Assignment¹ of the canine cadherin related 23 gene (CDH23) to chromosome 4q12→q13 by fluorescence in situ hybridization and 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

Cadherin related gene (CDH23), which encodes otocadherin is a novel member of the cadherin gene superfamily. CDH23 mutations were found in families with nonsyndromic autosomal recessive deafness (DFNB12), and in families with deafness associated with vestibular dysfunction and retinitis pigmentosa (USH1D), respectively (Bolz et al., 2001; Bork et al., 2001; Petit, 2001). DFNB12 has been located on HSA10q21 → q22 by linkage mapping (Chaib et al., 1996). In the waltzer mouse it was shown that mutations in the orthologous *Cdh23* gene cause disorganization of inner ear stereocilia leading to deafness (Di Palma et al., 2001). *Cdh23* expression was detected in the murine neurosensory epithelium. Otocadherin is a critical component of hair bundle formation and in *Cdh23* deficient mice the stereocilia organization was disrupted during early hair cell differentiation (Di Palma et al., 2001). Congenital sensorineural deafness in dogs may result from degeneration of the hair cells of the organ of Corti (Strain, 1996; Coppens et al., 2000; Poncelet et al., 2000). Therefore, CDH23 might be a suitable candidate gene for congenital nonsyndromic hearing loss in dogs. To facilitate future linkage studies we report here the assignment of the canine CDH23 gene to CFA4q12 → q13 by FISH and RH mapping.

Materials and methods

Isolation and characterization of the CDH23 clone

A genomic DNA clone (RPCI81_99C20) of approximately 150 kb from the canine RPCI-81 BAC library (Li et al., 1999) was isolated after screening high density BAC filters according to the RPCI protocols (<http://www.cho-ri.org/bacpac/>) with a ³²P-labelled insert of the IMAGE cDNA clone IMAGp998A186224, containing the human CDH23 mRNA, provided by the Ressource Center/Primary Database of the German Human Genome Project (<http://www.rzpd.de/>). DNA of the positive BAC clone was isolated using the Qiagen plasmid maxi kit (Qiagen, Hilden, Germany). BAC DNA termini were sequenced with the ThermoSequenase kit (AmershamBio-sciences, 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. (1999c). The BAC clone containing the canine CDH23 gene was labeled by nick translation using a Nick-Translation-Mix (Boehringer Mannheim, Germany). Identification of 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_99C20

Probe type: canine genomic BAC clone

Insert size: 150 kb

Vector: pBACe 3.6

Proof of authenticity: DNA hybridization

Gene reference of human CDH23: Bolz et al. (2001)

Radiation hybrid (RH) mapping

A pair of PCR primers for RH mapping (5'-TGGACTCTGGTTCCT-TCCAG-3' and 5'-CGGTCAGGCCTCTGAGTAAC-3') were designed from the SP6 sequence of the BAC clone and this 202-bp STS marker was typed on the RHDF5000 dog/hamster radiation hybrid panel (Vignaux et al., 1999). Amplification reaction was performed as previously described (Priat et al., 1998). The typing data, obtained in duplicate, were incorporated into the latest radiation hybrid map (Breen et al., 2001), using the MultiMap package (Matise et al., 1994).

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Fig. 1. Chromosome assignment of the canine CDH23 gene by FISH analysis of a canine metaphase spread. The digoxigenin labeled clone RPCI81_99C20 containing parts of the canine CDH23 gene was hybridized to metaphase chromosomes of a normal male dog. Double signals indicated by arrows are visible on both chromosomes 4q. The chromosomes were counterstained with propidium iodide and subsequently identified by DAPI staining.

Results and discussion

The canine BAC clone RPCI81_99C20 was retrieved from the BAC library (Li et al., 1999) using a human cDNA CDH23 probe. Sequencing of the BAC termini revealed a significant match of 95% identity over 76 bp between the SP6 BAC end sequence (EMBL acc. no. AJ428859) and the CDH23 exon 2 sequence (GenBank acc. no. AC016823) which is annotated to genomic DNA HSA10q22.1 clone RP11_472K8 (<http://www.ensembl.org/>). The chromosomal location of the canine CDH23 gene was determined by FISH analysis using the BAC clone hybridized to metaphase chromosomes (Fig. 1). This BAC clone has been assigned to CFA4q12→q13.

Mapping data:

Location: 4q12→q13

Number of cells examined: 40

Number of cells with specific signal: 0 (1), 1 (2), 2 (6), 3 (5), 4 (26) chromatids per cell

Most precise location: 4q12→q13

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

Moreover this BAC clone was localized on the canine RHDF5000 radiation hybrid panel (Vignaux et al., 1999). The marker used for RH mapping was designed from the SP6 BAC end sequence. Using the MultiMap package, two-point analysis revealed that CDH23 is linked to the marker AHT120 situated

on CFA4 with a Lod score of 9.7. The human ortholog is located on HSA10q22.1 (<http://www.ensembl.org/>), which corresponds well with the synteny data of the canine RH map (Breen et al., 2001). Therefore, CDH23 is the first mapped gene supporting the homology with HSA10 and otherwise confirmed by heterologous FISH painting (Breen et al., 1999b; Yang et al., 1999).

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