Assignment¹ of the canine myosin Va gene (MYO5A) to chromosome 30q14 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

Myosin Va belongs to the unconventional myosin family that has been identified in many non-muscle tissues of vertebrates and invertebrates. All known myosins bind actin and produce mechanical force through ATP hydrolysis. Myosin Va is only expressed in melanocytes and in cells of the neuronal system (Mercer et al., 1991). In melanocytes it is part of the melanosome transport complex (Wu et al., 1997). Mutations in the MYO5A gene cause the dilute phenotype in mice that is generally associated with severe neurological disorders (Mercer et al., 1991), the dilute-opisthotonus rat mutant (Futaki et al., 2000), and in human the rare Griscelli syndrome with neurological impairment (Pastural et al., 1997; Ménasche et al., 2000). This autosomal recessive disorder results in a phenotype with pigmentary dilution of skin and hair, the presence of large clumps of pigment in hairshafts and an accumulation of melanosomes in melanocytes in addition to neurological defects. The MYO5A gene has been located on HSA 15q21 → q22 and

on the homologous mouse and rat chromosomes 8 and 9, respectively (Hasson et al., 1996; Ohuo et al., 1996). In dogs affected with black hair follicular dysplasia (BHFD) abnormal hair follicles showing clumps of melanosomes in the hair matrix and an abnormal shape of the hairshaft are also observed (Schmutz et al., 1998). Therefore, MYO5A might be a suitable candidate gene for this canine disorder. To facilitate future linkage studies we report here the assignment of the canine MYO5A gene to CFA30q14 by FISH and RH mapping.

Materials and methods

Isolation and characterization of the MYO5A clone

A genomic DNA clone (RP81-108N13) of approximately 170 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.chori.org/bacpac/>) with a ³²P-labelled insert of the IMAGE cDNA clone IMAGp998O2410022, containing the human MYO5A cDNA, 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 and selected plasmid subclones were sequenced with the ThermoSequenase kit (AmershamBiosciences, Freiburg, Germany) on a LI-COR 4200L-2 automated sequencer (MWG Biotech, Ebersberg, Germany).

Fluorescence in situ hybridization (FISH) analysis

200 ng of BAC DNA from clone RP81-108N13 were labelled by nick translation to incorporate Spectrum Red-dUTP as described previously (Breen et al., 2001). 25 ng of the labeled clone were co-precipitated with 10 µg of sonicated canine genomic DNA as competitor. The probe plus competitor precipitate was resuspended in 15 µl of hybridization buffer comprising 50 % deionized formamide, 2× SSC, 10 % dextran sulphate and 0.1 % Tween-20. Probes were hybridized to canine metaphase spreads and post-hybridization

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Fig. 1. Chromosome assignment of the canine MYO5A gene by FISH analysis on a canine metaphase spread. The Spectrum Red labeled BAC clone RP81-108N13 containing the canine MYO5A gene was hybridized to metaphase chromosomes of a normal male dog. Hybridization signals are seen on both homologues of CFA30. Inset: Co-localization of the Spectrum Red labeled MYO5A clone was performed with the Spectrum Green labeled BAC clone RP81-109O1 containing the RAB27A gene. These data indicate that MYO5A is proximal to RAB27A on CFA30.

washing was performed as described elsewhere (Breen et al., 1999b). Chromosomes were counterstained in 80 ng/ml 4',6-diamidino-2-phenylindole (DAPI) and mounted in antifade solution (Vectashield, Vector Laboratories). Images were acquired and processed using a multi-color FISH workstation comprising a fluorescence microscope (Axioplan 2ie, Zeiss) equipped with narrow pass filter sets and a cooled CCD camera (CoolSnapHQ, Photometrics, Tucson, AZ) both driven by dedicated software (SmartCapture 2.3.1 Digital Scientific, Cambridge, U.K.). The digital image of each DAPI stained metaphase spread was processed using a high-pass spatial filter to reveal enhanced DAPI bands. The clone was assigned to CFA30q14 according to the DAPI banded nomenclature of Breen et al. (1999a).

Probe name: RP81-108N13

Probe type: canine genomic BAC clone

Insert size: 170 kb

Vector: pBACe 3.6

Proof of authenticity: DNA sequencing

Gene reference of human MYO5A: Hasson et al. (1996)

Radiation hybrid (RH) mapping

A pair of PCR primers for RH mapping (5'-GTCCAGCATGTCAA-CAATGG-3' and 5'-GCCTTCTGTTCCTCCCAAGATT-3') were designed from the partial sequence information of the BAC clone and a PCR product of 201 bp was obtained on dog genomic DNA. Amplification of the marker was done as described previously (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).

Results and discussion

The canine BAC clone RP81-108N13 was retrieved from the BAC library (Li et al., 1999) with a human cDNA MYO5A probe. Sequencing of some plasmid subclones of the BAC clone

confirmed that the canine MYO5A gene was contained in the BAC clone. The chromosomal location of the canine MYO5A gene was determined by FISH of the BAC clone to metaphase chromosomes (Fig. 1). This BAC clone has been assigned to CFA30q14. Subsequent co-localization of MYO5A with RAB27A (Philipp et al., 2003) indicated that MYO5A lies proximal to RAB27A on CFA30 (Fig. 1 inset).

Mapping data:

Location: CFA30q14

Number of cells examined: 25

Number of cells with specific signal: 0 (0), 1 (0), 2 (1), 3 (5), 4 (19) chromatids per cell

Most precise location: 30q14

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

Additionally, the localization of this BAC clone was verified using the canine RHDF5000 radiation hybrid panel (Vignaux et al., 1999). The PCR primers used for RH mapping were designed from the putative intron 24 sequence of the canine MYO5A gene. Using the MultiMap package, two-point analysis revealed that MYO5A is linked to the marker REN248F14 situated on CFA30 with a two point Lod score of 16.3. The human MYO5A ortholog is located on HSA 15q21 (Pastural et al., 1999), which corresponds well to the synteny data of the canine RH map (Breen et al., 2001). RAB27A was also shown to map to CFA30, linked to the marker FH2290 with a Lod score of 13.7 (Philipp et al., 2003). The RH data thus suggest that MYO5A is proximal to RAB27A and this is confirmed by our dual color FISH (Fig. 1 inset).

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