Brief Gene Mapping Report

Cytogenet Genome Res 108:363H (2005)
DOI: 10.1159/000081540

Cytogenetic and Genome Research

Assignment[1] of the bovine TYK2 and PDE4A genes to bovine chromosome 7q15 by fluorescence in situ hybridization and radiation hybrid mapping

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Manuscript received 12 July 2004; accepted in original form for publication by M. Schmid 20 August 2004.

[1] To our knowledge this is the first time these genes have been mapped in cattle.

Rationale and significance

The non-receptor tyrosine kinase TYK2 is a key component of the interferon signalling pathway. It can bind to the interferon α/β receptor where it mediates, together with another non-receptor tyrosine kinase called JAK1, the phosphorylation of downstream targets. Other cytokine receptors that mediate their signals via TYK2 include the receptors for IL-6, IL-11, CNTF, LIF, and oncostatin M (Briscoe et al., 1996). Functional studies with *Tyk2* deficient mice demonstrated that these mice are viable and show very specific changes in several cytokine responses. Surprisingly, interferon α/β signalling is only impaired but not completely abolished in these mice (Karaghiosoff et al., 2000, 2003). Naturally occurring mutations in the murine *Tyk2* gene are associated with varying susceptibilities to infectious and autoimmune disease (Shaw et al., 2003). The human TYK2 gene consists of 25 exons spanning about 30 kb on chromosome 19p13.2 (Firmbach-Kraft et al., 1990; NCBI map viewer, human genome build 35.1). The murine *Tyk2* gene also consists of 25 exons and is located on mouse chromosome 9A3 (NCBI map viewer, mouse genome build 32.1). Because of their important role for various immunological functions we started to characterize the non-receptor tyrosine kinase genes in livestock species. Following previous studies in pig (Kuiper et al., 2003; Leeb et al., 2004) we report here the assignment of the bovine TYK2 gene and the tightly linked PDE4A gene to BTA7q15 by FISH and RH mapping.

Materials and methods

Isolation and characterization of the bovine TYK2 clone

The bovine BAC library RPCI-42 (Warren et al., 2000) was screened for clones with the TYK2 gene according to the CHORI protocols (http://www.chori.org/bacpac/) with a 954-bp ^{32}P-labelled cDNA fragment corresponding to nucleotides +2251 to +3204 of the porcine TYK2 cDNA. A bovine genomic BAC clone designated RP42-545G13 of approximately 190 kb was isolated. BAC DNA was prepared from 100 ml overnight cultures using the Qiagen Midi plasmid kit according to the modified protocol for BACs (Qiagen, Hilden, Germany). BAC ends were sequenced using the ThermoSequenase kit (Amersham Biosciences, Freiburg, Germany) and a LI-COR 4200 automated sequencer. The BAC end sequences were deposited in the EMBL nucleotide database (Accessions AJ704778, AJ704779) and used in BLAST analyses against build 34 of the human genome.

Chromosome preparation

Bovine metaphase spreads for FISH on GTG-banded chromosomes were prepared using phytohemagglutinin stimulated blood lymphocytes. Cells were harvested and slides prepared using standard cytogenetic techniques. Prior to fluorescence in situ hybridization the chromosomes were GTG-banded and well-banded metaphase chromosomes were photographed using a highly sensitive CCD camera and IPLab 2.2.3 (Scanalytics, Inc.). Identification of chromosomes strictly followed the ISCNDB (2000) classification (Cribiu et al., 2001).

This work was supported by a grant of the German Research Council DFG to T.L. (LE1032/14-1) and by support from the EC BovGen project to J.L.W. (QLRT-CT-2002-02744). The authors thank Heike Klippert-Hasberg and Diana Seinige for expert technical assistance.

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Fluorescence in situ hybridization (FISH) analysis

The bovine BAC clone RP42-545G13 containing the bovine TYK2 gene was labelled with digoxigenin by nick translation using a Nick-Translation-Mix (Boehringer Mannheim, Mannheim, Germany). FISH on GTG-banded bovine chromosomes was performed using 750 ng of digoxigenin-labelled BAC DNA. 1 µg sheared total bovine DNA and 10 µg salmon sperm were used as competitors in this experiment. After hybridization overnight, 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. Thirty metaphases that had been photographed were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Probe name: RP42-545G13
Probe type: bovine genomic BAC clone
Insert size: 190 kb
Vector: pBACe 3.6
Proof of authenticity: DNA sequencing
Gene reference: Firmbach-Kraft et al. (2001)

Radiation hybrid (RH) mapping

To confirm the cytogenetic localization of the bovine TYK2 gene a bovine radiation hybrid panel was analyzed. The Roslin/Cambridge bovine RH panel was purchased from Research Genetics (Huntsville, Ala., USA). The RH panel of 94 clones was created by exposing a bovine fibroblast primary cell line to 3,000 rads of X-rays and fusing the irradiated cells with non-irradiated HPRT-deficient hamster recipient cells (Wg3H). A pair of bovine primers (PDE4A–F 5′-TAG GCC TTC CAG AGT CCA G-3′; PDE4A–R 5′- CTG GAA CCC TTC AGC TCA C-3′) for PCR amplification was designed based on the SP6 sequence of BAC clone RP42-545G13 to give a 327-bp fragment. This STS is located in intron 7 of the PDE4A gene. PCR reactions were performed in a total of 20 µl containing 25 ng of RH cell line DNA, 15 pmol of each primer and 0.75 U *Taq* polymerase (Qbiogene, Heidelberg, Germany). The reaction started with an initial denaturation at 94 °C for 4 min followed by 35 cycles under the following conditions: denaturation for 30 s at 94 °C, annealing for 60 s at 58 °C and extension for 30 s at 72 °C. PCR products were separated on 1 % agarose gels. Two separate PCRs were carried out and scoring of the presence or absence of products was carried out independently by two investigators. The RHMAP3.0 package (Lange et al., 1995) was used for a two-point analysis against approximately 1,400 bovine microsatellite markers typed previously on the panel (Williams et al., 2002).

Results and discussion

The bovine BAC clone RP42-545G13 was retrieved from the BAC library using a heterologous porcine TYK2 cDNA probe. Sequencing and BLAST analysis of the SP6 BAC end sequence (AJ704778) revealed a match within the human PDE4A gene at 10.429 Mb on HSA19 (build 35.1; BLAST E value 0.009). The BAC end sequence also showed 75 % identity over 758 bp with sequences from intron 7 of the porcine PDE4A gene (AJ632303). The T7 sequence of the BAC clone did not show any significant homology to the human genome. Consequently, the T7 BAC end sequence was used to query the publicly available bovine whole genome shotgun sequences of the TraceArchive. Thus, the T7 BAC end sequence was stepwise extended to a sequence contig of 3.5 kb. The extended T7 sequence contig gave a highly significant BLAST hit at 10.231 Mb on HSA19 (build 35.1; BLAST E value $2 \cdot 10^{-43}$). Therefore, the comparative mapping established that the 190-kb bovine BAC clone covered approximately 198 kb of human genomic DNA sequence with seemingly good overall conservation of synteny. The human interval that is covered by this bovine BAC clone contains the complete genes ICAM1, ICAM4, ICAM5, MGC19604, RAVER1, ICAM3, TYK2, CDC37, and the 5′ end of the PDE4A gene. Experimental evidence for the gene content of the bovine BAC clone is available for the TYK2 gene (hybridization probe during the library screen) and the PDE4A gene (partial DNA sequence). The chromosomal location of this bovine BAC clone was determined by fluorescence in situ hybridization (FISH) to bovine metaphase chromosomes (Fig. 1).

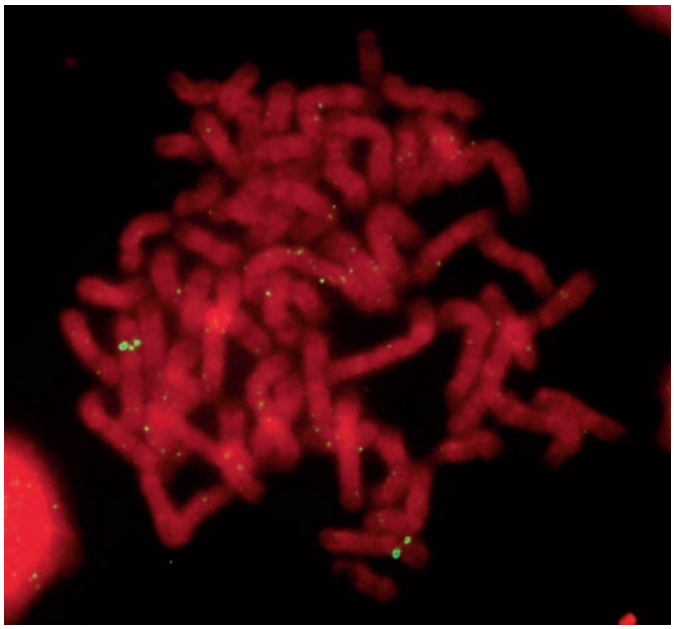

Fig. 1. Chromosomal assignment of the TYK2 and PDE4A genes by FISH analysis. The digoxigenin-labeled BAC clone RP42-545G13 containing the entire TYK2 gene and parts of the PDE4A gene was hybridized to GTG-banded metaphase chromosomes of a normal cow. Double signals are visible on both chromosomes 7q15. The chromosomes were counterstained with DAPI and propidium iodide and subsequently identified by GTG banding and DAPI staining.

Mapping data:
Location: 7q15
Number of cells examined: 30
Number of cells with specific signals: 0 (0), 1 (0), 2 (2), 3 (3), 4 (25) chromatids per cell
Most precise assignment: 7q15
Location of background signals (site with >2 signals): none observed

To confirm the chromosome location of the BAC clone the Roslin/Cambridge bovine RH panel was analyzed. The STS marker used for RH mapping was designed from the SP6 BAC end sequence located in intron 7 of the PDE4A gene. Two-point analysis revealed close linkage of PDE4A to the anonymous BTA7 marker RSJW410 at a distance of 0.0 cR (LOD score 18.0). The closest microsatellite marker is IDVGA62 at a distance of 10.4 cR (LOD 14.9), which has been physically mapped to BTA7q15 (Ferretti et al., 1997). Thus the RH results for the bovine TYK2/PDE4A BAC confirmed and refined the

results obtained by FISH. The chromosomal assignment of TYK2 and PDE4A to BTA7q15 is in good agreement with known conservation of synteny between HSA19p and BTA7 (Band et al., 2000).

References

Band MR, Larson JH, Rebeiz M, Green CA, Heyen DW, Donovan J, Windish R, Steining C, Mahyuddin P, Womack JE, Lewin HA: An ordered comparative map of the cattle and human genomes. Genome Res 10:1359–1368 (2000).

Briscoe J, Kohlhuber F, Müller M: JAKs and STATs branch out. Trends Cell Biol 6:336–340 (1996).

Cribiu EP, Di Berardino D, Di Meo GP, Eggen A, Gallagher DS, Gustavsson I, Hayes H, Iannuzzi L, Popescu CP, Rubes J, Schmutz S, Stranzinger G, Vaiman A, Womack J: International System for Chromosome Nomenclature of Domestic Bovids (ISCNDB 2000). Cytogenet Cell Genet 92:283–299 (2001).

Ferretti L, Urquhart BG, Eggen A, Olsaker I, Harlizius B, Castiglioni B, Mezzelani A, Solinas Toldo S, Thieven U, Zhang Y, Morgan AL, Teres VM, Schwerin M, Martin-Burriel I, Chowdhary BP, Erhardt G, Nijman IJ, Cribiu EP, Barendse W, Leveziel H, Fries R, Williams JL: Cosmid-derived markers anchoring the bovine genetic map to the physical map. Mamm Genome 8:29–36 (1997).

Firmbach-Kraft I, Byers M, Shows T, Dalla-Favera R, Krolewski JJ: tyk2, prototype of a novel class of non-receptor tyrosine kinase genes. Oncogene 5:1329–1536 (1990).

Karaghiosoff M, Neubauer H, Lassnig C, Kovarik P, Schindler H, Pircher H, McCoy B, Bogdan C, Decker T, Brem G, Pfeffer K, Müller M: Partial impairment of cytokine responses in Tyk2-deficient mice. Immunity 13:549–560 (2000).

Karaghiosoff M, Steinborn R, Kovarik P, Kriegshauser G, Baccarini M, Donabauer B, Reichart U, Kolbe T, Bogdan C, Leanderson T, Levy D, Decker T, Müller M: Central role for type I interferons and Tyk2 in lipopolysaccharide-induced endotoxin shock. Nat Immunol 4:471–477 (2003).

Kuiper H, Martins-Wess F, Lassnig C, Distl O, Müller M, Leeb T: Assignment of the porcine janus kinase 1 gene (JAK1) to chromosome 6q34 → q35 by fluorescence in situ hybridization and radiation hybrid mapping. Cytogenet Genome Res 101:92D (2003).

Lange K, Boehnke M, Cox DR, Lunetta KL: Statistical methods for polyploid radiation hybrid mapping. Genome Res 5:136–150 (1995).

Leeb T, Martins-Wess F, Kuiper H, Lassnig C, Distl O, Müller M: Molecular characterization of the porcine TYK2 gene on SSC 2q1.3 → q2.1. Cytogenet Genome Res 107:103–107 (2004).

Shaw MH, Boyartchuk V, Wong S, Karaghiosoff M, Ragimbeau J, Pellegrini S, Müller M, Dietrich WF, Yap GS: A natural mutation in the Tyk2 pseudokinase domain underlies altered susceptibility of B10.Q/J mice to infection and autoimmunity. Proc Natl Acad Sci USA 100:11594–11599 (2003).

Warren W, Smith TP, Rexroad CE, Fahrenkrug SC, Allison T, Shu L, Catanese J, de Jong P: Construction and characterization of a new bovine bacterial artificial chromosome library with 10 genome-equivalent coverage. Mamm Genome 11:662–663 (2000).

Williams JL, Eggen A, Ferretti L, Farr CJ, Gautier M, Amati G, Ball G, Caramorr T, Critcher R, Costa S, Hextall P, Hills D, Jeulin A, Kiguwa SL, Ross O, Smith AL, Saunier K, Urquhart B, Waddington D: A bovine whole-genome radiation hybrid panel and outline map. Mamm Genome 13:469–474 (2002).