Assignment¹ of the equine colony stimulating factor 1 receptor gene (*CSF1R*) to equine chromosome 14q15→q16 (ECA14q15→q16) by in situ hybridization and radiation hybrid panel mapping

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Manuscript received 30 August 2004; accepted for publication by M. Schmid 29 October 2004.

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

Rationale and significance

The receptor of the macrophage colony-stimulating factor 1 (*CSF1R*) belongs to a subfamily of receptor tyrosine kinases (Roberts et al., 1988; Yarden and Ullrich, 1988). The *CSF1R* gene is in close vicinity to the *PDGFR1* gene (platelet-derived growth factor receptor 1) which is located only a few hundred basepairs proximal in a head-to-tail orientation. Hampe et al. (1989) demonstrated that the human *CSF1R* gene consists of 21 small exons interrupted by introns ranging in size from 6.3 to less than 0.1 kb. Mutations in the *CSF1R* gene have been shown to be causative for a number of hereditary diseases and neoplastic transformations (Ridge et al., 1990; Boulton et al., 1991). We have isolated and characterized the complete equine *CSF1R* gene. In agreement with the human ortholog, the equine *CSF1R* gene harbours 21 exons coding for a protein of 968 amino acids and spans a region of approximately 30 kb.

Materials and methods

Screening of an equine BAC library

An equine BAC library (Godard et al., 1998) was screened by PCR using forward primer (5'-CATTTGGGTTTGCTATCACTG-3') and reverse primer (5'-AAGCCTTTGGCTTCTGAGT-3') (Brenig et al., 2004). A single recombinant clone (EBAB837F9) was isolated and completely sequenced. The BAC clone harbours the complete equine *CSF1R* gene including the 5' and 3' region.

Fluorescence in situ hybridization (FISH)

The equine BAC-clone harbouring *CSF1R* was used in fluorescence in situ hybridization experiments on equine metaphase spreads (prepared from peripheral lymphocytes) obtained from a normal, healthy stallion. Metaphase preparations and hybridization were carried out essentially as described previously by Lichter et al. (1990).

Probes (1 µg DNA) were labeled with digoxigenin-11-dUTP by nick translation using the DIG-Nick Translation Mix (Roche, Penzberg, Germany). Labelled probes were hybridized with 10× excess of equine Cot-DNA and 7-amino-actinomycin D. Immunodetection was performed using digoxigenin-antibodies conjugated to Cy3. Chromosomes were counterstained with DAPI and examined with a Zeiss Axioplan 2 microscope (Carl Zeiss, Jena, Germany). The G-like banding pattern generated by DAPI staining was used for chromosome identification and for regional assignment of the hybridization signals. FISH experiments were carried out twice, using duplicate slides. Thirty-nine well-spread metaphases were examined and 27 showed signals on ECA14q15→q16 on both chromatids of both chromosomes (Fig. 1).

Radiation hybrid (RH) mapping

The following primers were used to analyze the 5000 rad horse × hamster RH panel (Chowdhary et al., 2003):

CSF1R-primer: f: GAGTTTATGAAGGAGGTG and r: GTGTGAGAGTGATGTTAG (Tm: 52.7; size: 174 bp)

CGMP a-subunit: f: AGAGAAAAGAGAGTGTGG and r: GAATCTGCATTGTAACAAGG (Tm: 52.7; size: 211 bp).

This work was supported by a grant of the Erxleben Research and Innovation Council to B. Brenig (ERIC-BR1959-2002-02).

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Fig. 1. Fluorescence in situ hybridization of the equine *CSF1R* gene. Specific signals were detected on chromosome 14q15→q16 (arrows). An enlarged detail of chromosome 14 with signals on both chromatids is shown in the lower right corner.

Typing was carried out in duplicate and two point analysis was done as described earlier (Chowdhary et al., 2003).

Probe name: EBAB837F9

Probe type: Equine genomic BAC clone

Insert size: 142,625 bp

Vector: pBeloBAC11 (Shizuya et al., 1992)

Proof of authenticity: Sequence accession no. AJ698944

Gene reference: *CSF1R*

Results

The equine *CSF1R* gene maps to the second linkage group on ECA14. The gene order in this region is *CSF1R*–*SLC26A2*–*PDE6A* (phosphodiesterase 6A, cGMP-specific, rod, alpha) with *CSF1R* mapping 9.87 cR (lod > 3.0) and *PDE6A* 7.26 cR

(lod > 3.0) from *SPARC* (secreted protein acidic and rich in cysteine, osteonectin). The equine *SLC26A2* gene has been mapped previously to EC14q15→q21 (Brenig et al., 2004). Human *CSF1R* and *SPARC* have been assigned to chromosome 5q33.2→q33.3 and 5q31.3→q32 respectively and are therefore in agreement with this linkage group.

Mapping data:

Most precise location: ECA14q15→q16

No. of cells examined: 39

Number of chromosomes examined: 78

Number of cells with specific signal: 1 (0), 2 (11), 3 (1), 4 (27) chromatids per cell

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