Comparative mapping¹ of the POLG gene to cattle chromosome 21q17→q22 by FISH and confirmation by RH mapping

C. Drögemüller,^a H. Kuiper,^a G. Hauke,^a J.L. Williams^b and O. Distl^a

^aInstitute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover (Germany);

^bRoslin Institute (Edinburgh), Roslin, Midlothian (Scotland)

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

Rationale and significance

The DNA directed polymerase gamma gene (POLG), known to be located on human chromosome (HSA) 15q25 (Walker et al., 1997), encodes the only DNA polymerase responsible for mitochondrial DNA (mtDNA) replication. Mutations in human POLG have been identified in affected families with progressive external ophthalmoplegia (PEO) characterized by accumulation of large-scale mitochondrial DNA deletions (OMIM #157640; Van Goethem et al., 2001). This gene was identified as candidate gene for congenital bilateral convergent strabismus with exophthalmus (BCSE) in cattle (OMIA 000353; Distl, 1993; Distl and Gerst, 2000). This monogenic autosomal dominant inherited phenotype in cattle shows similarities to the heterogeneous dominant and recessive forms of PEO in man, e.g. eye muscle biopsies of affected humans and cattle demonstrated the presence of ragged red fibers and many mtDNA deletions (Li et al., 1999). Application of a comparative approach, using a human cDNA to screen a bovine BAC library revealed a POLG-containing bovine BAC

clone. To facilitate future linkage studies we report the physical mapping of the bovine POLG gene to cattle chromosome (BTA) 21q17→q22 by FISH and RH mapping.

Materials and methods

Isolation and characterization of the bovine POLG clone

To isolate the bovine POLG gene we screened a genomic BAC library (Warren et al., 2000) constructed in pBACe3.6 with a radioactively labelled heterologous probe according to standard protocols. The probe consisted of the human cDNA IMAGp998N048415 clone provided by the Resource Center/Primary Database of the German Human Genome Project (<http://www.rzpd.de/>) and sequencing of the 1.5-kb insert confirmed 100% identity to human POLG. This allowed the isolation of an approximately 180-kb bovine genomic DNA BAC clone (RCPI42_64G3). 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 with the ThermoSequenase kit (AmershamPharmacia, Freiburg, Germany) using a LI-COR 4200 automated sequencer. The BAC DNA was digested with *SacI* and separated by gel-electrophoresis on a single 0.8% agarose gel. The DNA from the gel was transferred to a nylon membrane, which was then hybridized positively with the insert of the appropriate human POLG IMAGE cDNA clone using the ECLTM direct labeling and detection kit (Amersham Biosciences, Freiburg, Germany). The BAC insert showed a positive hybridization signal.

Chromosome preparation and fluorescence in situ hybridization (FISH) analysis

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 FISH 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 strictly followed the ISCNDB (2000) classification. The BAC clone containing the bovine POLG gene was labelled by nick translation using a Nick-Translation-Mix (Boehringer Mannheim Corp.). Sonicated total bovine DNA and salmon sperm were used as competitor DNA in the FISH experiment. After hybridi-

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Corresponding author: Dr. Cord Drögemüller
Institute of Animal Breeding and Genetics
School of Veterinary Medicine Hannover
Bünteweg 17p, 30559 Hannover (Germany)
telephone: +49-511-9538866; fax: +49-511-9538582
e-mail: cord.droegemueller@tiho-hannover.de

zation 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. Previously GTG-banded metaphase spreads were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Probe name: RPCI-42_64G3

Probe type: bovine genomic BAC clone

Insert size: 180 kb

Vector: pBACe 3.6

Proof of authenticity: DNA sequencing

Gene reference: Walker et al. (1997)

Radiation hybrid (RH) mapping

For fine mapping, the bovine POLG BAC clone was localized on a bovine radiation hybrid panel (Williams et al., 2002). The Roslin/Cambridge bovine RH panel was purchased from Research Genetics (Huntsville, Ala., USA). Primers (F 5'-AAG ATA GTG GGG GCA AAA TGA C-3'; R 5'-TAA AGG AGA CAT CAA GTA G-3') for PCR amplification of a 104-bp fragment were 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 2% 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 1,200 bovine markers typed previously on the panel.

Results and discussion

The bovine BAC clone RPCI-42_64G3 was retrieved from the BAC library using a heterologous human cDNA POLG probe. This BAC clone hybridised to cattle chromosome (BTA) 21q17→q22 (Fig. 1).

Mapping data:

Location: 21q17→q22

Number of cells examined: 30

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

Most precise assignment: 21q17→q22

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

To confirm the chromosome location of the bovine POLG gene the Roslin/Cambridge bovine RH panel was analysed. The STS marker showed a retention frequency of 26.6 %. Two-point analysis revealed close linkage of POLG to BTA21 marker IDVGA45 at a distance of 9.3 cR (LOD score 16.6), and to the BTA21 markers BM103 and ETH131 at larger distances of 30.6 cR (LOD score 10.2), and 50.2 cR (LOD score 6.9), respectively. The RH results for the POLG BAC confirmed the results obtained by FISH. These three linked microsatellite markers were previously mapped on BTA21 by linkage mapping (<http://sol.marc.usda.gov/>). FISH mapping of an IDVGA45-containing cosmid clone on BTA21q15 anchored this linkage group on BTA21 (Mezzelani et al., 1995). Here we report evidence for the assignment of POLG to BTA21q17→q22 concordant to previous Zoo-FISH studies (Hayes, 1995; Solinas-Toldo et al., 1995; Chowdhary et al., 1996). Comparative RH mapping showed that the long arm of HSA 15 was shown to be homologous to a part of BTA10 and a part of BTA21 (Band et al.,

Fig. 1. Chromosome assignment of the bovine POLG gene by FISH. (A) GTG-banding of the bovine metaphase spread. (B) Double signals indicated by arrows are visible on both chromosomes 21q.

2000). The present assignment of the bovine POLG gene refines the assumed conservation of gene order between HSA 15q24→q26 and BTA21q. Therefore, the presented mapping of POLG provides the basis for future linkage studies that might clarify the role of POLG as a putative candidate gene for BCSE in cattle.

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