Assignment¹ of monoglyceride lipase (MGLL) gene to bovine chromosome 22q24 by in situ hybridization and confirmation by radiation 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

Monoglyceride lipase (MGLL) functions together with hormone-sensitive lipase (HSL) in the mobilization of fatty acids from the triglyceride stores of adipocytes (Holm et al., 1986). HSL catalyzes the hydrolysis of triglycerides to diglycerides and the subsequent hydrolysis of diglycerides and, to some extent, monoglycerides (Fredrikson et al., 1981). However, MGLL is required to obtain complete hydrolysis of monoglycerides. The MGLL gene has been cloned and sequenced in man (NM_007283) and in mouse (NM_011844). The MGLL gene has been mapped to human (HSA) chromosome 3 and to mouse (MMU) chromosome 6 (Karlsson et al., 2001), but as yet, the bovine MGLL gene has not been mapped. We used a bovine EST (BF046260) homologous to MGLL human gene sequence to design specific primers to partially amplify the bovine gene. We report the localization of the bovine MGLL gene on bovine chromosome BTA22q24 by fluorescence in situ hybridization and radiation hybrid mapping.

Materials and methods

Isolation of a specific bovine MGL fragment

In order to obtain a specific bovine MGL DNA fragment, PCR amplification was performed using the following set of bovine-specific primers:

MGLL.F: 5'-TGAGCGTCTTGCTCTGGCTC-3'

MGLL.R: 5'-GGCAACCAGCTGCTCAACA-3'

Primers were designed from the bovine EST (BF046260) sequence. They amplified a 156-bp fragment. PCR amplification was performed using 20 ng of genomic DNA. Cycling conditions were 94 °C for 30 s, 55 °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 the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/BLAST/>).

The isolated bovine sequence showed a high level of sequence homology with the human and mouse MGLL genes, 84 % and 83 %, respectively.

Screening of a bovine BAC library

Using the bovine-specific primers, a bovine BAC library (Eggen et al., 2001) was screened by PCR and a BAC clone containing the bovine MGLL gene was identified. Partial sequence analysis of the BAC clone (589D2) confirmed the presence of MGLL sequences (Accession number: AY251819).

Fluorescence in situ hybridization (FISH)

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

Probe name: 589D2

Probe type: genomic DNA

Vector: pBelo BAC11.

Insert size: 150 kb

Proof of authenticity: DNA sequencing.

Gene reference: AY251819

Radiation hybrid (RH) mapping

To confirm the cytogenetic localization obtained by FISH, a bovine × hamster radiation hybrid panel was analyzed. PCR reactions were performed on the 94 radiation hybrid cell lines that constitute the newly developed 3000 Rad bovine panel (Williams et al., 2002). Primers and PCR conditions for radiation hybrid mapping were the same as those used for screening the BAC library.

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Fig. 1. FISH mapping of MGLL using a bovine BAC clone containing the MGLL gene. A specific signal is observed on chromosome 22q24.

The Carthagene software (Schiex et al., 2002) was used to perform two-point and multipoint analyses of the radiation hybrid data and to provide a map of the region where the MGLL gene is located (Fig. 2). The distances between markers on the most likely map were calculated with the RHMAP3.0 software (Lange et al., 1995) under the equal retention probability model.

Results

Mapping data

Most precise location: 22q24

Number of cells examined: 25

Number of cells with specific signal: 1 (0) 2 (9), 3 (6), 4 (10) chromatids per cell

Location of background signals: none observed

Radiation hybrid mapping

Vector result: 00011 10101 00111 00111 00000 01010 11000 00001 01010 10001 00000 00000 00011 00100 00010 01001 00001 00000 0000. Most significantly linked marker by two-point analysis: BM4102 on BTA 22 (57 cRay; LOD= 6)

The localization of the MGLL gene on bovine chromosome 22 agrees totally with comparative mapping data between cattle and man since BTA22 corresponds to part of human chromosome 3.

References

Eggen A, Gautier M, Billaut A, Petit E, Hayes H, Laurent P, Urban C, Pfister-Genskow M, Eilertsen K, Bishop MD: Construction and characterization of a bovine BAC library with four genome-equivalent coverage. *Genet Sel Evol* 33:543–548 (2001).

Fredrikson G, Stralfors P, Nilsson NO, Belfrage P: Hormone-sensitive lipase of rat adipose tissue. Purification and some properties. *J Biol Chem* 256:6311–6320 (1981).

Hayes H, Di Meo GP, Gautier M, Laurent P, Eggen A, Iannuzzi L: Localization by FISH of 31 Texas nomenclature type I markers to both Q and R banded bovine chromosomes. *Cytogenet Cell Genet* 90:315–320 (2000).

Holm C, Fredrikson G, Belfrage P: Demonstration of the amphiphilic character of hormone-sensitive lipase by temperature-induced phase separation in Triton X-114 and charge-shift electrophoresis. *J Biol Chem* 261:15659–15661 (1986).

Karlsson M, Reue K, Xia YR, Lusis AJ, Langin D, Tornqvist H, Holm C: Exon-intron organization and chromosomal localization of the mouse monoglyceride lipase gene. *Gene* 272:11–18 (2001).

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

Schiex T, Chabrier P, Bouchez M, Milan D: Boosting EM for radiation hybrid and genetic mapping. *Theor Appl Genet*, in press (2002).

Williams JL, Eggen A, Ferreti L, Farr C, Gautier M, et al: A bovine whole genome radiation hybrid panel and outline map. *Mammal Genome* 13:469–474 (2002).

Fig. 2. RH mapping of MGLL gene to bovine chromosome 22. Genetic distances in centiRay around MGAM are shown on the right.