Brief Gene Mapping Report

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Assignment[1] of the equine solute carrier 26A2 gene (SLC26A2) to equine chromosome 14q15→q21 (ECA14q15→q21) by in situ hybridization and radiation hybrid panel mapping

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[1] To our knowledge this is the first time this gene has been mapped.

Rationale and significance

The SLC26A2 gene belongs to a family of anion exchangers that transport a variety of monovalent and divalent anions. Currently, this family consists of 10 members (Mount and Romero, 2004). Mutations in the SLC26A2 gene have been shown to be responsible for chondrodysplasias in humans (Rossi and Superti-Furga, 2001). A mutation in the bovine SLC26A2 gene has been reported to influence the sulfate uptake in fibroblastoid cells and therefore might have an effect on the development of leg weakness in cattle (Brenig et al., 2003). Recently, the bubaline SLC26A2 gene has been mapped to chromosome 9q26 (Kierstein et al., 2003). We have isolated and characterized the equine SLC26A2 gene and mapped it to chromosome 14q15→q21 (ECA14q15→q21) by fluorescence in situ hybridization and screening of an equine radiation hybrid panel (Chowdhary et al., 2003). This chromosomal location is in agreement with its human ortholog on chromosome 5q31→q34 (Hästbacka et al., 1994).

Materials and methods

Screening of an equine BAC library

An equine BAC library (Godard et al., 1998) was screened by PCR using heterologous primers derived from the bovine SLC26A2 gene (forward primer: 5′-CATTGGGTTTGCTATCACTG-3′; reverse primer: 5′-AAGCCTTTGGCTTCTGAGT-3′). A single recombinant clone (EBAB837F9) was isolated and completely sequenced. The cloned BAC harbors the complete equine SLC26A2 gene including the 5′ and 3′ region.

Fluorescence in situ hybridization (FISH)

The equine BAC-clone harbouring the SLC26A2 gene 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 30× excess of equine Cot-DNA and 6 μg of salmon sperm DNA. 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 well-spread metaphases were examined and 26 were showing signals on ECA14q15→q21 on both chromatids of both chromosomes (Fig. 1).

Probe name:	EBAB837F9
Probe type:	Equine genomic BAC clone
Insert size:	142625 bp
Vector:	pBeloBAC11 (Shizuya et al., 1992)
Proof of authenticity:	Sequence accession no. AJ698508; AJ698728
Gene reference:	SLC26A2

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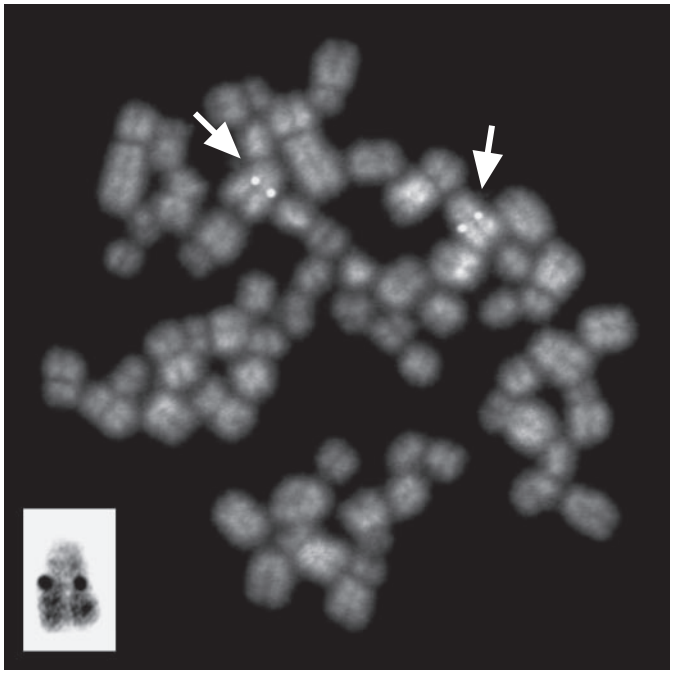

Fig. 1. Fluorescence in situ hybridization of the equine SLC26A2 gene. Specific signals were detected on chromosome 14q15→q21 (arrows). An enlarged detail of chromosome 14 with signals on both chromatids is shown in the lower left corner.

Results

The equine SLC26A2 gene maps to the second linkage group on ECA14. The gene order in this region is CSF1R-SLC26A2-cGMP-alpha subunit with CSF1R (colony stimulating factor 1 receptor) mapping 9.87 cR (lod >3.0) and cGMP-alpha subunit (rod photoreceptor cGMP phosphodiesterase alpha-subunit) 7.26 cR (lod >3.0) from SPARC (secreted protein acidic and rich in cysteine, osteonectin). SPARC has been assigned to human chromosome 5q31.3→q32 and is therefore in agreement with this linkage group.

Mapping data:
Most precise location: ECA14q15→q21
No. of cells examined: 30
Number of chromosomes examined: 60
Number of cells with specific signal: 1 (0), 2 (2), 3 (0), 4 (26) chromatids per cell

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