Assignment¹ of the porcine janus kinase 1 gene (JAK1) to chromosome 6q34→q35 by fluorescence in situ hybridization and radiation hybrid mapping

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Rationale and significance

The biological role of the non-receptor tyrosine kinase JAK1 was originally identified by its pivotal role in interferon signaling (Müller et al., 1993). It binds to the interferon α/β receptor where it mediates together with the janus kinase TYK2 the phosphorylation of downstream targets such as various transcription factors of the STAT family. JAK1 also binds to the interferon γ receptor where it acts together with JAK2 on downstream effector molecules. Subsequent studies including analysis of *Jak1*-deficient knock out mice revealed the participation of JAK1 in numerous cytokine and growth factor signaling pathways (Briscoe et al., 1996; Rodig et al., 1998). The human JAK1 gene consists of 24 exons spanning about 50 kb on chromosome 1p31.3 (Pritchard et al., 1992; GenBank contig accession no. NT_032977). Because of its important role for various immunological functions we started to characterize the JAK1 gene also in the pig. We report here the assignment of the porcine JAK1 gene to SSC6q34→q35 by FISH and RH mapping.

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Materials and methods

Isolation and characterization of the JAK1 clone

A genomic DNA clone (RP44-515H10) of approximately 210 kb was isolated from the porcine RPCI-44 BAC library (Fahrenkrug et al., 2001) after screening high density BAC filters according to the RPCI protocols (<http://www.chori.org/bacpac/>) with a ³²P-labelled insert of a porcine JAK1 cDNA clone (GenBank accession AF052689). DNA of the positive BAC clone was isolated using the Qiagen plasmid midi kit (Qiagen, Hilden, Germany). BAC ends were sequenced with the ThermoSequenase kit (AmershamBiosciences, Freiburg, Germany) on a LI-COR 4200L-2 automated sequencer (MWG Biotech, Ebersberg, Germany). The presence of the entire JAK1 gene on the clone was confirmed by DNA hybridization and by the BLAST hits of the BAC end sequences against the human genome sequence.

Fluorescence in situ hybridization (FISH) analysis

The porcine genomic BAC clone RP44-515H10 containing the porcine JAK1 gene was labeled with digoxigenin by nick translation using a Nick-Translations-Mix (Boehringer Mannheim, Mannheim, Germany). FISH on GTG-banded pig chromosomes was performed using 750 ng of digoxigenin labeled BAC DNA. In this experiment 24 μ g sheared total porcine DNA and 10 μ g salmon sperm were used as competitors. After hybridization over night, signal detection was performed using a Digoxigenin-FITC Detection Kit (Quantum Appligene, Heidelberg, Germany). The chromosomes were counterstained with DAPI and propidium iodide and embedded in antifade. 50 metaphases that were previously photographed were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Probe name: RP44-515H10

Probe type: porcine genomic BAC clone

Insert size: 210 kb

Vector: pTARBAC2

Proof of authenticity: DNA sequencing and gene-specific hybridization

Gene reference of human JAK1: Wilks et al. (1991)

Radiation hybrid (RH) mapping

A pair of PCR primers for RH mapping (5'-ACCCATCAGTACGAG-GATG-3' and 5'-GTGTTGCTCTCGCTGTTC-3') was designed from the putative intron 19 sequence of the porcine JAK1 gene. These primers generated a PCR product of 152 bp on pig genomic DNA. Amplification of the marker was done on the INRA-Minnesota Porcine Radiation Hybrid

Fig. 1. Chromosome assignment of the porcine JAK1 gene by FISH analysis on a porcine metaphase spread. The digoxigenin-labeled BAC clone RP44-515H10 containing the porcine JAK1 gene was hybridized to GTG-banded metaphase chromosomes of a normal pig. Double signals are visible on both chromosomes 6q34→q35. The chromosomes were counterstained with propidium iodide and subsequently identified by DAPI staining.

(IMpRH) panel (Yerle et al., 1998; Hawken et al., 1999; <http://imprh.toulouse.inra.fr/>) as described previously (Leeb and Rohrer, 2002). The mapping tool at the IMpRH Server (Milan et al., 2000) was used for the mapping of this new marker relatively to markers previously mapped on the IMpRH panel. The order of markers already mapped on the first generation map are considered as known (Hawken et al., 1999). The two point option of the RHMAP3.0 package (Lange et al., 1995) reveals two point distances with flanking markers.

Results and discussion

The porcine BAC clone RP44-515H10 has been retrieved from the RPCI-44 BAC library with a porcine JAK1 cDNA probe. DNA hybridization and analysis of the end sequences of the BAC clone confirmed that the porcine JAK1 gene was contained on the clone. The chromosomal location of the porcine JAK1 gene was determined by FISH of the BAC clone to metaphase chromosomes (Fig. 1). This JAK1 BAC clone has been assigned to SSC6q34→q35.

Mapping data:

Location: SSC6q34→q35

Number of cells examined: 50

Number of cells with specific signal: 0 (0), 1 (0), 2 (12), 3 (5), 4 (33) chromatids per cell

Most precise location: 6q34→q35

Location of background signal (sites with > 2 signals): none observed

Additionally, the localization of the JAK1 gene was verified by RH mapping on the porcine IMpRH panel. The RH results were submitted to the IMpRH database (<http://imprh.toulouse.inra.fr/>). Two-point analysis revealed that JAK1 is linked to the marker S0121 on SSC6 with a distance of 50 cR and a two point Lod score of 8.3. The RH results were thus consistent with the cytogenetic localization of JAK1 at SSC6q34→q35. The human JAK1 ortholog is located on HSA1p31.3 (Pritchard et al., 1992) and the murine *Jak1* gene is located on MMU4 (Gough et al., 1995). These localizations correspond well to the synteny data of the porcine RH map (Rink et al., 2002).

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Note added in proof

During the processing of this manuscript another report by Sun et al. (2002) has become available describing the localization of porcine JAK1 to SSC6q31→q35 by somatic cell hybrid mapping.