Assignment¹ of the bovine C10orf2 gene to bovine 26q13→q21 by fluorescence 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

Mutations in coding regions of the C10orf2 (chromosome 10 open reading frame 2) gene encoding the mitochondrial protein twinkl were shown to co-segregate in man with autosomal dominant progressive external ophthalmoplegia (adPEO) in several pedigrees of various ethnic origin (Spelbrink et al., 2001). A specific feature of the disease is accumulation of multiple deletions of mtDNA in tissues, mostly in the brain, muscle and heart, which results in a lack of the respiratory chain proteins thus leading to defective energy production and muscle weakness, most severely in the ocular muscles (Soumalainen et al., 1995). The human C10orf2 gene is located on HSA10q24.31 (<http://www.ensembl.org>). The objective of this study was to map the C10orf2 gene by fluorescence in situ hybridization and radiation hybrid mapping in cattle. C10orf2 appears as an appropriate candidate gene for bilateral convergent strabismus with exophthalmus (BCSE) in cattle, because the phenotype of BCSE in cattle shows striking similarities to the heterogeneous dominant and recessive forms of PEO in man (Distl, 1993). BCSE is similarly to adPEO characterized by a progressive symmetrical insufficient function of the eye muscles recti laterals and retractores. Furthermore, in German

Brown cattle a monogenic, autosomal dominant inheritance proved to be most probable using complex segregation analysis (Distl, 1993). Due to the wide dissemination of BCSE in many cattle breeds, the late onset in life not earlier than by an age of 1–2 years, and a high incidence of 0.9% of this inherited anomaly in German Brown cattle, the development of an effective genetic program based on molecular genetic tests is strongly warranted.

Materials and methods

Isolation and characterization of the bovine C10orf2 clone

For the isolation of the bovine BAC clones with the C10orf2 gene a genomic bovine BAC library (Warren et al., 2000) constructed in pBACe3.6 was screened according to the RPCI protocols (<http://www.chori.org/bac-pac/>) with a ³²P-labeled insert of the IMAGE cDNA IMAGp998D139950 clone (Ressource Center/Primary Database of the German Human Genome Project; <http://www.rzpd.de/>) containing 2.2 kb corresponding to the complete ORF of the human C10orf2 gene. A bovine genomic DNA BAC clone designed RPCI-42_291E8 of approximately 175 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 DNA termini were sequenced using the ThermoSequenase kit (AmershamPharmacia, Freiburg, Germany) and a LI-COR 4200 automated sequencer. The isolated bovine BAC clone containing C10orf2 was digested with the restriction enzyme *SacI* separated on a 0.8% agarose gel. The DNA from the gel was transferred to a nylon membrane, which was then hybridized with the insert of the IMAGp998D139950 cDNA clone labeled using the ECLTM direct labeling and detection kit (Amersham Biosciences, Freiburg, Germany). The BAC insert showed a positive hybridization signal.

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.). Chromosomes were arranged according to ISCNDB (2000).

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Fig. 1. Chromosomal assignment of the C10orf2 gene by FISH analysis. The digoxigenin labeled BAC clone RPCI-42_291E8 containing the C10orf2 gene was hybridized to GTG-banded metaphase chromosomes of a normal cow. The chromosomes were counterstained with DAPI and propidium iodide and subsequently identified by GTG-banding and DAPI staining. (A) GTG-banding of the bovine metaphase spread. (B) Double signals indicated by arrows are visible on both chromosomes 26q13→q21.

Fluorescence in situ hybridization (FISH) analysis

The 175-kb bovine BAC clone containing the bovine C10orf2 gene was labeled 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 embedded in propidium iodide, antifade, 35 metaphases that were previously photographed were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Probe name: RPCI-42_291E8

Probe type: bovine genomic BAC clone

Insert size: 175 kb

Vector: pBACe 3.6

Proof of authenticity: DNA sequencing

Gene reference: Spelbrink et al. (2001)

Radiation hybrid (RH) mapping

To confirm the cytogenetic localization of the bovine C10orf2 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 the bovine cell line to 3,000 rad of X-rays and fusion with non-irradiated HPRT deficient hamster recipient cells (Wg3H). A pair of bovine primers (C10orf2_F 5'-TCC TCC CCT CCT TCC TCA C-3'; C10orf2_R 5'-TGA GGT GGG GGT GAA CTT G-3') for PCR amplification of a 507-bp fragment of the C10orf2 gene was designed based on the SP6 terminus BAC clone sequence. Two independent PCR reactions were performed in a total of 25 µl using 25 ng of RH cell line DNA, 0.5 U of Qiagen *Taq* polymerase, and annealing at 59 °C. PCR products were separated on a 1% agarose gel. Scoring of PCR 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.

Results and discussion

The bovine BAC clone RPCI-42-291E8 was retrieved from the BAC library using a heterologous human cDNA C10orf2 probe. Sequencing of the SP6 BAC terminus (AJ440729) revealed a match of 83% identity over 313 bp (BLAST E-value <10⁻³⁶) to the 113-kb human genomic DNA chromosome 10q24.31 clone RP11-179B2 (GenBank acc. no. AL138762), which is located approximately 100 kb upstream to the annotated C10orf2 gene on HSA10q24.31 (<http://www.ensembl.org/>).

The chromosomal location of the bovine C10orf2 gene was determined by fluorescence in situ hybridization (FISH) of the BAC clone RPCI-42_291E8 to bovine metaphase chromosomes (Fig. 1).

Mapping data:

Location: 26q13→q21

Number of cells examined: 35

Number of cells with specific signals: 0 (1), 1 (1), 2 (10), 3 (10), 4 (13) chromatids per cell

Most precise assignment: 26q13→q21

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

To confirm the chromosome location of the bovine C10orf2 gene the Roslin/Cambridge bovine RH panel was analyzed. The STS marker used for RH mapping was designed from the SP6 BAC end sequence. Two-point analysis revealed close linkage of C10orf2 to the BTA26 marker CSKB74 at a distance of 25 cR (LOD score 8.5). This microsatellite marker has been

physically mapped on BTA26q14 (Byrne et al., 1997). The RH results for the bovine C10orf2 BAC confirmed the results obtained by FISH. This chromosomal assignment of C10orf2 to BTA26 is in good agreement with known conservation of synteny between HSA10q and BTA26 (Band et al., 2000).

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