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Cytogenetic and syntenic assignment of the bovine *platelet-activating factor receptor* (*PTAFR*) to cattle chromosome 2 (Brief report)

Zytogenetische und synthänische Kartierung des bovinen *Platelet-Activating Factor Receptor (PTAFR)* auf Rinderchromosom 2 (Brief Report)

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Background

The platelet-activating factor receptor (*PTAFR*) encoding gene, also known as *PAFR* or *PAFr*, belongs to the rhodopsin gene family. The receptor binds the platelet-activating factor (PAF) that has been implicated as a mediator in diverse pathologic processes. In cattle, *PTAFR* is associated to the reproduction process and is described as a receptor that is involved in inflammatory-like processes of the uterus associated with increased vascular permeability (TIEMANN *et al.* 2005). The gene sequence was recently annotated on *Bos taurus* (BTA) chromosome 2 at 129.4 megabases in NCBI *Bos taurus* build Btau_4.0. The presented data confirm this annotation by independent physical mapping methods and anchor the corresponding DNA segment to the chromosome. *PTAFR* was assigned by fluorescence in situ hybridization (FISH) and somatic hybrid cell (SHC) mapping.

Material and methods

Primer sequences for SHC mapping and BAC identification, PTAFR-F 5'-ACC TGG CTG GCT GCT TCT TCT-3' and PTAFR-R 5'-GGG ATG CTG CCC TTC TCG TAA, were designed from the bovine cDNA sequence: Genbank acc. no. AJ295321. Overlapping parts of this bovine cDNA sequence and the actual reference DNA sequence NM_001040538 for *PTAFR* in cattle NCBI *Bos taurus* build Btau_4.0 match 100 %.

Pools of the 5× genome covering bovine BAC library BBI_B750 (ZHU *et al.* 1999) were received from the former Resource Center of the German Human Genome Project (RZPD, Berlin, Germany) and screened by PCR to obtain a genomic DNA fragment specific bovine specific *PTAFR*. DNA sequencing of the PCR product was performed to verify the amplification of the bovine *PTAFR* DNA sequence. Genome database search using the NCBI BLAST tool identified similarity between the bovine PCR product and human genome data. DNA of the identified BAC clone was isolated and purified and used as probe DNA in FISH experiments. Metaphase spreads for FISH were prepared from a cattle embryonic male fibroblast culture by standard cytogenetic techniques. Chromosomes were G-banded by trypsin and Giemsa stained and digitized images of metaphase spreads were applied. Chromosomes were karyotyped according to the ISCNDB 2000 guidelines for bovine G-banded chromosomes (DI BERARDINO *et al.* 2001). Hybridization

*To our knowledge, this is the first physically chromosome assignment of this gene in cattle.

was performed essentially as described by (PINKEL *et al.* 1986). Briefly, probes were labeled with biotin-16-dUTP (Roche) by nick-translation. The DNA mixture for FISH was prepared using 0.2 µg of the probe, 5 µg bovine C_0t-1 DNA, and 20 µg salmon sperm DNA. The analysis of FITC fluorescence signals on propidium iodide colored chromosomes was performed with a fluorescence microscope (Nikon FXA). The overlay of digitized images from the same metaphases prior and after FISH identified the chromosome band with FITC signals.

For SHC mapping, chromosomal assignment of *PTAFR* has been carried out using a characterized hamster/bovine somatic hybrid cell panel (LAURENT *et al.* 2000). The PCR reaction in each cell line of the SHC panel was performed in a final volume of 10 µl with 100 ng DNA, 10 pmol of each primer, 200 µM dNTPs, 0.15 U *Taq* DNA Polymerase (Promega), and standard enzyme buffer conditions. Samples were initially denatured at 94 °C for 3 min followed by 35 PCR cycles. Each PCR cycle consisted of denaturation at 95 °C for 15 sec, annealing at 65 °C for 1 min and extension at 72 °C for 15 sec. A final elongation step for 7 min at 72 °C finished the PCR. PCR products were separated by electrophoresis on agarose gels stained with ethidium bromide.

Results

PCR screening in the bovine BAC library identified the *PTAFR* containing DNA fragment in BAC clone B750-L22158Q2. The large fragment clone has been used as probe for FISH and assigned the bovine *PTAFR* gene to BTA2q45 (Figure1). DNA sequencing of the 277 bp PCR product proved gene specificity. PCR using the *PTAFR* primers on the SHC panel detected a typical bovine-specific band in 12 of the 39 hybrid cell lines. The data vector obtained was: 11000010000001000100000000100101100111 (1=present in cell line; 0=not present in cell line). A concordance value of 0.94 linked *PTAFR* to the SHC microsatellite marker CSSM042 and assigned the gene to the BTA2 corresponding synteny group U17.

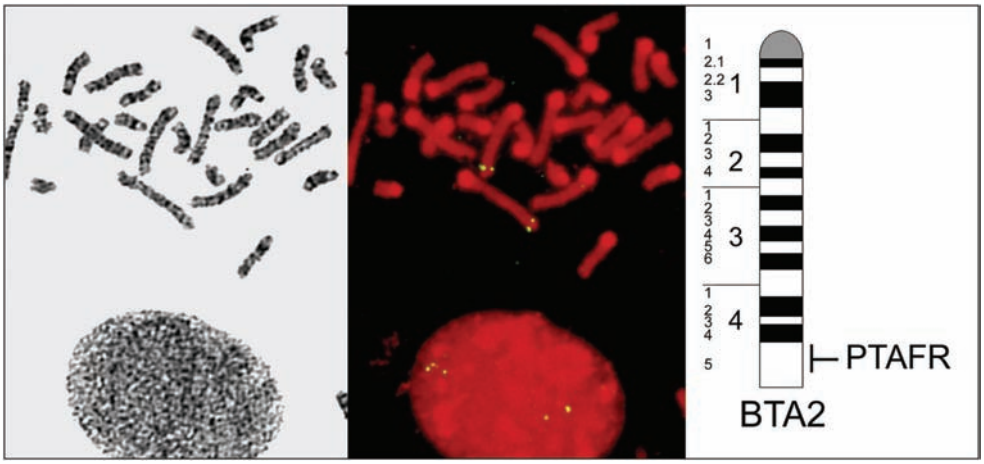

Figure 1
Assignment of *PTAFR* to cattle chromosome 2q45
Kartierung des PTAFR-Gens auf Rinderchromosom 2q45

The comparative mapping position of *PTAFR* in human is on *Homo sapiens* (HSA) chromosome region HSA1p35-p34.3. Bovine *PTAFR* is the first comparative cytogenetic anchor that identifies conserved synteny between HSA1p35.3-p34.3 and BTA2q45. Four more genes, *RHCE*, *RHD*, *ALPI*, and *CHRND*, with described cytogenetic assignments to BTA2q45 have cytogenetic mapping positions on HSA1p36.11 in human (*RHCE*, *RHD*) or proximal on HSA2p (*ALPI*, *CHRND*), respectively. Thus, the *PTAFR* locus in cattle is the most distal cytogenetic anchor indicating homology between BTA2 and HSA1.

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