The canine FRDA gene maps to CFA 1q31.1→q31.3

H. Kuiper,^a C. Drögemüller,^a S. Rak,^a T. Leeb,^a P. Quignon,^b C. André,^b and
O. Distl^a

^aInstitute of Animal Breeding and Genetics, School of Veterinary Medicine Hannover, Hannover (Germany);

^bUMR 6061 CNRS, Génétique et Développement, Faculté de Médecine, Rennes (France)

Rationale and significance

Friedreich's ataxia (FRDA) is the most common form of inherited ataxia in man (Campuzano et al., 1996; Montermini et al., 1997). It is a neuro-degenerative disease involving the central and peripheral nervous system and is clinically characterized by a progressive ataxia of the limb and gaits, and dysarthria. The patients show skeletal deformations e.g. skeletal scoliosis, pes cavus and hammertoe, absence of lower limb tendon reflexes, a positive Babinski sign and decreased vibration and proprioceptive senses. Diabetes and cardiomyopathy are characteristic signs in the later course of the disease (Harding 1981). Mean age of onset is usually before adolescence and death occurs after variable time, usually in the late thirties (Harding, 1981; Durr et al., 1996). Histopathology reveals a systemic disease of the central nervous system, predominantly a central axonopathy.

The cause of Friedreich's ataxia in 97–98% of patients is a GAA triplet expansion in the first intron of the FRDA gene on

chromosome 9q13. About 92–97% of patients are homozygous for the GAA expansion. The remainder are either compound heterozygotes carrying a GAA repeat expansion in one FRDA allele and a point mutation in the other or are only carriers of point mutations (Campuzano et al., 1996; Durr et al., 1996; Bidichandani et al., 1997; Cossee et al., 1999). The severity of FRDA in patients is correlated with the length of the GAA expansion that varies from 90 to 1,800. The large expansion can arise from premutation alleles (large normal alleles) with 32–90 GAA repeats.

The FRDA gene consists of six exons spanning 95 kb and encodes a widely expressed 210-aa protein, frataxin (Campuzano et al., 1996). Frataxin is located in the mitochondrial membrane and is severely reduced in FRDA patients (Campuzano et al., 1997). Lack of frataxin is associated with mitochondrial iron accumulation which leads to oxidative stress and cell death (Rotig et al., 1997).

The phenotypic and histologic alterations found in Friedreich's ataxia in man show similarity to an inherited form of ataxia in the Parson Jack Russell Terrier dog. In this dog breed, the symmetric generalized ataxia is characterized by hypermetric and spastic movements. The dogs usually show clinical signs before the age of six months (Chrisman, 1991). The disease is progressive and after a short time of suffering, most of the canine patients are killed painlessly. Inherited forms of ataxia are also known in the Fox terrier and the Gordon setter. In both breeds, the disease appears to be inherited as an autosomal recessive trait (Björk et al., 1957; Cork et al., 1981).

Due to the similarities between Friedreich's ataxia and the ataxia in Parson Jack Russell terrier and other dog breeds, it was our objective to map the FRDA gene in the dog as a good candidate gene for inherited canine ataxias and to facilitate future studies. Furthermore, the dog could provide an appropriate animal model for the human disease in addition to the knockout mouse investigated by Puccino et al. (2001).

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Corresponding author: Dr. Heidi Kuiper
Institute of Animal Breeding and Genetics
School of Veterinary Medicine Hannover
Bünteweg 17p, 30559 Hannover (Germany)
telephone: +49-511-9538802; fax: +49-511-9538582
e-mail: heidi.kuiper@tiho-hannover.de

Fig. 1. (A) GTG-banded metaphase chromosome spread of a normal male dog. **(B)** Chromosome assignment of the canine FRDA gene by FISH analysis. The digoxigenin labeled clone RPCI81_270H19 containing parts of the canine FRDA gene was hybridized to metaphase chromosomes. Double signals indicated by arrows are visible on both chromosomes 1q31.1→q31.3. The chromosomes were counterstained with DAPI and propidium iodide.

Materials and methods

Isolation and characterization of the canine FRDA clone

A human cDNA clone of FRDA (IMAGp998L098497), provided by the Ressource Center/Primary Database of the German Human Genome Project (<http://www.rzpd.de/>) was used to screen the canine RPCI-81 BAC library (Li et al., 1999). After screening the high density BAC filters according to the RPCI protocols (<http://www.chori.org/bacpac/>) with the ³²P-labeled cDNA probe a 180-kb clone (RPCI81_270H19) was isolated. DNA of the positive BAC clone was isolated using the Qiagen plasmid maxi 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. To ensure that the isolated canine BAC clone contains the FRDA gene, the BAC DNA was *SacI* digested and separated by gel-electrophoresis on a single 0.8% agarose gel. The DNA from the gel was transferred to a nylon membrane, which was then hybridized positively with the insert of the mentioned human IMAGE cDNA clone using the ECLTM direct labeling and detection kit (Amersham Biosciences, Freiburg, Germany).

Chromosome preparation

Canine metaphase spreads were prepared using phytohemagglutinin stimulated blood lymphocytes and standard cytogenetic techniques. Fluorescence in situ hybridization was carried out on GTG-banded chromosomes and metaphases were photographed prior to hybridization using a highly sensitive CCD camera and IPLab 2.2.3 (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., 1999).

Fluorescence in situ hybridization (FISH) analysis

FISH on GTG-banded chromosomes was performed using digoxigenin labeled BAC DNA (Nick-Translation-Mix, Boehringer Mannheim). Sonicated total canine DNA and salmon sperm were used as competitors in this experiment. After hybridization overnight, signal detection was performed using a Digoxigenin-FITC Detection Kit (Quantum Appligene). The chromosomes were counterstained with DAPI and embedded in propidium iodide/antifade. Metaphase spreads were re-examined after hybridization with a Zeiss Axioplan 2 microscope equipped for fluorescence.

Probe name: RPCI81_270H19

Probe type: canine genomic BAC clone

Insert size: 180 kb

Vector: pBACe 3.6

Proof of authenticity: DNA sequencing

Gene reference: Campuzano et al. (1996)

Radiation hybrid (RH) mapping

The localization of this BAC clone was confirmed by radiation hybrid mapping on the canine RHDF5000 panel (Vignaux et al., 1999). The sequence data for the T7 BAC end (EMBL AJ459258) was used to design canine-specific primers for RH mapping (5'-TTTGCAATAGGCATG-GACTC-3' and 5'-GAACGTGTCCAGTGAATGGTT-3') and a 193-bp PCR product was obtained on dog genomic DNA. The marker was typed in duplicate on the 126 cell lines DNA, composing the RH panel. PCR reactions were performed in a total of 10 µl 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: 8 min 94 °C, followed by 20 cycles of 30 s 94 °C, 30 s 61 °C decreasing of 0.5 °C per cycle, 30 s 72 °C and 15 cycles of 30 s 94 °C, 30 s 51 °C, 30 s 72 °C and a final extension of 2 min 72 °C. PCR products were migrated on 2% agarose gels. The typing data were computed on the previously published map (Breen et al., 2001) and two-point analysis was done using the MultiMap package (Matise et al., 1994).

Results and discussion

Mapping data:

Location: CFA1q31.1→q31.3

Number of cells examined: 50

Number of cells with specific signals: 0 (1), 1 (0), 2 (7), 3 (19), 4 (23) chromatids per cell

Most precise assignment: CFA1q31.1→q31.3

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

First the chromosomal location of the canine FRDA gene was determined by fluorescent in situ hybridization (FISH) of the BAC to canine metaphase chromosomes (Fig. 1). The canine BAC clone RPCI81_270H19 was retrieved from the BAC library using a heterologous human cDNA FRDA probe. Sequencing of BAC termini revealed a match of 94% identity over 100 bp (BLAST E-value 2×10^{-6}) to the human clone AL590238 located on HSA9q21.11, approximately 100 kb downstream to the FRDA gene (<http://www.ensembl.org>).

Second this canine BAC clone containing FRDA was mapped by the radiation hybrid method to CFA1q31.1→q31.3.

Fig. 2. Comparative map of CFA1q31.1→q31.3. The position of FRDA1 on the DAPI banded CFA1 ideogram (Breen et al. 2001) is demonstrated. HSA9q13 represents the homologous human chromosome segment. In both species TJP2 and HuEST-133987 are mapped in the same chromosomal region close to FRDA1.

The assignment of the canine FRDA gene corresponds well to the previously established synteny of HSA9q13 and CFA1q25.3→q32, which was found by mapping the markers TJP2 from HSA9q13→q21.11 and HuEST-L33987 from HSA9qtel to this chromosomal region (Fig. 2) (Breen et al., 2001; <http://www-recomgen.univ-rennes1.fr/cgi-dog/display-cfa.prog?cfa=CFA01>). The RH results confirmed the chromosomal location obtained by FISH. The two-point analysis revealed that FRDA is linked to the marker TJP2 (tight junction protein 2; zona occludens 2) situated on CFA1 with a lod score of 17.4.

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