Assignment¹ of the RAB27A gene to canine chromosome 30q15.1 by fluorescence in situ hybridization and radiation hybrid mapping

U. Philipp,^a A. Scott,^b P. Quignon,^c C. André,^c M. Breen,^b and T. Leeb^a

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

^bDepartment of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh NC (USA);

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

¹ To our knowledge this is the first time this gene has been mapped in dogs.

Rationale and significance

The Ras related gene RAB27A which encodes the Ras like GTP-binding protein 27A belongs to the Rab gene family. Members of the Rab family are nontransforming monomeric membrane-bound GTP-binding proteins of the Ras superfamily that contain highly conserved regions involved in GTP binding and hydrolysis, which is necessary for vesicular fusion and trafficking. RAB27A mutations were found in families with Griscelli syndrome and in the corresponding mouse mutant *ashen* (Ménasche et al., 2000, Wilson et al., 2000). This autosomal recessive disorder results in pigmentary dilution of skin and hair, the presence of large clumps of pigment in hairshafts and an accumulation of melanosomes in melanocytes. Most patients develop an uncontrolled T-lymphocyte and macrophage activation syndrome (haemophagocytic syndrome). Further investigation showed that Rab27A is also expressed in cytotoxic T lymphocytes (CTLs) and plays a key role in exocytosis of cytolytic granules (Stinchcombe et al., 2001). RAB27A

has been located on HSA15q15→q21.1 (Tolmachowa et al., 1999) and on the homologous mouse chromosome 9.

RAB27A might also be a suitable candidate gene for similar disorders in dogs with color dilution phenotypes. To facilitate future linkage studies we report here the assignment of the canine RAB27A gene to CFA30q15.1 by FISH and RH mapping.

Materials and methods

Isolation and characterization of the RAB27A clone

A genomic DNA clone (RP81-109O1) of approximately 140 kb was isolated from the canine RPCI-81 BAC library (Li et al., 1999) after screening high density BAC filters according to the RPCI protocols (<http://www.chori.org/bacpac/>) with a ³²P-labeled insert of the IMAGE cDNA clone IMAGp998N107814, containing a fragment from the human RAB27A cDNA, provided by the Resource 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 some plasmid shotgun 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 RCPI-81 109-O1 were labeled 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 ml 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 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,

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Corresponding author: Dr. Tosso Leeb
Institute of Animal Breeding and Genetics
School of Veterinary Medicine Hannover
Bünteweg 17p, 30559 Hannover (Germany)
telephone: +49-511-9538877; fax: +49-511-9538582;
e-mail: Tosso.Leeb@tiho-hannover.de

Fig. 1. Chromosome assignment of the canine RAB27A gene by FISH analysis on a canine metaphase spread. The Spectrum Red-labeled clone RP81-109O1 containing the canine RAB27A gene was hybridized to metaphase chromosomes of a normal male dog. Double signals indicated by arrows are visible on both homologues of chromosome 30.

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, UK.) 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 CFA30q15.1 according to the DAPI banded nomenclature of Breen et al. (1999a).

Probe name: RP81-109O1

Probe type: canine genomic BAC clone

Insert size: 140 kb

Vector: pBACe 3.6

Proof of authenticity: DNA sequencing

Gene reference of human RAB27A: Tolmachova et al. (1999)

Radiation hybrid (RH) mapping

A pair of PCR primers for RH mapping (5'-TGGTGAACCAACTG-TACCACA-3' and 5'-AGTTTGGAGGAGGGGCTAAG-3') were designed from the partial sequence information of the BAC clone. A PCR product of 188 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 RP81-109O1 has been retrieved from the BAC library (Li et al., 1999) with a human RAB27A cDNA probe and sequencing of some plasmid subclones of the

BAC clone confirmed that the canine RAB27A gene was contained in the BAC clone. The chromosomal location of the canine RAB27A gene was determined by FISH of the BAC clone to metaphase chromosomes (Fig. 1). This BAC clone has been assigned to CFA30q15.1.

Mapping data:

Location: CFA30q15.1

Number of cells examined: 30

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

Most precise location: 30q15.1prox

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

Moreover, the localization of this BAC clone was verified on the canine RHDF5000 radiation hybrid panel (Vignaux et al., 1999). The primers used for RH mapping were located in intron 3 of the RAB27A gene. Using the MultiMap package, two-point analysis revealed that RAB27A is linked to the marker FH2290 situated on CFA30 with a two point Lod score of 13.7. The human ortholog is located on HSA15q15→q21.1 (Tolmachova et al, 1999), which corresponds well to the synteny data of the human–canine comparative map (Breen et al., 2001).

References

- Breen M, Langford CF, Carter NP, Holmes NG, Dickens HF, Thomas R, Suter N, Ryder EJ, Pope M, Binns MM: FISH mapping and identification of canine chromosomes. *J Hered* 90:27–30 (1999a).
- Breen M, Bullerdiek J, Langford CF: The DAPI banded karyotype of the domestic dog (*Canis familiaris*) generated using chromosome-specific paint probes. *Chrom Res* 7:401–406 (1999b).
- Breen M, Jouquand S, Renier C, Mellersh CS, Hitte C, Holmes NG, Cheron A, Suter N, Vignaux F, Bristow AE, Priat C, McCann E, Andre C, Boundy S, Gitsham P, Thomas R, Bridge WL, Spriggs HF, Ryder EJ, Curson A, Sampson J, Ostrander EA, Binns MM, Galibert F: Chromosome-specific single-locus FISH probes allow anchorage of an 1800-marker integrated radiation-hybrid/linkage map of the domestic dog genome to all chromosomes. *Genome Res* 11:1784–1795 (2001).
- Li R, Mignot E, Faraco J, Kadotani H, Cantanese J, Zhao B, Lin X, Hinton L, Ostrander EA, Patterson DF, de Jong PJ: Construction and characterization of an eight-fold redundant dog genomic bacterial artificial chromosome library. *Genomics* 58:9–19 (1999).
- Matise TC, Perlin M, Chakravarti A: Automated construction of genetic linkage maps using an expert system (MultiMap): a human genome linkage map. *Nature Genet* 6:384–390 (1994).
- Ménasché G, Pastural E, Feldmann J, Gertain S, Ersoy F, Dupuis S, Wulfraat N; Bianchi D, Le Deist F, Saint Basile de G: Mutations in Rab27A cause Griscelli syndrome associated with haemophagocytic syndrome. *Nature Genet* 25:173–176 (2000).
- Priat C, Hitte C, Vignaux F, Renier C, Jiang Z, Jouquand S, Cheron A, Andre C, Galibert F: A whole-genome radiation hybrid map of the dog genome. *Genomics* 54:361–378 (1998).
- Stinchcombe JC, Barral DC, Mules EH, Booth S, Hume AN, Machesky LM, Seabra MC, Griffiths GM: Rab27a is required for regulated secretion in cytotoxic T lymphocytes. *J Cell Biol* 152:825–834 (2001).
- Tolmachova T, Ramalho JS, Anant JS, Schultz RA, Huxley CM, Seabra MC: Cloning, mapping and characterization of the human RAB27A gene. *Gene* 239:109–116 (1999).
- Vignaux F, Hitte C, Priat C, Chuat JC, Andre C, Galibert F: Construction and optimization of a dog whole-genome radiation hybrid panel. *Mammal Genome* 10:888–894 (1999).
- Wilson SM, Yip R, Swing DA, O'Sullivan TN, Zhang Y, Novak EK, Swank RT, Russell LB, Copeland NG, Jenkins NA: A mutation in RAB27A causes the vesicle transport defects observed in ashen mice. *Proc natl Acad Sci, USA* 97:7933–7938 (2000).