Expanded Repeat in Canine Epilepsy

Hannes Lohi,[1]* Edwin J. Young,[1]* Susan N. Fitzmaurice,[2] Clare Rusbridge,[3] Elayne M. Chan,[1] Mike Vervoort,[1] Julie Turnbull,[1] Xiao-Chu Zhao,[1] Leonarda Ianzano,[1] Andrew D. Paterson,[1] Nathan B. Sutter,[4] Elaine A. Ostrander,[4] Catherine André,[5] G. Diane Shelton,[6] Cameron A. Ackerley,[1] Stephen W. Scherer,[1] Berge A. Minassian[1]†

Epilepsy afflicts 1% of humans and 5% of dogs. More than 5% of purebred miniature wirehaired dachshunds (MWHDs) in the United Kingdom suffer an autosomal recessive progressive myoclonic epilepsy (PME), which we show to be Lafora disease (EPM2) (*1*), the severest form of teenage-onset epilepsy in humans (*2*). EPM2 is caused by mutations in the chromosome 6q24 *EPM2A* or 6p22 *EPM2B* genes (*3*). Using homozygosity and linkage analysis, we mapped the MWHD disease locus to canine chromosome 35 (*1*), which is syntenic in its entirety to human 6p21-25. We then cloned canine *Epm2b* (*1*). Polymerase chain reaction (PCR) failed in affected dogs across the 5′ half of the gene's single exon. The normal sequence in this region contains two consecutive identical dodecamers (D) and a third copy differing by a single nucleotide (T) (Fig. 1A). The corresponding region in other species is not repetitive and is shorter by 12 nucleotides, the length of one D repeat (Fig. 1A). Concerted modifications (*1*) of PCR conditions ultimately enabled sequencing across the repeat region in affected dogs and revealed bi-allelic expansion of the dodecamer repeat with 19 to 26 copies of the D sequence (Fig. 1B) (*1*). Comparing the amount of *Epm2b* mRNA in skeletal muscle from three affected dogs and two controls with quantitative reverse transcription–PCR (*1*) showed that affected mRNA levels were more than 900 times reduced (Fig. 1C).

To determine whether the extra D sequence is specific to MWHDs, we sequenced *Epm2b* from two normal unrelated dogs from each of 128 breeds. Sixty percent of their chromosomes had three repeats (2 D's and 1 T) and 40%, two repeats (1 D and 1 T). Almost all breeds had examples of both variants, in homozygous or heterozygous state. We tested the next non-MWHD PME case to present to the clinic, a basset hound, and found a homozygous 14-copy expansion of the repeat (Fig. 1B).

In the presence of the normal allele, PCR of the expanded allele was impossible. Deaminating carrier DNA cytosines (*1*, *4*) before PCR allowed amplification of the mutant allele and reliable carrier detection (Fig. 1B). In affected dog DNA, every C nucleotide in the expansion deaminated, ruling out expansion methylation as a mechanism for the absent mRNA. The actual mechanism may be adoption of RNA polymerase-obstructive secondary hairpin structures by the large GC-pure DNA expansion (*5*).

A single recurring cystatin-B promoter expansion causes most human PME (EPM1), rather than the more than 60 mutations in EPM2 genes. To our knowledge, EPM1 has never been described in dogs, likely because they do not have the cystatin-B dodecamer repeat. EPM2, on the other hand, is regularly reported (basset hounds, miniature and standard poodles, pointers, corgis, beagles, dachshunds, etc.) (*6*), likely because of recurrent *Epm2b* expansion events plus inbreeding.

The presence of the dodecamer repeat across canine breed barriers suggests that its origin predates dogs, and it might therefore be present in related species. Sequencing the *Epm2b* gene in 32 different carnivores (*1*) showed that the repeat is present and polymorphic across *Canidae* (wolves, dogs, foxes, coyotes, jackals, etc.) but not their closest relatives, the *Arctoidae* (bears, raccoons, otters, skunks, etc.). Whether the variable four–amino acid lengthening of the middle portion of malin, the *Epm2b* gene product (Fig. 1A), confers a property to this ubiquitin E3 ligase advantageous to canids remains to be seen. *Arctoidae* have a single copy of the D sequence (Fig. 1A) (*1*), indicating that its duplication occurred sometime between the canid-arctoid split 50 million years ago (Ma) and the appearance of extant canids 10 Ma. The D sequence is missing in *Felidae* (Fig. 1A) (*1*), suggesting it first appeared after the earlier feline divergence 60 Ma (*7*).

We have described a canine epilepsy mutation that represents a tandem repeat expansion outside humans and devised a test to detect and counteract it through controlled breeding. Affected animals outnumber human EPM2 patients and afford us valuable experience in treating this disease. Additional searches for large coding repeats may reveal other expansions central to inherited diseases.

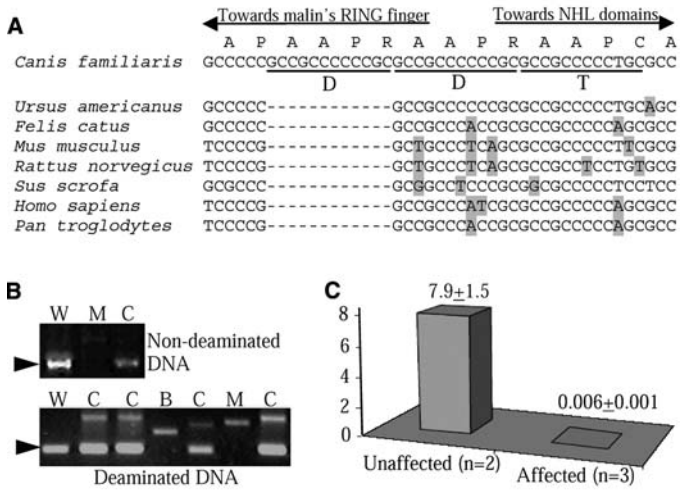

Fig. 1. (**A**) The canine *Epm2b* dodecamer repeat sequence and orthologs. (**B**) The expansion mutation. W, wild-type; M, affected MWHD; C, carrier; B, affected basset hound; arrowheads, normal alleles; other bands, mutant alleles. (**C**) Muscle *Epm2b* mRNA amounts normalized against *Gapdh* (*1*).

References and Notes

1. Materials and methods are available as supporting material on *Science* Online.
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Supporting Online Material

www.sciencemag.org/cgi/content/full/307/5706/81/DC1

Materials and Methods, SOM Text, Figs. S1 to S3, References and Notes, Movie S1

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[1]The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada. [2]Wey Referrals, Woking, Surrey GU21 5BP, UK. [3]Stone Lion Veterinary Centre, Wimbledon, London SW19 5AU, UK. [4]Fred Hutchinson Cancer Research Center, Seattle, WA 98109–1024, USA. [5]CNRS Génétique et Développement, 35043 Rennes, France. [6]University of California, San Diego, CA 92093, USA.

*These authors contributed equally to this work.
†To whom correspondence should be addressed. E-mail: bminass@sickkids.ca