breeds. The allele distribution revealed that the *G* allele was only present in Large White (frequency 0.15), Piétrain (0.25), and was fixed in Meishan breed (1.0). Thus, the *G* allele is likely of Asian origin and its presence in European domestic breeds can be explained by the introgression of Asian germplasm (Kijas & Andersson 2001; Alves *et al.* 2003).

Finally, we amplified a specific porcine *STAT5B* fragment in the IMpRH panel to determine the precise location of this gene. The analysis of the results with the IMpRH mapping tool (Milan *et al.* 2000) revealed that the *STAT5B* gene was located on porcine chromosome 12 (SSC12) at 51 cR of the microsatellite marker *SW943* (LOD = 7.92) (Figure 2). This result is consistent with the *STAT5A* gene chromosomal location on pig SSC12 (Sardina *et al*. 2006). As it has been previously reported in mouse, *STAT5* genes are tightly linked on the same chromosomal region suggesting recent tandem gene duplication for the origin of these genes (Copeland *et al.* 1995).

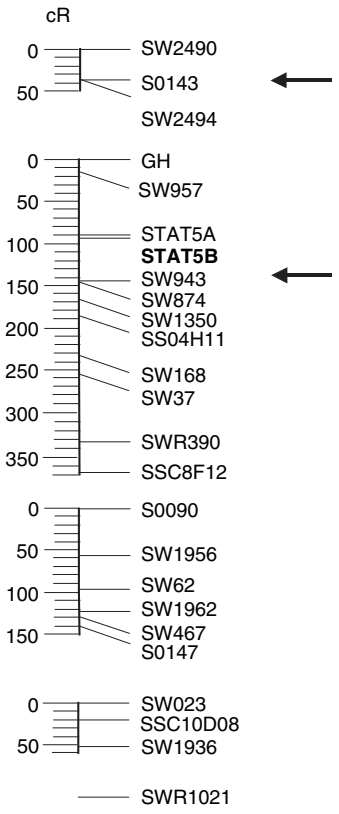

Figure 2 Chromosomal location of the *STAT5B* locus on SSC12. Arrows indicate the markers flanking the fatty acid composition QTL. Units in the radiation hybrid map are in centiRays.

A quantitative trait loci (QTL) located on chromosome 12 for fatty acid composition and metabolic ratios in an Iberian × Landrace cross has been previously described (Clop *et al.* 2003). *STAT5* genes are located in the confidence interval for this QTL these genes being positional and functional candidates for this QTL.

Acknowledgements

We are grateful to D. Milan for providing the irradiated pig/hamster somatic cell hybrid panel. M. Ballester received a research scholarship (FI) from AGAUR (Generalitat de Catalunya).

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