Assignment¹ of the canine tectorin alpha gene (TECTA) to CFA5q12→q13 by FISH 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 dogs.

Rationale and significance

The mammalian tectorial membrane is an extracellular matrix of the inner ear which contacts the stereocilia bundles of specialized sensory hair cells. This membrane of the cochlear duct is positioned above these hair cells and provides a mass against which the stereocilia can bend and leads to fluctuations in hair-cell membrane potential, transducing sound into electrical signals (Steel and Kros, 2001). The gelatinous tectorial membrane contains two noncollagenous glycoproteins, alpha- and beta-tectorin (Legan et al., 1997). Mutations in the human tectorin alpha gene (TECTA), which has been mapped to HSA11q22→q24 (Hughes et al., 1998), cause autosomal dominant hearing impairment DFNA8 and DFNA12 in man (Verhoeven et al., 1998). Alpha-tectorin mutations can also be responsible for recessive forms of deafness (DFNB21; Mustapha et al., 1999). Mice homozygous for a targeted deletion in alpha-tectorin have tectorial membranes that are detached from the cochlear epithelium and lack all noncollagenous ma-

trix (Legan et al., 2000). Alpha-tectorin may be involved in the development of hereditary deafness in dogs which has been described in many canine breeds (Strain, 1996, 1999). Several case reports showed congenital deafness in dogs with histological alterations of the tectorial membrane (Branis and Burda, 1985; Hiraide and Paparella, 1988; Coppens et al., 2000). Therefore, the presented mapping of a canine BAC clone containing the TECTA gene to chromosome CFA5q12→q13 by FISH and RH mapping provides the basis for future linkage studies that might clarify the role of TECTA as a putative candidate gene for deafness in dogs.

Materials and methods

Isolation of the canine TECTA gene-containing BAC clone

For the isolation of a canine TECTA-containing genomic DNA clone the comparative approach, i.e. to use appropriate human or murine heterologous screening probes for the identification of gene-specific clones in animals, has been used. The human IMAGE cDNA clone IMAGp998M121786 was provided by the Resource Center/Primary Database of the German Human Genome Project (<http://www.rzpd.de/>) and sequencing of the 0.8-kb cDNA probe confirmed 100% identity to human tectorin alpha (TECTA) mRNA (GenBank NM_005422). High-density clone filters of the 8.1-fold canine genomic BAC library RPCI-81 (Li et al., 1999) constructed in pBACe 3.6 were screened according to the RPCI protocols (<http://www.chori.org/bacpac/>). The insert of the human TECTA cDNA clone were labeled with ³²P and used as probe. DNA of a positive BAC clone (RPCI81_59C2) was isolated using the Qiagen plasmid maxi kit (Qiagen, Hilden, Germany). BAC DNA termini were sequenced with the ThermoSequenase kit (Amersham-Biosciences, Freiburg, Germany) on a LI-COR 4200L-2 automated sequencer (MWG Biotech, Ebersberg, Germany).

Fluorescence in situ hybridization (FISH) analysis

Canine metaphase spreads for FISH on GTG-banded chromosomes were prepared as described by Breen et al. (1999b). The BAC clone containing the canine TECTA gene was labeled for FISH by nick translation using a Nick-Translation-Mix (Boehringer Mannheim, Germany). Identification of

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Fig. 1. Chromosome assignment of the canine TECTA gene by FISH. (A) GTG-banding of the canine metaphase spread. (B) Detection of double signals indicated by arrows are visible on both chromosomes 5q.

the chromosomes was done according to the established GTG-banded and DAPI-banded karyotype of the domestic dog (Reimann et al., 1996; Breen et al., 1999a).

Probe name: RPCI81_59C2

Probe type: canine genomic BAC clone

Insert size: 150 kb

Vector: pBACe 3.6

Proof of authenticity: DNA hybridization

Gene reference for the human TECTA gene: Verhoeven et al. (1998)

Radiation hybrid (RH) mapping

A pair of PCR primers for RH mapping (5'-CCGCATAAACATGCT-GACAC-3' and 5'-ACATGGGGTGTGGTAGGAA-3') were designed from the generated SP6 sequence of the BAC clone (EMBL AJ428861) and a PCR product of 194 bp was obtained using dog genomic DNA. The marker was mapped on the RHDF5000 panel (Vignaux et al., 1999) according to the described protocols (Priat et al., 1998). After typing the RHDF5000 panel in duplicate, the genotyping data were computed with the MultiMap software (Matise et al., 1994) on the latest radiation hybrid map (Breen et al., 2001).

Results and discussion

The canine BAC clone RPCI81_59C2 was retrieved from the BAC library (Li et al., 1999) using a human cDNA TECTA probe. Sequencing of the canine T7 BAC terminus (EMBL AJ428860) revealed a match of 77% identity over 639 bp (BLAST E-value < 10⁻⁵⁷) to the end of the 168-kb human genomic DNA chromosome 11q24.1 clone RPP11_709H4 sequence (GenBank AP000826), which contains the human TECTA gene approximately 40 kb downstream (<http://www.ensembl.org/>).

The chromosomal location of the canine TECTA gene was determined by FISH using the BAC clone to metaphase chromosomes (Fig. 1). This BAC has been assigned to CFA5q12 → q13.

Mapping data

Location: 5q12 → q13

Number of cells examined: 40

Number of cells with specific signal: 0 (0), 1 (1), 2 (8), 3 (11), 4 (20) chromatids per cell

Most precise assignment: 5q12 → q13

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

Moreover, the localization of this BAC clone was performed on the canine RHDF5000 radiation hybrid panel (Vignaux et al., 1999). The STS marker used for RH mapping was designed from the SP6 BAC end sequence. Using the MultiMap package, two-point analysis revealed that TECTA is linked to the marker Ren42N13 situated on CFA5 with a Lod score of 19.67. This chromosome assignment of TECTA to CFA5 is in good agreement with known homology between HSA11q and CFA5q (Thomas et al., 1999). The marker Ren42N13 is linked on the RH map of CFA5 proximal to the Thy-1 cell surface antigen gene (THY1) and the CD3E antigen, epsilon polypeptide (TiT3 complex) gene (CD3E) (Breen et al., 2001). THY1 has been mapped to HSA11q22.3 → q23 (Seki et al., 1985) and annotated on HSA11q23.3 (<http://www.ensembl.org/>). CD3E was physically mapped on HSA11q23 (Gold et al., 1987). Therefore, the order of these genes on CFA5q seems to be conserved with respect to the order of genes on the corresponding segment of HSA11q23 → q24.

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