Assignment of the calcium channel, voltage-dependent, P/Q type, alpha 1A subunit gene (*CACNA1A*), and the ataxin 7 gene (*ATXN7*) to canine chromosome 20 by fluorescence in situ hybridization and confirmation by radiation hybrid mapping

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Manuscript received 23 December 2004; accepted for publication by M. Schmid on 7 April 2005.

Rationale and significance

CACNA1A (SCA6, Spinocerebellar ataxia type 6), and *ATXN7* (SCA7, Spinocerebellar ataxia type 7) belong to a group of genes involved in autosomal dominant cerebellar ataxia in humans. The autosomal dominant cerebellar ataxias are a heterogeneous group of neurodegenerative disorders characterized by progressive degeneration of the cerebellum, brain stem and spinal cord. The pathogenetic mechanism is the same in all SCA genes and is based on the inheritance of an unstable, expanded CAG trinucleotide repeat in the coding region, resulting in an extended polyglutamine sequence within the protein. The function of the proteins (ataxin 6 and 7) is not known (Klockgether and Evert, 1998; Lebre and Brice, 2003). The human *CACNA1A* gene maps at 13.47 Mb on HSA19p13.2→p13.1 and contains 47 exons spanning 39 kb, whereas the human *ATXN7* gene maps at 64.63 Mb on HSA3p14 and consists of 14 exons spanning 136 kb (NCBI map viewer, human genome build 35.1). The mapping of these two genes in the dog is the first step for further investigations in inherited ataxia in the dog which is a well known problem in several dog breeds (Wessmann et al., 2004).

Materials and methods

Isolation and characterization of the canine *CACNA1A*, *ATXN7* BAC clones

Human cDNA clones of *CACNA1A* (IMAGp998A165345), and *ATXN7* (IMAGp998O077419), provided by the Resource Center/Primary Database of the German Human Genome Project (<http://www.rzpd.de/>) were used to screen the canine RPCI-81 BAC library (Li et al., 1999) according to the RPCI protocols (<http://www.chori.org/bacpac/>) with the ³²P-labeled cDNA probes. Positive BAC clones containing the *CACNA1A* and *ATXN7* genes were designated RP81-59P20 and RP81-32D10, respectively.

DNA was prepared from 100 ml overnight cultures of the positive BAC clones using the Qiagen Midi plasmid kit according to the modified protocol for BACs (Qiagen, Hilden, Germany). BAC DNA termini were sequenced with the ThermoSequenase kit (AmershamPharmacia, Freiburg, Germany) using a LI-COR 4200 automated sequencer (LI-COR, Inc., Lincoln, Nebraska, USA). The RP81-59P20 and RP81-32D10 T7 BAC end sequences were deposited in the EMBL nucleotide database (AJ845127; AJ845129). A BLASTN comparison of the canine T7 end sequence of RP81-59P20 with build 35.1 of the human genome sequence revealed a significant match (BLAST E-value 10^{-109} over 402 bp, identity = 78%) starting at 13,316,200 bp of HSA19 approximately 181 kb upstream to human *CACNA1A*. The canine T7 end sequence of RP81-32D10 revealed a significant match (BLAST E-value 0.000 over 927 bp, identity = 91%) starting at 64,145,800 on chromosome 3 in intron 4 of the human *ATXN7* gene.

Additionally a BLAST comparison of the BAC end sequence using the recently published canine genome assembly (<http://pre.ensembl.org>) revealed a significant match to CFA20 (BLAST E-value $2.1 \cdot 10^{-115}$ over 637 bp, identity 90.42%) for the T7 end of RP81-59P20 starting at 51,933,252 bp and for RP81-32D10 with a BLAST E-value of $9 \cdot 10^{-126}$ over 650 bp (identity 93.38%) starting at 30,106,973 bp.

Chromosome preparation and fluorescence in situ hybridization (FISH) analysis

Canine metaphase spreads for FISH on GTG-banded chromosomes were prepared using phytohemagglutinin stimulated blood lymphocytes. Cells were harvested and slides prepared using standard cytogenetic techniques. Prior to FISH the chromosomes were GTG-banded and well-banded metaphase chromosomes were photographed using a highly sensitive CCD

Supported by a grant of the Gesellschaft zur Förderung kynologischer Forschung (GKF) e.V., Bonn. Supported by funds from the Conseil Régional de Bretagne. R.G. is supported by funds from the American Kennel Club.

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Fig. 1. Chromosomal assignment of the canine *CACNA1A*, and *ATXN7* genes by FISH analysis. G-banded metaphase spreads of a normal male dog before (left) and after hybridization (right) with the digoxigenin-labelled canine BAC clones RP81-59P20 (**A**), and RP81-32D10 (**B**). Double signals are indicated by arrows.

camera and IPLab 3.5 (Scanalytics, Inc.). 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). The BAC clones were labelled by nick translation using a Nick-Translation-Mix (Boehringer Mannheim Corp., Germany). Sonicated total canine DNA and salmon sperm were used as competitor. After hybridization overnight, signal detection was performed using a Digoxigenin-FITC Detection Kit (Quantum Appligene, Heidelberg, Germany). The chromosomes were counterstained with DAPI and propidium and embedded in antifade solution. Previously photographed metaphase spreads were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Probe names: RP81-59P20 for *CACNA1A*; RP81-32D10 for *ATXN7*

Probe type: canine genomic BAC clones for all probes

Insert size: 165 kb for RP81-59P20; 150 kb for RP81-32D10

Vector: pBACe 3.6

Proof of authenticity: hybridization and DNA sequencing

Gene reference for the human genes: Ophoff et al., 1996 (*CACNA1A*); Michalik et al., 1999 (*ATXN7*)

Radiation hybrid (RH) mapping

Two pairs of primers were designed based on the T7 sequence of the BAC clones. The PCR primer pair for *CACNA1A* (5'-GAGGAGGGCGTCCT-GAGT-3' and 5'-GGATCGTCTGGGTTGTGTCT-3') generated a PCR product of 200 bp, the PCR primer pair for *ATXN7* (5'-TACTTTAGTGG-GGCTTTTATTTC-3' and 5'-GCATTCTCCTCCCCCTAAC-3') generated a PCR product of 183 bp on dog genomic DNA. Amplification of the marker was tested using standard conditions on dog and hamster DNA and on a mixture (1:3) of dog and hamster DNA. Amplification was performed in 10 µl reactions containing 50 ng DNA, 0.3 µM of each primer, 250 µM of each dNTP (Pharmacia), 2 mM MgCl₂, 1× AmpliTaq Buffer and 0.5 U AmpliTaq Gold (Perkin-Elmer). PCR reactions were carried out in PTC-200 PCR machines (MJ Research) with the following program: 7 min 95°C, followed by 20 cycles of 30 s 94°C, 30 s 61°C decreasing 0.5°C per cycle, 1 min 72°C and 15 cycles of 30 s 94°C, 30 s 51°C, 1 min 72°C and a final extension of 2 min at 72°C. The marker was dog specific and could be readily

typed on the radiation hybrid panel RHDF5000-2 composed of 118 cell lines (Vignaux et al., 1999) using the above PCR conditions. PCR products were loaded on 2% agarose gels containing EtBr and run in 0.5× TBE at 120 V for 30 min. Products were visualized under UV light, images were recorded and results were scored in terms of present, absent or ambiguous in the 118 hybrid cell lines.

Statistical analysis

The typing data were incorporated into the latest radiation hybrid map made of 3,270 markers (Guyon et al., 2003), using the two-point analysis of the Multimap package (Matisse et al., 1994).

Results and discussion

Mapping data:

Location: *CACNA1A* CFA20q16→q17

ATXN7 CFA20q14

Number of cells examined: 40 for each BAC clone

Number of cells with specific signals:

RP81-59P20: 0 (0), 1 (0), 2 (4), 3 (5), 4 (31) chromatids per cell

RP81-32D10: 0 (0), 1 (2), 2 (5), 3 (5), 4 (28) chromatids per cell

First the chromosomal location of the canine genes was determined by fluorescence in situ hybridization (FISH) of the BACs RP81-59P20 and RP81-32D10 to canine metaphase chromosomes (Fig. 1). To confirm the FISH mapping of the *CACNA1A* and *ATXN7* BAC clones the markers were typed on the 118 cell lines RH panel and computed on the 3,270-marker

version of the RH map. *CACNA1A* was localized on CFA20, close to marker REN249D14 with a lod score of 13.79. *ATXN7* was also localized on CFA20, close to markers FH3109 and BAC_380-H2 with a lod score of 21 for both.

The assignment of the canine *CACNA1A* and *ATXN7* genes on CFA20 agrees with the comparative mapping data, which shows conserved synteny between CFA20 and HSA3 and HSA19 (Breen et al., 1999b).

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