Assignment¹ of solute carrier family 26 (sulfate transporter), member 2 (SLC26a2) to river buffalo (*Bubalus bubalis*, 2n = 50) chromosome 9q26 (BBU9q26) by fluorescence in situ hybridization and R-banding

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¹ To our knowledge this is the first time this gene has been mapped in domestic water buffalo.

Rationale and significance

To investigate the possible cause of a bone dysplastic phenotype in a Brazilian water buffalo population we used a candidate gene approach. The SLC26a2 gene has been associated with diastrophic dysplasia (DTD) in human and was shown to code for a novel sulfate transporter, thus symbolized as DTDST (Hästbacka, 1994). Mutations in the SLC26a2 gene, beside other genes like those encoding cartilage oligomeric matrix protein (COMP), type IX collagen (COL9A1, COL9A2, and COL9A3) and matrilin (MATN3) have been shown to cause pseudoachondroplasia and multiple epiphyseal dysplasia. Both syndromes constitute a bone dysplasia family, which is genetically and phenotypically heterogeneous (Briggs and Chapman, 2002).

Materials and methods

A bubaline λ FIX[®] II library (Silva et al., 1999) was screened by PCR using oligonucleotides based on the bovine sequence of the SLC26a2 gene (Brenig et al., 2003). Phage DNA was isolated with a Qiagen Plasmid Purifi-

cation Kit according to the manufacturer's instructions and was subcloned into pGEM[®]-4Z (Promega GmbH, Mannheim) vector. Subsequent sequencing was carried out on a LI-COR DNA Sequencer Model 4000L (MWG) as described previously (Middendorf et al., 1992).

Concavalin A stimulated peripheral blood lymphocytes were cultured for three days in RPMI medium at 37.8°C (CO₂ incubator) and treated with 5-BrdU (15 μ g/ml) and Hoechst 33258 (30 μ g/ml) 6 h before harvesting (late incorporation) to obtain R-banding pattern preparations. Colcemid treatment (0.05 μ g/ml) lasted for 1 h. After hypotonic treatment (20 min with 0.5% KCl at 37.5°C) and three fixations (the third overnight) in methanol/acetic acid 3:1, cell suspension was dropped on cleaned, wet and cool slides. A day later, slides were stained with Hoechst 33258 (20 μ g/ml) for 10 min, then washed with distilled water, mounted in 2 $\times$ SSC (pH 7.0) with a glass coverslip, exposed to UV light for 30 min and washed with distilled water. Slides were then treated for FISH-technique with a BAC-clone containing SLC26a2 for about three days (weekend) in presence of bovine COT-1 DNA (with 1 h of annealing step at 37.5°C before the in situ hybridization) and allocated in a moist chamber. Biotin-14-dATP was incorporated into 1 μ g of probe DNA by nick-translation (Gibco-BRL, BioNick). After the detection step with FITC-avidin and anti-avidin antibody (Oncor), slides were mounted with Antifade/Hoechst 33258 dye (3 μ g/ml). Both R-banded metaphases (RBH-banding) and fluorescent FITC-signals were separately captured by a CCD-camera (Sensys, Photometrics) and processed by superimposing FITC-signals on RBH-banded preparations. Chromosome identification and banding followed the river buffalo standard karyotype (Iannuzzi, 1994).

Probe name(s): λ 6-1

Probe type: bubaline genomic λ FIX[®] II phage

Insert size: app. 20 kb

Vector: pGEM[®]-4Z (Promega GmbH, Mannheim)

Proof of authenticity: sequence accession no. AY350740

Gene reference: SLC26a2

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Fig. 1 .FISH mapping of the bubaline SLC26a2 gene. Specific signals were detected on chromosome 9q26 (arrow).

Results

Mapping data

Most precise location: BBU9q26

Number of cells examined: 60

Number of cells with specific signal: 32

1 (0), 2 (13), 3 (14), 4 (5) chromatids per cell

Location of background signals (sites with >2 signals): 0

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