Assignment¹ of mitochondrial glycerol-3-phosphate acyltransferase (GPAM) gene to bovine chromosome 26 (26q22) by in situ hybridization and confirmation by somatic cell hybrid mapping

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¹ To our knowledge this is the first time this gene has been mapped in cattle.

Rationale and significance

Glycerol-3-phosphate acyltransferase catalyzes the first step of glycerolipid biosynthesis. It plays a key role in the regulation of cellular triacylglycerol and phospholipid levels. There are two isoforms of glycerol-3-phosphate acyltransferase in mammals, a mitochondrial and a cytosolic form. The mitochondrial form (GPAM) prefers saturated fatty acyl-CoA as a substrate, whereas the cytosolic enzyme uses both saturated and unsaturated fatty acyl-Co. The GPAM gene has been cloned and sequenced in rat (NM017274) and mouse (NM008149). Human GPAM has been located on chromosome 10 (HSA10q24→q26) and mouse *Gpam* on MMU19 (Welch et al., 1998) whereas the bovine GPAM gene remains unassigned. We used the mouse *Gpam* gene sequence to design specific primers in order to partially amplify the bovine gene. We report the localization of the bovine GPAM gene on bovine (BTA) chromosome 26q22 by fluorescence in situ hybridization and somatic cell hybrid analysis.

Materials and methods

Isolation of a specific bovine GPAM fragment

In order to obtain a specific bovine DNA fragment, PCR amplification was performed using one set of primers:

GPAM.F: 5'-AGCACCAGCAATTCATCACC-3'

GPAM.R: 5'-TTCTGCAGGTACTCAGACTC-3'

Primers designed from mouse *Gpam* sequence amplified a 120-bp sequence. PCR amplification was performed using 20 ng of genomic DNA. Cycling conditions were 94 °C for 30 s, 53 °C for 30 s and 72 °C for 30 s for 35 cycles. PCR products were cloned and sequenced. Homology searches were performed with BLAST programs at National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>). The isolated bovine sequence showed a high identity with GPAM in other species (82 % with mouse and rat).

Screening of a bovine BAC library

The above primers were used to screen a bovine BAC library (Eggen et al., 2001). Two BAC clones containing the bovine GPAM gene were identified, 522B11 and 789H09.

Fluorescence in situ hybridization (FISH)

In situ hybridization was carried out on RBP-banded bovine chromosome preparations, as described previously (Hayes et al., 2000).

Probe name: 522B11

Probe type: Genomic DNA

Vector: pBelo BAC11

Insert size: 150 kb

Proof of authenticity: DNA sequencing

Gene reference: AF469047

Somatic cell hybrid mapping

Somatic cell hybrid analysis was performed using a panel of 38 hamster-bovine hybrid clones constructed by Heuertz and Horst-Cayla (1981) and

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Fig. 1. (a) Chromosome mapping of GPAM by FISH. A specific signal is observed on chromosome 26q22. (b) Results of the screening of the somatic cell hybrid panel. Lanes 3, 10, 32, 39, 40 and 42 show the specific fragment. Lanes 24 and 43 are bovine controls. Lanes 25 and 44 are hamster controls. Lane 46 is a human control. This confirms the localization of the GPAM gene on BTA26.

characterized by Laurent et al. (2000). Since primers GPAM.F and GPAM.R amplified both hamster and bovine DNA resulting in a fragment of similar size, other primers flanking an intron were designed and used to amplify a 700-bp fragment from the GPAM bovine sequence.

GPAM3: 5'-GTCACAATCACCCACACGAG-3'
GPAM4: 5'-ACTTCCCAGGGCTTCTGTTT-3'

Results

Mapping data

Most precise location: 26q22

Number of cells examined: 15

Number of cells with specific signal: 1 (0), 2 (4), 3 (1), 4 (10) chromatids per cell

Location of background signals: none observed

Figure 1a shows a partial metaphase spread with signals on both bovine chromosomes 26.

Mapping by somatic cell hybrid analysis

Correlation coefficient: 0.90

Somatic cell hybrid analysis is shown in Fig. 1b. Correlation coefficient is 0.90 with the reference markers RW6 and RW11, which are located on BTA26. The results of FISH and somatic cell hybrid analysis confirm the assignment of bovine GPAM to BTA 26 which is consistent with comparative mapping information since GPAM maps in human to HSA10 and in mouse to MMU19 which both share conserved regions with BTA26.

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