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# A Truncated Retrotransposon Disrupts the *GRM1* Coding Sequence in Coton de Tulear Dogs with Bandera's Neonatal Ataxia

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**Background:** Bandera's neonatal ataxia (BNAt) is an autosomal recessive cerebellar ataxia that affects members of the Coton de Tulear dog breed.

**Objective:** To identify the mutation that causes BNAt.

**Animals:** The study involved DNA from 112 Cotons de Tulear (including 15 puppies with signs of BNAt) and 87 DNA samples from dogs of 12 other breeds.

**Methods:** The BNAt locus was mapped with a genome-wide association study (GWAS). The coding exons of positional candidate gene *GRM1*, which encodes metabotropic glutamate receptor 1, were polymerase chain reaction (PCR)-amplified and resequenced. A 3-primer PCR assay was used to genotype individual dogs for a truncated retrotransposon inserted into exon 8 of *GRM1*.

**Results:** The GWAS indicated that the BNAt locus was in a canine chromosome 1 region that contained candidate gene *GRM1*. Resequencing this gene from BNAt-affected puppies indicated that exon 8 was interrupted by the insertion of a 5′-truncated retrotransposon. All 15 BNAt-affected puppies were homozygous for the insert, whereas all other Cotons de Tulear were heterozygotes (n = 43) or homozygous (n = 54) for the ancestral allele. None of the 87 dogs from 12 other breeds had the insertion allele.

**Conclusions and Clinical Importance:** BNAt is caused by a retrotransposon inserted into exon 8 of *GRM1*. A DNA test for the *GRM1* retrotransposon insert can be used for genetic counseling and to confirm the diagnosis of BNAt.

**Key words:** Cerebellar ataxia; Genome-wide association study; Metabotropic glutamate receptor 1; Synaptic plasticity.

Bandera's neonatal ataxia (BNAt) (Bandera was the name of one of the first affected puppies to be clinically evaluated), also known as Bandera's syndrome and neonatal cerebellar ataxia, is a previously described autosomal recessive disease of Coton de Tulear dogs.[1] The ataxia of affected Coton de Tulear puppies became apparent as soon as their littermates developed coordinated movements. The affected puppies exhibited titubation of the head and intention tremors. Most were unable to walk but could scoot in sternal recumbency as a means of purposeful locomotion. This locomotion often was interrupted when the pups fell to lateral recumbency with subsequent paddling and decerebellate posturing. Spinal reflexes were intact, but righting reflexes were delayed and proprioceptive positioning was severely decreased or absent. Affected puppies had vision but lacked a menace response and exhibited fine vertical ocular tremors at rest and saccadic dysmetria together with an upbeat nystagmus during dorsal positioning. Repeated neurologic examinations failed to show either progression or remission of clinical signs.[1]

**Abbreviations:**

| | |
|---|---|
| BNAt | Bandera's neonatal ataxia |
| CFA1 | canine chromosome 1 |
| GWAS | genome-wide association study |
| LINE | long interspersed nuclear element |
| mGluR1 | metabotropic glutamate receptor 1 |
| SINE | short interspersed nuclear element |
| SNPs | single-nucleotide polymorphisms |

Analysis of affected puppies by CBC, serum biochemistry, and urinalysis produced normal results.[1] Brain images produced by computed tomography and magnetic resonance imaging also were normal, as were electromyography results, motor nerve conduction parameters, and auditory brainstem-evoked responses. Cerebrospinal fluid samples from 2 affected pups were analyzed and 1 had slightly increased protein concentrations. Although postmortem examination of the brains from affected puppies failed to identify any gross or light microscopic abnormalities, ultrastructural abnormalities were observed in the molecular layer of the cerebellum from a 4-month-old affected Coton de Tulear. Compared with an age-matched control, the affected cerebellar molecular layer contained a decreased number of presynaptic parallel fiber varicosities and an increased number of Purkinje cell dendritic spines containing postsynaptic densities unpaired with presynaptic terminals. Pedigree analysis suggested an autosomal recessive mode of inheritance for BNAt.[1] An apparently distinct, later-onset cerebellar ataxia of Coton de Tulear puppies with granule cell degeneration also has been described.[2]

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**Table 1.** PCR primer sequences and amplicon lengths

| Target | Forward Primer/Reverse Primer Sequences | Amplicon Length (bp) |
|---|---|---|
| Exon 2—5′-end | GTCGGGCATCTGTCTCGGTT/ACACTTCCTCTCGGGCACCT | 302 |
| Exon 2—middle | CAGAATGGACGGAGATGTCATCA/TCACTCCCGCAATAGGCTT | 362 |
| Exon 2—3′-end | AGGGACTCTCTGATTTCCATTCG/ATCATGCAACGGACTCGCTTT | 387 |
| Exon 3 | CAGATCTTCCTTCAGTTCCAGT/TAGAAACATATGTGGGCCAA | 490 |
| Exon 4 | ATGCTGAGATGTATTTGTGAGAC/AACAAGTTGCAATGGGGCTA | 366 |
| Exon 5 | GAGACCCCTGAAAAGCATC/TTGCCATCTGTCTAGCTCA | 402 |
| Exon 6 | ATTCTAGCTTTTATCTCCGTTC/CTTTACATAGGCCCATGCAAC | 355 |
| Exon 7 | CAGAGACCATCTTTAAAGGCAT/GCATATTGAGAAGGCCCAT | 227 |
| Exon 8—5′-end | CATTTGGCTTCATGATGGGTA/GCACACTAATCAGAATTGAGG | 500 |
| Exon 8—middle | ACCAACCGAATTGCACGCATC/CCAGGGCCACCGTTACACTGA | 448 |
| Exon 8—3′-end | AAGCAACAACCAGCTGTGCAT/ATGCACAGCTGGTTGTTGCTT | 462 |
| Exon 9 | ACTTTGGGAGCAACTACAAGA/TTCCTACGGTAAGAAAATGTCA | 398 |
| Exon 10 | CACGTGTATAACCACTCACTC/TGGCACGTAAATGGTTCCTC | 263 |
| Exon 11—5′-end | GGGGATCAAATTGTATTGAGC/GTCTCGTCTGTGGTCAGGT | 349 |
| Exon 11—middle | CCTCCCAGCAGCCCTTCCATGGT/TCCGTGTTCCCCTCCCGCTCGTA | 444 |
| Exon 11—3′-end | CTGCAGCTGAGCACCTTCG/CAGCTGCCCGCACACCTCC | 421 |

PCR, polymerase chain reaction.

We here report that a genome-wide association study (GWAS) found strong associations between the BNAt phenotype and single-nucleotide polymorphisms (SNPs) on canine chromosome 1 (CFA1). In addition, we report the resequencing of positional candidate gene *GRM1*, which showed that BNAt-affected Coton de Tulear puppies were homozygous for a 62-bp truncated retrotransposon insert in exon 8.

## Materials and Methods

DNA was isolated from EDTA blood samples from 112 Cotons de Tulear and from 87 randomly selected representatives of 12 other dog breeds as described previously.[3] The blood samples were shipped to the University of Missouri between 1996 and 2010, and the isolated DNA was stored frozen as part of the University of Missouri Animal DNA Repository. Although the Coton de Tulear samples were submitted for a variety of reasons, the majority of them were sent because they or their close relatives exhibited clinical signs of BNAt.

A case control GWAS was performed with a genotyping assay[a] and DNA samples from 12 BNAt-affected cases and 12 adult Coton de Tulear control dogs. Clinically normal siblings or other close relatives of the affected dogs were selected as controls to minimize population stratification. Associations between SNP alleles and phenotypes were calculated under an autosomal recessive model of inheritance as described previously.[4] Permutation analysis with 10,000 label-swapping permutations was used to estimate genome-wide significances (http://pngu.mgh.harvard.edu/~purcell/plink/).

DNA samples from normal Cotons de Tulear and from BNAt-affected puppies were polymerase chain reaction (PCR)-amplified with primers flanking the coding regions and intron-exon junctions of consecutive *GRM1* exons from exon 2, which contains the initiator methionine codon, to exon 11, which contains the termination codon. Table 1 lists the PCR primer sequences and the expected sizes of the resulting amplicons. The PCR amplifications were done with a kit[b] and the thermocycling consisted of 40 cycles of 95°C for 30 seconds, 60°C for 30 seconds, followed by an additional 30 seconds at 72°C. The amplicons were purified with a spin column[c] and sequenced in both directions with an automated DNA sequencer.[d] DNA sequence analysis software[e] was used to compare the nucleotide sequences from individual dogs to one another and to build 2.1 of the NCBI canine reference sequence (http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9615).

We initially attempted to genotype individual DNA samples with respect to a 62-bp exon 8 insertion by PCR amplification with flanking primers 5′-ACCAACCGAATTGCACGCATC-3′ (sense primer 1) and 5′-CCAGGGCCACCGTTACACTGA-3′ (antisense primer 1). As expected, these primers produced a single 510-bp amplicon with DNA from BNAt-affected puppies and a 448-bp amplicon with DNA from unrelated control dogs, but only the 448-bp amplicon was detected when DNA samples from obligate carriers were analyzed. When the sense primer 1 was replaced with 5′-GCCTTCAAGACCCGCAACGTAAAA-3′ (sense primer 2, designed to detect only the insertion allele), PCR amplification produced a single 234-bp amplicon with DNA from affected dogs and obligate carriers, but no product was observed when DNA samples from unrelated normal dogs were analyzed. By adjusting primer concentrations, we were able to include all 3 primers in a PCR assay that unambiguously identified each dog's genotype (Figs 1, 2). For this assay, the 25 μL PCR reaction mixtures contained 2.5 mM $MgCl_2$, 0.215 mM each of the 4 dNTPs, betaine,[f] 1× PCR buffer[b] with antibody-complexed Taq DNA polymerase,[b] 0.56 μM sense primer 1, 0.56 μM antisense primer 1, and 3.2 μM sense primer 2. Forty cycles of 95°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds were used for amplification. The resulting amplicons were evaluated with a microcapillary electrophoresis system.[g]

## Results

The GWAS produced the strongest associations between the BNAt phenotype and SNPs on CFA1 (Fig 3) although the peak association did not approach genome-wide significance when corrected for multiple testing ($P = .242$). Nonetheless, inspection of the individual CFA1 genotypes showed that all 12 affected dogs were homozygous for the same 39-marker haplotype between BICF2G630721382 (http://www.broadinstitute.org/ftp/pub/papers/dog_genome/) at 40,068,882 bp (numbered as in the NCBI canine genome reference sequence build 2.1 [http://www.ncbi.nlm.nih.gov/projects/mapview/map_search.cgi?taxid=9615]) and BICF2G630721833 at 40,737,343 bp, whereas none of the control dogs were homozygous for

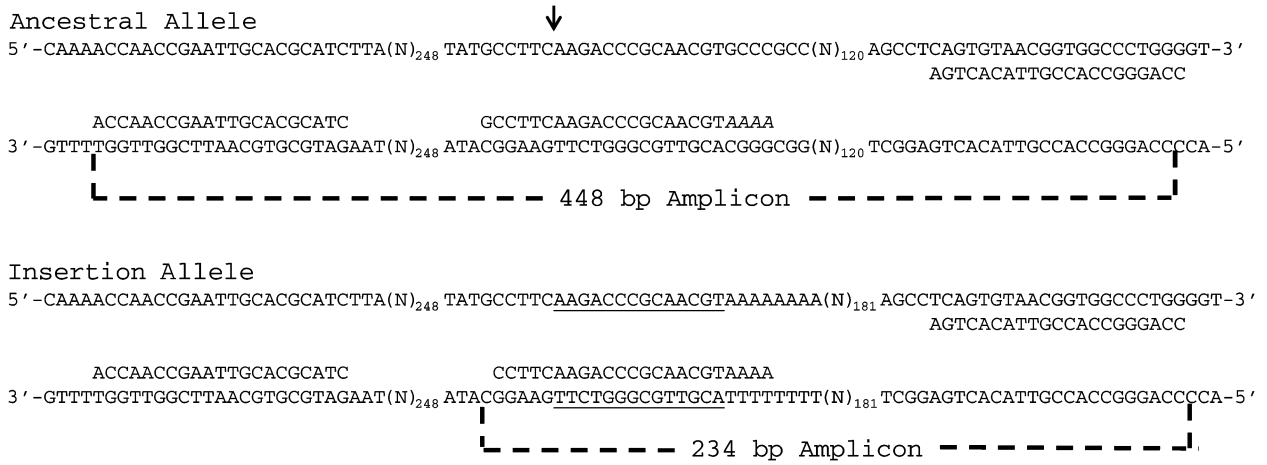


**Fig 1.** Sequences of the sense and antisense DNA strands of the *GRM1* ancestral allele (top) show the annealing sites for the 3 polymerase chain reaction (PCR) primers in the genotyping assay. Sense primer 1 (left) produces a 448-bp PCR amplicon with antisense primer 1 (right). A 4-bp mismatch shown in italics at the 3′-end of sense primer 2 (middle) prevents it from producing an amplicon from the ancestral allele. Arrow shows the insertion site for the retrotransposon. Sequences of the sense and antisense DNA strands of the *GRM1* mutant allele (bottom) show the annealing sites for the 3 PCR primers. Sense primer 2 is completely complementary to a segment of the mutant allele allowing it to produce a 234-bp amplicon with antisense primer 1. The inserted 14-bp direct repeat is underlined.

this haplotype. Four BNAt-affected dogs were heterozygous at the centromeric flanking marker BICF2P1285497 at 40,058,600 bp and 1 affected dog was heterozygous at the telomeric flanking marker BICF2G630721843 at 40,771,282 bp. These markers restricted the BNAt locus to a 713 kb region that contained part or all of 4 annotated genes: *FBXO30*, *SHPRH*, *GRM1*, and *RAB32*.

We resequenced the coding regions and intron-exon borders of all 10 *GRM1* coding exons (exons 2 through 11) from 2 BNAt-affected Cotons de Tulear. Comparison of these sequences to the NCBI canine genome reference sequence indicated sequence variation at 4 sites. Two

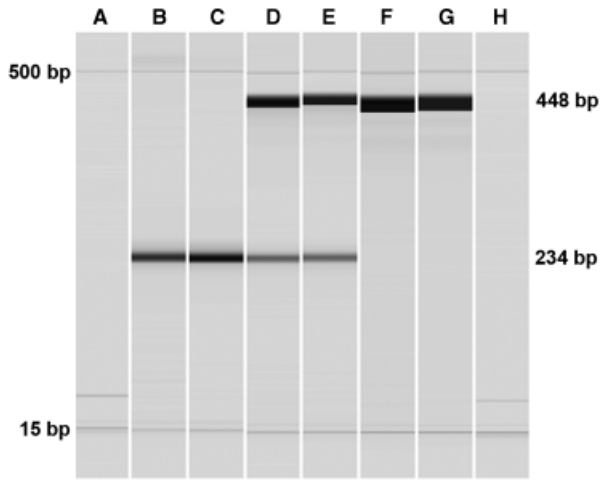


**Fig 2.** Microcapillary electrophoretograms of amplicons produced with the 3-primer assay from mutant-allele homozygotes (lanes B and C), heterozygotes (lanes D and E), and homozygotes for the ancestral allele (lanes F and G). Lanes A and H are no-template controls. The bands from the 15- and 500-bp internal molecular weight markers are labeled on the left and the bands from the 448- and 234-bp amplicons are labeled on the right.

single-base substitutions in exon 5, c.1227G>A (numbered as in GenBank accession XM_845272.1) and c.1305C>T, were synonymous mutations. A single-base substitution in exon 9, c.3369G>C, alters the predicted amino acid sequence at position p.1123 from glutamic acid to aspartic acid. We resequenced *GRM1* exon 9 with DNA from adult Cotons de Tulear and identified healthy individuals that were c.3396C homozygotes.

The 4th sequence variant was a 62-bp adenosine-rich insertion in exon 8 between c.2316 and c.2317 (Fig 4). The 1st 14 bp of the insertion was an exact copy of the 14 bp that followed the insertion and the motif for 6 consecutive bases starting 2 bp 5′- to the insertion site was TC:AAGA (http://www.broadinstitute.org/ftp/pub/papers/dog_genome/) which is similar to the TT:AAAA consensus substrate motif for the long interspersed nuclear element 1 (LINE1) endonuclease (Fig 4).[5] We used a 3-primer PCR assay (Figs 1, 2) to genotype each of the 112 Coton de Tulear DNA samples in our collection for this insertion. All 15 Coton de Tulear puppies with BNAt-like clinical signs tested homozygous for the 62-bp insertion allele, whereas the remaining 97 samples from clinically normal Cotons de Tulear either were heterozygous (n = 43) or homozygous for the ancestral allele (n = 54). There was a highly significant association between homozygosity for the insertion allele and BNAt ($P = 6.4 \times 10^{-19}$; Fisher's exact test). Among all Cotons de Tulear represented in our DNA collection, the insertion allele frequency was 0.33. We also genotyped 87 DNA samples from dogs of 12 other breeds and found that all were homozygous for the ancestral allele.

## Discussion

A 12-case and 12-control GWAS restricted the BNAt locus to a 0.7 Mb region on CFA1. Among the 4 annotated genes within this region, *GRM1* was considered to

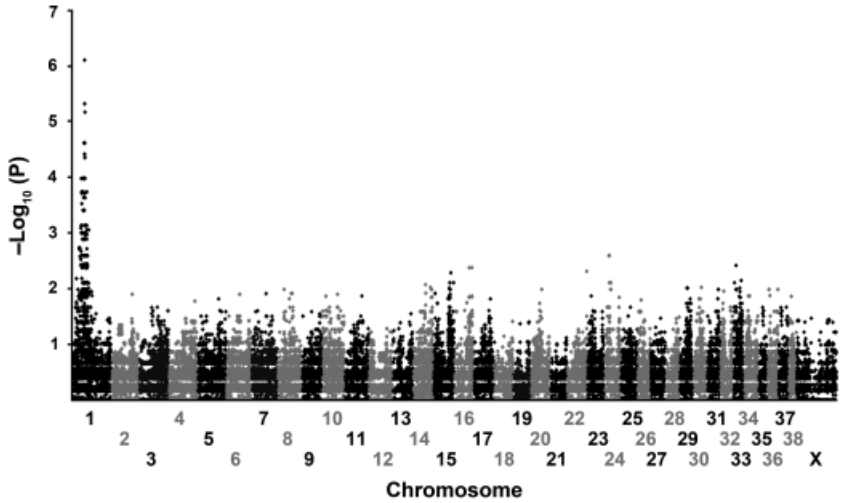


**Fig 3.** A Manhattan plot of $-\mathrm{Log}_{10}(P)$ values from a 12 case × 12 control genome-wide association study for the Bandera's neonatal ataxia locus calculated under a recessive model of inheritance.

be the most likely candidate to contain the BNAt-causing mutation because naturally occurring and experimentally induced *Grm1* deficiencies produced disease phenotypes in mice that resembled that of the BNAt-affected canine puppies.[6–9] Resequencing *GRM1* from affected dogs identified a 62-bp adenosine-rich insertion in exon 8 that appears to be the cause of BNAt. The 15 DNA samples in our collection from BNAt-affected Coton de Tulear puppies was all homozygous for this insertion, whereas none of our 97 DNA samples from unaffected Cotons de Tulear was homozygous for the insertion allele.

The adenosine-rich portion of the *GRM1* exon 8 insertion is flanked by a 14-bp direct repeat and the TC:AAGA insertion site motif is found commonly at retrotransposon insertion sites in human and murine DNA and differs only slightly from the TT:AAAA consensus substrate motif for LINE1 endonuclease.[5] Thus, it is very likely that the 62-bp insertion is a 5′-truncated retrotransposon inserted by LINE1 endonuclease/transposonase.[10] This enzyme can facilitate the retrotransposition not only of LINE1 transcripts but also transcripts of short interspersed nuclear elements (SINES) and messenger RNAs.[11] At least 6 other heritable canine diseases have been attributed to retrotransposon insertions (Table 2).[12–17] All 6 of these retrotransposons were 5′-truncated, and only 5 of them had sufficient sequence preceding the poly-A tail to identify their origin as being from a LINE or SINE. On the other hand, the retrotransposon in canine *GRM1* is more severely 5′-truncated and the only recognizable structure besides the poly-A tail is a potential poly-A signal (AATAAA),[18] making it impossible to determine if the insert is from the 3′-end of a LINE, SINE, or retrogene. An even shorter retrotransposon was reported to cause cone-rod retinal dystrophy in Miniature Longhaired Dachshunds[16] and other breeds[19] and a similar truncated retrotransposon inserted in the antisense orientation into intron 3 of *SPINK1* in Miniature Schnauzers and may be a risk factor for pancreatitis.[20]

Like the BNAt-affected puppies, mice with spontaneous and induced *GRM1* mutations exhibit intention tremors and cerebellar ataxia.[6–8] These mutant mice appear to have normal gross and microscopic neuroanatomy except for subtle abnormalities in the molecular layer of the cerebellum which may result from incomplete developmental pruning of the multiple climbing fibers that innervate Purkinje cells in the neonate.[21,22] Similar pathology may explain the subtle ultrastructural abnormalities reported in the cerebellar molecular layer from a 4-month-old puppy with BNAt.[1]

Electrophysiologic experiments with brain sections from *Grm1* knockout mice have demonstrated deficiencies in long-term depression in the cerebellum and deficiencies in long-term potentiation in the hippocampus.[6,7,23] Long-term depression and long-term potentiation are widely believed to be the bases for learning and memory.[24,25] The

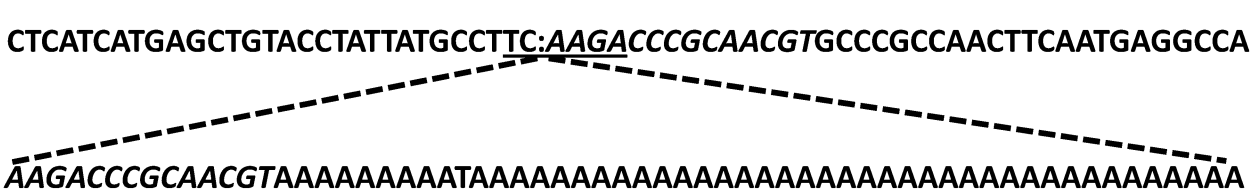


**Fig 4.** The ancestral canine *GRM1* sequence from c.2287 to c.2353 is shown at the top. The colon between c.2316 and c.2317 marks the insertion site. The 6-bp long interspersed nuclear element endonuclease substrate motif is underlined, the 62-bp insertion sequence is shown at the bottom, and the 14-bp direct repeats are shown in italics.

**Table 2.** Heritable canine diseases attributed to retrotransposon insertions

| Disease | Gene | Retrotransposon | Insertion Site | Affected Dog Breed |
|---|---|---|---|---|
| Narcolepsy[12] | *HCRTR2* | SINE | Intron 3 | Doberman Pincher |
| Hemophilia B[13] | *F9* | LINE | Intron 5 | German Wirehair Pointer |
| Centronuclear myopathy[14] | *PTPLA* | SINE | Exon 2 | Labrador Retriever |
| Merle Coat syndrome[15] | *SILV* | SINE | Exon 11 | Several breeds |
| Cone-rod retinal dystrophy[16] | *RPGRIP1* | Unknown | Exon 2 | Miniature Dachshund |
| Lamellar icthyosis[17] | *TGM1* | LINE | Intron 9 | Jack Russell Terrier |

LINE, long interspersed nuclear element; SINE, short interspersed nuclear element.

diminished conditioned eye blink and decreased adaptability of horizontal optokinetic responses observed in G*RM1* knockout mice may be in vivo manifestations of their decreased cerebellar long-term depression[6,26,27] and are likely related to the lack of a menace response observed in BNAt-affected puppies.[1] Further learning and memory deficits in the G*rm1* knockout mice were detected by the context-dependent fear conditioning test, the Morris hidden platform test, the social transmission of food preference test, the novel-object—recognition memory test, and the prepulse inhibition of the startle response test.[7,26,28] Similar deficits in BNAt-affected puppies may have gone unrecognized because of their severe ataxia or because they were euthanized at an early age. More extensive examination of the BNAt disease phenotype in individuals maintained to an older age could lead to new insights about the role of metabotropic glutamate receptor 1 (mGluR1) in learning and memory. Furthermore, careful evaluation of heterozygous BNAt carriers may identify subtle behavioral changes because of *GRM1* haploinsufficiency.

Our finding that a mutation in canine *GRM1* can cause neonatal cerebellar ataxia in dogs is consistent with earlier reports that neonatal cerebellar ataxias in mice have resulted from *GRM1* mutations.[8,9] These findings suggest that mutations in human *GRM1* may be responsible for some of the human nonprogressive cerebellar ataxias of unknown etiology.[29] Nonetheless, no *GRM1* mutations have been reported so far in DNA from ataxic infants.[30] On the other hand, rare cases of adult-onset subacute cerebellar ataxia have been reported in patients with paraneoplastic or autoimmune anti-mGluR1 autoantibodies in their cerebral spinal fluid and blood plasma.[31,32] Passive transfer of some of these human anti-mGluR1 antibodies has evoked transient cerebellar ataxia in mice.[31]

We have shown that homozygosity for a truncated retrotransposon inserted into exon 8 of *GRM1* is strongly associated with BNAt and is most likely the cause of this disease. The frequency of the mutant allele in our collection, 0.33, is likely to be much higher than of the overall Coton de Tulear population, because many of our DNA samples were acquired from affected puppies and their close relatives. Nonetheless, the current Coton de Tulear breeding stock may include a substantial number of BNAt carriers. DNA tests for this insertion now can be used to confirm the diagnosis of BNAt in ataxic Coton de Tulear neonates. These tests also can identify heterozygous carriers of the retrotransposon insert for Coton de Tulear breeders wishing to avoid litters with affected puppies. Studies of BNAt-affected puppies could shed light on the roles of mGluR1 in learning and memory.

## Footnotes

[a] CanineHD Whole-genome genotyping kit, Illumina, San Diego, CA
[b] GoTaq enzyme and buffer, Promega, Madison, WS
[c] QIAquick PCR Purification Kit, Qiagen Inc, Alameda, CA
[d] Model 3730xl, Applied Biosystems, Foster City, CA
[e] Sequencher 4.10.1, Gene Codes Corporation, Ann Arbor, MI
[f] MasterAmp PCR Enhancer, Epicentre Biotechnologies, Madison, WI
[g] eGene, now sold by Qiagen Inc

## References

1. Coates JR, O'Brien DP, Kline KL, et al. Neonatal cerebellar ataxia in Coton de Tulear dogs. J Vet Intern Med 2002;16:680–689.

2. Tipold A, Fatzer R, Jaggy A, et al. Presumed immune-mediated cerebellar granuloprival degeneration in the Coton de Tulear breed. J Neuroimmunol 2000;110:130–133.

3. Katz ML, Khan S, Awano T, et al. A mutation in the *CLN8* gene in English Setter dogs with neuronal ceroid-lipofuscinosis. Biochem Biophys Res Commun 2005;327:541–547.

4. Farias FH, Johnson GS, Taylor JF, et al. An *ADAMTS17* splice donor site mutation in dogs with primary lens luxation. Invest Ophthalmol Vis Sci 2010;51:4716–4721.

5. Jurka J. Sequence patterns indicate an enzymatic involvement in integration of mammalian retroposons. Proc Natl Acad Sci USA 1997;94:1872–1877.

6. Aiba A, Kano M, Chen C, et al. Deficient cerebellar long-term depression and impaired motor learning in mGluR1 mutant mice. Cell 1994;79:377–388.

7. Conquet F, Bashir ZI, Davies CH, et al. Motor deficit and impairment of synaptic plasticity in mice lacking mGluR1. Nature 1994;372:237–243.

8. Conti V, Aghaie A, Cilli M, et al. *crv4*, a mouse model for human ataxia associated with kyphoscoliosis caused by an mRNA splicing mutation of the metabotropic glutamate receptor 1 (*Grm1*). Int J Mol Med 2006;18:593–600.

9. Sachs AJ, Schwendinger JK, Yang AW, et al. The mouse mutants *recoil wobbler* and *nmf373* represent a series of *Grm1* mutations. Mamm Genome 2007;18:749–756.

10. Moran JV, Holmes SE, Naas TP, et al. High frequency retrotransposition in cultured mammalian cells. Cell 1996;87:917–927.

11. Esnault C, Maestre J, Heidmann T. Human LINE retrotransposons generate processed pseudogenes. Nat Genet 2000; 24:363–367.

12. Lin L, Faraco J, Li R, et al. The sleep disorder canine narcolepsy is caused by a mutation in the *hypocretin (Orexin) receptor 2* gene. Cell 1999;98:365–376.

13. Brooks MB, Gu W, Barnas JL, et al. A Line 1 insertion in the factor IX gene segregates with mild hemophilia B in dogs. Mamm Genome 2003;14:788–795.

14. Pelé M, Tiret L, Kessler JL, et al. SINE exonic insertion in the *PTPLA* gene leads to multiple splicing defects and segregates with the autosomal recessive centronuclear myopathy in dogs. Hum Mol Genet 2005;14:1417–1427.

15. Clark LA, Wahl JM, Rees CA, Murphy KE. Retrotransposon insertion in *SILV* is responsible for merle patterning of the domestic dog. Proc Natl Acad Sci USA 2006;103:1376–1381.

16. Mellersh CS, Boursnell ME, Pettitt L, et al. Canine *RPGRIP1* mutation establishes cone-rod dystrophy in Miniature Longhaired Dachshunds as a homologue of human Leber congenital amaurosis. Genomics 2006;88:293–301.

17. Credille KM, Minor JS, Barnhart KF, et al. Transglutaminase 1-deficient recessive lamellar ichthyosis associated with a LINE-1 insertion in Jack Russell Terrier dogs. Br J Dermatol 2009;161:265–272.

18. Colgan DF, Manley JL. Mechanism and regulation of mRNA polyadenylation. Genes Dev 1997;11:2755–2766.

19. Miyadera K, Kato K, Aguirre-Hernández J, et al. Phenotypic variation and genotype-phenotype discordance in canine cone-rod dystrophy with an *RPGRIP1* mutation. Mol Vis 2009;15:2287–2305.

20. Bishop MA, Xenoulis PG, Levinski MD, et al. Identification of variants of the *SPINK1* gene and their association with pancreatitis in Miniature Schnauzers. Am J Vet Res 2010;71: 527–533.

21. Kano M, Hashimoto K, Kurihara H, et al. Persistent multiple climbing fiber innervation of cerebellar Purkinje cells in mice lacking mGluR1. Neuron 1997;18:71–79.

22. Kano M, Hashimoto K. Synapse elimination in the central nervous system. Curr Opin Neurobiol 2009;19:154–161.

23. Aiba A, Chen C, Herrup K, et al. Reduced hippocampal long-term potentiation and context-specific deficit in associative learning in mGluR1 mutant mice. Cell 1994;79:365–375.

24. Citri A, Malenka RC. Synaptic plasticity: Multiple forms, functions, and mechanisms. Neuropsychopharmacology 2008;33: 18–41.

25. Kotaleski JH, Blackwell KT. Modelling the molecular mechanisms of synaptic plasticity using systems biology approaches. Nat Rev Neurosci 2010;11:239–251.

26. Kishimoto Y, Fujimichi R, Araishi K, et al. mGluR1 in cerebellar Purkinje cells is required for normal association of temporally contiguous stimuli in classical conditioning. Eur J Neurosci 2002;16:2416–2424.

27. Shutoh F, Katoh A, Kitazawa H, et al. Loss of adaptability of horizontal optokinetic response eye movements in mGluR1 knockout mice. Neurosci Res 2002;42:141–145.

28. Brody SA, Conquet F, Geyer MA. Disruption of prepulse inhibition in mice lacking mGluR1. Eur J Neurosci 2003;18:3361–3366.

29. Steinlin M, Zangger B, Boltshauser E. Non-progressive congenital ataxia with or without cerebellar hypoplasia: A review of 34 subjects. Dev Med Child Neurol 1998;40:148–154.

30. Rossi PIA, Vaccari CM, Terracciano A, et al. The metabotropic glutamate receptor 1, *GRM1*: Evaluation as a candidate gene for inherited forms of cerebellar ataxia. J Neurol 2010;257: 598–602.

31. Sillevis Smitt P, Kinoshita A, De Leeuw B, et al. Paraneoplastic cerebellar ataxia due to autoantibodies against a glutamate receptor. N Engl J Med 2000;342:21–27.

32. Marignier R, Chenevier F, Rogemond V, et al. Metabotropic glutamate receptor type 1 autoantibody-associated cerebellitis: A primary autoimmune disease? Arch Neurol 2010;67:627–630.